SoIPa

SOCIETÀ ITALIANA DI PARASSITOLOGIA

XXVIII CONGRESSO NAZIONALE

ROMA, 24-27 giugno 2014



LETTURE
RELAZIONI DEI SIMPOSI
COMUNICAZIONI SCIENTIFICHE

Casa dell'Aviatore, Viale dell'Università, 20 - Roma

INDICE

PRESENTAZIONE CONGRESSO 3
NOTA EDITORIALE 5
COMITATO ORGANIZZATORE - COMITATO SCIENTIFICO 7
GENERAL INFORMATION 8
SPONSORS 10
PROGRAMMA IN SINTESI 11
PROGRAMMA DETTAGLIATO 13
CERIMONIA INAUGURALE 33
SIMPOSIO 1 39
SIMPOSIO 2 49
SIMPOSIO 3 63
SIMPOSIO 4 71
TAVOLA ROTONDA 1 79
TAVOLA ROTONDA 2 81
SESSIONE PARALLELA O-01 91
SESSIONE PARALLELA O-02 109
SESSIONE PARALLELA O-03 123
SESSIONE PARALLELA O-04 133
SESSIONE PARALLELA O-05 147
SESSIONE PARALLELA O-06 161
SESSIONE PARALLELA O-07 175
SESSIONE PARALLELA O-08 187
SESSIONE PARALLELA O-09 203
SESSIONE PARALLELA O-10 219
SESSIONE PARALLELA O-11A 237
SESSIONE PARALLELA O-11B 247
SESSIONE PARALLELA O-12 255
POSTER 01 269
POSTER 02 281
POSTER 03 289
POSTER 04 293
POSTER 05 303
POSTER 06 311
POSTER 07 325
POSTER 08 345
POSTER 09 359
POSTER 10 371
POSTER 11A 383
POSTER 11B 389
POSTER 12 393
AUTHOR INDEX 401

PRESENTAZIONE CONGRESSO

Il Congresso Nazionale della Società Italiana di Parassitologia torna, dopo 16 anni, a Roma, principale sede di attività di ricerca degli scienziati che, nel lontano 1959, fondarono la Società: E. Biocca, A. Corradetti e O. Starkoff. In 55 anni siamo giunti alla XXVIII edizione, organizzata dai parassitologi del Dip. di Sanità Pubblica e Malattie Infettive dell'Università "Sapienza" e del Dip. di Medicina Sperimentale e Chirurgia dell'Università "Tor Vergata". L'evento è preceduto da un "Corso di base sull'analisi di dati genetico-molecolari" che desidera offrire strumenti teorici di base ed opportunità pratiche per analizzare dati genetico-molecolari.

Il Congresso si svolge, nel solco della tradizione della SoIPa, nell'ottica "One Health" e, per promuovere il dialogo tra comunità scientifica, istituzioni e personale sanitario, coinvolge nella discussione delle varie tematiche anche esponenti di Food Agricultural Organization, Comunità Europea, Ministero Affari Esteri, Ministero dell'Istruzione, Università e Ricerca, Istituto Superiore di Sanità, Istituti Zooprofilattici, Società Italiana di Medicina Veterinaria Preventiva, Società Italiana di Medicina Tropicale, Società Italiana di Medicina delle Migrazioni, Società Spagnola e Società Serba di Parassitologia, Medici e Veterinari Senza Frontiere, Caritas e Fondazione de Carneri.

Sono state organizzate sessioni tematiche, simposi, sessioni plenarie e workshops in un programma che ha tenuto in considerazione gli interessi più attuali del settore, per aggiornare le diverse professionalità su epidemiologia, ecologia, patogenesi, rapporto parassita-ospite, genetica e biologia molecolare, filogenesi, genomica e proteomica, trasmissione, diagnosi e sviluppo di nuovi farmaci.

Spero che il Congresso risponda alle aspettative di ciascuno di noi e rappresenti una opportunità per scambiare esperienze e avviare nuove collaborazioni.

Ringrazio i componenti del Comitato Organizzatore per l'impegno e l'entusiasmo profuso nel collaborare. A nome di tutti sono grata sia al Comitato Direttivo della Società italiana di Parassitologia per aver promosso la partecipazione di giovani ricercatori, sia a coloro che a diverso titolo e in varia misura hanno contribuito alla organizzazione di questa edizione del Congresso Nazionale. Ma ancor più mi preme ringraziare tutti i Partecipanti a questo evento per la loro entusiastica risposta all'invito di adesione (oltre ai soci, anche circa 60 non-soci SoIPa), ulteriormente dimostrata attraverso l'invio di numerosissimi abstracts che ci hanno consentito di organizzare gruppi di lavoro di estremo interesse.

Il Presidente del Comitato Organizzatore
Prof.ssa Gabriella Cancrini



NOTA EDITORIALE

La cerimonia inaugurale del XXVIII Congresso Nazionale della Società Italiana di Parassitologia si apre con l'annuncio dell'istituzione di due Fondi Storici attivati presso l'unità di ricerca di Storia della Medicina di "Sapienza", che raccolgono le opere dei professori Biocca e Coluzzi come riconoscimento dell'eredità scientifica e culturale lasciata da questi due scienziati che hanno fortemente caratterizzato la Parassitologia romana. Dopo un breve ricordo dell'opera del prof. Mario Coluzzi, la cerimonia si conclude con la lettura magistrale del prof. Bruno Gottstein su "Immunomodulation of host immunity by larval helminths".

Il programma scientifico del Congresso, cui partecipano anche molti altri illustri ospiti stranieri, si articola in quattro Simposi, due Tavole Rotonde e tredici Sessioni di comunicazioni scientifiche e posters.

I Simposi, realizzati attraverso trentuno relazioni su invito, rappresentano un aggiornamento sui seguenti argomenti:

Hot spots in medical & veterinary entomology: from Italy to Europe

Italian Malaria Network

Parassitosi cardio-polmonari del cane e del gatto: uno scenario in continua evoluzione

Zoonosi da alimenti: un approccio globale e nuove prospettive

Nelle Tavole Rotonde, alle quali -oltre a tutti i presenti- sono stati invitati a partecipare venti esperti di settori diversi, si discute a proposito di due temi molto attuali: "La parassitologia italiana nella Cooperazione internazionale" e "Migrazioni e tutela sanitaria", di cui restano tracce nei riassunti di otto relazioni programmate.

In base agli abstracts pervenuti, relativi a 143 comunicazioni orali e 97 posters, sono state organizzate le seguenti tredici sessioni scientifiche:

01. Infestazioni da elminti (15 comunicazioni orali e 10 posters)
02. Parassitosi trasmesse da artropodi (12 comunicazioni orali e 6 posters)
03. Farmacoresistenza (8 comunicazioni orali e 1 poster)
04. Controllo di parassitosi (12 comunicazioni orali e 7 posters)
05. Entomologia medica e veterinaria (12 comunicazioni orali e 4 posters)
06. Parassiti della fauna acquatica (11 comunicazioni orali e 11 posters)
07. Biologia molecolare e filogenesi applicate a parassiti e vettori (10 comunicazioni orali e 17 posters)
08. Infezioni da protozoi (13 comunicazioni orali e 11 posters)
09. Controllo e monitoraggio di vettori (13 comunicazioni orali e 9 posters)
10.Parassiti della fauna selvatica (15 comunicazioni orali e 10 posters)
11a - Infezioni da miceti (7 comunicazioni orali e 4 posters)
11b - Immunità e immunopatologia delle infezioni parassitarie (5 comunicazioni orali e 1 poster)
12. Parassiti degli animali da compagnia e rischio zoonosico (10 comunicazioni orali e 6 posters)

Il presente volume raccoglie, oltre alla lettura magistrale e ai riassunti delle relazioni presentate alla cerimonia inaugurale e ai simposi, gli abstracts delle comunicazioni orali e dei posters. Il materiale viene presentato sia in ordine di successione temporale che per sede di svolgimento. Quindi, per ogni giorno si riporta il programma che si svolgerà in ciascuna delle tre sale Congressuali.

A nome del Comitato Organizzatore ringrazio il Comitato Direttivo SoIPa e tutti i soci per aver consentito a "Sapienza" e "Tor Vergata" di organizzare il XXVIII Congresso della Società Italiana di Parassitologia

Il Presidente del Comitato Organizzatore
Prof.ssa Gabriella Cancrini



COMITATO ORGANIZZATORE - COMITATO SCIENTIFICO

COMITATO ORGANIZZATORE

Prof.ssa Gabriella Cancrini (Presidente) - "Sapienza", Università di Roma
Bruno Arcà, Pamela Avellino, Federica Berrilli, Beniamino Caputo, Serena Cavallero
Paolo Cipriani, Stefano D'Amelio, Alessandra della Torre, David di Cave, Simona Gabrielli
Fabrizio Lombardo, Emiliano Mancini, Valentina Mangano, Simonetta Mattiucci, David Modiano,
Vincenzo Petrarca, Marco Pombi, Rita Romano, Federica Santolamazza

COMITATO SCIENTIFICO

Comitato Organizzatore
Moderatori di simposi e sessioni scientifiche

DIRETTIVO SoIPa

Prof. Mario Pietrobelli (Presidente) - Università di Padova
Paola Beraldo - Università di Udine
Ferroglio Ezio - Università di Torino
Antonio Frangipane di Regalbono - Università di Padova
Annunziata Giangaspero - Università di Foggia
Stefano D'Amelio - Università di Roma
Domenico Otranto - Università di Bari
Giovanni Poglayen - Università di Bologna
Simone Cacciò - Istituto Superiore di Sanità
David Di Cave - Università di Roma

SEGRETERIA ORGANIZZATIVA

MV CONGRESSI
Meeting & Association Management

Via Marchesi 26 D - 43126 PARMA - Italia
Tel. +39-0521 290191 Fax +39-0521 291314
www.mvcongressi.it info@mvcongressi.it

GENERAL INFORMATION

POSTER PRESENTATIONS

In **preparing your poster presentation** please use the following guidelines:
• Size: 70cm x 100cm - in portrait orientation
• Written in English
• If figures are included, these should be clearly labelled and legible

Please check in with the onsite Secretariat Registration Desk when you pick up your registration materials. They will direct you to the poster area for your session.
Posters will be displayed in groups by Session. Your poster session and number can be found by checking the Author index.

Authors may begin putting up their posters at the morning of the correspondent session and they will have to take it off at the end of the same day. You can pick up the supplies you need to fix your poster to the boards from the Registration Desk. Also, check with them if you need further assistance regarding your poster.

We strongly suggest that authors be at their posters to discuss their work and answer questions during breaks and lunch on the day of their session.

Printing and transport of the poster is at author's charge and care.

ORAL PRESENTATION

In **preparing your oral presentation** please remember to:
• Use clear and comprehensible language (discussion in Italian, slides in English)
• Create relevant graphs, photos, tables using standard software (OS: windows XP; Software: Microsoft Office Power Point 2000/2003/2007 or OS: Macintosh; Software: Keynote)
• Save slide data (.PPT/ KEYNOTE) on a USB flash memory drive (previously checked using your own PC)
NOTE: There is no system for editing the slide in our session rooms.

Receiving the slide data:
Please bring your USB flash drive to the Technical Desk of the Congress room at least 3 hours before the beginning of the relevant session. A technician will upload your slide presentation and return your flash drive. The copied slides will be erased after each speech.
Please note it is not possible to use your own PC.
Authors must present themselves to the Chairperson of the session at least 10 minutes before the session starts in order to acquaint themselves with the equipment.
Authors are kindly requested to respect the indicate timings in the program.

CERTIFICATE OF ATTENDANCE

A Certificate of Attendance will be given at the end of the Congress. Please ask the Registration Desk.

DISCLAIMER

The Congress Committee reserves the right to change the scientific program at any time without notice.

NAME BADGES

For security purposes, and to facilitate collaboration, delegates, speakers, sponsors, and exhibitors are asked to wear their name ages to all sessions.
If you misplace your name badge, please enquire at the Registration Desk to organize a replacement.

TICKETS

No entrance allowed at social dinner without entrance ticket, if purchased.
Social Dinner Thursday, June 26th

XXVIII Congresso Nazionale SoIPa, Roma 24-27 giugno 2014

GENERAL INFORMATION

PROGRAMME

Every effort has been made to produce an accurate Program. If you are presenting at the Congress, please ensure you double check your presentation time as indicated in this Programme.

REGISTRATION DESK

The Registration Desk is located next to the Main Hall.
It will be open at the following times:
Tuesday, 24 June 2014 15.00 - 18.00
Wednesday, 25 June 2014 8.00 - 17.30
Thursday, 26 June 2014 8.00 - 19.00
Friday, 27 June 2014 8.00 - 13.30

SPEAKERS

Please ensure you are available in the conference room at least 15 minutes before the start of the session. Speakers, please visit your speech room to confirm your audiovisual requirements at least 3 hours prior to the start of your session.

HOW TO REACH CONGRESS VENUE

Casa dell'Aviatore, Viale dell'Università 20 - Rome
Railway Station: Stazione Termini, 500 m.
Subway: Stazione Termini
Bus Stop ATAC: no. 310
Tram Stop ATAC: no.19, no.3

PARKING

The internal Parking is reserved to the Casa dell'Aviatore Members. Next to the venue there are the Policlinico Umberto I Hospital and the Sapienza University Parkings.

SMOKING

Please be advised that smoking is not permitted in any public buildings.

SPECIAL DIETARY REQUIREMENTS

If you have advised the Congress Secretariat of special dietary requirements, please speak to a member of the catering staff at catered events to receive your special meal.

SOCIAL ACTIVITIES

Tuesday, 24 June 2014 - Nobile Accademia dei Farmacisti, Via Miranda 10, Foro Romano
20.00-21.00 Welcome Cocktail
21.00-23.00 Opening Ceremony

Wednesday, 25 June 2014 - Città Universitaria, "Sapienza" University of Rome
20.00-22.00 "Serata enogastronomica Italy"

Thursday,26 June 2014 - Location: Rome
20.00-23.00 Social Dinner

SPONSORS

CON IL PATROCINIO DI

Policlinico Umberto 1°
Fondazione PTV Policlinico Tor Vergata
Ordine provinciale di Roma dei Medici Chirurghi e degli Odontoiatri (OMCEO)
Ordine dei Medici Veterinari della Provincia di Roma
Ordine Nazionale dei Biologi (ONB)
Federazione Nazionale degli Ordini Veterinari Italiani (FNOVI)
Roma Capitale

MAJOR SPONSORS

ALTRI SPONSORS

EDILMON

MERIDIAN BIOSCIENCE EUROPE

TECNOCHIMICA MODERNA

PROGRAMMA COLPO D'OCCHIO

XXVIII CONGRESSO NAZIONALE SOIPA			
Martedì 24 giugno 2014			
15.00-18.00	*Registrazioni (Casa dell'Aviatore)*		
20.00-21.00	*Cocktail di benvenuto (Nobile Accademia dei Farmacisti, Foro Romano)*		
21.00-23.00	*Cerimonia inaugurale (Nobile Accademia dei Farmacisti, Foro Romano)*		
Mercoledì 25 giugno 2014			
	SALA BARACCA	SALA SOCI	SALA BALBO
08.30-11.00	**O1: Infestazioni da elminti**	**O2: Parassitosi trasmesse da artropodi**	**O3: Farmacoresistenza**
11.00-11.30	*Pausa caffè e visita posters*		
11.30-13.30	**S1: Hot spots in medical & veterinary entomology: from Italy to Europe**		
13.30-14.30	*Colazione di lavoro*		
14.30-17.00	**O4: Controllo delle parassitosi**	**O5: Entomologia medica e veterinaria**	**O6: Parassiti della fauna acquatica**
17.00-17.30	Pausa caffè e visita posters		
17.30-19.00		Assemblea Soipa	
20.00-21.00	*"Serata enogastronomica Italy" (Città Universitaria)*		
Giovedì 26 giugno 2014			
	SALA BARACCA	SALA SOCI	SALA BALBO
08.30-11.00	**O7: Biologia e filogenesi molecolare applicate a parassiti e vettori**	**O8: Infezioni da protozoi**	**O9: Controllo e monitoraggio di vettori**
11.00-11.30	Pausa caffè e visita posters		
11.30-13.30	**S2: Italian Malaria Network**	**S3: Parassitosi cardio-polmonari del cane e del gatto: uno scenario in continua evoluzione**	
13.30-14.30	*Colazione di lavoro*		
14.30-16.45	**TV1:La parassitologia italiana nella Cooperazione internazionale**		
16.45-17.00	Pausa caffè e visita posters		
17.00-19.00	**TV2: Migrazioni e tutela sanitaria**		
20.00-23.00	*Cena Sociale*		
Venerdì 27 giugno 2014			
	SALA BARACCA	SALA SOCI	SALA BALBO
08.30-10.00	**O10: Parassiti della fauna selvatica**	**O11a: Infezioni da miceti**	**O12: Parassiti degli animali da compagnia e rischio zoonosico**
10.00-11.00		**O11b: Immunità e immunopatologia delle infezioni parassitarie**	
11.00-11.30	*Pausa caffè e visita posters*		
11.30-13.30	**S4: Zoonosi da alimenti: un approccio globale e nuove prospettive**		
	Arrivederci al XXIX Congresso SoIPa		

PROGRAMMA DETTAGLIATO

Martedì 24 Giugno 2014

Cerimonia inaugurale

20:00 - 23:00

20:00 Cocktail di benvenuto

21:00 CI.1 ETTORE BIOCCA, MARIO COLUZZI: TWO ARCHIVES FOR THE HISTORY OF ITALIAN PARASITOLOGY
Conforti M.*[1]
[1]Dip. di Medicina Molecolare, Unità di Storia della Medicina e Bioetica, "Sapienza" Università di Roma

21:40 CI.2 MARIO COLUZZI (1938-2012): EPISTEMOLOGIST OF MOSQUITOES
Corbellini G.*[1]
[1]Museo di storia della medicina, Dipartimento di Medicina Molecolare, Sapienza Università di Roma

22:20 CI.3 IMMUNOMODULATION OF HOST IMMUNITY BY LARVAL HELMINTHS
Gottstein B.*[1]
[1]Institute of Parasitology, Vetsuisse Faculty, University of Bern, Switzerland

Mercoledì 25 Giugno 2014

SESSIONE PARALLELA O-01 Infestazioni da elminti

08:30 - 11:00

Moderatori: S. Otaševic, A. Scala, S. Giannetto

O-01.1 THE SEROINCIDENCE OF HYDATIDOSIS IN SOUTHEASTEN SERBIA: A LOT HAS BEEN DONE - A LOT OF WORK REMAINS
Otaševic S.*[1], Miladinovic Tasic N.[1], Ignjatović Ćupina A.[1]
[1]University of Niš ~ Niš ~ Serbia

O-01.2 UPDATE ON DIAGNOSTIC METHODS IN HUMAN PARASITOLOGY: WHEN TECHNOLOGY HELPS TRAINING
Barda B.*[1], Albonico M.[2], Levecke B.[3], Genchi M.[4], Vercruysse J.[3], Utzinger J.[5], Magnino S.[4], Cringoli G.[6], Montresor A.[7]
[1]San Raffaele Hospital ~ Milan ~ Italy, [2]Ivo de Carneri Foundation ~ Milan ~ Italy, [3]Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine ~ Merelbeke ~ Belgium, [4]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Ubertini ~ Pavia ~ Italy, [5]Swiss Tropical and Public Health Institute ~ Basel ~ Switzerland, [6]Section of Veterinary Parasitology and Parasitic Diseases, University of Naples Federico II ~ Naples ~ Italy, [7]Department of Neglected Tropical Diseases, World Health Organization ~ Geneva ~ Switzerland

O-01.3 ACCURACY OF THE MINI-FLOTAC METHOD FOR THE DIAGNOSIS OF SOIL-TRANSMITTED HELMINTHS IN PRESERVED STOOL SAMPLES: HOW LONG DO THE EGGS RESIST?
Barda B.*[5], Albonico M.[1], Ianniello D.[4], Utzinger J.[2], Keiser J.[2], Speich B.[2], Rinaldi L.[4], Cringoli G.[4], Burioni R.[5], Montresor A.[3]
[1]Ivo de Carneri Foundation ~ Milan ~ Italy, [2]Swiss Tropical and Public Health Institute ~ Basel ~ Switzerland, [3]Department of Neglected Tropical Diseases, World Health Organization ~ Geneva ~ Switzerland, [4]Section of Veterinary Parasitology and Parasitic Diseases ~ Naples ~ Italy, [5]Laboratory of Microbiology, San Raffaele Hospital ~ Milan ~ Italy

O-01.4 INCREASED PREVALENCE OF TRICHINELLA PSEUDOSPIRALIS DUE TO THE MORE WIDESPREAD USE OF DIGESTION TESTS
Pozio E.*[1], Marucci G.[1], Interisano M.[1], Tonanzi D.[1], La Rosa G.[1]
[1]Istituto Superiore di Sanità ~ Rome ~ Italy

O-01.5 GENETIC CHARACTERIZATION OF HUMAN AND PIG ASCARIS SPP. FROM ITALY
Cavallero S.*[1], Gambetta B.[1], Gabrielli S.[1], Dutto M.[2],Perrone V. [3], D'Amelio S.[1]
[1]Department of Public Health and Infectious Diseases ~ Rome ~ Italy, [2]Department of Prevention Local Health Unit ASL CN1 ~ Cuneo ~ Italy [3]Asl Roma B ~ Italy

O-01.6 DUPLEX-PCR FOR THE SIMULTANEOUS DETECTION AND DIFFERENTIATION BETWEEN TROGLOSTRONGYLUS BREVIOR AND AELUROSTRONGYLUS ABSTRUSUS
Annoscia G.*[3], Latrofa M.S.[3], Campbell B.E.[3], Giannelli A.[3], Ramos R.A.N.[3], Brianti E.[1], Dantas Torres F.[2], Otranto D.[3]
[1]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina, Polo Universitario Annunziata ~ Messina ~ Italy, [2] Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães, Recife, Brazil; Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [3]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy

O-01.7 STUDY OF AELUROSTRONGYLUS ABSTRUSUS IN A SPONTANEOUS INFECTION IN CATS
Viglietti A.[1], Fonti P.[2], De Francesco I.[3], Genchi C.[3], Piazza C.[1], Ferrari N.[3], **Genchi M.***[4]
[1]Veterinary Clinic ~ Veterinary Clinic, Carloforte, Carbonia-Iglesias ~ Italy, [2]Centro Veterinario Specialistico ~ Roma ~ Italy, [3] Dipartimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di Milano ~ Milano ~ Italy, [4]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Ubertini" ~ Pavia ~ Italy

O-01.8 PAST OCCURRENCE OF THE FELINE LUNGWORMS AELUROSTRONGYLUS ABSTRUSUS AND TROGLOSTRONGYLUS SPP. IN ENDEMIC AREAS
Di Cesare A.[1], Di Francesco G.[2], Frangipane Di Regalbono A.[3], Eleni C.[4], De Liberato C.[4], Marruchella G.[2], Iorio R.[1], Malatesta D.[1], Romanucci M.R.[1], Bongiovanni L.[1], Cassini R.[3], **Traversa D.***[1]
[1]Faculty of Veterinary Medicine ~ Teramo ~ Italy, [2]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise ~ Teramo ~ Italy, [3] Department of Animal Medicine, Production and Health ~ Padua ~ Italy, [4]Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana ~ Roma ~ Italy

O-01.9 CLINICAL ANGIOSTRONGYLOSIS: DIAGNOSIS AND CONTROL IN A TERRIER KENNEL
Di Cesare A.*[1], Miotti C.[2], Venco L.[3], Pampurini F.[4], Centaro E.[5], Traversa D.[1]
[1]Faculty of Veterinary Medicine ~ Teramo ~ Italy, [2]Veterinary Practice "Dr. Carlo Miotti, Dr. Marco Miotti" ~ Gallicano ~ Italy, [3] Veterinary Hospital "Città di Pavia" ~ Pavia ~ Italy, [4]Bayer Sanità Animale ~ Milano ~ Italy, [5]Idexx Laboratories ~ Milano ~ Italy

O-01.10 ONCHOCERCA LUPI: CUTANEOUS DISTRIBUTION AND CIRCADIAN RHYTHM OF MICROFILARIAE IN DOGS
Giannelli A.*[1], Dantas Torres F.[2], Abramo F.[3], Ignjatović Ćupina A.[4], Petric D.[4], Cardoso L.[7], Mutafchiev Y.[5], Cortes H.[6], Otranto D.[8]
[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Ba ~ Italy, [2]Department of Immunology, Aggeu Magalhães Research Institute ~ Recife ~ Brazil, [3]Dipartimento di Scienze Veterinarie, Università di Pisa ~ Pisa ~ Italy, [4]University of Novi Sad, Faculty of Agriculture ~ Novi Sad ~ Serbia, [5]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences ~ Sofia ~ Bulgaria, [6]Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora ~ Evora ~ Portugal, [7]Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD) and Instituto de Biologia Molecular e Celular, Universidade do Porto ~ Villareal and Oporto ~ Portugal, [8]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy

O-01.11 ABERRANT OCULAR INFECTION BY ANGIOSTRONGYLUS VASORUM IN A DOG
Lia R.P.*[1], Traversa D.[2], D'Anna N.[3], Giannelli A.[1], Dantas Torres F.[4], Otranto D.[1]
[1]Dipartimento di Medicina Veterinaria Università degli Studi di Bari ~ Bari ~ Italy, [2]Facoltà di Medicina Veterinaria Università degli Studi di Teramo ~ Teramo ~ Italy, [3]Clinica Veterinaria Roma Sud ~ Roma ~ Italy, [4]Department of Immunology, Aggeu Magalhães Research Centre ~ Recife ~ Brazil

O-01.12 ASSESSMENT OF SOME POTENTIAL RISK FACTORS FOR FASCIOLA HEPATICA INFECTION IN GOATS BY USING A CAPTURE ELISA
Pérez A.[1], Díaz P.[1], López C.[1], Martínez Sernández V.[2], Panadero R.[1], Ubeira F.[2], Morrondo P.[1], **Díez Baños P.***[1]
[1]University of Santiago de Compostela ~ Lugo ~ Spain, [2]University of Santiago de Compostela ~ Santiago de Compostela ~ Spain

O-01.13 POOLING FAECAL SAMPLES IN SHEEP FOR THE ASSESSMENT OF GASTRO-INTESTINAL STRONGYLE INFECTION INTENSITY AND ANTHELMINTIC DRUG EFFICACY USING MCMASTER AND MINI-FLOTAC
Rinaldi L.*[1], Vercruysse J.[2], Bosco A.[3], Levecke B.[2], Ianniello D.[3], Pepe P.[3], Charlier J.[2], Cringoli G.[1]
[1]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania); Inter-University Center for Research in Parasitology (CIRPAR) ~ Naples ~ Italy, [2]Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University ~ Merelbeke ~ Belgium, [3]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania) ~ Naples ~ Italy

O-01.14 PREVALENCE AND RISK FACTORS OF GASTROINTESTINAL PARASITES IN CALVES FROM CATTLE HERDS OF TUSCANY
Matia M.[1], Stefanelli S.[3], Banchi A.[2], **Perrucci S.***[1]
[1]Dipartimento di Scienze Veterinarie-University of Pisa ~ Pisa ~ Italy, [2]Freelance Sociologist ~ Pisa ~ Italy, [3]Istituto Zooprofilattico delle Regioni Lazio e Toscana ~ Pisa ~ Italy

O-01.15 ARE SMALL STRONGYLES (CYATHOSTOMINAE) INVOLVED IN HORSE COLIC OCCURRENCE?
Stancampiano L.*[1], Marigo A.[1], Spadari A.[1], Rinnovati R.[1]
[1]Dipartimento di Scienze Mediche Veterinarie - Università di Bologna ~ Ozzano dell'Emilia (Bologna) ~ Italy

SESSIONE PARALLELA O-02 Parassitosi trasmesse da artropodi

08:30 - 11:00

Moderatori: L. Gradoni, E. Ferroglio

O-02.1 DERMACENTOR MARGINATUS TICKS AND ASSOCIATED PATHOGENS IN WILD BOAR POPULATION OF COLLI EUGANEI AREA, NORTHERN ITALY
Cassini R.*[1], Drigo M.[1], Signorini M.[1], Mondin A.[1], Tessarin C.[1], Da Rold G.[2], Pizzocaro M.L.[3], Gallo M.[3], Martini M.[1]
[1]Dipartimento MAPS - Università di Padova ~ Legnaro (PD) ~ Italy, [2]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [3]Parco Regionale dei Colli Euganei ~ Este (PD) ~ Italy

O-02.2 RHIPICEPHALUS SANGUINEUS GROUP AS VECTOR OF PATHOGENS THROUGHOUT THE WORLD
Latrofa M.S.*[2], Dantas Torres F.[1], Giannelli A.[2], Otranto D.[2]
[1]Department of Immunology, Centro de Pesquisas Aggeu Magalhães, Recife, Brazil; Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [2]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy

O-02.3 DEVELOPMENT OF A SURVEILLANCE SYSTEM ON TICKS AND TICK-BORNE ZOONOSES IN PIEDMONT
Pintore M.D.*[1], Tomassone L.[2], Ceballos L.[2], Pautasso A.[1], Francese D.R.[1], Bardelli M.[3], Rizzo F.[1], Mandola M.L.[1], Peletto S.[1], Torina A.[4], Casalone C.[1], Mannelli A.[2], Iulini B.[1]
[1]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta ~ Turin ~ Italy, [2]Department of Veterinary Sciencies, University of Turin ~ Turin ~ Italy, [3]ASL VCO ~ Verbania ~ Italy, [4]Istituto Zooprofilattico Sperimentale della Sicilia ~ Palermo ~ Italy

O-02.4 DEVELOPMENT OF NOVEL SEROLOGICAL TOOLS FOR DIAGNOSIS OF IXODES RICINUS BITE
Sassera D.*[1], Plantard O.[2], Bardoni A.[1], Fumagalli M.[1], Epis S.[3], Mariconti M.[4], Salvini R.[1], Viglio S.[1], Bazzocchi C.[3], Bandi C.[3]
[1]Universita' degli Studi di Pavia ~ Pavia ~ Italy, [2]UMR INRA-Oniris 1300 Biologie, Epidémiologie, Analyse de Risque en santé animale ~ Nantes ~ France, [3]Universita' degli Studi di Milano ~ Milano ~ Italy, [4]Fondazione IRCCS Policlinico S. Matteo ~ Pavia ~ Italy

O-02.5 FIRST DETECTION OF BABESIA MICROTI IN PEOPLE LIVING IN THE PLURINATIONAL STATE OF BOLIVIA
Gabrielli S.*[1], Macchioni F.[2], Totino V.[1], Suniga F.[3], Rojas P.[3], Lara Y.[4], Roselli M.[5], Bartoloni A.[5], Cancrini G.[1]
[1]Università Sapienza ~ Roma ~ Italy, [2]Università di Pisa ~ Pisa ~ Italy, [3]Distrito de Salud Cordillera ~ Santa Cruz ~ Bolivia, Plurinational State of, [4]Hospital S. Antonio de Los Sauces ~ Monteagudo ~ Bolivia, Plurinational State of, [5]Università di Firenze ~ Firenze ~ Italy

O-02.6 MOLECULAR AND SEROLOGICAL DETECTION OF TICK-BORNE PATHOGENS IN DONKEYS (EQUUS ASINUS) IN CENTRAL ITALY
Morganti G.*[1], Ravagnan S.[2], Veronesi F.[1], Capelli G.[2], Giusepponi V.[3], Diaferia M.[1], Moretti A.[1], Piergili Fioretti D.[1]
[1]Department of Veterinary Medicine ~ Perugia ~ Italy, [2]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [3]DVM ~ Perugia ~ Italy

O-02.7 MOLECULAR DETECTION OF TOSCANA VIRUS (BUNYAVIRIDAE: PHLEBOVIRUS) IN SANDFLIES (DIPTERA: PSYCODIDAE) FROM REGGIO EMILIA PROVINCE
Maioli G.[1], Calzolari M.[1], Defilippo F.[1], Pinna M.[1], Massirio I.[2], **Dottori M.***[1], Bonilauri P.[1]
[1]IZSLER ~ Reggio Emilia ~ Italy, [2]Ausl ~ Reggio Emilia ~ Italy

O-02.8 INCIDENCE OF CERCOPITHIFILARIA BAINAE IN DOGS AND PROBABILITY OF CO-INFECTION WITH OTHER TICK-BORNE PATHOGENS
Ramos R.A.N.*[1], Giannelli A.[1], Lia R.P.[1], Brianti E.[2], Tarallo V.D.[1], Stanneck D.[3], Breitschwerdt E.[4], Dantas Torres F.[5], Otranto D.[1]
[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [2]Dipartimento di Sanità Pubblica Veterinaria, Università degli Studi di Messina ~ Messina ~ Italy, [3]Bayer Animal Health GmbH ~ Leverkusen ~ Germany, [4]Intracellular Pathogens Research Laboratory, North Carolina State University ~ Raleigh ~ United States, [5]Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães ~ Recife ~ Brazil

O-02.9 TEMPORAL AND SPATIAL PATTERNS OF DIFFERENT LINEAGES AND STRAINS OF WEST NILE VIRUSES IN NORTH-EASTERN ITALY IN THE MOSQUITO VECTOR CULEX PIPIENS
Ravagnan S.*[1], Cazzin S.[1], Da Rold G.[1], Salviato A.[1], Palei M.[2], Russo F.[3], Montarsi F.[1], Marangon S.[1], Capelli G.[1]
[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [2]Veterinary Public Health Service Friuli Venezia Giulia region ~ Udine ~ Italy, [3]Promotion and Development Hygiene and Public Health Service, Veneto Region ~ Venezia ~ Italy

O-02.10 CAN IXODES RICINUS AND DERMACENTOR RETICULATUS BE A RESERVOIR FOR FRANCISELLA TULARENSIS?
Genchi M.*[1], Prati P.[1], Manfredini A.[1], Vicari N.[1], Bragoni R.[1], Sacchi L.[2], Epis S.[3], Fabbi M.[1]
[1]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Ubertini" ~ Pavia ~ Italy, [2]Dipartimento di Biologia Animale, Università degli Studi di Pavia ~ Pavia ~ Italy, [3]Diaprtimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di Milano ~ Milano ~ Italy

O-02.11 ON A CASE OF HUMAN VISCERAL LEISHMANIASIS IN UMBRIA
Di Giuli C.[1], **Piergili Fioretti D.***[2], Tozzi S.[1], Palumbo M.[1], Frongillo F.R.[1]
[1]Clinic of Infectious Diseases, Terni Hospital, University of Perugia ~ Terni ~ Italy, [2]Department of Veterinary Medicine, University of Perugia ~ Perugia ~ Italy

O-02.12 DEVELOPMENT OF ACANTHOCHEILONEMA RECONDITUM (SPIRURIDA, ONCHOCERCIDAE) IN THE CAT FLEA CTENOCEPHALIDES FELIS (SIPHONAPTERA, PULICIDAE)
Napoli E.*[2], Brianti E.[2], Falsone L.[2], Gaglio G.[2], Radicchi G.[5], Abramo F.[4], Annoscia G.[1], Dantas Torres F.[3], Giannetto S.[2], Otranto D.[1]
[1]Dipartimento di Medicina Veterinaria, Università di Bari ~ Bari ~ Italy, [2]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina ~ Messina ~ Italy, [3]Department of Immunology, Aggeu Magalhães Research Centre, Oswaldo Cruz Foundation ~ Recife ~ Brazil, [4]Dipartimento di Scienze Veterinarie, Università di Pisa ~ Pisa ~ Italy, [5]AbLab Laboratorio di analisi istopatologiche veterinarie ~ La Spezia ~ Italy

SESSIONE PARALLELA O-03 Farmacoresistenza

08:30 - 11:00

Moderatori: F. Severini, C. Genchi

O-03.1 DRUGS FOR NEGLECTED TROPICAL DISEASES: THE CHALLENGE OF IMPROVING ACCESS WHILE SUSTAINING EFFICACY
Albonico M.*[1], Gabrielli A.F.[2], Montresor A.[2], Savioli L.[2]
[1]Fondazione Ivo de Carneri ~ Milan ~ Italy, [2]World Health Organization ~ Geneva ~ Switzerland

O-03.2 GENETIC AND MOLECULAR MECHANISMS OF OXAMNIQUINE RESISTANCE IN SCHISTOSOMES.
Cioli D.*[1], Pica Mattoccia L.[1], Guidi A.[1], Basso A.[1]
[1]CNR, Institute of Cell Biology and Neurobiology ~ Roma ~ Italy

O-03.3 WHY DID THE VETS SHOULD NOT USE THE ANTI-LEISHMANIA DRUGS CURRENTLY USED FOR HUMANS?
Oliva G.*[1], Gradoni L.[2]
[1]Department of Veterinary Medicine and Animal Production, University Federico II ~ Naples ~ Italy, [2]MIPI Department, Istituto Superiore di Sanità ~ Rome ~ Italy

O-03.4 EQUINE STRONGYLID EGG RE-APPEARANCE PERIOD AFTER IVERMECTIN OR MOXIDECTIN TREATMENT IN ITALY
Traversa D.*[1], Frangipane Di Regalbono A.[2], Di Cesare A.[1], Otranto D.[3], Lia R.P.[3], Beraldo P.[4], Besognet B.[5], Geurden T.[6]
[1]Faculty of Veterinary Medicine ~ Teramo ~ Italy, [2]Department of Veterinary Medicine, Production and Health ~ Padua ~ Italy, [3]Department of Veterinary Medicine ~ Bari ~ Italy, [4]Department of Food Science ~ Udine ~ Italy, [5]Zoetis ~ Paris ~ France, [6]Zoetis ~ Zaventem ~ Belgium

O-03.5 EVALUATION OF THE DISTRIBUTION OF PLASMODIUM FALCIPARUM N86Y POLYMORPHISM IN PFMDR1 GENE IN UGANDA
Cosentino V.*[1], Paganotti G.M.[1], Tabacchi F.[1], Russo G.[1], Coluzzi M.[1], Romano R.[1]
[1]Department of Public Health and Infectious Diseases, "Sapienza" University of Rome ~ Italy

O-03.6 MOLECULAR SURVEILLANCE OF PLASMODIUM VIVAX DHFR AND DHPS MUTATIONS IN ISOLATES FROM IRAN
Fuda I.[1], Menegon M.[1], Rezanezhad H.[2], **Severini C.***[3]
[1]Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità ~ Rome ~ Italy, [2]Department of Microbiology, Jahrom University of Medical Sciences ~ Jahrom ~ Iran, Islamic Republic of, [3]Department of Infectious, Parasitic and Immunomediated Diseases and WHO Collaborating Centre for Research and Training in Tropical Diseases Control, Istituto Superiore di Sanità ~ Rome ~ Italy

O-03.7 THE MAINTENANCE OF ANTHELMINTIC EFFICACY IN SHEEP IN THE CAMPANIA REGION (SOUTHERN ITALY)
Rinaldi L.*[1], Bosco A.[2], Coles G.[3], Morgan E.[3], Parrilla M.[2], Cringoli G.[1]
[1]1Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania); Inter-University Center for Research in Parasitology (CIRPAR) ~ Naples ~ Italy, [2]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania) ~ Naples ~ Italy, [3]School of Veterinary Sciences, University of Bristol, Langford House ~ Bristol ~ United Kingdom

O-03.8 GASTROINTESTINAL NEMATODES OF DAIRY GOATS IN NORTHERN ITALY: ANTHELMINTIC RESISTANCE AND PRACTICES OF PARASITE CONTROL.
Zanzani S.A.[1], Gazzonis A.L.[1], Di Cerbo A.R.[1], **Manfredi M.T.***[1]
[1]Department of Veterinary Science and Public Health, University of Milan ~ Milano ~ Italy

SIMPOSIO S1-Hot Spots in medical & veterinary entomology: from Italy to Europe

11:30 - 13:30

Moderatori: A. Della Torre, D. Otranto

S1.1 THE CHANGING LANDSCAPE FOR VECTOR-BORNE DISEASE
Wall R.*[1]
[1]Veterinary Parasitology & Ecology Group, School of Biological Sciences, University of Bristol, Bristol,UK

S1.2 SIMULIIDS: A RE-EMERGING THREAT TO ANIMAL AND HUMAN HEALTH ACROSS EUROPE
Ignjatović Ćupina A.*[1], Kúdela M.[2], Ciadamidaro S.[3], Maiolini B.[4], Brúderová T.[2], Giannelli A.[5], Otranto D.[5], Petrić D.[1], Rivosecchi L.[6]
[1]Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia, [2]Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia, [3]ENEA, Saluggia Research Centre, Italy, [4]Fondazione Edmund Mach, Research and Innovation Centre (CRI), San Michele all´Adige, Italy, [5]Department of Veterinary Medicine, University of Bari, Valenzano (Bari), Italy, [6]Istituto Superiore di Sanità, Roma, Italy (retired researcher)

S1.3 THE RHIPICEPHALUS SANGUINEUS GROUP: NEW SCENARIOS IN DISEASE TRANSMISSION
Dantas Torres F.*[1], Dantas Torres F.[2], Latrofa M.S.[2], Otranto D.[2]
[1]Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães, Recife, Brazil, [2]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Italy Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Italy

S1.4 RISK OF TRANSMISSION OF NEW TICK-BORNE PATHOGENS: A REVIEW
Rizzoli A.*[1]
[1]Fondazione Edmund Mach, San Michele all'Adige (TN), Italy

S1.5 THE SIGNIFICANCE OF ENTOMOLOGICAL SURVEYS FOR ENDEMIC AND AT RISK OF INTRODUCTION MOSQUITO-BORNE PATHOGENS
Capelli G.*[1]
[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy

S1.6 CULICOIDES BITING MIDGES AND RELATED ARBOVIRUSES.
Goffredo M.*[1]
[1]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Campo Boario - Teramo (Italy)

S1.7 PHLEBOTOMINE SAND FLIES AND THE RISK OF TRANSMISSION OF VIRAL DISEASES
Maroli M.*[1], Ciufolini M.G.[1], Gramiccia M.[1], Gradoni L.[1]
[1]Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, Rome, Italy

SESSIONE PARALLELA O-04 Controllo delle parassitosi

14:30 - 17:00
Moderatori: M.T. Manfredi, M. Albonico

O-04.1 A FIELD CLINICAL STUDY EVALUATING THE EFFICACY AND SAFETY OF MILBEMAX® (NOVARTIS ANIMAL HEALTH) IN THE CHEMOPREVENTION OF DOG SUBCUTANEOUS FILARIOSIS
Di Cesare A.[1], P**aoletti B.***[1], Bartolini R.[1], Aquilino V.[1], La Torre F.[2], Drake J.[3], Braun G.[4], Traversa D.[1]
[1]Faculty of Veterinary Medicine ~ Teramo ~ Italy, [2]Novartis Animal Health S.p.A ~ Origgio (VA) ~ Italy, [3]Novartis Animal Health S.p.A ~ Greensboro (NC) ~ United States, [4]Klifovet ~ Munchen ~ Germany

O-04.2 SUSTAINABLE AND LONG-TERM CONTROL OF TICK AND FLEA INFESTATIONS IN DOGS THROUGH THE USE OF SLOW RELEASE COLLAR CONTAINING IMIDACLOPRID (10%) AND FLUMETHRIN (4.5%)
Brianti E.*[1], Falsone L.[1], Napoli E.[1], Gaglio G.[1], Prudente C.[1], Aprile S.[2], Giannetto S.[1]
[1]Dipartimento di Scienze Veterinarie, Università di Messina ~ Messina ~ Italy, [2]Medico Veterinario libero professionista ~ Siracusa ~ Italy

O-04.3 A PILOT TRIAL EVALUATING THE EFFICACY OF A 10% IMIDACLOPRID/2.5% MOXIDECTIN SPOT-ON FORMULATION IN THE TREATMENT OF NATURAL NASAL EUCOLEOSIS IN DOGS
Veronesi F.*[1], Morganti G.[1], Diaferia M.[1], Di Cesare A.[2], Schaper R.[3], Traversa D.[2]
[1]Department of Veterinary Medicine, University of Perugia ~ Perugia ~ Italy, [2]Faculty of Veterinary Medicine, University of Teramo ~ Teramo ~ Italy, [3]Bayer Animal Health GmbH ~ Leverkusen ~ Germany

O-04.4 PRELIMINARY ASSESSMENT OF THE EFFICACY OF PARASITICIDES IN THE TREATMENT OF TROGLOSTRONGYLUS BREVIOR INFECTION IN CATS
Crisi P.E.*[1], Boari A.[1], Di Cesare A.[1], Iorio R.[1], Santori D.[1], Seghetti M.[2], Di Costanzo R.[3], Dimitri C.F.[4], Traversa D.[1]
[1]Faculty of Veterinary Medicine ~ Teramo ~ Italy, [2]Ambulatorio Veterinario Riviera ~ Grottammare ~ Italy, [3]Clinica Veterinaria L'Arca ~ Teramo ~ Italy, [4]Ambulatorio Veterinario "Carmelo Flavio Dimitri" ~ Isola del Gran Sasso, Teramo ~ Italy

O-04.5 EFFICACY OF ORAL SPINOSAD (COMFORTIS®) TO CONTROL CAT FLEAS IN A FELINE SHELTER
Longhini A.[1], Colnago G.[2], Manuguerra B.[1], **Kramer L.H.***[1]
[1]Dipt. Scienze medico Veterinarie ~ Parma ~ Italy, [2]Elanco Animal Health Italy ~ Sesto Fiorentino ~ Italy

O-04.6 PILOT STUDY ON EFFICACY AND SAFETY OF TINIDAZOLE AGAINST GIARDIA LAMBLIA IN NATURALLY INFECTED CATS
Napoli E.*[1], Gaglio G.[1], Gallo D.[2], Falsone L.[1], Brianti E.[1], Giannetto S.[1], Pennisi M.[1]
[1]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina, ~ Messina ~ Italy, [2]Medico Veterinario libero professionista ~ Messina ~ Italy

O-04.7 DIMINISHING ROLE OF TRADITIONAL HEALERS IN THE MANAGEMENT OF MALARIA IN MALINDI, KENYA, IN THE CONTEXT OF DECLINING MALARIA
Kibe L.[2], **Habluetzel A.***[2], Kamau A.[1], Jones C.[1], Mbogo C.[1]
[1]Kenya Medical Research Institute, Centre for Geographic Medicine Research-Coast ~ Malindi ~ Kenya, [2]School of Pharmacy, University of Camerino ~ Camerino ~ Italy

O-04.8 MAPPING INFECTION PROBABILITIES FOR PARASITOLOGICAL SURVEILLANCE IN LIVESTOCK USING A BAYESIAN KRIGING MODEL
Musella V.*[1], Rinaldi L.[2], Catelan D.[3], Biggeri A.[3], Cringoli G.[2]
[1]Department of Health Sciences, University of Catanzaro Magna Graecia ~ Catanzaro ~ Italy, [2]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania); Inter-University Center for Research in Parasitology (CIRPAR) ~ Naples ~ Italy, [3]Department of Statistics, Informatics and Applications "G. Parenti" University of Florence ~ Florence ~ Italy

XXVIII Congresso Nazionale SoIPa, Roma 24-27 giugno 2014
PROGRAMMA DETTAGLIATO

O-04.9 EVALUATION OF THE EFFICACY OF ANTHELMINTHIC TREATMENTS IN SHEEP AND HORSES OF SARDINIA, ITALY
Scala A.*[1], Varcasia A.[1], Pipia A.P.[1], Tamponi C.[1], Muntoni S.[1], Dore F.[1], Sanna G.[1]
[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari ~ Sassari ~ Italy

O-04.10 COMPARATIVE EFFICACY OF TWO PYRANTEL ORAL FORMULATIONS (PASTE AND GRANULATE) IN DONKEYS NATURALLY INFECTED WITH INTESTINAL STRONGYLIDAE.
Veneziano V.*[1], Smaldone G.[1], Mariani U.[2], Neola B.[1], Roncoroni C.[3], Gokbulut C.[4]
[1]Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Italy ~ Napoli ~ Italy, [2]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy ~ Portici ~ Italy, [3]Istituto Zooprofilattico Sperimentale Lazio e Toscana,Italy ~ Roma ~ Italy, [4]dDepartment of Medical Pharmacology, Balikesir University Turkey ~ Balikesir ~ Turkey

O-04.11 METAPHYLACTIC TREATMENT WITH TOLTRAZURIL AND DICLAZURIL FOR THE CONTROL OF COCCIDIOSIS IN BUFFALO CALVES
Bosco A.*[1], Rinaldi L.[2], Cappelli G.[1], Santaniello M.[1], Morgoglione M.E.[1], Guariglia I.[1], Cringoli G.[2]
[1]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania) ~ Naples ~ Italy, [2]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania); Inter-University Center for Research in Parasitology (CIRPAR) ~ Naples ~ Italy

O-04.12 THE SUPPLY OF DRUGS FOR NEGLECTED TROPICAL DISEASES IN ITALY: CRITICAL ISSUES AND POSSIBLE SOLUTIONS
Angheben A.*[1], Bisoffi Z.[1]
[1]Ospedale Sacro Cuore - Don Calabria, Centro per le Malattie Tropicali ~ Negrar ~ Italy

SESSIONE PARALLELA O-05 Entomologia medica e veterinaria

14:30 - 17:00
Moderatori: R. Romi, A. Iori

O-05.1 CULICOIDES INDOOR WINTER ACTIVITY IN CENTRAL ITALY
Magliano A.[1], Scaramozzino P.[1], Cincinelli G.[2], Moni A.[3], Silvestri P.[4], **De Liberato C.***[1]
[1]Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana ~ Roma ~ Italy, [2]ASL8 Arezzo, Zona Valdarno ~ Arezzo ~ Italy, [3]ASL 1, Massa Carrara ~ Massa ~ Italy, [4]ASL Rieti Distretto Mirtense ~ Poggio Mirteto (Ri) ~ Italy

O-05.2 ECOLOGICAL NICHE MODEL OF P. PERNICIOSUS (DIPTERA: PSYCHODIDAE) IN NORTH-EASTERN ITALY
Signorini M.*[1], Cassini R.[1], Frangipane Di Regalbono A.[1], Pietrobelli M.[1], Babiker A.M.A.[2], Stensgaard A.S.[3], Stansgaard A.S.[4]
[1]1Dipartimento di Medicina Animale, Produzioni e Salute, Università degli Studi di Padova ~ Legnaro, Padova ~ Italy, [2]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro, Padova ~ Italy, [3]3Center for Macroecology, Evolution and Climate, Department of Biology, University of Copenhagen, Universitetsparken 15 ~ Copenhagen ~ Denmark, [4]Section for Parasitology and for Aquatic Diseases, University of Copenhagen ~ Copenhagen ~ Denmark

O-05.3 DIVERSITY AND SEASONALITY OF PHLEBOTOMINE SAND FLIES IN A MILITARY TRAINING AREA IN NORTH-EASTERN BRAZIL
Moura A.C.D.A.[1], Da Silva K.G.[1], Miranda D.E.D.O.[1], Ramalho M.[1], De Moura M.R.A.[1], Da Silva F.J.[1], Brandão Filho S.P.[1], Otranto D.[2], **Dantas Torres F.***[1]
[1]Aggeu Magalhães Research Centre ~ Recife ~ Brazil, [2]University of Bari ~ Valenzano ~ Italy

O-05.4 A REAL-TIME PCR FOR THE CHARACTERIZATION OF MULTIPLE BLOOD SOURCES IN FIELD-COLLECTED SAND FLIES
Da Silva K.G.[1], De Morais R.C.S.[1], Miranda D.E.D.O.[1], Moura A.C.D.A.[1], Brandão Filho S.P.[1], Otranto D.[2], De Paiva Cavalcanti M.[1], **Dantas Torres F.***[1]
[1]Aggeu Magalhães Research Centre ~ Recife ~ Brazil, [2]University of Bari ~ Valenzano ~ Italy

O-05.5 MATING BEHAVIOR OF ANOPHELES GAMBIAE IN ENCLOSED ENVIRONMENT: CHALLENGES AND OPPORTUNITIES
Facchinelli L.*[1], Valerio L.[1], Oliva C.[2], Lees R.S.[2], Persampieri T.[1], Collins M.C.[3], Crisanti A.[1], Benedict M.Q.[1]
[1]Department of Experimental Medicine, University of Perugia ~ Perugia ~ Italy, [2]Polo d'Innovazione Genomica, Genetica e Biologia S.C.a.R.L. ~ Perugia ~ Italy, [3]Department of Life Sciences, Imperial College London ~ London ~ United Kingdom

O-05.6 IMPACT OF REPEATED NEEMAZAL® TREATED BLOOD MEALS ON THE FITNESS OF ANOPHELES STEPHENSI MOSQUITOES
Dembo E.[1], Abay S.M.[2], Lupidi G.[2], Lucantoni L.[2], Ogboi J.S.[2], Dahiya N.[2], Esposito F.[2], **Habluetzel A.***[2]
[1]Department of Pharmacy, College of Medicine, University of Malawi ~ Blantyre ~ Malawi, [2]School of Pharmacy, University of Camerino ~ Camerino ~ Italy

O-05.7 BITING RATE AND HOST PREFERENCE OF CULEX PIPIENS IN AN AREA REPEATEDLY AFFECTED BY WEST NILE VIRUS
Montarsi F.*[1], Danesi P.[1], Martini S.[2], Drago A.[2], Cazzin S.[1], Frangipane Di Regalbono A.[3], Capelli G.[1]
[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [2]Entostudio snc ~ Brugine (PD) ~ Italy, [3]University of Padua ~ Padua ~ Italy

O-05.8 DISTRIBUTION AND ABUNDANCE OF A POTENTIAL RIFT VALLEY FEVER VIRUS VECTOR, AEDES VEXANS, IN CENTRAL AND NORTHERN ITALY
Magliano A.[1], Montarsi F.[2], Di Domenico M.[1], **De Liberato C.***[1]
[1]Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana ~ Roma ~ Italy, [2]Entomologist Consulting ~ Padova ~ Italy

O-05.9 HOST PREFERENCE OF THE INVASIVE MOSQUITO AEDES KOREICUS [HULECOETEOMYIA KOREICA]
Cazzin S.*[2], Ciocchetta S.[1], Ravagnan S.[2], Drago A.[3], Carlin S.[2], Capelli G.[2], Montarsi F.[2]
[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy; QIMR Berghofer Medical Research Institute ~ Brisbane ~ Australia, [2]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [3]Entostudio snc ~ Brugine (PD) ~ Italy

O-05.10 STUDIES OF VECTOR COMPETENCE OF ITALIAN AEDES ALBOPICTUS POPULATIONS BY EXPERIMENTAL INFECTIONS WITH CHIKUNGUNYA VIRUS
Fortuna C.*[1], Remoli M.E.[1], Di Luca M.[2], Severini F.[2], Toma L.[2], Benedetti E.[1], Bucci P.[1], Ciufolini M.G.[1], Boccolini D.[2], Romi R.[2]
[1]Unit of Viral diseases and attenuated vaccine, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy, [2]Unit of Vector-borne Diseases and International Health, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy

O-05.11 STUDIES OF VECTOR COMPETENCE OF CULEX PIPIENS POPULATIONS BY EXPERIMENTAL INFECTIONS WITH WEST NILE VIRUS
Fortuna C.*[1], Remoli M.E.[1], Di Luca M.[2], Severini F.[2], Toma L.[2], Benedetti E.[1], Bucci P.[1], Platonov A.E.[3], Capelli G.[4], Nicoletti L.[1], Boccolini D.[2], Romi R.[2], Ciufolini M.G.[1], Fedorova M.V.[3]
[1]Unit of Viral diseases and attenuated vaccine, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy, [2]Unit of Vector-borne Diseases and International Health, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy, [3]Central Institute of Epidemiology ~ Moscow ~ Russian Federation, [4]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy

SESSIONE PARALLELA O-06 Parassiti della fauna acquatica

14:30 - 17:00

Moderatori: M. Caffara, P. Merella

O-06.1 HISTOLOGICAL EXAMINATION, BIO-MOLECULAR ANALYSIS AND ULTRASTRUCTURAL OBSERVATIONS OF MICROSPORIDIA IN WHITE-CLAWED CRAYFISH AUSTROPOTAMOBIUS PALLIPES (LEREBOULLET, 1858) IN LOMBARDY (ITALY).
Berton V.*[1], Tosi F.[1], Montesi F.[1], Manfrin A.[1], Terregino C.[1], Pretto T.[1]
[1]Istituto Zooprofilattico sperimentale delle Venezie ~ Legnaro (PD) ~ Italy

O-06.2 PARASITE FAUNA OF THE HEAD OF JUVENILE THUNNUS THYNNUS (OSTEICHTHYES: SCOMBRIDAE) FROM THE WESTERN MEDITERRANEAN SEA
Mele S.*[1], Culurgioni J.[2], Rodríguez Llanos J.[3], Palacio Abella J.[3], Mazzotta P.[1], Garau S.[2], Sanna N.[2], Macías D.[4], Addis P.[2], Garibaldi F.[5], Garippa G.[1], Merella P.[1], Montero F.E.[3]
[1]Parassitologia e Malattie Parassitarie, Dipartimento di Medicina Veterinaria, Università di Sassari ~ Sassari ~ Italy, [2]Dipartimento di Scienze della Vita e dell'Ambiente, Università di Cagliari ~ Cagliari ~ Italy, [3]Unidad de Zoología Marina, Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València ~ Valencia ~ Spain, [4]Centro Oceanográfico de Málaga, Instituto Español de Oceanografía ~ Malaga ~ Spain, [5]Laboratorio di Biologia Marina, DIP. TE. RIS., Università di Genova ~ Genova ~ Italy

O-06.3 GASTROINTESTINAL HELMINTHS INFECTION AND DIGESTIVE HORMONES IN THE FISH-PARASITE SYSTEMS
Bosi G.[1], Giari L.[2], **Sayyaf Dezfuli B.***[2]
[1]Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, St. Trentacoste 2, 20134 Milan ~ Milan ~ Italy, [2]Department of Life Sciences and Biotechnology, University of Ferrara ~ Ferrara ~ Italy

O-06.4 METACERCARIAE OF ASCOCOTYLE SP. AND HETEROPHYES SP. (TREMATODA: HETEROPHYIDAE) IN GREY MULLETS (OSTEICHTHYES: MUGILIDAE) FROM SARDINIA BRACKISH WATERS (WESTERN MEDITERRANEAN SEA)
Masala S.*[1], Garippa G.[1], Piras M.C.[1], Merella P.[1], Scholz T.[2], Kostadinova A.K.[2]
[1]Parassitologia e Malattie Parassitarie, Dipartimento di Medicina Veterinaria, Università di Sassari ~ Sassari ~ Italy, [2]Institute of Parasitology Academy of Sciences of the Czech Republic ~ Ceske Budejovice ~ Czech Republic

O-06.5 EUSTRONGYLIDES SP. LARVAE IN MUSCLE OF EUROPEAN PERCH, PERCA FLUVIATILIS: HISTOPATHOLOGY AND RISK OF ZOONOTIC DISEASES
Sayyaf Dezfuli B.*[1], Pironi F.[1], Lorenzoni M.[2], Giari L.[1]
[1]Department of Life Sciences and Biotechnology, University of Ferrara ~ Ferrara ~ Italy, [2]Department of Cellular and Environmental Biology, University of Perugia ~ Perugia ~ Italy

O-06.6 PRESENCE OF ANISAKIS SPP. (NEMATODA: ANISAKIDAE) IN LOCAL AND ATLANTIC FISH SOLD IN SARDINIAN MARKET
Piras M.C.*[1], Garippa G.[1], Masala S.[1], Muglia S.[2], Frau F.[2], Spanu V.[2], Merella P.[1]
[1]Parassitologia e Malattie Parassitarie, Dipartimento di Medicina Veterinaria, Università di Sassari ~ Sassari ~ Italy, [2]Ispezione degli Alimenti di Origine Animale, Dipartimento di Medicina Veterinaria, Università di Sassari ~ Sassari ~ Italy

O-06.7 DISTRIBUTION AND INFECTION LEVELS BY ANISAKIS PEGREFFII AND A. SIMPLEX (S. S.) LARVAE (NEMATODA : ANISAKIDAE) IN FISH TISSUES OF MERLUCCIUS MERLUCCIUS FROM TYRRHENIAN SEA AND NE ATLANTIC WATERS (ATLANTIC COST OF SPAIN): IMPLICATIONS FOR FOOD SAFETY
Cipriani P.*[1], Acerra V.[2], Smaldone G.[3], Bellisario B.[2], Anastasi A.[3], Palma G.[4], Nardi V.[2], Nascetti G.[2], Mattiucci S.[1]
[1]Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, Italy ~ Roma ~ Italy, [2]Department of Ecology and Biology (DEB), Tuscia University, Viterbo, Italy ~ Viterbo ~ Italy, [3]Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Napoli ~ Napoli ~ Italy, [4]Federazione Nazionale delle Impresa di Pesca - Federpesca ~ Roma ~ Italy

O-06.8 REAL TIME PCR USING DNA PROBES, BASED ON MITOCHONDRIAL COX2 GENE, FOR THE SPECIFIC IDENTIFICATION OF LARVAL ANISAKIDS OF THE GENERA ANISAKIS AND PSEUDOTERRANOVA
Paoletti M.*[1], Nascetti G.[1], Mattiucci S.[2]
[1]Department of Ecology and Biology (DEB), Tuscia University, Viterbo, Italy ~ Viterbo ~ Italy, [2]Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, Italy ~ Roma ~ Italy

O-06.9 PUTATIVE HYBRIDS OF TWO ANISAKIS CRYPTIC SPECIES IN STENELLA COERULEOALBA FROM THE MEDITERRANEAN SEA
Cavallero S.*[1], Costa A.[2], Caracappa G.[2], Currò V.[2], Gambetta B.[1], Amprimo V.[1], D'Amelio S.[1]
[1]Department of Public Health and Infectious Diseases ~ Rome ~ Italy, [2]Istituto Zooprofilattico Sperimentale della Sicilia ~ Palermo ~ Italy

O-06.10 PROGENESIS IN TREMATODES
Di Cave D.*[1], Coiante V.[2], Ronci L.[2], Berrilli F.[1], Gustinelli A.[3], Pintus D.[2], Setini A.[2]
[1]University of Rome "Tor Vergata", Department of Experimental Medicine and Surgery, [2]University of Rome "La Sapienza", Department of Biology and Biotechnology "Charles Darwin", [3]University of Bologna , Department of Veterinary Medicine

O-06.11 SPIRORCHIIDIASIS IN STRANDED LOGGERHEAD CARETTA CARETTA IN NORTHERN ADRIATIC SEA (ITALY)
Marcer F.*[1], Marchiori E.[2], Tessarin C.[1], Danesi P.[3], Poppi L.[2]
[1]Dipartimento di Medicina Animale Produzioni e Salute - Università di Padova ~ Legnaro (PD) ~ Italy, [2]Dipartimento di Biomedicina Comparata e Alimentazione - Univerisità di Padova ~ Legnaro (PD) ~ Italy, [3]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy

Giovedì 26 Giugno 2014

SESSIONE PARALLELA O-07 Biologia e filogenesi molecolare applicate a parassiti e vettori

08:30 - 11:00

Moderatori: S.M. Cacciò,C. Bandi

O-07.1 ISOLATION AND CHARACTERIZATION OF PARAMYOSIN FROM ONCHOCERCA LUPI, A NEGLECTED ZOONOTIC NEMATODE
Campbell B.E.*[1], Giannelli A.[1], Annoscia G.[1], Dantas Torres F.[2], Cortes H.[3], Cardoso L.[4], Otranto D.[1]
[1]Universita di Bari Aldo Moro ~ Bari ~ Italy, [2]Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães (Fiocruz-PE) ~ Recife ~ Brazil, [3]Victor Caeiro Laboratory of Parasitology, Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Evora ~ Evora ~ Portugal, [4]Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real ~ Vila Real ~ Portugal

O-07.2 ANALYSIS OF RIBOSOMAL DNA INTERNAL TRANSCRIBED SPACER SEQUENCES OF LEISHMANIA DONOVANI ISOLATES FROM SUDANESE PATIENTS
Babiker A.M.A.*[3], Ravagnan S.[3], Porcellato E.[3], Signorini M.[1], Hassan M.M.[2], Cassini R.[1], Cattoli G.[3], Capelli G.[3]
[1]Università degli Studi di Padova ~ Padova ~ Italy, [2]Tropical Medicine Research Institute ~ Khartoum ~ Sudan, [3]istituto zooprofilattico sperimentale delle venezie ~ Padova ~ Italy

O-07.3 CHARACTERIZATION OF THE GLYCEROL-3-PHOSPHATE DEHYDROGENASE (GG3PD) OF GIARDIA DUODENALIS AND ITS INTERACTION WITH THE MULTIFUNCTIONAL G14-3-3 PROTEIN.
Finelli R.[1], Cecchetti S.[1], Pozio E.[1], **Lalle M.***[1]
[1]Istituto Superiore di Sanità ~ Roma ~ Italy

O-07.4 DISTINCT PROPERTIES OF THE EGRESS-RELATED OSMIOPHILIC BODIES IN SEXUAL STAGES OF THE RODENT MALARIA PARASITE PLASMODIUM BERGHEI.
Olivieri A.*[1], Bertuccini L.[2], Deligianni E.[3], Franke Fayard B.[4], Currà C.[1], Siden Kiamos I.[3], Hanssen E.[5], Superti F.[2], Janse C.[4], Ponzi M.[1]
[1]Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Parassitarie ed Immunomediate ~ Roma ~ Italy, [2]Istituto Superiore di Sanità, Dipartimento di Tecnologia e Salute ~ Roma ~ Italy, [3]Institute of Molecular Biology and Biotechnology, FORTH ~ Heraklion ~ Greece, [4]Leiden Malaria Research Group, Department of Parasitology, Centre for Infectious Diseases, Leids Universitair Medisch Centrum (LUMC) ~ Leiden ~ Netherlands, [5]Bio21 Molecular Science and Biotechnology Institute, Electron Microscopy Unit and Department of Biochemistry and Molecular Biology, University of Melbourne ~ Melbourne ~ Australia

O-07.5 POSITIVE SELECTION DRIVES ACCELERATED EVOLUTION OF MOSQUITO SALIVARY GENES ASSOCIATED WITH BLOOD FEEDING
Arcà B.*[1], Struchiner C.[2], Pham V.[3], Sferra G.[1], Lombardo F.[1], Pombi M.[1], Ribeiro J.[3]
[1]Dipartimento di Sanità Pubblica e Malattie Infettive, Sezione di Parassitologia, Sapienza Università di Roma ~ Roma ~ Italy, [2]Escola Nacional de Saude Publica, Fundaçao Oswaldo Cruz ~ Rio de Janeiro ~ Brazil, [3]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases ~ Rockville, MD ~ United States

O-07.6 PHYLOGENETIC EVIDENCE FOR THE ORIGINS OF WOHLFAHRTIA MAGNIFICA ISOLATES FROM SHEEP IN ITALY
Marangi M.*[1], Giangaspero A.[1], Gaglio G.[2], Paul D R.[3], Hall M.J.R.[3]
[1]University of Foggia ~ Foggia ~ Italy, [2]University of Messina ~ Messina ~ Italy, [3]Natural History Museum ~ London ~ United Kingdom

O-07.7 EMERGING PATHOGENS IN VERTEBRATES: BIOLOGY, GENOMICS AND INFECTIVITY OF BACTERIA ASCRIBED TO THE FAMILY MIDICHLORIACEAE
Bazzocchi C.*[1], Cafiso A.[1], Petroni G.[2], Lanzoni O.[2], Sassera D.[3], Epis S.[1], Mariconti M.[4], Bandi C.[1]
[1]DIVET, University of Milan ~ Milan ~ Italy, [2]Department of biology, University of Pisa ~ Pisa ~ Italy, [3]DBB, University of Pavia ~ Pavia ~ Italy, [4]IRCCS; Policlinico S. Matteo ~ Pavia ~ Italy

O-07.8 BACTERIAL SYMBIOTIC CONTROL OF MOSQUITO VECTORS: FROM BENCH TO FIELD
Mancini M.V.*[1], Bozic J.[1], Capone A.[1], Cappelli A.[1], Damiani C.[1], Epis S.[2], Rossi P.[1], Valzano M.[1], Bandi C.[2], Ricci I.[1], Favia G.[1]
[1]Scuola di Bioscienze e Medicina Veterinaria, Università degli Studi di Camerino ~ Camerino ~ Italy, [2]Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria, Università degli Studi di Milano ~ Milano ~ Italy

O-07.9 USING SYMBIOTIC YEASTS ASSOCIATED TO MOSQUITOES TO PREVENT PLASMODIAL INFECTION INMALARIA VECTORS: CURRENT STATUS AND FUTURE STRATEGIES FOR SYMBIOTIC CONTROL OF MOSQUITO BORN DISEASES
Bozic J.*[1], Capone A.[1], Valzano M.[1], Cappelli A.[1], Damiani C.[1], Rossi P.[1], Mancini M.V.[1], Favia G.[1], Ricci I.[1]
[1]Scuola di Bioscenze e Medicina Veterinaria, Università degli studi di Camerino ~ Camerino ~ Italy

O-07.10 ENGINEERING OF THE YEAST WICKERHAMOMYCES ANOMALUS, SYMBIONT OF MOSQUITO SPECIES RELEVANT TO PUBLIC HEALTH, FOR PARATRANSGENIC CONTROL STRATEGIES.
Capone A.*[1], Bozic J.[1], Cappelli A.[1], Damiani C.[1], Rossi P.[1], Valzano M.[1], Epis S.[2], Favia G.[1], Ricci I.[1]
[1]Scuola di Bioscienze e Medicina Veterinaria, Università degli Studi di Camerino ~ Camerino ~ Italy, [2]Dipartimento di Patologia Animale, Igiene e Santità Pubblica Veterinaria, Università degli Studi di Milano ~ Milano ~ Italy

SESSIONE PARALLELA O-08 Infezioni da protozoi

08:30 - 11:00
Moderatori: D. Piergili-Fioretti, M. Gramiccia

O-08.1 EPIDEMIOLOGICAL STUDY OF BESNOITIA BESNOITI INFECTION AMONG CATTLE IN INSULAR AND NORTH-WESTERN ITALY.
Gazzonis A.L.*[1], Alvarez Garcia G.[2], Zanzani S.A.[1], Maggioni A.[1], Garippa G.[3], Rossi L.[4], Maggiora M.[4], Inverizzi A.[7], Luini M.V.[8], Dini V.[5], Tranquillo V.[6], Ortega Mora L.[2], Manfredi M.T.[1]
[1]Department of Veterinary Science and Public Health, University of Milan ~ Milano ~ Italy, [2]SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid ~ Madrid ~ Spain, [3]Department of Veterinary Medicine, University of Sassari ~ Sassari ~ Italy, [4]Department of Veterinary Sciences, University of Turin ~ Torino ~ Italy, [5]Unità Sanitaria Locale Savonese 2 ~ Savona ~ Italy, [6]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna ~ Bergamo ~ Italy, [7]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna ~ Milano ~ Italy, [8]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna ~ Lodi ~ Italy

O-08.2 SPREADING SURVEY OF BESNOITIA BESNOITI IN ITALIAN CATTLE POPULATION
D'Avino N.[1], Maresca C.[1], Costarelli S.[1], Filippini G.[1], Gentile A.[2], Felici A.[1], Dettori A.[1], Sebastianelli M.[1], Broccatelli S.[1], Cordovani E.[1], **Righi C.***[1]
[1]Istituto Zooprofilattico Sperimentale Umbria e Marche ~ Perugia ~ Italy, [2]Dipartimento Clinico Veterinario Università di Bologna ~ Bologna ~ Italy

O-08.3 PREVALENCE OF BABESIA SPP. POTENTIALLY PATHOGENIC FOR HUMANS IN IXODES RICINUS TICKS OF NORTH-EASTERN ITALY
Falcaro C.[1], Granato A.[1], Babiker A.M.A.[1], Cassini R.[2], Capelli G.[1], Montarsi F.[1], Pietrobelli M.[2], **Ravagnan S.***[1]
[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [2]Department of Animal Medicine, Production and Health ~ University of Padua ~ Italy

O-08.4 OVERVIEW ON DOURINE OUTBREAK IN ITALY; SURVEILLANCE, RESULTS OF CLINICAL AND LABORATORY MONITORING OF INFECTED HORSES, LESIONS AND DEVELOPMENT OF LABORATORY DIAGNOSIS.
Pascucci I.*[1], Cammà C.[1], Di Provvido A.[1], Di Domenico M.[1], Luciani M.[1], Narcisi V.[1], Giansante D.[1], Di Francesco G.[1], Podaliri Vulpiani M.[1], Scacchia M.[1]
[1]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" Campo Boario 64100 ~ Teramo ~ Italy

O-08.5 CYCLOSPORA CAYETANENSIS IN ENVIRONMENTAL SAMPLES IN ITALY
Giangaspero A.*[1], Marangi M.[1], Lacasella V.[1], Lonigro A.[2]
[1]University of Foggia ~ Foggia ~ Italy, [2]University of Bari ~ Bari ~ Italy

O-08.6 EVAGREEN® REALTIME PCR (RT-PCR), PLASMID-BASED CONTROLS AND HIGH-RESOLUTION MELTING (HRM) ASSAY FOR ANALYSIS OF TOXOPLASMA GONDII AND CYCLOSPORA CAYETANENSIS IN ENVIRONMENTAL AND FOOD SAMPLES
Marangi M.*[1], Giangaspero A.[1]
[1]University of Foggia ~ Foggia ~ Italy

O-08.7 SEROPREVALENCE AND RISK FACTORS FOR TOXOPLASMA GONDII IN SMALL RUMINANTS IN NORTHERN ITALY
Gazzonis A.L.*[1], Veronesi F.[2], Zanzani S.A.[1], Molineri G.[1], Moretta I.[2], Moretti A.[2], Piergili Fioretti D.[2], Invernizzi A.[3], Manfredi M.T.[1]
[1]Department of Veterinary Science and Public Health, University of Milan ~ Milan ~ Italy, [2]Department of Bio-Pathological Sciences and Hygiene of Animal and Food Production, University of Perugia ~ Perugia ~ Italy, [3]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna ~ Milano ~ Italy

O-08.8 TOXOPLASMOSIS IN HAEMATOPOIETIC STEM CELLS TRANSPLANTED PATIENTS . IMPROVING STRATEGIES FOR DIAGNOSIS AND FOLLOW-UP
Meroni V.*[1], Genco F.[2], Colombo A.A.[3], Ripamonti F.[3], Alessandrino P.E.[3], Di Matteo A.M.[4]
[1]Dipartimento Terapia Medica e Medicina Interna università degli studi di Pavia ~ pavia ~ Italy, [2]SC Microbiologia e Virologia Fondazione IRCCS Policlinico San Matteo PAVIA ~ pavia ~ Italy, [3]Unità Trapianto, Clinica Ematologica, Fondazione IRCCS Policlinico San Matteo Pavia ~ pavia ~ Italy, [4]Dipartimento Malattie Infettive Fondazione IRCCS Policlinico San Matteo Pavia ~ pavia ~ Italy

O-08.9 EVALUATION OF A NOVEL RAPID ANTIGEN DETECTION TEST COMPARED TO IN HOUSE REAL-TIME PCR SYSTEM FOR DIAGNOSIS OF AMEBIASIS IN CLINICAL SPECIMENS
Formenti F.*[1], Perandin F.[1], Bonafini S.[1], Degani M.[1], Bisoffi Z.[1]
[1]Ospedale Sacro Cuore Don Calabria ~ Negrar - Verona ~ Italy

O-08.10 MOLECULAR DIAGNOSTICS OF INTESTINAL PROTOZOA IN PATIENTS WITH IRRITABLE BOWEL SYNDROME
Tosini F.[2], Biasutto D.[1], Badiali D.[1], Pozio E.[2], **Cacciò S.M.***[2]
[1]Policlinico Umberto I ~ Roma ~ Italy, [2]Istituto Superiore di Sanita ~ Roma ~ Italy

O-08.11 INTESTINAL PROTOZOA AND HUMAN GUT MICROBIOTA IN CÔTE D'IVOIRE
Berrilli F.[1], Schippa S.[2], Iebba V.[2], Santangelo F.[2], Totino V.[2], Di Cave D.[1], Di Cristanziano V.[3], **D'Alfonso R.***[4]
[1]Department of Experimental Medicine and Surgery, Tor Vergata University ~ Rome ~ Italy, [2]Department of Public Health and Infectious Diseases, Sapienza University ~ Rome ~ Italy, [3]Institute of Virology, University of Cologne ~ Cologne ~ Germany, [4] Department of Systems Medicine, Tor Vergata University ~ Rome ~ Italy

O-08.12 INTESTINAL PARASITES IN TWO RURAL COMMUNITIES LIVING IN THE CORDILLERA PROVINCE (PLURINATIONAL STATE OF BOLIVIA)
Macchioni F.*[1], Suniga Rios F.[2], Totino V.[3], Gabrielli S.[3], Rojas Gonzales P.[2], Roselli M.[4], Bartoloni A.[4], Cancrini G.[3]
[1]Dipartimento di Scienze Veterinarie ~ Pisa ~ Italy, [2]Distrito de Salud Cordillera ~ Santa Cruz ~ Bolivia, Plurinational State of, [3] Dipartimento di Sanità Pubblica e Malattie Infettive, Università "Sapienza" ~ Roma ~ Italy, [4]Dipartimento di Medicina Sperimentale e Clinica ~ Firenze ~ Italy

O-08.13 DETECTION AND GENOTYPING OF FREE-LIVING AMOEBAE OF THE GENUS ACANTHAMOEBA IN WATER SAMPLES
Di Cave D.*[1], D'Alfonso R.[2], Santoro M.[1], Montalbano Di Filippo M.[1], Capolongo C.[3], Cinquepalmi V.[3], Monno R.[3], Berrilli F.[1]
[1]Department of Experimental Medicine and Surgery, Tor Vergata University ~ Rome ~ Italy, [2]Department of Systems Medicine, Tor Vergata University ~ Rome ~ Italy, [3]Department of Basic Medical Science, Neuroscience and Sense Organ, University of Bari ~ Bari ~ Italy

SESSIONE PARALLELA O-09 Controllo e monitoraggio di vettori

08:30 - 11:00
Moderatori: G. Favia, C. De Liberato

O-09.1 MONITORING PHLEBOTOMUS SERGENTI IN URBAN, PERIURBAN AND RURAL SITES OF CATANIA PROVINCE, SICILY, AND SAND FLY POPULATION DYNAMICS
Bongiorno G.*[1], Vaccalluzzo V.[2], Severini F.[1], Lisi O.[2], Khoury C.[1], Di Muccio T.[1], D'Urso V.[2], Maroli M.[1], Gradoni L.[1], Gramiccia M.[1]
[1]Unit of Vector-borne Diseases and International Health, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy, [2]Department of Biological, Geological and Environmental Sciences, Section of Animal Biology "M. La Greca", University of Catania ~ Catania ~ Italy

O-09.2 EVALUATION OF COLLECTION METHODS FOR PHLEBOTOMUS-BORNE VIRUSES DETECTION: ISOLATION AND VIRAL RNA INTEGRITY PERFORMANCE
Remoli M.E.*[1], Bongiorno G.[2], Fortuna C.[1], Marchi A.[1], Bianchi R.[2], Khoury C.[2], Ciufolini M.G.[1], Gramiccia M.[2]
[1]Unit of Viral diseases and attenuated vaccine, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy, [2]Unit of Vector-borne Diseases and International Health, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy

O-09.3 COSTS EVALUATION AND BENEFITS OF THE ENTOMOLOGICAL MONITORING: THE EXPERIENCE OF REGIONAL SURVEILLANCE PROGRAMS FOR WEST NILE DISEASE IN NORTH-EASTERN ITALY
Montarsi F.*[1], Ravagnan S.[1], Ciocchetta S.[2], Omodeo S.G.[1], Giuliato I.[1], Mazzucato M.[1], Russo F.[3], Palei M.[4], Marangon S.[1], Capelli G.[1]
[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [2]1 Mosquito Control Laboratory, QIMR Berghofer Medical Research Institute; 2 Istituto Zooprofilattico Sperimentale delle Venezie ~ Brisbane ~ Australia, [3]Promotion and Development Hygiene and Public Health Service, Veneto Region ~ Venice ~ Italy, [4]Veterinary Public Health Service, Friuli Venezia Giulia region ~ Udine ~ Italy

O-09.4 IMMEDIATE AND RESIDUAL ANTI-FEEDING EFFICACY OF A DINOTEFURAN-PERMETHRIN-PYRIPROXYFEN TOPICAL ADMINISTRATION AGAINST SANDFLY (PHLEBOTOMUS PERNICIOSUS) INFESTED DOGS UNDER LABORATORY CONDITIONS
Varloud M.[1], Warin S.[1], **Ferrari G.***[2], Columba M.[3], Mcgrath S.[3], Martin M.[3]
[1]Ceva ~ Libourne ~ France, [2]Ceva Salute Animale S.p.A ~ Agrate Brianza ~ Italy, [3]Charles River Laboratories Preclinical Services Ireland Ltd ~ Ballina, Co. Mayo ~ Ireland

O-09.5 IMMEDIATE AND RESIDUAL ANTI-FEEDING AND INSECTICIDAL EFFICACY OF A DINOTEFURAN-PERMETHRIN-PYRIPROXYFEN TOPICAL ADMINISTRATION AGAINST STABLE FLY (STOMOXYS CALCITRANS) CHALLENGES ON DOGS
Varloud M.[1], Fourie J.[2], **Crippa A.***[3]
[1]Ceva ~ Libourne ~ France, [2]ClinVet International (Pty) Ltd ~ Bloemfontein ~ South Africa, [3]Ceva Salute Animale S.p.A ~ Agrate Brianza ~ Italy

O-09.6 EFFICACY AND RESIDUAL ACTIVITY OF SUNLIGHT-ACTIVATABLE PORPHYRIN FORMULATIONS, NEEM PREPARATIONS AND PORPHYRIN-NEEM COMBINATIONS AGAINST LARVAE OF ANOPHELES GAMBIAE S.L.
Ouedraogo R.[1], Yerbanga S.[1], Damiano S.[2], Sawadogo S.[1], Fabris C.[3], Lucantoni L.[2], Diabate A.[1], Coppellotti O.[3], **Lupidi G.***[3], Jori G.[3], Ouedraogo J.B.[1], Habluetzel A.[2], Dabire R.[1]
[1]Institut de Recherche en Science de la Sante/Centre Muraz ~ Bobo-Dioulasso ~ Burkina Faso, [2]School of Pharmacy, University of Camerino ~ Camerino ~ Italy, [3]Department of Biology, University of Padova ~ Padova ~ Italy

O-09.7 EFFICACY OF DISINFESTATION AGAINST ADULT OF CULEX PIPIENS MOSQUITOES IN WEST NILE VIRUS POSITIVE SITES OF NORTH-EASTERN ITALY
Carlin S.*[1], Martini S.[6], Signorini M.[2], Scremin M.[1], Nicolardi L.[3], Foroni M.[4], Russo F.[5], Capelli G.[1], Montarsi F.[1]
[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro - Padova ~ Italy, [2]Department of Animal Medicine, Production and Health, Padua University ~ Padova ~ Italy, [3]Dipartimento di Prevenzione, ULSS 10 ~ San Donà di Piave - Venezia ~ Italy, [4]Dipartimento di Prevenzione, ULSS 22, Bussolengo, Italy, [5]Promotion and Development Hygiene and Public Health Service, Veneto Region ~ Venezia ~ Italy, [6]Entostudio srl ~ Brugine - Padova ~ Italy

O-09.8 EVALUATION OF INSECTICIDE TREATMENTS AGAINST AEDES ALBOPICTUS IN THE CAMPUS OF UNIVERSITY OF ROME SAPIENZA
Caputo B.*[1], Ienco A.[1], Manica M.[2], Rosà R.[2], Bruni A.[3], Fascetti R.[3], Petrarca V.[4], Della Torre A.[1]
[1]Dipartimento di Scienze di Sanità Pubblica e Malattie Infettive, Università di Roma "Sapienza" ~ Roma ~ Italy, [2]Environment and Natural Resources Area, IASMA Research and Innovation Centre, Edmund Mach Foundation ~ S. Michele all'Adige ~ Italy, [3]Sogea srl ~ Roma ~ Italy, [4]Dipartimento di Biologia e Biotecnologie "Charles Darwin" ~ Roma ~ Italy

O-09.9 EVALUATION OF A NOVEL SAMPLING TOOL TO DETECT ABUNDANCE AND INFECTIVE STATUS OF MALARIA VECTOR SPECIES OF THE ANOPHELES GAMBIAE COMPLEX
Pombi M.*[1], Guelbeogo W.M.[2], Calzetta M.[1], Kreppel K.[3], Traoré A.[2], Sanou A.[2], Ranson H.[4], Ferguson H.M.[3], Mancini E.[1], Sagnon N.[2], Della Torre A.[1]
[1]Dipartimento di Sanità Pubblica e Malattie Infettive, Università di Roma "Sapienza" ~ Roma ~ Italy, [2]Centre National de Recherche et Formation sur le Paludisme ~ Ouagadougou ~ Burkina Faso, [3]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow ~ Glasgow ~ United Kingdom, [4]Vector Group, Liverpool School of Tropical Medicine ~ Liverpool ~ United Kingdom

O-09.10 A KILLER YEAST STRAIN IS HARBORED IN MALARIA VECTORS: NEW INSIGHTS IN THE MOSQUITO BIOLOGY AND POSSIBLE IMPLICATIONS IN THE MALARIA TRANSMISSION BLOCKING
Valzano M.*[1], Cappelli A.[1], Ulissi U.[1], Damiani C.[1], Capone A.[1], Bozic J.[1], Cecarini V.[1], Favia G.[1], Ricci I.[1]
[1]Università di Camerino ~ Camerino ~ Italy

O-09.11 PATHOGENICITY OF A NATIVE STRAIN OF BEAUVERIA BASSIANA ON RHIPICEPHALUS SANGUINEUS GROUP TICKS UNDER LABORATORY CONDITION
Immediato D.*[1], Cafarchia C.[1], Ramos R.A.N.[1], Lia R.P.[1], Iatta R.[1], Figueredo L.A.[2], Dantas Torres F.[2], Otranto D.[1]
[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [2]Department of Immunology, Aggeu Magalhães Research Centre, Recife-PE, Brazil; Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy

O-09.12 MODELS FOR THE CONTROL AND THE THERAPY OF VECTOR-BORNE DISEASES: KILLER YEASTS AND KILLER PEPTIDES
Epis S.*[1], Damiani C.[2], Giovati L.[3], Ricci I.[2], Favia G.[2], Polonelli L.[3], Kramer L.H.[4], Bandi C.[1]
[1]Department of Veterinary Science and Public Health ~ Milan ~ Italy, [2]School of Biosciences and Biotechnology ~ Camerino ~ Italy, [3]Department of Pathology and Laboratory Medicine ~ Parma ~ Italy, [4]Department of Animal Health ~ Parma ~ Italy

O-09.13 DEVELOPMENT OF A PCR ASSAY TO DETECT IXODIPHAGUS HOOKERI (HYMENOPTERA, ENCYRTIDAE) IN TICKS
Campbell B.E.*[1], Giannelli A.[1], Ramos R.A.N.[1], Whittle A.[2], Dantas Torres F.[3], Wall R.[2], Otranto D.[1]
[1]Universita di Bari Aldo Moro ~ Bari ~ Italy, [2]University of Bristol ~ Bristol ~ United Kingdom, [3]Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães (Fiocruz-PE) ~ Recife ~ Brazil

SIMPOSIO S2 Italian Malaria Network

11:30 - 13:30
Moderatori: A. Crisanti, D. Modiano

S2.1 PREDICTION OF PLASMODIUM FALCIPARUM PROTEIN INTERACTION NETWORK: THE MEMBRANE MICRODOMAIN INTERACTOME.
Sferra G.[1], Santoni D.[2], Ponzi M.[1], **Pizzi E.***[1]
[1]Dipartimento di Malattie Infettive, Parassitarie ed Immunomediate, Istituto Superiore di Sanità, Roma, Italy, [2]Istituto di Analisi dei Sistemi ed Informatica "Antonio Ruberti", Consiglio Nazionale delle Ricerche, Roma, Italy

S2.2 DIFFERENT STRATEGIES FOR THE DESIGN AND SYNTHESIS OF INNOVATIVE ANTIMALARIALS: DEALING WITH PARASITE RESISTANCE AND SEVERE MALARIA
Gemma S.*[1], Taramelli D.[2], Blackman M.J.[3], Martin R.[4], Croft S.[5], Brun R.[6], Craig A.[7]
[1]Dipartimento di Biotecnologie, Chimica e Farmacia, Universita' di Siena, Italy, [2]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università di Milano, Italy, [3]Division of Parasitology, MRC National Institute for Medical Research, London, UK, [4]Research School of Biology, The Australian National University, Canberra, Australia, [5]London School of Hygiene and Tropical Medicine, London, UK, [6]Swiss Tropical and Public Health Institute, Basel, Switzerland, [7]Liverpool School of Tropical Medicine, Liverpool, UK

S2.3 NOVEL ASSAYS FOR DRUGS AGAINST PLASMODIUM FALCIPARUM GAMETOCYTES
Camarda G.[1], Silvestrini F.[1], Cevenini L.[2], Siciliano G.[1], Michelini E.[2], Calabretta M.[2], Signore M.[1], Bona R.[1], Kumar Tiruppadiripuliyur S.[3], Branchini B.C.[4], Cara A.[1], Fidock D.A.[3], Roda A.[2], **Alano P.***[1]
[1]Dipartimenti di Malattie Infettive, Parassitarie ed Immunomediate e di Farmaco, Istituto Superiore di Sanità, Rome, Italy, [2]Dipartimento di Chimica, Università di Bologna, Bologna, Italy, [3]Department of Microbiology and Immunology, Columbia University, New York, NY, USA, [4]Connecticut College, New London CT, USA

S2.4 NEW IN VITRO ASSAYS TO SCREEN COMPOUNDS AGAINST P. FALCIPARUM GAMETOCYTES: A PROGRESS TOWARD THE IDENTIFICATION OF TRANSMISSION BLOCKING AGENTS
D'alessandro S.*[1], Basilico N.[2], Parapini S.[1], Corbett Y.[1], Misiano P.[1], Camarda G.[3], Siciliano G.[3], Michelini E.[4][5], Cevenini L.[4], Roda A.[4], Alano P.[3], Taramelli D.[1]
[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università di Milano, Italy, [2]Dipartimento di Scienze Biomediche, Chirurgiche ed Odontoiatriche, Università di Milano, Milano, Italy, [3]Dipartimento di Malattie Infettive, Parassitarie, Immunomediate, Istituto Superiore di Sanità, Roma, Italy, [4]Dipartimento di Chimica "G. Ciamician", Università di Bologna, Bologna, Italy, [5]INBB, Istituto Nazionale di Biostrutture e Biosistemi, Roma, Italy

S2.5 TRANSMISSION BLOCKING ACTIVITY OF VERNONIA AMYGDALINA ON PLASMODIUM BERGHEI AND PLASMODIUM FALCIPARUM FIELD ISOLATES
Abay S.M.*[1], Dahiya N.[1], Dembo E.[1], Dori G.[2], Esposito F.[1], Lucantoni L.[1], Lupidi G.[1], Ogboi S.[1], Ouédraogo K.R.[1], Ouédraogo K.R.[3], Ouédraogo J.B.[3], Sinisi A.[4], Taglialatela-Scafati O.[4], Yerbanga R.[3], Habluetzel A.[1]
[1]University of Camerino, Camerino, Italy, [2]University of Naples "Federico II", Naples, Italy, [3]Institut de Recherche en Sciences de la Santé ~ Bobo-Dioulasso ~ Burkina Faso, [4]Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso

S2.6 IN VITRO AND IN VIVO ACTIVITY OF NEEMAZAL® AND AZADIRACHTIN A AGAINST EARLY SPOROGONIC STAGES OF PLASMODIUM BERGHEI
Dahiya N.*[1], Abay S.M.[1], Chianese G.[2], Esposito F.[1], Habluetzel A.[1], Lupidi G.[1], Ogboi S.[1], Taglialatela-Scafati O.[2], Lucantoni L.[1]
[1]School of Pharmacy, University of Camerino, Camerino, Italy, [2]Department of Pharmacy, University of Naples "Federico II", Naples, Italy

S2.7 ANTIMALARIAL POTENTIAL OF SYNTHETIC QUINONE DERIVATIVES DESIGNED ON THE MODEL OF THE APLIDINONES, NATURAL MARINE THIAZINOQUINONES.
Imperatore C.*[1], Aiello A.[1], D'aniello F.[1], Luciano P.[1], Fattorusso C.[1], Persico M.[1], Taramelli D.[2], Parapini S.[2], Cebrián-Torrejón G.[3], Doménech-Carbó A.[3], Menna M.[1]
[1]The NeaNat Group, Department of Pharmacy, University of Naples "Federico II", Napoli, Italy, [2]Scienze Farmacologiche e Biomolecolari Università di Milano, Milan, Italy, [3]Departament de Química Analítica, Facultat de Química, Universitat de València, Burjassot, Valencia, Spain.

S2.8 ANTIMALARIAL 1,2-DIOXANES INSPIRED BY MARINE NATURAL PRODUCTS
Chianese G.*[1], Fattorusso E.[1], Taglialatela-Scafati O.[1], Persico M.[1], Fattorusso C.[1], Parapini S.[2], Corbett Y.[2], Taramelli D.[2], Basilico N.[3], Quintavalla A.[4], Trombini C.[4], Lombardo M.[4]
[1]Dipartimento di Farmacia, Università di Napoli "Federico II", Napoli, Italy, [2]Dipartimento di Scienze Farmacologiche e Biomolecolari Università di Milano, Milano, Italy, [3]Dipartimento di Scienze Mediche, Chirurgiche e Odontoiatriche, Università di Milano, Milano, Italy, [4]Dipartimento di Chimica "Giacomo Ciamician", Alma Mater Studiorum, Università di Bologna, Bologna, Italy

S2.9 BIOACTIVE MOLECULES OF MALARIAL PIGMENT HEMOZOIN AND THEIR PATHOMECHANISTIC ROLE IN MALARIA
Skorokhod O.[1], Uyoga S.[1], Marrocco T.[1], Aguilar R.[2], Gremo G.[1], Barrera V.[1], Gallo V.[1], Davalos D.[1], Schwarzer E.[1], **Arese P.***[1]
[1]Dipartimento di Oncologia, University of Torino Medical School, Torino, Italy, [2]Centre de Recerca en Salut Internacional de Barcelona (CRESIB, Hospital Clínic-Universitat de Barcelona), Barcelona, Spain

S2.10 AUTOIMMUNITY LOCI PLAY A ROLE IN THE SUSCEPTIBILITY TO PLASMODIUM FALCIPARUM INFECTION AND UNCOMPLICATED DISEASE

Mangano V.D.* [1-2], Bougouma E.C. [3], Verra F. [1], Sepulveda N. [4-5], Rockett K.A. [6-7], Kabore Y. [3], Diarra A.[3], Bisseye C. [1-3], Nebie I. [3], Kwiatkowski D.P. [6-7], Sirima B.S. [3], Modiano D. [1-2] & The MalariaGEN Consortium [6-7]
[1]Dept. of Public Health and Infectious Diseases, Sapienza University of Rome, Italy, [2]Istituto Pasteur - Fondazione Cenci Bolognetti, Sapienza University of Rome, Italy, [3]Centre National de Recherche et Formation sur le Paludisme, Ouagadougou, Burkina Faso, [4] London School of Hygiene and Tropical Medicine, United Kingdom, [5]Center of Statistics and Applications of University of Lisbon, Lisbon, Portugal, [6]Wellcome Trust Centre for Human Genetics, University of Oxford, United Kingdom, [7]Wellcome Trust Sanger Institute, Hinxton, United Kingdom

S2.11 ROLE OF MICROBIAL COMMUNITY OF BREEDING HABITATS IN LARVAL NICHE PARTITIONING OF ANOPHELES GAMBIAE MALARIA VECTORS IN BURKINA FASO.

Pombi M.*[1], Totino V.[1], Iebba V.[1], Santangelo F.[1], Bassole I.H.N.[2], Guelbeogo W.M.[3], Sagnon N.[3], Della Torre A.[1], Schippa S.[1], Costantini C.[4]
[1]Dipartimento di Sanità Pubblica e Malattie Infettive, Università di Roma "Sapienza" ~ Roma ~ Italy, [2]Université de Ouagadougou, Laboratoire de Biochimie Alimentaire Enzymologie Biotechnologie Industrielle et Bioinformatique ~ Ouagadougou ~ Burkina Faso, [3]Centre National de Recherche et Formation sur le Paludisme ~ Ouagadougou ~ Burkina Faso, [4]Institut de Recherche pour le Développement, UMR MIVEGEC (UM1, UM2, CNRS 5290, IRD 224), Centre IRD France-Sud ~ Montpellier ~ France

S2.12 ANOPHELES GAMBIAE SALIVARY PROTEINS AS A TOOL TO EVALUATE SPATIAL AND TEMPORAL VARIATION OF HUMAN EXPOSURE TO MALARIA VECTORS

Lombardo F.*[1], Ronca R.[2], Rizzo C.[1], Mangano V.[1], Sirima B.[3], Nebié I.[3], Bousema T.[4], Drakeley C.[4], Modiano D.[1], Arcà B.[1]
[1]Dept of Public Health and Infectious Diseases, Parasitology Section, Sapienza University, Rome, Italy, [2]Dept of Biology, Federico II University, Naples, Italy, [3]Centre National Recherche et Formation sur Le Paludisme, Ouagadougou, Burkina Faso, [4]Dept of Immunity and Infection, London School of Hygiene and Tropical Medicine, London, UK

S2.13 SYMBIONTS AND MOSQUITO VECTORS: WORK IN PROGRESS AT UNICAM.

Ricci I.* [1], Damiani C. [1], Rossi P.[1], Capone A. [1], Valzano M. [1], Cappelli A. [1], Bokzic J. [1], Mancini M.V. [1], Favia G.[1]
[1]School of Biosciences and Veterinary Medicine, University of Camerino, Italy

SIMPOSIO S3 Parassitosi cardio-polmonari del cane e del gatto, uno scenario in continua evoluzione

11:30 - 13:30
Moderatori: L. Kramer, D. Traversa

S3.1 EMERGING CARDIO-RESPIRATORY CANINE AND FELINE NEMATODES IN ITALY

Di Cesare A.*[1], Brianti E.[2], Traversa D.[1]
[1]Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy, [2]Department of Veterinary Sciences, University of Messina, Messina, Italy

S3.2 LUNG AND NASAL CAPILLARIOSIS OF COMPANION ANIMALS AS EMERGING DISEASES

Veronesi F.*[1], Morganti G.[1]
[1]Department of Veterinary Medicine of Perugia, Italy

S3.3 CLINICAL FEATURES OF CARDIOPULMONARY PARASITIC DISEASES IN DOGS AND CATS

Venco L.*[1]
[1]Veterinary Hospital Città di Pavia, Italy

S3.4 NEW INSIGHTS ON THE BIOLOGY, EPIDEMIOLOGY AND DIAGNOSIS OF TROGLOSTRONGYLUS SPP. INFECTION IN CATS

Giannelli A.*[1], Brianti E.[2], Ramos R.A.N.[1], Giannetto S.[2], Dantas Torres F.[1][3], Otranto D.[1]
[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Italy, [2]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina, Polo Universitario Annunziata, Italy, [3]Department of Immunology, Aggeu Magalhães Research Institute, Brazil

S3.5 ANIMAL DIROFILARIOSIS IN THE IBERIAN PENINSULA

Simón F.*[1], Simón L.[1], Morchón R.[1], González-Miguel J.[1]
[1]Laboratory of Parasitology, Faculty of Pharmacy, University of Salamanca, Spain

XXVIII Congresso Nazionale SoIPa, Roma 24-27 giugno 2014

PROGRAMMA DETTAGLIATO

TV1 Tavola rotonda su: La parassitologia italiana nella Cooperazione internazionale

14:30 - 16:45
Moderatori: D. Modiano, R. Cassini, Z. Bisoffi

1) Gentile B.(MAE-DGCS)
2) Bartoloni A. (Università di Firenze – SIMET)
3) Esposito F. (Università di Camerino)
4) Maiori G. (ISS)
5) Crisanti A. (Università di Perugia)
6) Albonico M. (Fondazione De Carneri)
7) De Balogh K.(FAO)
8) De Meneghi D. (Università di Torino)
8) Corsi M. (Sigma-Tau)
9) Broglia A. (presidente VSF Italia e VSF International)
10) Ghirotti M. (MAE)

TV2 Tavola rotonda su: Migrazioni e tutela sanitaria

17:00 - 19:00
Moderatori: V. Vullo, G. Taliani, G. Cancrini

TV2.1 MIGRATION AND HEALTH IN THE PERSPECTIVE OF PUBLIC HEALTH

Marceca M.*[1]
[1]Dep. of Public Health and Infectious Diseases "Sapienza" University, Rome. Italian Society of Migration Medicine

T-V2.2 TUTELA DEI MIGRANTI PRESENTI IN ITALIA AFFETTI DALLA "MALATTIA INVISIBILE" DI CHAGAS

Angheben A.*[1]
[1]Società Italiana di Medicina Tropicale, Rome; Centre for Tropical Diseases, Hospital Sacro Cuore, Negrar)

TV2.3 HEALTH PROTECTION, IN SPAIN, OF MIGRANTS AFFECTED BY CHAGAS DISEASE

Osuna A.*[1], Marti Gimenez M.[2], Munoz Calabuig E.[2], Parada C.[3], Rodrigues Garcia M.[4]
[1]Instituto de Biotecnología, Universidad de Granada, Spain, [2]Hospital La Fé. Valencia, Spain, [3]Centro de Transfusion de Comunidad Valenciana, Spain, [4]Hospital General Universitario Valencia, Spain

TV2.4 PROSSIMITÀ, GRATUITÀ E RETI PER LA SALUTE DEGLI IMMIGRATI

Geraci S.*[1]
[1]Area sanitaria Caritas Roma, Società Italiana di Medicina delle Migrazioni

TV2.5 THE MSF EXPERIENCE AS PRIMARY HEALTH CARE PROVIDER FOR MIGRANTS IN ITALY: MAIN FINDINGS AND LESSONS LEARNT

Egidi S.*[1]
[1]Medici Senza Frontiere

TV2.6 LABORATORY UNIT FOR THE DIAGNOSIS OF INTESTINAL PARASITIC INFECTIONS OF MIGRANTS IN CAMPANIA REGION

Galdiero M.*[1], Cringoli G.*[2]
[1]Department of Experimental Medicine of the Second University of Naples, [2]Department of Veterinary Medicine University of Naples "Federico II"

TV2.7 HEALTH CARE SERVICE FOR MIGRANTS IN POLICLINICO UMBERTO I HOSPITAL: EVOLUTION AND CURRENT ACTIVITY OF A DEDICATED SERVICE

Massetti A.P.*[1]
[1]Department of Public Health and Infectious Diseases, "Sapienza" University, Rome, Italy

TV2.8 PARASITES AND IMMIGRANTS: THE ROLE OF THE SCIENTIFIC COMMUNITY FROM THE ITALIAN PERSPECTIVE

Berrilli F.[1]
[1]Department of Experimental Medicine and Surgery, University of Rome Tor Vergata

Venerdì 27 Giugno 2014

SESSIONE PARALLELA O-10 Parassiti della fauna selvatica

08:30 - 11:00

Moderatori: P. Lanfranchi, L. Rossi

O-10.1 MOLECULAR DETECTION OF TICK-BORNE PATHOGENS IN SICILIAN WILD ANIMALS
Torina A.[1], Crucitti D.[1], Di Pasquale C.[1], Marino F.[1], De Maria C.[1], Tarantino V.[1], Randazzo K.[1], Blanda V.[1], **Caracappa S.***[1]
[1]Istituto Zooprofilattico Sperimentale della Sicilia ~ Palermo ~ Italy

O-10.2 SURVEY OF ZOONOTIC VISCERAL LEISHMANIASIS AMONG FOXES AND HUNTING DOGS LIVING IN SAME AREA OF THE CAMPANIA REGION OF ITALY
Piantedosi D.*[1], Veneziano V.[1], Di Muccio T.[2], Foglia Manzillo V.[1], Fiorentino E.[2], Scalone A.[2], Neola B.[1], D'Alessio N.[3], Gradoni L.[2], Oliva G.[1], Gramiccia M.[2]
[1]Department of Veterinary Medicine and Animal Production, University Federico II, Naples ~ Napoli ~ Italy, [2]Unit of Vector-borne Diseases and International Health, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy, [3]Istituto Zooprofilattico del Mezzogiorno, Avellino Section ~ Avellino ~ Italy

O-10.3 COPROLOGICAL AND MOLECULAR STUDY ON THE GASTRO-INTESTINAL PARASITES OF RED FOXES IN NORTH-EASTERN ITALY
Omodeo S.G.*[1], Cazzin S.[1], Danesi P.[1], Biasion L.[1], Zannini F.[2], Simonato G.[2], Francione E.[3], Pietrobelli M.[2], Capelli G.[1]
[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [2]Department of Animal Medicine, Production and Health, Padua University ~ Legnaro (PD) ~ Italy, [3]Istituto Zooprofilattico Sperimentale delle Venezie ~ Trento ~ Italy

O-10.4 A FOCUS OF ECHINOCOCCUS MULTILOCULARIS IN FOXES OF NORTH-EASTERN ITALY: AFTER TEN YEARS IS STILL THERE
Dellamaria D.[1], Trevisiol K.[1], Francione E.[1], Citterio C.[1], Simonato G.[2], Cazzin S.[1], Casulli A.[3], Deplazes P.[4], Marangon S.[1], **Capelli G.***[1]
[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro ~ Italy, [2]Department of Animal Medicine, Production and Health, Padua University ~ Legnaro ~ Italy, [3]MIPI Department, Istituto Superiore di Sanità ~ Rome ~ Italy, [4]Vetsuisse-Faculty, University of Zurich ~ Zurich ~ Switzerland

O-10.5 PREVALENCE OF EUCOLEUS BOEHMI INFECTION IN RED FOX (VULPES VULPES) FROM ITALY
Morganti G.*[1], Veronesi F.[1], Di Cesare A.[2], Ferroglio E.[3], Piergili Fioretti D.[1]
[1]Department of Veterinary Medicine ~ Perugia ~ Italy, [2]Faculty of Veterinary Medicine ~ Teramo ~ Italy, [3]Department of Veterinary Sciences ~ Turin ~ Italy

O-10.6 ANALYSIS OF THE HELMINTHOFAUNA OF EUROPEAN WILD CAT IN FRIULI VENEZIA GIULIA
Beraldo P.*[1], Massimo M.[1], Pascotto E.[1]
[1]Division of Veterinary Pathology, Department of Food Science, University of Udine ~ Udine ~ Italy

O-10.7 EUROPEAN WILDCATS (FELIS SILVESTRIS SILVESTRIS) AS SPREADERS OF LUNGWORMS
Falsone L.*[4], Brianti E.[4], Gaglio G.[4], Napoli E.[4], Anile S.[1], Mallia E.[3], Giannelli A.[2], Poglayen G.[5], Giannetto S.[4], Otranto D.[2]
[1]Dipartimento di Biologia Animale "Marcello La Greca", Univesità degli Studi di Catania ~ Catania ~ Italy, [2]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [3]Parco Regionale Gallipoli Cognato e Piccole Dolomiti Lucane, Basilicata ~ Matera ~ Italy, [4]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina ~ Messina ~ Italy, [5]Dipartimento di Scienze Mediche Veterinarie,Università degli Studi di Bologna ~ Bologna ~ Italy

O-10.8 OCCURRENCE OF ENDOPARASITES IN ROE DEER (CAPREOLUS CAPREOLUS) IN SPAIN: INFLUENCE OF AGE.
Morrondo P.*[1], Pérez A.[1], Pajares G.[2], Prieto A.[1], Cabanelas E.[1], Panadero R.[1], López C.[1], Fernández G.[1], Díaz P.[1], Díez Baños P.[1]
[1]University of Santiago de Compostela ~ Lugo ~ Spain, [2]Asociación del Corzo Español (ACE) ~ Madrid ~ Spain

O-10.9 DIRECT LIFE CYCLE ENDOPARASITES IN ROE DEER (CAPREOLUS CAPREOLUS) FROM SPAIN: INFLUENCE OF CLIMATIC FACTORS ON PREVALENCE AND INTENSITY OF ELIMINATION
Morrondo P.*[1], Pérez A.[1], Díaz P.[1], Prieto A.[1], Panadero R.[1], López C.[1], Fernández G.[1], Pajares G.[2], Díez Baños P.[1]
[1]University of Santiago de Compostela ~ Lugo ~ Spain, [2]Asociación del Corzo Español (ACE) ~ Madrid ~ Spain

O-10.10 COMPARISON BETWEEN SEROLOGICAL AND MOLECULAR DIAGNOSTIC APPROACH TO TOXOPLASMA GONDII INFECTION IN ALPINE RED DEER (CERVUS ELAPHUS)
Formenti N.*[1], Gaffuri A.[2], Trogu T.[1], Ferrari N.[1], Pedrotti L.[3], Viganò R.[4], Lanfranchi P.[1]
[1]Dipartimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di Milano ~ Milano ~ Italy, [2]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, sezione di Bergamo ~ Bergamo ~ Italy, [3]Consorzio Parco Nazionale dello Stelvio ~ Bormio (Sondrio) ~ Italy, [4]Medico Veterinario – AlpVet ~ Verbania ~ Italy

O-10.11 VARIABILITY AT NEUTRAL AND ADAPTIVE MARKERS IN THE ENDANGERED TATRIC CHAMOIS
Cavallero S.*[1], Vichova B.[2], Chovancová B.[3], Paule L.[4], Snabel V.[2], D'Amelio S.[1]
[1]Department of Public Health and Infectious Diseases ~ Rome ~ Italy, [2]Institute of Parasitology, Slovak Academy of Sciences ~ Kosice ~ Slovakia, [3]Tatra National Park Research Centre ~ Tatranská Lomnica ~ Slovakia, [4]Technical University ~ Zvolen ~ Slovakia

O-10.12 DETECTION OF CRYPTOSPORIDIUM AND GIARDIA BY ELISA ASSAY IN ALPINE CHAMOIS (RUPICAPRA R. RUPICAPRA)
Trogu T.*[1], Formenti N.[1], Ferrari N.[1], ViganÒ R.[2], Lanfranchi P.[1]
[1]Dipartimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di Milano ~ MILANO ~ Italy, [2]Medico Veterinario – AlpVet ~ VERBANIA ~ Italy

O-10.13 INTESTINAL PROTOZOANS IN RUPICAPRA SPP. POPULATIONS: BASELINE DATA IN THE FRAMEWORK OF THE RELEVANT ITALIAN PROJECT (PRIN)
De Liberato C.*[1], Trogu T.[2], Ferretti F.[3], Berrilli F.[4], Marani I.[4], Putignani L.[5], Santoro M.[5], Marangi M.[6], D'Amelio S.[7], Giangaspero A.[6]
[1]IZS Lazio e Toscana ~ Roma ~ Italy, [2]University of Milan ~ Milan ~ Italy, [3]University of Siena ~ Siena ~ Italy, [4]University of Tor Vergata ~ Rome ~ Italy, [5]OPBG ~ Rome ~ Italy, [6]University of Foggia ~ Foggia ~ Italy, [7]University Sapienza ~ Rome ~ Italy

O-10.14 DOES DECREASED BODY CONDITION CAUSE INCREASED SUSCEPTIBILITY TO PARASITIC INFECTION OR HIGH PARASITE INTENSITY CAUSE DECREASED BODY CONDITION? DEALING WITH THIS DILEMMA BY OBSERVATIONAL DATA
Ferrari N.*[1], Formenti N.[1], Trogu T.[1], Sartorelli P.[1], Citterio C.[2], Tarantola M.[3], Lanfranchi P.[1]
[1]DIVET, Università degli Studi di Milano ~ Milano ~ Italy, [2]Istituto Zooprofilattico Sperimentale delle Venezie ~ Belluno ~ Italy, [3]Dipartimento di Scienze Veterinarie Università di Torino ~ Grugliasco ~ Italy

O-10.15 PRE-ANALYTICAL BIAS MAY SEVERELY IMPACT ON THE RELIABILITY OF COPROMICROSCOPY DATA IN CHAMOIS AND OTHER MOUNTAIN DWELLING UNGULATES
Rossi L.*[1], Victoriano Llopis I.[1], Rambozzi L.[1], Demeneghi D.[1], Diaz Alcazar E.[1]
[1]Dept. Veterinary Sciences ~ TORINO ~ Italy

SESSIONE PARALLELA O-11a Infezioni da miceti

08:30 - 10:00

Moderatori: C. Cafarchia, A. Moretti

O-11a.1 MOLECULAR EVIDENCE OF POLYMORPHISM IN PNEUMOCYSTIS FROM WILD MICE AND SHREWS IN THE EASTERN ALPS, ITALY
Da Rold G.[1], Rizzoli A.[2], Hauffe H.C.[2], Arnoldi D.[2], Tagliapietra V.[2], Demanche C.[3], Guillot J.[4], Capelli G.[1], **Danesi P.***[1]
[1]Istituto Zooprofilattico delle Venezie, Parasitology Laboratory ~ Legnaro (PD) ~ Italy, [2]Fondazione E. Mach, Research and Innovation Centre, Department of Biodiversity and Molecular Ecology, S. Michele all'Adige ~ Trento ~ Italy, [3]Parasitology Dept, Faculty of Biological and Pharmaceutical Sciences ~ Lille ~ France, [4]Parasitology-Mycology Dept, Dynamyc research group, National Veterinary College of Alfort ~ Maisons-Alfort ~ France

O-11a.2 SURVEY ON YEASTS IN MUCOSAE OF DOGS WITH AND WITHOUT WEAKENING PATHOLOGIES
Galuppi R.*[1], Garutti S.[1], Fracassi F.[1], Capitani O.[1], Tampieri M.P.[1]
[1]Dipartimento di Scienze Mediche Veterinarie - Università Di Bologna ~ Bologna ~ Italy

O-11a.3 MALASSEZIA AND CANDIDA SPECIES INDUCING HUMAN BLOODSTREAM INFECTION
Iatta R.*[1], Otranto D.[1], Montagna M.T.[2], Cafarchia C.[1]
[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [2]Dipartimento di Scienze Biomediche e Oncologia Umana, Università degli Studi di Bari ~ Bari ~ Italy

O-11a.4 ANTIFUNGAL SUSCEPTIBILITY OF MALASSEZIA FURFUR FROM BLOODSTREAM INFECTIONS
Iatta R.*[1], Figueredo L.A.[2], Otranto D.[1], Cafarchia C.[1]
[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [2]Department of Immunology, Aggeu Magalhães Research Centre, Recife-PE, Brazil; Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy

O-11a.5 THERAPEUTIC ACTIVITY OF A KILLER PEPTIDE AGAINST MALASSEZIA PACHYDERMATIS OTITIS IN DOGS
Immediato D.*[1], Di Paola G.[1], Mogliani W.[2], Ciociola T.[2], Conti S.[2], Polonelli L.[2], Otranto D.[1], Cafarchia C.[1]
[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [2]Dipartimento di Scienze Biomediche, Biotecnologiche e Traslazionali, Università degli Studi di Parma ~ Parma ~ Italy

O-11a.7 EVALUATION OF TOXICITY OF EUCALYPTUS SPP. ESSENTIAL OILS ON BEAUVERIA BASSIANA
Oliveira Arruda De Abreu G.[1], Cafarchia C.[2], Otranto D.[2], Dantas Torres F.[1], Pinto Brandão Filho S.[1], **Aguiar Figueredo L.***[1]
[1]Centro de Pesquisas Aggeu Magalhães, Departamento de Imunologia. Fundação Oswaldo Cruz – FIOCRUZ ~ RECIFE ~ Brazil, [2] Dipartimento Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy

O-11a.8 GENOTYPE AND MATING TYPE CHARACTERISATION OF CRYPTOCOCCUS NEOFORMANS ISOLATES FROM ITALIAN VETERINARY ISOLATES
Danesi P.*[1], Capelli G.[1], Cogliati M.[2], Peano A.[3], Cafarchia C.[4], Granato A.[1], Meyer W.[5]
[1]Istituto Zooprofilattico delle Venezie, Parasitology Laboratory ~ Legnaro (PD) ~ Italy, [2]Medical Mycology Laboratory, Department of Scienze Biomediche per la Salute ~ Milan ~ Italy, [3]4Departmen of Veterinary Medicine, University of Turin ~ Turin ~ Italy, [4]Department of Veterinary Medicine, University of Bari ~ Bari ~ Italy, [5]Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Sydney Medical School - Westmead Hospital,The University of Sydney, Westmead Millennium Institute ~ Sydney ~ Australia

SESSIONE PARALLELA O-11b Immunità e immunopatologia delle infezioni parassitarie

10:00 - 11:00

Moderatori: f. Mancianti, F. Bruschi

O-11b.1 B CELL EPITOPES OF HEMOGLOBIN IN ANISAKIS

González Fernández J.[1], **Daschner A.***[2], Natalie N.[3], Cuéllar C.[1]

[1]Departamento de Parasitología, Facultad de Farmacia, Universidad Complutense ~ Madrid ~ Spain, [2]Servicio de Alergia, Instituto de Investigación Sanitaria, Hospital Universitario de la Princesa ~ Madrid ~ Spain, [3]Department of Immunology, Max Planck Institut für Infektionsbiologie ~ Berlin ~ Germany

O11b.2 HUMAN SENSITIZATION TO ANISAKIS PEGREFFII-ANTIGENS: COMPARISON OF TWO IMMUNOLOGICAL METHODS IN ALLERGIC ANISAKIASIS DIAGNOSIS

Mori Ubaldini F.*[1], Crisafi B.[2], Fazii P.[3], Bruschi F.[1], Mattiucci S.[2]

[1]Department of Translational Research,N.T.M.S., Università di Pisa ~ Pisa ~ Italy, [2]Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, Italy ~ Roma ~ Italy, [3]Hospital "Spirito Santo" ~ Pescara ~ Italy

O-11b.3 PROTECTIVE ROLE OF HAEMOGLOBIN S AND C AGAINST ASYMPTOMATIC PLASMODIUM FALCIPARUM PARASITAEMIA

Mangano V.*[1], Kabore Y.[2], Bougouma E.C.[2], Verra F.[1], Sepulveda N.[3], Bisseye C.[1], Santolamazza F.[1], Avellino P.[1], Diarra A.[2], Nebié I.[2], Sirima S.[2], Modiano D.[1]

[1]Sapienza University of Rome ~ Rome ~ Italy, [2]Centre National de Recherche et Formation sur le Paludisme ~ Ouagadougou ~ Burkina Faso, [3]London School of Hygiene and Tropical Medicine ~ London ~ United Kingdom

O-11b.4 OUTBREAK OF TRICHINELLOSIS IN GARFAGNANA (TUSCANY): CLINICAL AND LABORATORY OBSERVATIONS

De Gennaro M.[1], **Bruschi F.***[2], Luciana R.[1], Mattei R.[1], Gomez Morales M.A.[3], Pozio E.[3], Luchi S.[1]

[1]Ospedale di Lucca ~ Lucca ~ Italy, [2]Università di Pisa ~ Pisa ~ Italy, [3]EURLP-Istituto Superiore di Sanità ~ Roma ~ Italy

O-11b.5 INDUCTION OF TH1 SHIFT IN IMMUNE RESPONSE IMPROVES HEALTH CONDITION OF DOGS WITH CHRONIC CANINE LEISHMANIASIS

Rossi G.*[1], Colizzi V.[2], Loria G.R.[3], Montesano C.[2], Scarpona S.[1]

[1]School of Veterinary Medical Sciences University of Camerino ~ Camerino ~ Italy, [2]Department of Biology Faculty of Science University of Tor Vergata ~ Roma ~ Italy, [3]Experimental Zooprophylactic Institute (IZS) of Sicily National Reference Center for Leishmaniasis and Anisakiasis ~ Palermo ~ Italy

SESSIONE PARALLELA O-12 Parassiti degli animali da compagnia e rischio zoonosico

08:30 - 11:00

Moderatori: G. Garippa, A. Frangipane

O-12.1 ENVIRONMENTAL CONTAMINATION BY PET FAECES AND PARASITIC RISK IN THE CITY OF SASSARI (SARDINIA, ITALY)

Tamponi C.*[1], Varcasia A.[1], Del Giudice F.[1], Serra C.[1], Sanna G.[1], Dore F.[1], Pipia A.P.[1], Brianti E.[2], Scala F.[1]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari ~ Sassari ~ Italy, [2]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina ~ Messina ~ Italy

O-12.2 CANINE FAECAL CONTAMINATION IN UDINE AND EVALUATION OF HEALTH RISK

Beraldo P.*[2], Candusso S.[2], Mingotto F.[2], Arzese A.[1]

[1]Experimental Clinical Medicine, University of Udine ~ Udine ~ Italy, [2]Division of Veterinary Pathology, Department of Food Science, University of Udine ~ Udine ~ Italy

O-12.3 ARE WE PERFORMING CORRECT CONTROL PROGRAMS AGAINST CANINE HELMINTHS? REMARKS ON OWNED DOG POPULATIONS FROM ROME AND PADUA PROVINCES

Frangipane Di Regalbono A.*[1], Traversa D.[2], La Torre F.[3], Cassini R.[1], Zanardello C.[4], Omodeo S.G.[4], Mutarello M.[5], Pietrobelli M.[1]

[1]Dipartimento di Medicina Animale, Produzioni e Salute, Università degli Studi di Padova ~ Padova ~ Italy, [2]Facoltà di Medicina Veterinaria, Università di Teramo ~ Teramo ~ Italy, [3]Novartis Animal Health ~ Origgio (Varese) ~ Italy, [4]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (Padova) ~ Italy, [5]Practitioner ~ Bassano del Grappa (Vicenza) ~ Italy

O-12.4 GIARDIA DUODENALIS IN SHELTER AND PRIVATELY OWNED DOGS IN NORTH-EASTERN ITALY

Simonato G.*[1], Marcer F.[1], Tessarin C.[1], Traversa D.[2], Zanardello C.[3], Ravagnan S.[3], Pietrobelli M.[1]

[1]Dipartimento di Medicina Animale, Produzioni e Salute, Università degli Studi di Padova ~ Padova ~ Italy, [2]Facoltà di Medicina Veterinaria, Università di Teramo ~ Teramo ~ Italy, [3]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (Padova) ~ Italy

O-12.5 ZOONOTIC PARASITES IN FECAL SAMPLES AND FUR FROM DOGS

Paoletti B.*[1], Di Cesare A.[1], Iorio R.[1], De Berardinis A.[1], Bartolini R.[1], Gatti A.[1], Traversa D.[1]

[1]Faculty of Veterinary Medicine ~ Teramo ~ Italy

O-12.6 FLOTAC & PETS: A SERVICE FOR PARASITOLOGICAL DIAGNOSIS IN VETERINARY PRACTICE

Maurelli M.P.*[1], Musella V.[2], Del Prete L.[3], Pepe P.[1], Noviello E.[3], Pennacchio S.[1], Rinaldi L.[4], Cringoli G.[4]

[1]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania) ~ Naples ~ Italy, [2]Department of Experimental and Clinical Medicine, University Magna Graecia of Catanzaro ~ Catanzaro ~ Italy, [3]Private Practitioner ~ Naples ~ Italy, [4]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania); Inter-University Center for Research in Parasitology (CIRPAR) ~ Naples ~ Italy

O-12.7 CROSS-SECTIONAL SURVEY ON LEISHMANIA INFANTUM INFECTION IN DOGS IN NORTHERN SARDINIA

Pipia A.P.[1], **Varcasia A.***[1], Tosciri G.[1], Pitzalis F.[1], Cadeddu R.[1], Sanna G.[1], Dore F.[1], Tamponi C.[1], Manunta M.L.[1], Brianti E.[2], Scala A.[1], Vitale F.[3], Piazza M.[3]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari ~ Sassari ~ Italy, [2]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina ~ Messina ~ Italy, [3]Centro di Referenza Nazionale per le Leishmaniosi (C.Re.Na.L.), Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy

O-12.8 A QUANTITATIVE REAL-TIME PCR PROTOCOL FOR THE DIAGNOSIS OF CANINE LEISHMANIOSIS

Romano A.*[1], Bruno B.[2], Spina S.[1], Mignone W.[1], Ingravalle F.[1], Barzanti P.[1], Zanatta R.[2], Goria M.[1]

[1]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, ~ Torino ~ Italy, [2]Dipartimento di Scienze Veterinarie ~ Grugliasco, Torino ~ Italy

O-12.9 MOLECULAR IDENTIFICATION OF RICKETTSIA PATHOGENS FROM STRAY CATS AND DOGS AND THEIR FLEAS

Torina A.[1], Alaimo A.[1], **Blanda V.***[1], Giudice E.[2], D'Agostino R.[1], D'Agostino R.[1], D'Agostino R.[1], Caracappa S.[2], Lelli R.[1]

[1]Istituto Zooprofilattico Sperimentale della Sicilia ~ Palermo ~ Italia, [2]Faculty of Veterinary Medicine, Universitá degli Studi di Messina ~ Messina ~ Italy, [4]Dip. di Medicina Sperimentale e Clinica ~ Firenze ~ Italy

O-12.10 DOGS AS SOURCE OF TRYPANOSOMA CRUZI INFECTION IN RURAL AREAS OF THE CHACO REGION (PLURINATIONAL STATE OF BOLIVIA)

Totino V.*[1], Macchioni F.[2], Gabrielli S.[1], Fraulo M.[1], Segundo E.[3], Rojas Gonzales P.[3], Roselli M.[4], Bartoloni A.[4], Cancrini G.[1]

[1]"Sapienza" University of Rome ~ Rome ~ Italy, [2]University of Pisa ~ Pisa ~ Italy, [3]Distr. de Salud Cordillera ~ Santa Cruz ~ Bolivia, Plurinational State of, [4]Dip. di Medicina Sperimentale e Clinica ~ Firenze ~ Italy

SIMPOSIO S4 Zoonosi da alimenti: un approccio globale e nuove prospettive

11:30 - 13:30

Moderatori: E. Pozio, A. Giangaspero

S4.1 TOXOPLASMOSIS IN A CHANGING WORLD: THE SOUTH-EAST EUROPEAN PERSPECTIVE

Djurković-Djaković O.*[1]

[1]National Reference Laboratory for Toxoplasmosis, Institute for Medical Research, University of Belgrade

S4.2 SOLVED AND UNSOLVED PROBLEMS IN THE DIAGNOSIS OF TOXOPLASMOSIS

Meroni V.*[1], Meroni V.[2], Genco F.[2]

[1]Dipartimento di Clinica Medica e Medicina Interna Università degli Studi di Pavia, Pavia, [2]SC Microbiologia e Virologia Fondazione IRCCS Policlinico San Matteo, Pavia

S4.3 ANISAKIS AND ALLERGY: A PHENOMENON OF GLOBALIZATION?

Daschner A.*[1]

[1]Servicio de Alergia. Instituto de Investigación Sanitaria- Hospital Universitario de la Princesa, Madrid, Spain.

S4.4 ALLERGY AND FOOD-BORNE PARASITES: NOT ONLY ANISAKIS

Bruschi F.*[1], D'elios M.M.[2]

[1]Department of Translational Research, N.T.M.S., Università di Pisa,Pisa, Italy, [2]Department of Experimental and Clnical Medicine, Università degli Studi di Firenze, Firenze, Italy

S4.5 FRESHWATER FISH-BORNE PARASITIC ZOONOSES IN ITALY

Fioravanti M.L.*[1], Gustinelli A.[1], Caffara M.[1], Menconi V.[1], Prearo M.[2]

[1]Department of Veterinary Medical Sciences, Alma Mater Studiorum University of Bologna, Italy, [2]State Veterinary Institute of Piedmont, Liguria and Valle d'Aosta, Italy

S4.6 THE ROLE OF ACCREDITATION FOR FOOD SAFETY

Tramontin S.*[1]

[1]Director of the Dept. of Testing Labs for Food Safety (ACCREDIA)

S4.7 VETERINARY PREVENTION AND PARASITIC ZOONOSES: AN EXAMPLE OF ONE-HEALTH MEDICINE

Grasselli A.*[1], **Perrone V.***[1]

[1]SIMeVeP ~ Roma ~ Italy

CERIMONIA INAUGURALE

CI.1
ETTORE BIOCCA, MARIO COLUZZI: TWO ARCHIVES FOR THE HISTORY OF ITALIAN PARASITOLOGY

Conforti M.*[1]

[1]*Dip. di Medicina Molecolare, Unità di Storia della Medicina e Bioetica, "Sapienza" Università di Roma*

INTRODUCTION:
The Library for the History of medicine of Sapienza, Rome University, has recently acquired, inventoried and preserved a meaningful part of the scientific archive of Ettore Biocca (1912-2001), until 2012 kept at the Istituto di Parassitologia he had founded at Sapienza, and directed for several decades. An introduction to Biocca's life, works and scientific achievements is probably unnecessary for many of those who will be present to the opening ceremony. However, it will perhaps be useful to remind that he has been more than a celebrated parasitologist: he also claims his place as one of the best field anthropologists in Italian Novecento. After his graduation at Rome University in 1936 -in the years of a triumphant Fascist regime- Biocca, whose political passion is still legendary among the many pupils and fellow scientist who have met him, worked at the Rockefeller Foundation, first in New York and then in Budapest, finally moving to Latin America, in Brazil, at the Butantan Institute in Sao Paulo. Back in Italy, he was professor at Sassari and in Rome. He has worked on trypanosomiasis, on ancylostomiasis, on the parasitology of alpine fauna, on human dermatitis caused by the Schistosoma bovis (1960). Between 1956 and 1963 he organized several expeditions in Latin America, and especially in Western Amazonia; the expeditions were meant for parasitological research, but they also allowed Biocca to encounter and study the previously unknown cultures of the Tucano, Desano, Wanano, Makù tribes and to record their music. Biocca's archive contains documents -objects and papers- on scientific expeditions, on the organization of the Istituto di Parassitologia and of the International Society of Parasitology. We will conclude with a brief description of another archive from the Istituto di Parassitologia, also now at the Library for the History of Medicine, the one of Mario Coluzzi (1938-2012), the author of outstanding researches on malaria parasites and vectors, as well as on public health. Coluzzi's papers have not yet been ordered and filed, but the two archives stand as a memento of the importance of the preservation and understanding of scientific heritage in Italy, both for historians as for scientists, as for the national community as a whole.

CI.2
MARIO COLUZZI (1938-2012): EPISTEMOLOGIST OF MOSQUITOES

Corbellini G.*[1]

[1]*Museo di storia della medicina, Dipartimento di Medicina Molecolare, Sapienza Università di Roma*

INTRODUCTION:
<<Coluzzi is the man who knows what a mosquito think>>, said one of the most important US immuno-parasitologists about Mario Coluzzi, who was an entomologist and a malariologist of world renown, and who died last Sunday at the age of nearly seventy-four years.
It was 1990 when I knew him, and we started to collaborate in order to study and to enhance the historical tradition of Italian malariology. The << Professor Coluzzi >>, as he was called – and he took some pride in having obtained the title for proved renown and in absence of a degree (he never graduated <<to not sit for the examination of chemistry>>) – twenty-two years ago was at the top of his splendor as scientist and teacher. Therefore I had the extraordinary and exclusive luck to be guided by him, and by his – hence not always rational too – scientific way to investigate everything, in my approach to the subjects and to the problems of medical history. Coluzzi had an intuitive awareness of the heuristic stimuli, which one can get from the comparison between the contents of the borderlands in biomedical research and the historical facts or the epistemological ideas of fundamental and applied research.
Moreover he attached an enormous importance to the scientific popularization, since he knew how much it was precarious the survival of scientific rationality in a human world which is mainly moved by Pleistocenic instincts. I can imagine how he would horrify in front of the obscurantist and tribal sentence of the L'Aquila court, about which one is talking by a week.
Nevertheless, there was much more. Coluzzi felt himself to be part of a medical-scientific school with a secular tradition: the Italian school of malariology, which at the end of the nineteenth century dragged a young and poor nation on the international stage, by contributing in an essential way to the historical detection of parasites and mechanisms of malaria infection's transmission. A tradition that entered in a sleeping phase after the eradication of the infection and that he brought again to the world heights of scientific competitiveness. He made it by always investing and betting on young people, hence by motivating them with enthusiasm to the learning and the use of the scientific method. His interest for malaria history was also dictated by the awareness, never paraded but plainly practiced, of being part of that history.
He started favored. He absorbed the malariological science taken by the hand by the father Alberto in the immediate second postwar period and in the middle of the campaign of eradication of malaria. But at the beginnings of the sixties he was already an internationally known entomologist for the studies on the working mechanism of the residual action insecticide, hence on the effects of DDT on the mosquitoes' behavior. His first genial idea was that of applying and renewing the analysis of polytene chromosomes to the study of African malaria mosquitoes, by discovering the possibility to cytogenetically differentiate as separate mosquito's species that are identical at the adult stage.
While he was developing cytogenetic techniques to be applied on the field for classifying malaria vectors in Africa, techniques turned out to be essential for improving the antimalarial strategies, he had another formidable idea. As an intimate expert of evolutionary genetics, he thought of applying it in order to attempt to explain the relations between the ecological and the population distribution of malaria vectors, and the intensity of transmission of the infection. At that point a scientific adventure started, from which it arose a fundamental chapter on evolutionary knowledge about the origins and the adaptations between malaria parasite, mosquitoes and man that characterizes a millenary ecosystem as the one of sub-Saharan malaria. Generally malaria, as Coluzzi and his school have first demonstrated, is a formidable model to understand Darwinian basis of biological evolution. Coluzzi's authority as a malaria entomo-epidemiologist was such that when << Science >> published Anopheles gambiae genoma, he was invited with his group to publish their genetic-evolutionary data.
Coluzzi's engagement was exemplar also on the political-sanitary front, hence in attempting to transfer scientific knowledge to reduce the impact of malaria in Africa. Beyond the activity carried out in collaboration with the Foreign Affairs Ministry to run field research and to train up local personnel in Mali and Burkina Faso, he was tutor and prompter for dozens of African researchers and public health experts, who today see the malaria decline thanks to the measures supported by OMS also on the basis of his authoritative and listened suggestions.
In the context of this engagement, it further came into play his interest for the history of malaria. In fact, he believed that the Italian success had nothing to teach to Africans on the problem's ecology plane, but that it was important as a political-cultural key. Malaria was overcame in Italy, he always explained me, also since it was constantly present a basic and applied research and since the illness was the object of political attention. Coluzzi understood before all colleagues that only a local scientific-cultural redemption could make possible the solution of the malaria problem, together with others in the sub-Saharan Africa.
Mario, as who associated with him knows, opened his face and became bright-eyed when he focused some idea which attracted his intellectual curiosity and confirmed some of his hypothesis. While the long and cruel illness implacably cemented his body, by imprisoning more and more his thought and his words, his look continued for years to transmit that critical and rational light that for someone, and certainly for me, was an intellectual lighthouse reference point, which will remain such forever in the memory.

* This text was published in Italian as an obituary in the Sunday cultural supplement of the Sole24Ore (28/10/2012). The English translation is by Barbara Coluzzi

CI.3
IMMUNOMODULATION OF HOST IMMUNITY BY LARVAL HELMINTHS

Gottstein B.*[1]

[1]Institute of Parasitology, Vetsuisse Faculty, University of Bern, Switzerland

INTRODUCTION:

The list of helminthic parasites infecting humans is long and rich in species diversity. Although the most prominent fundamental niche parasitized by adult stage helminths is the gastro-intestinal (GI) tract, a series of larval stages of helminths have by their evolution learnt to systemically invade the actual host organism, and to select specific organs or host cell types as predilection site to reside, maturate or even proliferate, until life-cycle continues in another host. Some of these helminths enter the host via the GI, but subsequently they will continue their pathway by penetrating first into the intestinal epithelium and lamina propria, after which they will be transported via blood or lymph to the anticipated predilection site. Echinococcus multilocularis, after leaving the intestinal tissue, will reach the next anatomical capillary system represented by the liver, thus metacestode development will primarily occur in the liver parenchyma. The newborn larval stage of the nematode Trichinella spiralis will find its home in striated skeletal muscle tissue. While the larval cestode principally remain at an extracellular stage of parasitism, Trichinella larvae have become successful as intracellular parasites and thus needed to evolve complex strategies for remodeling their niche of residence into one they can occupy for many years. Both Echinococcus multilocularis and Trichinella spiralis parasitize in the organ or the host cell by avoiding the best way possible to cause harm to the host, although both parasites are facing a developing host immunity that needs to be controled, provided the parasite wants to survive. The present article should shed some light into the parasite strategy developed to survive in the host organ or cell, respectively, and into the periparasitic host reaction that may help the host to control the parasite, but which may also be responsible for immunopathological events harmful to the host himself. Finally, for both parasites selected, the murine host appears the optimal model for carrying out experimental studies, as for both parasites rodents as well as humans become infected in the parasites natural life cycle.

Trichinella spiralis

After a newborn Trichinella larva enters its host muscle cell, this larva will actively orchestrate a cellular reconstruction and transformation of the host cell ending in the formation of a nurse cell. The phenomenon of nurse cell transformation has been tackled experimentally, in view of determining how the parasite modulates these events. Thus, the addition of excretory/secretory (E/S) products from newborn larvae or muscle larvae into a culture of myocytes was shown to elicit morphological changes in myotubes, such as the formation of nodular structures that contain numerous cavities, probably due to enzymatic digestion by parasitic proteases, including serine protease (Romaris et al., 2002, Biochem Parasitol, 122:149–60; Nagano et al., 2003, J Parasitol, 89: 92–8) and a metalloproteinase (Lun et al., 2003, Parasitol Res, 90:27–37). In the murine immune response to infection, some key components include the cooperative interplay between IL-10 (Beiting et al., 2004, Inf. Immunity, 72:3129-37), TGF-β and Teffs and Tregs that ensures parasite survival while protecting the host from inflammatory disease (Beiting et al., 2007, J Immunol, 178: 1039-47).

Results obtained from C57BL/6 mice showed that T. spiralis derived antigens have the capacity to induce bone marrow-derived dendritic cells to acquire an incompletely mature phenotype that promotes a significant proliferation of naive CD4+ T cells and a mixed Th1/Th2 cytokine profile with the predominance of Th2 cytokines (Ilic et al., 2011, Parasite Immunol, 33:572–82). Increased production of IL-4, IL-9, IL-10 and IL-13 accompanied increased IFN-γ. Furthermore, dendritic cells pulsed with T. spiralis antigens did not induce an increase in the population of Foxp3+ T regulatory cells. Although other helminth antigens have demonstrated the capacity to induce de novo generation of Foxp3+ T regulatory cells (such as E. multilocularis as discussed below), the T. spiralis in vitro studies provided no evidence that these parasite antigens possess this capacity.

Echinococcus multilocularis

The metacestode that causes alveolar echinococcosis (AE) consists of a cluster of tightly mingled fluid-filled vesicles. The outer acellular surface of these vesicles is formed by the laminated layer, a carbohydrate-rich structure synthesized by the parasite. The laminated layer plays a crucial role in the survival strategy of the parasite by modulating immunological and physiological reactions on part of the host. The E. multilocularis metacestode reproduces asexually, by exogenous formation and budding of daughter vesicles, which overall resembles progressive tumour-like growth. Metastases formation may occur in other organs due to release of parasite micro-vesicles into the blood or lymph system. While groowing and proliferating, the metacestode induces a strong periparasitic cellular immune reaction. The type/nature of this reaction turned out to be crucial with regard to either control or fail to control parasite proliferation and thus disease (reviewed by Vuitton and Gottstein, 2010, J Biomed Biotechnol, 2010:923193). T cell-deficient mice (Dai et al., 2004, Immunol, 112:481-8) and as well as HIV-co-infected patients (Sailer et al.,1997, J Pediatr, 130: 320-3; Zingg et al., 2002, Infection, 32:299-302) exhibit high susceptibility to infection and disease, herewith suggesting that the host cell mediated immune response plays an important role in suppressing the larval growth. In recent mouse experiments, Wang et al.(PLoS One, in press) showed that the combined cytokine profile associating IL-12α, IFN-γ but also the “starter-Th2 cytokine”, IL-4, is established very early in the periparasitic infiltrate (rather Th1 orientation at early stage of infection), and that subsequent decrease in IL-12α and TNF-α is accompanied by tolerogenic profile, IL-10, IL-5 and TGF-β (rather mixed Th2-orientation at late stage of infection).

A closer look at the immunological events characterizing the different stages of infection may yield the following picture: Basically, the larval infection with E. multilocularis begins with the intrahepatic postoncospheral development of a metacestode. In certain cases, an appropriate host innate or acquired immune response can inhibit parasite proliferation, which may provide the explanation for the relatively large part of human individuals exposed to infection but resistant to disease. For those cases where the metacestode survives, the parasite obviously seems to have been able to modulate the host immune response for its own advantage, and the working hypothesis would be that the parasite actively interferes in the immunological pathway selection such as to abrogate those mechanisms that could efficiently control or kill the parasite. This hypothesis was strongly supported by experiments carried out in T cell deficient mouse strains (Dai et al., 2004, Immunol. 112, 481-488). It has been claimed for long that parasite metabolites yield those immunomodulatory effects that allow the parasite to survive in the host. In such a context it was shown that mouse macrophages (derived from E. multilocularis-infected mice) exhibit a reduced ability (as APCs) of maturation and antigen presentation (Dai and Gottstein, 1999, Immunol, 97:107-16; Dai et al., 2003, Immunol, 10:238-44; Mejri and Gottstein, 2006, Parasite Immunol, 28:373-85). Then it was also shown that E. multilocularis infection affected peritoneal DCs such as to remain in an immature or resting state as well, characterized by low expression of co-stimulatory molecules and MHC class II (I-a) molecules (Mejri et al., 2011, Parasite Immunol, 33: 471-82). A concomitant high level of TGF-β-expression classifies these DCs within cells with suppressive features. Together, impairment of MØ and DC maturation and antigen presentation indicated that a successful E. multilocularis infection induces an anergix c status of immunity in the host. Subsequent experiments at the T cell level documented that regulatory T cells (Tregs) interfere in the complex immunological host response to infection (Mejri et al., 2011, Parasitol. Int, 60:45-53). Indeed, a subpopulation of regulatory CD4+CD25+ T cells isolated from E. multilocularis-infected mice reduced ConA-driven proliferation of CD4+ T cells. The high expression levels of Foxp3 mRNA by CD4+ and CD8+ T cells suggested that subpopulations of regulatory CD4+Foxp3+ and CD8+Foxp3+ T cells were involved in negatively modulating the immune responses of E. multilocularis-infected mice (Mejri et al., 2011, Parasite Immunol., 33: 471-482). Our most recent data demonstrated now that a novel CD4+CD25+ Treg effector molecule FGL2 contributes to the outcome of E. multilocularis infection by promoting Treg cell functions (Wang et al., 2014, PLoS One, in press); they give evidence for a role of IL-17 in FGL2 regulation, and suggest that targeting FGL2 could be used for the development of novel treatment approaches in this parasitic disease.

XXVIII Congresso Nazionale SoIPa, Roma 24-27 giugno 2014

CERIMONIA INAUGURALE

NOTES

SIMPOSIO 1

HOT SPOTS IN MEDICAL & VETERINARY ENTOMOLOGY: FROM ITALY TO EUROPE

S1.1
THE CHANGING LANDSCAPE FOR VECTOR-BORNE DISEASE

Wall R.*[1]

[1]Veterinary Parasitology & Ecology Group, School of Biological Sciences, University of Bristol, Bristol,UK

During the last decades of the 20th Century and the first decades of the 21st Century, a global emergence and resurgence of many direct arthropod parasites and arthropod vector borne diseases has been evident in human and animal populations (Gubler, 1998, Emerg. Infect. Dis., 4: 442-450; Gratz, 1999, Ann Rev Entomol, 44: 51-75). North America has seen the importation of and subsequent rapid spread of West Nile viral encephalitis (Lanciotti et al., 1999, Science, 286: 2333-2337). Europe has witnessed unexpected outbreaks of bluetongue virus and Schmallenberg virus in northern countries. There has been a continued spread and establishment of the exotic mosquito Aedes albopictus, a major vector of dengue fever, to most of the Mediterranean region (facilitated by the global trade in used tyres which act as carriers of mosquito eggs), and the emergence of chikungunya virus in Italy. Crimean-Congo haemorrhagic fever virus and its tick vector, Hyalomma, have also emerged in parts of Turkey, and for the first time caused clinical disease in humans in both Turkey and Greece. Usutu virus has emerged in Austria, Hungary and Spain and outbreaks of West Nile virus continue to appear in France, Hungary and Romania and has emerged in Italy and Greece (Medlock & Jameson, 2010, Emerg. Health Threats J. doi: 10.3134/entj.10.002). Tick-borne encephalitis has been found recently for the first time in mountainous regions of the Czech Republic (Daniel et al., 2004, Parasitol, 129: 329-352), and has expanded its range in Scandinavia, and many new tick-transmitted rickettsial pathogens have been identified of concern to both human and veterinary health. In cats and dogs specifically, in recent years, babesiosis has been recorded in northern Germany and The Netherlands, canine monocytic ehrlichiosis (CME) in southern Europe, Anaplasma platys anaplasmosis in France, and A. phagocytophilum anaplasmosis in cattle, horses, dogs and cats in northern Europe (Beugnet & Marié, 2009. Vet. Parasitol, 163: 298-305).

The increasing rate and scale of emergence and resurgence of vectors and vector borne disease that has occurred in recent decades, exemplified by the global spread of invasive mosquitoes and large-scale outbreak of West Nile virus in North America, are indicative of the global nature of this change. Such changes are the result of a complex interaction of factors, such as increasing global movement of people and animals, climate and habitat change, each of which may carry a different weight and play a different role under specific local circumstances. Understanding the interactions between these factors is essential if the increase in disease incidence is to be halted or managed and predictions made in relation to future trends.

Of all the various contributors to changing patterns of vector-borne disease, the impact of climate change is an area of particular current interest, with wide range of direct and indirect effects expected. However, these effects are complex and difficult to predict. Undoubtedly, increased or decreased temperature and rainfall at certain times of year will impact directly on arthropod vectors and the pathogens they transmit, since the rates of physiological processes in arthropods are highly dependent on ambient temperature. Hence, superficially, long-term changes in climate would be expected to have a direct effect on their distribution and abundance in addition to their ability to mature pathogen infection. However, depending on the relative effects of temperature on development and mortality at different stages of development, the outcome in terms of abundance are likely to vary dramatically, especially when seasonality is taken into account. For most vectors, the greatest effect of climate change on transmission is likely to be observed at the extremes of the range of temperatures at which transmission occurs. Population growth and changes in social and economic conditions may also have an important influence on observed disease incidence patterns. Unplanned urbanization, in combination with inadequate housing and poor quality water, sewage, and waste management systems, produce conditions in which high densities of hosts and poor animal and public health allow for increased transmission of vector-borne diseases in and between human and companion animal populations. This in turn may be affected by climate change through changes in human behaviours. These might include altered animal husbandry practices, as well as changes in the approach to chemical prophylaxis and reactive treatment. A change in the perception of disease risk may also lead to changes in approach to intervention, with perhaps a greater willingness to treat prophylactically or to intervene with treatment earlier. This is likely to further complicate the identification of a link between climate and disease risk. The factors affecting human responses are often as complex as those affecting the biology of the vectors or disease agents, and need to be taken into account when attempting to predict the effects of climate change.

Arthropod-borne diseases of animals remain considerably less well researched than diseases of human concern. A major contributor to this is the fact that veterinary services/surveillance and diagnostic services are often relatively poorly developed. In particular, there are limited survey data on the distribution of arthropods and arthropod-borne disease in many areas of the world, in part as a result of the difficulties in diagnosis. Hence, changes in distribution, particularly if subtle or at the early stages of an epidemic curve, are hard to spot. As a result, for the future, it would be important that more effective surveillance systems are established for most arthropod-borne diseases across different countries to allow a detailed risk analysis, including the evaluation of the potential spread to new areas or the new introduction of exotic species or diseases. This is particularly important given the risks linked to climate change and changes in land use patterns. This will require a clear and exhaustive knowledge of the distribution of arthropod-borne diseases in different areas, monitoring of new strains or unrecognized disease agents transmitted by arthropods, continuous monitoring of insecticide resistance and the development of management strategies to minimise its onset. However, in difficult economic times, investment in such surveillance has been reduced in some European countries, certainly in relation to veterinary medicine. It is essential, therefore, that policy makers become more aware of the potential risks of arthropod parasites and vector-borne disease and that this is given greater priority in policy/funding decisions.

S1.2
SIMULIIDS: A RE-EMERGING THREAT TO ANIMAL AND HUMAN HEALTH ACROSS EUROPE

Ignjatović Ćupina A.*[1], Kúdela M.[2], Ciadamidaro S.[3], Maiolini B.[4], Brúderová T.[2], Giannelli A.[5], Otranto D.[5], Petrić D.[1], Rivosecchi L.[6]

[1]Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia, [2]Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia, [3]ENEA, Saluggia Research Centre, Italy, [4]Fondazione Edmund Mach, Research and Innovation Centre (CRI), San Michele all´Adige, Italy, [5]Department of Veterinary Medicine, University of Bari, Valenzano (Bari), Italy, [6]Istituto Superiore di Sanità, Roma, Italy (retired researcher)

Simuliids, commonly referred to as blackflies (Diptera: Simuliidae), are widely distributed insects which breed in different types of running waters. Majority of species are of medical concern, due to the haematophagous feeding behaviour of females, which cause nuisance to animals, including humans and may also transmit pathogens, such as Leucocytozoon spp. haemotropic protozoa of birds and Onchocerca spp. filarial worms.

The main medical importance of simuliids in Europe is primarily attributed to nuisance and their biting activity, which cause itching, painful swellings, erythema and cutaneous lesions persisting from several days to weeks. Massive attacks of simuliids may affect animal welfare and livestock production, ultimately causing death of animals. In the last few decades, outbreaks of different simuliid species and bite-related problems have been recorded in different parts of Europe. Some countries have been particularly affected, such as: Lithuania, by Simulium maculatum, Simulium ornatum and Simulium reptans (Bernotiene, 2001, Norw. J. Entomol. 48:115-120); Poland by Simulium erythrocephalum, Simulium pusillum, Simulium nigrum, Simulium morsitans, Simulium noelleri, S. ornatum, S. reptans and Simulium equinum (Wegner, 2006, Acta Entomol. Serb., Suppl:155-159); France by S. ornatum complex, Simulium posticatum, Simulium variegatum, Simulium bezzii and S. erythrocephalum (Beaucournu et al., 1992, Ann. Parasitol. Hum. Comp., 67, 6:202-208); Spain by S. ornatum complex and S. erythrocephalum (Gállego et al., 1994, Parasite, 1:288; Valle Trujillo and Escosa, 2009, Proc. 5th EMCA workshop:115-116).

In northern Italy, mass attacks of S. reptans, S. ornatum complex, S. variegatum and S. bezzii complex to cattle were followed by numerous deaths of animals (Zanin & Rivosecchi, 1974, Atti Soc. Ital. Sc. Vet. 28:865-868; Zanin et al., 1983, Atti Soc. Ital. Buiatria 15:513-524; Zanin et al. 1992, Atti Soc. Ital. Buiatria, 24:635-640; Car, 2001, Stud. Dipterol. 8:613-620). In addition, S. erythrocephalum has been recognized as responsible for bites to humans (Rivosecchi, 1986, Riv. Parass., 3:1-15). Spreading of this species was confirmed by it´s recent founds in the Lazio region (Ciadamidaro, 2010, PhD thesis, 189 pp). In central Italy, after changes in faunal composition, manifested by decrease of populations of the subgenus Wilhelmia and spreading of ornatum group, attacks to humans were recorded in Marche, Lazio and Sardinia regions (Rivosecchi, 1997, Riv. Parass., 14:329-337; Rivosecchi, 2005, Ann. Ist. Super. Sanità, 41:415-419; Ciadamidaro et al., 2009, Proc. 5th EMCA workshop:113-114). As reported by Rivosecchi (2005), similar replacement scenario occurred throughout Italy due to alteration of agricultural practices and landscape aspects. On the other hand, preliminary study conducted in Basilicata Region in 2013 demonstrated the presence of abundant population of Simulium pseudequinum in conditions of well preserved nature, with traditional animal production (unpublished data).

Due to the repeated outbreaks of simuliids, economical losses and health problems, Serbia was considered as the most threatened European country in the last century. Before damming of the Danube in the 60´s, Simulium colombaschense caused enormous losses of livestock in the region of the Iron Gate, eastern Serbia (Baranov, 1926, Neue Beitr. Syst. Insektenk. 3:183-194; Zivkovich, 1970, Cah. Off. Rech. Sci. Tech.Outre-Mer (Ent.Med), 8:113-120). Significant losses in poultry production were caused by S. maculatum (Živković, 1958, Acta Vet. 8:7–14), while outbreaks of S. erythrocephalum caused severe health problems in humans (Živković and Burány, 1972, Acta Vet., 22:133–142). Since 1999, outbreaks of S. erythrocephalum and bite consequences in humans (Fig. 1 and 2) re-occurred (Ignjatović Ćupina et al., 2006, Acta Entomol. Serb., Suppl.:97-114; Ignjatović Ćupina and Petrić, 2010, Biljni lek., 3:208-217). In the lowland part of the Danube river (the region of Novi Sad), where S. reptans represented the dominant species in the past (Živković, 1971, Acta Vet., 17:433-438), recent studies have demonstrated significant reduction in number of species (from 8 to 4) and remarkable dominancy of S. erythrocephalum, while in small tributaries among 11 identified species, S. ornatum complex became dominant (Ignjatović Ćupina, 2011 PhD thesis, 372+lxxiii). Downstreams, in the Iron Gate region, recent studies did not confirm the presence of simuliids in the Danube, but a rich diversity represented by 21 mainly mammophilic species was recorded in the confluent streams, eight species were new to the region and one (Simulium vulgare) new for Serbia (Kúdela et al., 2012, Proc. 5th Int. Simuliid Symp.:15). Nevertheless, low population density of the notorious S. colombaschense was detected in the Nera and Cerna rivers, within the radius of 30 km from the Iron Gate, proving the species adaptation to breed outside of the Danube (unpublished data). Following the historical data on the breeding foci of S. colombaschense/voilense in Italy (Rubtzov, 1964, Mem. Soc. Entomol. Ital., 43:5-128; Rivosecchi, 1978, Ed. Calderini, 533 pp), the tracking was extended to Trentino Alto Adige and Piedmont regions. In both areas immatures of S. colombaschense/voilense were identified in association with S. reptans and other species (unpublished data).

The current situation of re-emerging health threats caused by simuliids in Europe, suggests the need of extensive studies directed to: a) updating of fauna composition, with special attention to pest species; b) monitoring of populations, species biology and behaviour (e.g. number of generations, fligth capacity, host preference); c) recognition of specific ecological factors that might provoke outbreaks and d) development of efficient and environment friendly knowledge based control strategies.

ACKNOWLEDGEMENTS: The study was supported by the Ministy of Science Education and Technological Development, Republic of Serbia (Projects: TR31084 and III43007) and by Merial, France.



Fig. 1. S. erythrocephalum female (photo: Ignjatović Ćupina, 2006)



Fig. 2. Extreme bite consequences caused by S. erythrocephalum (photo:Vujanović, 2006)

Keywords: Simuliidae, outbreaks

HOT SPOTS IN MEDICAL & VETERINARY ENTOMOLOGY: FROM ITALY TO EUROPE

SIMPOSIO 1

S1.3
THE RHIPICEPHALUS SANGUINEUS GROUP: NEW SCENARIOS IN DISEASE TRANSMISSION

Dantas Torres F.*[1], Dantas Torres F.[2], Latrofa M.S.[2], Otranto D.[2]

[1]Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães, Recife, Brazil, [2]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Italy Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Italy

Ticks (Ixodida: Ixodidae) are vectors of numerous pathogenic microorganisms that may put the health and wellbeing of animals and humans in jeopardy (Dantas-Torres et al., 2012, Trends Parasitol., 28:437–446). Ticks belonging to the so-called Rhipicephalus sanguineus group have attracted the interest from the scientific community since the 1800s (Latreille, 1806, Parisiis et Argentorati: Amand Koenig, bibliopolam) and gained more attention in the early 1900s (Neumann, 1911, Berlin: Das Tierreich), the dawn of tick taxonomy. Recently, there has been much debate about the taxonomy of this tick group, due to the vagueness regarding the definition of Rhipicephalus sanguineus sensu stricto (s.s.), whose type specimen is currently unavailable (Gray et al., 2013, Ticks Tick Borne Dis., 4:171–180). Morphological, biological and molecular data from recent studies have clearly demonstrated the existence of a complex of species within the name “R. sanguineus”, identifying at least four distinct taxa among specimens collected from dogs in different continents (Dantas-Torres et al., 2013, Parasite Vectors, 6:213). In addition, a recent study suggested Rhipicephalus rossicus as the main tick species on dogs in the steppe region of southeastern Romania (Dumitrache et al., 2014, Parasite Vectors, in press). Altogether, these data indicate that dogs may be infested by morphologically similar, but taxonomically different tick species misidentified as ‘R. sanguineus’, whose vector role has not been fully assessed. As an example, while there is limited evidence implicating R. sanguineus sensu lato from Brazil as a vector of H. canis (Demoner et al., 2013, Ticks Tick Borne Dis., 4:542–546), ticks from southern Italy (designated Rhipicephalus sp. I; Dantas-Torres et al., 2013, Parasite Vectors, 6:213) are certainly competent vectors of this protozoon (Giannelli et al., 2013, Vet. Parasitol., 196:1–5). Incidentally, these ticks are morphologically and genetically distinct from specimens from Brazil, for example (Dantas-Torres et al., 2013. Parasite Vectors 6:213). There has also been speculation on the vector competence of two distinct tick populations, namely tropical (or northern lineage) and temperate (or southern lineage) species, for Ehrlichia canis (Burlini et al., 2010, Exp. Appl. Acarol., 50:361–374; Moraes-Filho et al., 2011, Acta Trop., 117:51–55; Nava et al., 2012, Vet. Parasitol., 190:547–555). Indeed, it has been suggested that between the above tick populations, only the tropical species is a competent vector for E. canis. Nonetheless, further studies will be necessary to better appreciate the vector competence of different taxa within the R. sanguineus group. The quintessential question is: what is ‘R. sanguineus’? Without answering to this question, the role of different tick species of the R. sanguineus group and even their real identity will remain in the realms of speculation. Hence, the next step should be redescription of R. sanguineus s.s. and the description of eventual new species. Afterwards, studies on the vector competence of such species should be rigorously conducted in order to better appreciate the whole puzzle regarding these ticks and the new scenarios in disease transmission to dogs and to humans as well.

S1.4
RISK OF TRANSMISSION OF NEW TICK-BORNE PATHOGENS: A REVIEW

Rizzoli A.*[1]

[1]Fondazione Edmund Mach, San Michele all'Adige (TN), Italy

Among vector borne diseases, those transmitted by ticks (TBDs) are increasingly important for veterinary and human public health in the EU. The great variability of ecological, climatic and socio-economical conditions that characterize Europe, provide a wide heterogeneous set of conditions affecting the risk of infection and determining which diseases are more likely to emerge and spread in certain areas rather than in others.

The risk of infection with a TB pathogens is in fact determined by a complex set of biotic and abiotic condition which determine on one side the vector occurrence, abundance, infection rate with various pathogens and the host seeking questing activity while, on the other side, the exposure of susceptible hosts, including human, which will determine the final disease burden.

Among the possible drivers of TBD emergence, climate is an important geographic determinant of vector distribution, survival and questing activity but climatic factor alone cannot explain the considerable spatial heterogeneity in the incidence observed for the majority of the TBDs in Europe, despite observed uniform patterns of climate change. Other potential causal pathways of changes in TBDs distribution and infection risk include changing in land use patterns, increased density of tick feeding hosts, habitat expansion for the pathogen reservoir hosts, alterations in recreational and occupational human activity including public awareness other than increase in commercial exchanges of goods and live animals among countries.

Beyond the risk posed by tick borne encephalitis (TBE) and Lyme Borreliosis (LB), other TBDs are of immediate concern in the EU.

In the case of those transmitted by the wood tick Ixodes ricinus, Anaplasma phagocytophilum the agent of the Human Granulocytic Anaplasmosis, is currently considered the most medically important emerging TBD in the EU. In Europe, this disease is known to cause fever in goats, sheep, and cattle and it has emerged as a human disease in 1996. A. phagocytophilum has also been detected in a wide spectrum of free-living vertebrates, birds and lizards including rodents and free-living ungulates. According to the latest studies, the intraspecific genetic variability of A. phagocytophilum probably plays an important role in the ecology of this pathogen but also in the variable pathogenicity for humans and animals associated to different strains. In addition, the ecology of A. phagocytophilum is much more complex than was originally assumed: the pathogen has now shifted to new geographical habitats throughout Europe, and many aspects are as yet poorly investigated, such as the role played by many wild vertebrate species, including birds.

Other tick-transmitted infections of emerging concern in the EU include those caused by Babesia spp pathogens of the spotted fever group rickettsioses transmitted by I. ricinus as R. helvetica. The observed increase in incidence may be due to improved diagnostic techniques but also to changes in wild reservoirs distribution and abundance.

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne RNA virus (main vector Hyalomma marginatum) provoking outbreaks of severe hemorrhagic fever in humans. CCHF has emerged and/or re-emerged in several Balkan countries, Turkey, southwestern regions of the Russian Federation during the last decade. CCHF virus circulates in nature in an enzootic tick-vertebrate-tick cycle and migrating birds and livestock transferred from endemic to non-endemic areas may carry large numbers of infected ticks, which could spread the CCHF virus into currently uninfected areas of the EU. Possible reasons for re-emergence of CCHF include climate and/or anthropogenic factors such as changes in land use, agricultural practices or hunting activities, movement of livestock, all of which may influence host-tick-virus dynamics.

The recent findings of Hyalomma marginatum rufipes on cattle in Hungary are suggestive of the moulting success of this Afro-Mediterranean tick species in a continental climate in Central Europe (Hornok and Horváth Parasites & Vectors 2012, 5:170). Potential CCHF circulation area in Western Paleartic were recently estimated (Estrada Pena et al, 2012. Journal of Applied Microbiology, 114: 278-286)

Acknowledgements

The study was funded by the European Union grant FP7-261504 EDENext

S1.5
THE SIGNIFICANCE OF ENTOMOLOGICAL SURVEYS FOR ENDEMIC AND AT RISK OF INTRODUCTION MOSQUITO-BORNE PATHOGENS

Capelli G.*[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy

Background

Mosquito-borne pathogens (MBPs) include viruses, protozoa and helminths affecting animals and humans worldwide (Engler et al., 2013, Int J Environ Res Public Health,10:4869-95; Otranto et al., 2013, Parasit Vectors, 6:16). In Italy some MBPs are endemic, with different spatial and temporal distribution, as Dirofilaria immitis and D repens, Setaria spp., West Nile (WNV), Usutu virus (USUV) and protozoa circulating among birds (Plasmodium, Haemoproteus). When a vector is established in an area there is a real and predictable risk of introduction of new MBPs or expansion of old ones, due to the global movement of goods, animals and humans. The recent outbreak of Chikungunya virus in Emilia-Romagna (Angelini et al, 2007, Euro Surveill., 12:E070906.1), the emergence of WNV in northern Italy (Busani et al., 2011, Epidemiol Infect.,139:818-25) and the expansion of D.immitis in southern regions (Otranto et al.,2009, Parasit Vectors., 2 Suppl1:S2) are paradigmatic examples of such occurrences. The global trade also enhanced the likelihood to import alien species, which pose new threats for the transmission of local and exotic MBPs. The surveillance of MBPs includes the entomological monitoring, which has different aims and strategies according to the target mosquito and pathogen.

In general the entomological monitoring has the following main aims:

1. mosquito species composition, relative density and seasonal dynamics
2. early detection of endemic pathogens
3. mosquito rate of infection
4. detection on new pathogens
5. detection of new mosquito species

The reachability of these aims are affected by the spatial and temporal intensity of the monitoring, the skills of the people involved and the budget availability.

Endemic pathogens and Culex pipiens

North-eastern Italy is particularly suitable for mosquito survival, due to its climate, landscape and abundance of wild/domestic animals. After the emergence of WNV in 2008, several entomological studies were implemented, including long term monitoring systems and short term studies to answer specific questions. For long term monitoring, a range of 24-66 CDC-CO2 traps were activated weekly or fortnightly from May to October 2009-2013. The mosquitoes were screened by RT-PCR for Flaviviridae. More than 700,000 mosquitoes were collected, with Cx.pipiens the most abundant (80%) and the only vector of WNV and USUV. The phylogenetic analyses showed the occurrence of different strains of WNV lineage 1 and 2, and indicated the capability of these viruses to become endemic and to rapidly evolve and emerge in different sites (Capelli et al., 2013,Vet Ital., 49:263-8). Mapping, modelling and spatial analyses were done using the entomological data to identify correlations with climate, landscape, animal and human infections (Mulatti et al, 2014, Parasit Vectors.,7:26). The results showed for the first time that the contribution of density-dependence in regulating vector population growth was greater than any environmental factor on its own. Overall the most significant predictors of Cx.pipiens dynamics included length of daylight, population density and temperature in the 15 days prior to sampling. Precipitation, number of rainy days and humidity had limited importance. Linear models detected significant relationships between WNV in humans and mosquitoes. Spatial analysis detected clusters of WNV occurrences for all the hosts, identifying an area where to focus surveillance and promptly detect WNV re-activation (Mulatti et al, 2013, Zoonoses Public Health., 60:375-82).

Beside annual monitoring, short term studies were organized. In one site, captures with CDC and gravid traps were done every 2hrs for 24hrs all over the season. Diel activity showed that Cx.pipiens changed its host searching activity according to the season and highlighted the period of the day at major risk of Cx. pipiens biting (Montarsi et al, J Med Entomology, submitted). In other three sites pre- and post-disinfestation captures were done. The success of disinfestation measures in reducing Cx.pipiens density varied according to the methods used, calling for the need of harmonic guidelines. The host preference of Cx.pipiens was assessed by PCR blood meal analysis. Cx.pipiens fed preferentially on birds (76%), mainly blackbird, sparrow, magpie and collared dove, indicating possible bird targets for surveillance.

Pathogens at risk of introduction and Aedes albopictus

In Italy every year a variable number of human imported Dengue and Chikungunya cases are reported. If these cases occur in summer in an area infested by the tiger mosquito the risk of transmission is real and increases according to the density of the mosquitoes. Consequently the knowledge of the degree of the infestation in an area is pivotal to promptly intervene with the disinfestation actions according to the National Program of Surveillance. The best examples of a large scale entomological monitoring is the one set up in the Emilia Romagna, where urban areas are constantly monitored by more than 2,700 ovitraps (Carrieri et al., 2011, J Vector Ecol.,36:108-16). Using the series of historical data, the mean number of eggs laid in ovitraps were compared to different estimates of adult mosquito abundance, human landing collection, n°of bites declared by citizens. It was then possible to calculate a disease risk threshold in terms of n° of eggs per ovitrap above which an arbovirus epidemic may occur (Carrieri et al., 2011, J Vector Ecol.; 36: 108-16).

Detection of new species

In Veneto region the entomological monitoring for the tiger mosquito allowed the detection of Ae. koreicus, a new invasive mosquito species (Capelli et al., 2011, Parasit Vectors., 4:188; Montarsi et al., 2013, Parasit Vectors., 6:292), potential vector of arboviruses and filariae. The vector competence of this mosquito is now under study.

The retrospective studies

When a vector monitoring system is set up for years a consistent bank of samples is usually stored. A sub-sample of the mosquitoes collected in previous years was screened for Bunyaviridae and Tahyna virus was isolated once in Ochlerotatus caspius. Using the DNA extracted from mosquitoes in 2010 it was possible to map the distribution of D.immitis and D.repens in mosquitoes in Veneto, giving new insights on the risk of transmission for animals and humans (Latrofa et al., 2012, Parasit Vectors., 5:76). Another beautiful example of the usefulness of retrospective studies is the evidence of the Schmallenberg virus (SBV) into Culicoides stored in 2012 in Italy. It was possible to demonstrate, that SBV has circulated in at least three Italian provinces since early September 2011, nearly 5 months prior the first detected case in 2012 (Goffredo et al.,2013, Prev Vet Med., 111:230-6).

S1.6
CULICOIDES BITING MIDGES AND RELATED ARBOVIRUSES

Goffredo M.*[1]

[1]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Campo Boario - Teramo (Italy)

More than 1,200 species of Culicoides (Diptera: Ceratopogonidae) have been described worldwide, almost all blood-suckers feeding on mammals, birds, humans and reptiles. These small pests, 1 to 3 mm in size, are able to cause seasonal dermatitis in horses and to bother humans with heavy attacks in some areas. Furthermore, Culicoides biting midges act as vectors of protozoa, filarial nematodes and arboviruses. At least 30 species of Culicoides have been associated with transmission of viral diseases in livestock, worldwide. Their capability to transmit viruses depends on the vector competence and capacity of each Culicoides species.
Bluetongue (BTV) and Schmallenberg viruses (SBV) affect ruminants and are currently circulating in Italy and Europe. In addition, the risk that other Culicoides-borne diseases might spread in Europe could not be ignored, first of all African Horse Sickness. The most common species implicated as vectors of these arboviruses belong to the subgenera Culicoides (Avaritia) and Culicoides (Culicoides).
BTV belongs to the genus Orbivirus (family Reoviridae) and circulates in Italy since 2000, whereas SBV is a "new" Orthobunyavirus (family Bunyaviridae), isolated for the first time in Northern Europe in 2011 and then reported also in Italy.
African horse sickness virus (AHSV) belongs, as BTV, to the genus Orbivirus and affects equids. Nine serotypes are recognised so far. All are endemic in sub-Saharan Africa but sporadically serotypes 4 and 9 have also occurred in Northern Africa, in the Middle East and Europe. Because of the global warming and the intensification of the global trade, the incursion in Italy of this orbivirus could occur, with a possible circulation of the virus through indigenous vector species as Culicoides imicola, Culicoides obsoletus and Culicoides scoticus. C. imicola is a known vector of AHSV in Africa, whereas the competence of the other two species has been recently evaluated under laboratory conditions.
Other Orbivirus transmitted by Culicoides biting midges are Epizootic Haemorragic Disease (EHDV) and Equine Encephalosis (EEV) viruses.
EHDV shares many characteristics with other Orbivirus as AHSV and BTV. The disease, historically affecting wild cervids (particularly white tailed deer of North America), affects wild and domestic ruminants. It is reported in North America, Australia, Asia, Africa and, recently, in the Mediterranean Basin.
EEV is reported in Southern Africa, where the clinical disease may be confused with AHS.
Culicoides are able to transmit also viruses of the family Rhabdoviridae, as the virus of Bovine Ephemeral Fever (BEF), which affects cattle and water buffaloes in Africa, Asia and Australia.
In Italy a National Entomological Surveillance Plan is currently operating for Bluetongue, all over the Country. The entomological activities, performed in the framework of this Plan, are also useful for other Culicoides-borne diseases, such as SBV. In addition, the information about vector distribution and abundance could be used to evaluate the risk of introduction of new vector borne viruses, as AHSV or EHDV.
Some highlights on Culicoides and Culicoides-borne diseases will be presented, as a result of about 10 years of surveillance and research:

- update on Culicoides vector species in Italy and Europe: "old" historical vector C. imicola and "new" species implicated in BTV and SBV transmission, belonging to the subgenera Culicoides (Avaritia) and Culicoides (Culicoides);
- current knowledge about C. imicola in the Mediterranean Basin;
- cryptic species of the C. obsoletus complex: taxonomy and identification, distribution and abundance in Italy, ecology and vector competence studies;
- Schmallemberg experience in Italy: field studies in Northern Italy and Sardinia;
- risk of AHSV spreading in Italy.

S1.7
PHLEBOTOMINE SAND FLIES AND THE RISK OF TRANSMISSION OF VIRAL DISEASES

Maroli M.*[1], Ciufolini M.G.[1], Gramiccia M.[1], Gradoni L.[1]

[1]Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, Rome, Italy

Phlebotomine sand flies (Diptera, Psychodidae) are haematophagous insects proven to transmit Leishmania parasites affecting humans and animals in many countries worldwide. These tiny insects are also vectors of other human pathogens such as Bartonella (Carrión's disease) and a number of viral agents causing sand fly fever, summer meningitis, vesicular stomatitis, and Chandipura virus disease (Maroli et al., 2013, Med. Vet. Entomol., 27:123-147). Noteworthy, both cutaneous and visceral leishmaniasis are endemic in most of the regions where sand fly-associated phleboviruses occur. Very recently, the epidemiological link between human leishmaniasis and phleboviral infections, assumed for long time, has been statistically demonstrated in France between L. infantum and Toscana virus (TosV) (Bichaud et al., 2011, PLoS Negl. Trop. Dis., 5, e1328). The risk of infection with sand fly-transmitted phleboviruses has been shown to cover very large areas of the Old World (southern Europe, Africa, Middle-East, central and western Asia) in connection with the presence of the sand fly vectors (Tesh et al., 1976, Am. J. Trop. Med. Hyg., 26:282-287). Recent investigations have also indicated that virus diversity within the Mediterranean areas is higher than initially suspected, and that populations living south and east of Mediterranean countries have a high risk of infection during their lifetime. In this note we focus on phleboviruses transmitted in southern Europe by members of the subgenus Phlebotomus (Larroussius) that includes also proven L.infantum vector species.
The genus Phlebovirus (family Bunyaviridae) is divided into nine antigenic complexes which includes 37 classified viruses. Further 16 serotypes are unclassified and are considered to be tentative members of the genus (King et al., 2012, 9th Rep. ICTV, Amsterdam: Elsevier). Four phleboviruses have been isolated in Italy: (i) Sand fly fever Naples (SFNV) and (ii) Sand fly fever Sicilian (SFSV) viruses were detected from sick soldiers during World War II; they are the causative agents of transient febrile illnesses in humans and have been associated to transmission by P.papatasi; (iii) TosV and (iv) Arbia (ArbV) viruses have been isolated from P.pernicious and P.perfiliewi (Verani et al., 1988, Am. J. Trop. Med. Hyg., 38:433-439). TosV and SFNV viruses belong to the species Sandfly fever Naples virus that currently includes also Tehran and Karimabad viruses, as well as newly recognized viruses. Surveillance studies (2009-2011) performed in other Mediterranean countries highlighted new phleboviruses, e.g. Punique (isolated from P.perniciosus and P.longicuspis), Massilia (from P.perniciosus), Adria and the reassortant Granada viruses (both from unidentified sand flies), suggesting that other viruses in this group remain to be discovered. Indeed, another putative novel Phlebovirus (Fermo) has been very recently isolated from P. perfiliewi in central Italy (Remoli et al., 2014, Am. J. Trop. Med. Hyg., 90:760-763, doi: 10.4269/ajtmh.13-0457).
TosV was originally isolated in 1971 from P. perniciosus collected in Monte Argentario (Grosseto province, central Italy) (Verani et al., 1982, Ann. Ist. Super. Sanità, 18:397-399) TosV detection in pools of male flies strongly suggests its vertical and/or sexual transmission in nature, as reported in Italy (Verani et al., 1988, ibidem) and Spain (Sanbonmatsu-Gámez et al., 2005, Emerg. Infect. Dis., 11:1701-1707). Vertical and venereal transmission of TosV in P.perniciosus, via the second gonotrophic cycle, have been demonstrated in laboratory studies by Maroli et al. (1993, Med. Vet. Entomol., 7:283-286). The reservoir of TosV is most likely the phlebotomine vector itself, although overwintering of the virus in hibernating larvae remains to be demonstrated. So far, natural vertebrate reservoirs of TosV are unknown. Neither mammals nor birds have been recognized as a potential reservoir, although few studies have been carried out on mammals and almost none on birds. Verani et al. (1988, ibidem) examined different species of wild vertebrates (wild mouse, stone marten, bank vole, coypus, porcupine, hedgehog, fox and bat) through serologic testing and viral isolation. The sole strain of TosV isolated from the brain of Pipistrellus kuhli only suggests a possible role of this species as an occasional host in the ecology of the virus. Whether humans can play a role in the virus cycle by infecting phlebotomine sand flies is not known.
TosV is among the three most prevalent viruses in meningitis during the warm seasons, together with herpesviruses and enteroviruses. It has a tropism for central nervous system and is a major cause of summer meningitis in the endemic countries. During the last decades it was recognized that TosV had a much larger geographic distribution than initially believed. In addition to Italy, it was present in most of the Western European countries (Portugal, Spain, France, Greece, Croatia) as well as eastern countries such as Turkey. TosV has been proved to be also present in many Mediterranean islands (Elba, Cyprus, Sardinia and Sicily), or it is suspected to circulate in Majorca and Corsica. The focus on the risk of transmission of this viral disease is documented by the increasing number of scientific publications in the last decade. A bibliographic search using "Toscana virus" as keyword in the PubMed database retrieved an increasing number of scientific publications, being 54, 166, and 254 research and review articles, in 2005 (Charrel et al., 2005, Emerg. Infect. Dis., 11:1657-1663), in 2012 (Charrel et al., 2012, World J. Virol., 1:135-141) and in 2014, respectively.
In summary, laboratory and epidemiological studies in the Mediterranean areas indicate that (i) geographic distribution of sand fly-associated phleboviruses is much larger than initially believed, (ii) number of phleboviruses that are circulating in phlebotomines could be higher, and (iii) relationships between sand fly-borne phleboviruses and Leishmania parasites are tighter than initially believed. In light of the above it is necessary to promote interdisciplinary researches aimed to study various aspects such as: (i) the epidemiology of the infection in human and animal populations; (ii) the role of other phleboviruses co-circulating with TosV in the epidemiology and transmission; (iii) the ecological factors that influence the distribution of phlebotomine vectors; and (iv) the transmission routes of TosV within sand flies through field studies in nature and experimental studies taking advantage of sand fly laboratory colonies.
Work partially supported by FP7 UE EDENext collaborative project, Contract No. 261504.

NOTES

SIMPOSIO 2

ITALIAN MALARIA NETWORK

S2.1
PREDICTION OF PLASMODIUM FALCIPARUM PROTEIN INTERACTION NETWORK: THE MEMBRANE MICRODOMAIN INTERACTOME

Sferra G.[1], Santoni D.[2], Ponzi M.[1], **Pizzi E.*[1]**

[1]Dipartimento di Malattie Infettive, Parassitarie ed Immunomediate, Istituto Superiore di Sanità, Roma, Italy, [2]Istituto di Analisi dei Sistemi ed Informatica "Antonio Ruberti", Consiglio Nazionale delle Ricerche, Roma, Italy

Lipid-rafts are specialized membrane compartments potentially involved in critical biological processes. It has been demonstrated that in Plasmodium falciparum these micro-domains play a role in several and diverse processes such as protein trafficking, invasion, host cell remodeling . This suggests a dynamical re-arrangement of the compartment during the life-cycle of the parasite to exert stage-specific activities.
The increasing amount of completely sequenced genomes and the availability of post-genomic data for P. falciparum makes possible the application of computational/statistical predictive methods. In particular, in recent years, several computational methods have been developed to predict protein-protein interactions at a genome-wide level exploiting data from high-throughput experiments.
In this work we propose an approach based on both computational and experimental methods to study the dynamics of the lipid-raft interactome in P. falciparum.
We integrated data from different sources (genomic and transcriptomic) to reconstruct the global probabilistic interaction network of P. falciparum. Proteomic data related to lipid-rafts have been produced at different Plasmodium-stages and utilized to derive the corresponding interactomes.
By a Bayesian approach, we generated de novo phylogenetic profiles and rosetta-stone data, covering 100% of P. falciparum proteome. We processed already available transcriptomic data in order to obtain a single profile covering the entire erythrocytic parasite life-cycle.
An experimental procedure was developed to isolate lipid-rafts from different erythrocyte stages of P. falciparum (trophozoites, early and late schizonts, gametocytes). Protein content was established by mass spectrometry .
We adopted a combination of computational and experimental approaches to derive the lipid-raft interactomes of P. falciparum. A novel strategy to derive phylogenetic profiles was proposed, in particular an assessment was carried out to compare our method with those usually utilized. A new set of rosetta-stone fusion data was generated. Furthermore available transcriptomic data have been organized to obtain a profile covering the entire erythrocytic life cycle (asexual and sexual stages). Through the combinatorial integration of these genomic and transcriptomic data, a global PPI network was predicted.
We experimentally identified lipid raft proteomein non-invasive (trophozoites), invasive (schizonts) and sexual forms (gametocytes), then we mapped the identified proteins on the global PPI network of P. falciparum to derive lipid-raft interactomes at the different developmental stages.
A dynamic model of lipid-raft interactome in P. falciparum was proposed by means of a combination of computational and experimental strategy.By means of well established mathematical tools derived from the graph theory, essential proteins were identified as potential targets for future KO experiments.

S2.2
DIFFERENT STRATEGIES FOR THE DESIGN AND SYNTHESIS OF INNOVATIVE ANTIMALARIALS: DEALING WITH PARASITE RESISTANCE AND SEVERE MALARIA

Gemma S.*[1], Taramelli D.[2], Blackman M.J.[3], Martin R.[4], Croft S.[5], Brun R.[6], Craig A.[7]

[1]Dipartimento di Biotecnologie, Chimica e Farmacia, Universita' di Siena, Italy, [2]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università di Milano, Italy, [3]Division of Parasitology, MRC National Institute for Medical Research, London, UK, [4]Research School of Biology, The Australian National University, Canberra, Australia, [5]London School of Hygiene and Tropical Medicine, London, UK, [6]Swiss Tropical and Public Health Institute, Basel, Switzerland, [7]Liverpool School of Tropical Medicine, Liverpool, UK

Malaria afflicts the populations of at least 102 countries, with about one billion persons at risk of infection in tropical and subtropical areas. The history of malaria teaches that the parasite is extremely resourceful and although in the last five years the research activity, capacity building and cooperation with endemic countries has been boosted by both USA and European authorities, the persistence of resistance and the limited number of therapeutic tools is compromising the way to elimination and then eradication of the disease. Our group is involved since several years in the development and optimization of novel classes of antimalarials targeting specific metabolic pathways of the parasite erythrocitic phase.
In silico ligand-based and structure-based drug design, and virtual screening approaches were used for the identification of small molecules or peptidomimetics able to interact with key molecular entities involved in the P. falciparum development within the erythrocite. Synthesis of libraries of compounds for structure-activity relationships evaluation was realized through liquid phase synthesis and through microwave-assisted solid-phase synthesis. Biological activity of the identified compounds was assessed through a number of methodologies ranging from in vitro enzymatic assays to evaluation of antiplasmodial activity in vitro (P. falciparum strains) and in vivo (P. berghei, P. chabaudi), to assessment of protein-protein interactions.
We developed innovative antimalarials targeting the heme detoxification process (Gemma S. et al. J. Med. Chem. 2012, 55, 6948; Gemma S. et al. J. Med. Chem. 2012, 55, 10387; Gemma S. et al. Tetrahedron Lett. 2013, 54, 1233; Gemma S. et al. J. Med. Chem. 2011, 54, 5949), we identified novel chemical entities inhibiting the egress and invasion mechanism of merozoites (Gemma S. et al. Bioorg. Med. Chem. Lett. 2012, 5317), and we studied small-molecule inhibitors of cytoadhesion as potential adjunct therapies for severe malaria (Gemma S. et al. RSC Advances 2014, 4, 4769).
Our continuous effort in the field of drug discovery and development for malaria disease led to the identification of new classes of potential drugs structurally based on novel pharmacophores and with low potential to develop resistance.

S2.3
NOVEL ASSAYS FOR DRUGS AGAINST PLASMODIUM FALCIPARUM GAMETOCYTES

Camarda G.[1], Silvestrini F.[1], Cevenini L.[2], Siciliano G.[1], Michelini E.[2], Calabretta M.[2], Signore M.[1], Bona R.[1], Kumar Tiruppadiripuliyur S.[3], Branchini B.C.[4], Cara A.[1], Fidock D.A.[3], Roda A.[2], **Alano P.*[1]**

[1]Dipartimenti di Malattie Infettive, Parassitarie ed Immunomediate e di Farmaco, Istituto Superiore di Sanità, Rome, Italy, [2] Dipartimento di Chimica, Università di Bologna, Bologna, Italy, [3]Department of Microbiology and Immunology, Columbia University, New York, NY, USA, [4]Connecticut College, New London CT, USA

Targeting malaria transmission is increasingly recognized as a crucial step towards malaria elimination and eradication. Nevertheless, with the exception of primaquine, no drugs are currently available to efficiently target Plasmodium falciparum gametocytes. An important reason for this major gap is that in vitro assays to screen for gametocytocidal activity of existing antimalarials and of libraries of new compounds are still suboptimal. In particular available assays still fail 1) to specifically measure compound inhibitory effects against different stages of gametocyte development and 2) to reliably monitor viability of the terminally differentiated mature gametocyte. For this reason we developed cell based sensitive, robust and reliable assays to be used in high throughput screenings for gametocyte-blocking drugs.
In order to specifically assess activity of compounds against early and late gametocytes we expressed for the first time in malaria parasites novel luciferase reporter genes with distinguishable emission properties under the control of sexual stage specific regulatory sequences switched on early and late in gametocytogenesis, which allowed us to develop a dual luciferase gametocyte assay. In the course of this work we identified P. falciparum gene regulatory sequences able to turn on expression of reporter genes in mature gametocytes at a higher efficiency than the currently used late gametocyte promoter from gene pfs28.
In order to measure viability of the mature gametocytes, we developed a phenotypic imaging-based assay which is able to quantitatively measure ability of stage V gametocytes to undergo the first step in the maturation into gametes (“rounding up”). This assay was adapted to the 384w format and used to screen a set of 50 selected antimalarials from the Medicine for Malaria Venture Validation set.
Both assays are presently in the position to be used in HTS both to measure ability of existing anti-asexual stage compounds to inhibit gametocyte development as well as to undertake primary screenings for novel gametocytocidal compounds.

S2.4
NEW IN VITRO ASSAYS TO SCREEN COMPOUNDS AGAINST P. FALCIPARUM GAMETOCYTES: A PROGRESS TOWARD THE IDENTIFICATION OF TRANSMISSION BLOCKING AGENTS

D’alessandro S.*[1], Basilico N.[2], Parapini S.[1], Corbett Y.[1], Misiano P.[1], Camarda G.[3], Siciliano G.[3], Michelini E.[4][5], Cevenini L.[4], Roda A.[4], Alano P.[3], Taramelli D.[1]

[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università di Milano, Italy, [2]Dipartimento di Scienze Biomediche, Chirurgiche ed Odontoiatriche, Università di Milano, Milano, Italy, [3]Dipartimento di Malattie Infettive, Parassitarie, Immunomediate, Istituto Superiore di Sanità, Roma, Italy, [4]Dipartimento di Chimica “G. Ciamician”, Università di Bologna, Bologna, Italy, [5]INBB, Istituto Nazionale di Biostrutture e Biosistemi, Roma, Italy

INTRODUCTION:
Drugs able to inhibit P.falciparum gametocytes and thus malaria transmission are strongly needed to achieve the goals of the malaria elimination/eradication agenda. Here, we describe two microtiter screening assays, based on different viability markers and read-outs. The pLDH assay is based on the spectroscopic detection of the gametocyte lactate dehydrogenase (pLDH) activity, the LUC assay is based on the use of a parasite strain which express a novel luciferase enzyme (LUC1-G) under the control of a promoter specific for gametocytes. The aim of our work was to screen the library “MMV-Malaria box” in order to compare the two methods and identify drugs against late stage gametocytes.
MATERIALS AND METHODS:
Gametocytogenesis of two strains of P. falciparum, the wild type 3D7 and the 3D7pfelo1-LUC1-G, was induced in vitro, asexual parasites were removed by N-acetylglucosamine treatment, and gametocytes were cultures up to stage IV-V.
A primary screening with the single dose of 3.7 µM was performed with the pLDH assay. Viability was measured at two different time points, 72h post-treatment (72 h assay) and 144 h, In the latter case, the drugs were removed from the cultures, which were incubated for further 72h (72+72 h assay). The dual checkpoint assay allows the identification of gametocytocidal compounds with fast versus slow speed of action. Based on the results of the primary screening, 36 compounds were selected for dose-response experiments and IC50 determination. The pLDH assay was performed on the 3D7 strains following the same time protocol as for the primary screening, the LUC assay was performed on the 3D7pfelo1-LUC1-G strain with 72h of drug treatment.
RESULTS:
The pLDH and LUC assay have been successfully applied to screen the 400 compounds of the MMV- Malaria Box. At the dose of 3.7 µM, seven compounds inhibited gametocyte viability by more than 50% already after the first 72h incubation; 15 compounds showed an activity higher than 75% at 144h. A good signal to background ratio and a Z’ were obtained.
The dose-response experiments, performed on the 36 compounds with activity higher than 50% at 144h, confirmed the primary screening results. Seven compounds showed an IC50 lower than 1µM with both assays. A good correlation between the IC50 results with the two assays was obtained
CONCLUSIONS:
The pLDH and LUC assay can be used as a fast and cheap screening method to find potential gametocitocydal drugs and to investigate their mode of action.
Acknowledgments: MMV for providing the malaria box and BMGF for funding. Details of pLDH assay in D’Alessandro et al 2013, JAC,68,2048.

S2.5
TRANSMISSION BLOCKING ACTIVITY OF VERNONIA AMYGDALINA ON PLASMODIUM BERGHEI AND PLASMODIUM FALCIPARUM FIELD ISOLATES

Abay S.M.*[1], Dahiya N.[1], Dembo E.[1], Dori G.[2], Esposito F.[1], Lucantoni L.[1], Lupidi G.[1], Ogboi S.[1], Ouédraogo K.R.[1], Ouédraogo K.R.[3], Ouédraogo J.B.[3], Sinisi A.[4], Taglialatela-Scafati O.[4], Yerbanga R.[3], Habluetzel A.[1]

[1]University of Camerino, Camerino, Italy, [2]University of Naples "Federico II", Naples, Italy, [3]Institut de Recherche en Sciences de la Santé ~ Bobo-Dioulasso ~ Burkina Faso, [4]Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso

Artemisinin based drug combinations (ACT) cure malaria patients by effectively killing asexual blood stages but do not eliminate mature gametocytes which remain in the circulation and are able to infect the mosquito vectors for several days. Post ACT supplementary treatment of malaria patients with compounds able to inhibit sporogonic development of malaria parasites in the mosquito midgut may add the value of reducing malaria transmission.

This study aimed to assess whether Vernonia amygdalina, a plant traditionally used for the treatment of malaria fever in several African countries, contains secondary metabolites that interfere with early sporogonic development of the Plasmodium parasite, namely with micro- and macrogamete formation, zygote formation and/or ookinete maturation.

Plasmodium berghei CTRPp.GFP, a parasite strain expressing green fluorescent protein, was used to determine in vitro activity of fractions and isolated molecules from V. amygdalina leaves against early sporogonic stages. Gametocytemic blood from Balb/c mice was incubated in microplates with the test substances; and zygote and ookinete formation was scored after 40h of incubation under the fluorescent microscope (400x). The fractions, found active on P. berghei, were then examined on P. falciparum field isolates in Burkina Faso. Gametocytemic blood from human volunteers was supplemented with V. amygdalina fractions (100 ppm) and membrane fed to Anopheles coluzzii mosquitoes. A week later, mosquito midguts were dissected and examined for the presence and density of oocysts.

Organic fractions from the methanol extract of V. amygdalina leaves proved to be strongly active against P. berghei early sporogonic stages. In particular, fraction 11 (eluted with n-hexane:ethylacetate 1:1 and 7:13) and fraction 13 (eluted with ethylacetate) suppressed sporogonic development by 98-100% at a concentration of 50µg/ml. In the experiments with P. falciparum field isolates, control mosquitoes displayed an oocyst prevalence ranging from 30 - 50%, whereas that of mosquitoes membrane fed with gametocytemic blood treated with fraction 11 and 13 at 100 ppm ranged from 0 to 32% and 0 - 8%, respectively. The oocyst density among oocyst-positive mosquitoes varied from 3.5 - 4.2 per mosquito in controls, to 1.8 - 2.4 and 1.3 - 1.8 in fraction 11 and 13, respectively.

Subsequent chemical analysis revealed rearranged sesquiterpenes vernolide and vernodalol to be the major components of fraction 11 and 13, respectively. The isolated molecules confirmed their effects on early sporogonic stages, with vernodalol displaying a relatively stronger in vitro inhibitory activity than vernolide on P. berghei's transmissible stages.

Taken together, the results obtained with vernolide and vernodalol rich fractions from V. amygdalina leaves hold promise for the development of medicines impacting on the transmissible stages of the malaria parasites.

Keywords:
Vernonia amygdalina, transmission blocking, malaria

S2.6
IN VITRO AND IN VIVO ACTIVITY OF NEEMAZAL® AND AZADIRACHTIN A AGAINST EARLY SPOROGONIC STAGES OF PLASMODIUM BERGHEI

Dahiya N.*[1], Abay S.M.[1], Chianese G.[2], Esposito F.[1], Habluetzel A.[1], Lupidi G.[1], Ogboi S.[1], Taglialatela-Scafati O.[2], Lucantoni L.[1]

[1]School of Pharmacy, University of Camerino, Camerino, Italy, [2]Department of Pharmacy, University of Naples "Federico II", Naples, Italy

NeemAzal® is a commercial ethanolic extract from seed kernels of Azadiarachta indica (Meliaceae), with a wide spectrum of biological activities attributed to limonoid secondary metabolites that it contains (Trifolio-M GmbH). The product is particularly rich in azadirachtins A-K, (52% of the product), azadirachtin A (Aza A) being the main constituent. The Aza A was shown to inhibit microgamete formation of Plasmodium (Billker et al., 2002, J. Eukariot Microbiol 49: 489-497) and, not surprisingly, NeemAzal® was found to completely inhibit the transmission of Plasmodium berghei to Anopheles stephensi females when administered to gametocytemic mice at an equivalent Aza A dose of 50 mg/kg (Lucantoni et al., 2010, Malaria Journal 9: 66).

The present study aimed at clarifying whether Aza A is the unique molecule responsible for the transmission blocking activity observed with NeemAzal® or other extract components contribute to the effects.

Bioavailability experiments. Infected BALB/c mice, with mature P. berghei gametocytes circulating in their peripheral blood, were treated i.p. with pure Aza A at the doses of 50 mg/kg and 100 mg/kg and with NeemAzal® (Trifolio-M GmbH) at the corresponding Aza A concentrations. Bioavailability of compounds inhibiting early sporogonic development was estimated by counting, at various time points after treatment, the so-called "exflagellation centers", formed by microgametocytes in process of releasing flagellated gametes.

In vitro assay. The direct effects of NeemAzal® and Aza A on gamete to ookinete development were measured by incubating gametocytemic blood with various concentrations of test substances in microplates.

Exflagellation tests performed ex vivo with blood sampled from NeemAzal® and Aza A treated mice revealed quicker and prolonged bioavailability of NeemAzal® compounds as compared to pure Aza A. After NeemAzal® treatment at an Aza A dose of 100 mg/kg, exflagellation was almost completely inhibited for up to 3 h after treatment. A 50% reduction of exflagellation centers with respect to pre-treatment values could be observed for up to 7 h after treatment. With Aza A at 100 mg/kg, just a short lived and moderate inhibitory activity of 60% was recorded around 1 h after treatment.

Similarly, adding NeemAzal® or Aza A to microplate wells containing gametocytemic blood, the former showed a stronger activity than the latter against early sporogonic stages. The IC50 values were 4.77+3.18 µg/mL and 12.55 +1.49 µg/mL for NeemAzal® and Aza A, respectively.

Since other members of the azadirachtin family different from Aza A, namely Aza B, D, and I are present in NeemAzal® at very low concentrations (around 1%), the increased activity observed with the commercial neem product is rather unlikely to be due to a direct anti-plasmodial effect of these limonoids. It appears more likely that azadirachtins B, D and I or other NeemAzal® components, are influencing indirectly in vitro activity and bioavailability of Aza A.

Keywords:
Plasmodium, transmission blocking, azadirachtin

S2.7
ANTIMALARIAL POTENTIAL OF SYNTHETIC QUINONE DERIVATIVES DESIGNED ON THE MODEL OF THE APLIDINONES, NATURAL MARINE THIAZINOQUINONES

Imperatore C.*[1], Aiello A.[1], D'aniello F.[1], Luciano P.[1], Fattorusso C.[1], Persico M.[1], Taramelli D.[2], Parapini S.[2], Cebrián-Torrejón G.[3], Doménech-Carbó A.[3], Menna M.[1]

[1]The NeaNat Group, Department of Pharmacy, University of Naples "Federico II", Napoli, Italy, [2]Scienze Farmacologiche e Biomolecolari Università di Milano, Milan, Italy, [3]Departament de Química Analítica, Facultat de Química, Universitat de València, Burjassot, Valencia, Spain.

INTRODUCTION: There is an urgent need to discover new antimalarials, due to the spread of chloroquine resistance and the limited number of available drugs. A number of quinones have been shown to be effective antimalarials; the observed effects are most likely related to the most prominent chemical feature of these kind of molecules, that is their ability to undergo redox cycling. Antiplasmodial activity of quinone structures of marine origin has been reported. Examples are xestoquinone and halenaquinone (Laurent et al., 2006, Bioorg. Med. Chem., 14:4477-82), their derivative orlaquinone (Longeon et al., 2010, Bioorg. Med. Chem., 18:6006-11), and ketoadociaquinones (Shmitz et al., 1988, J. Org. Chem., 53:3922-25; Cao et al., 2005, Bioorg. Med. Chem., 13:999-1003), isolated from Xestospongia sponges, and thiaplakortones (Davis et al., 2013, J. Org. Chem., 78: 9608-13), isolated from Plakortis lita. It has been also evidenced that the presence of a dioxothiazine moiety enhances the antiplasmodial activity (Figure 1).

Within our search for bioactive compounds from Mediterranean ascidians, we have previously isolated a large group of thiazinoquinones (Menna et al., 2013, Eur. J. Org. Chem,, 16:3241-46; Aiello et al., 2005, Eur. J. Org. Chem., 23:5024-30; Aiello et al., 2005, J. Med. Chem., 48:3410-16; Aiello et al., 2003, Eur. J. Org. Chem., 5: 98-900). Some of these compounds have been used as lead structures for the synthesis of simplified analogues for pharmacological screening (Aiello et al., 2010, Bioorg. Med. Chem., 18: 719-27).

Based on the structural analogy with the reported antimalarial quinone structures, the synthetic derivatives prepared using the natural thiazinoquinones aplidinones A and B as model structures (1-12, Figure 2) have been tested in vitro against D10 (chloroquine-sensitive) and W2 (chloroquine-resistant) strains of P. falciparum. The synthetic derivatives have shown a significant antiplasmodial activity and many structural requirements, critical for their activity, have been evidenced.

Conformational (MD and MM) and electronic (tautomeric form, pka values, percentage of neutral/ionized forms at different pH values) analysis have been performed by computational means. The propensity for electron acceptance of the active compounds has been explored by LUMO orbital and anionic radical analysis. A correlation between electrochemistry ("direct" and in the presence of Fe(III)-Heme) of the studied compounds and their antiplasmodial effects has been evidenced by using solid-state electrochemical assays (Doménech-Carbó et al., 2013, Anal. Chem., 85: 4014-21).

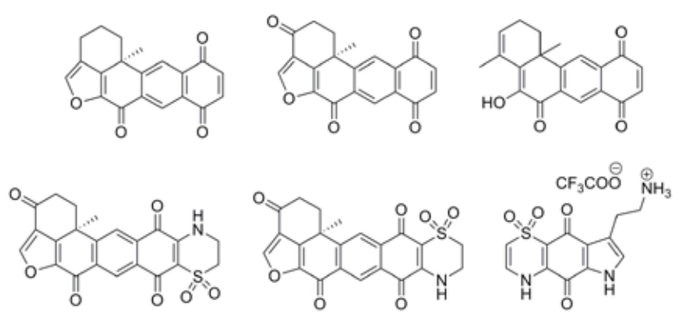
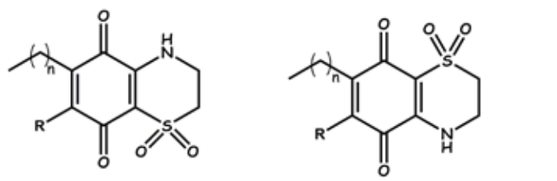


Figure1: Structures of natural antimalarial quinones isolated from marine organisms



1 R = -OMe; n=1
2 R = -OMe; n=7
3 R = -OMe; n=13
4 R = -NHCOC(CH$_3$)$_3$; n=1
5 R = -NHCOC(CH$_3$)$_3$; n=4
6 R = -NHCOC(CH$_3$)$_3$; n=10

7 R = -OMe; n=1
8 R = -OMe; n=7
9 R = -OMe; n=13
10 R = -NHCOC(CH$_3$)$_3$; n=1
11 R = -NHCOC(CH$_3$)$_3$; n=4
12 R = -NHCOC(CH$_3$)$_3$; n=10

Figure2: Structures of synthetic analogues of aplidinones A and B

Keywords:
antimalarial, quinones, redox

S2.8
ANTIMALARIAL 1,2-DIOXANES INSPIRED BY MARINE NATURAL PRODUCTS

Chianese G.*[1], Fattorusso E.[1], Taglialatela-Scafati O.[1], Persico M.[1], Fattorusso C.[1], Parapini S.[2], Corbett Y.[2], Taramelli D.[2], Basilico N.[3], Quintavalla A.[4], Trombini C.[4], Lombardo M.[4]

[1]Dipartimento di Farmacia, Università di Napoli "Federico II", Napoli, Italy, [2]Dipartimento di Scienze Farmacologiche e Biomolecolari Università di Milano, Milano, Italy, [3]Dipartimento di Scienze Mediche, Chirurgiche e Odontoiatriche, Università di Milano, Milano, Italy, [4]Dipartimento di Chimica "Giacomo Ciamician", Alma Mater Studiorum, Università di Bologna, Bologna, Italy

The present communication will report our results on the antimalarial activity of natural and synthetic 1,2-dioxanes. In the course of our ongoing search for antimalarial hits from marine sources, we have reported that plakortins, endoperoxide-containing polyketides isolated from the sponge Plakortis simplex, possess a significant in vitro antimalarial activity on CQ-resistant P. falciparum (Pf) strains and no cellular toxicity (Fattorusso et al., 2002, J Antimicrob Chemother, 50:883–888). A multidisciplinary approach identified the essential pharmacophoric requirements for antimalarial activity, indicated the crucial role of the endoperoxide functionality, and suggested the importance of the "western" alkyl side chain and of conformation-dependent features (Taglialatela-Scafati et al. 2010, Org. Biomol. Chem., 8:846–56). Further information on the structure-activity relationships have been recently gained through the chemical investigation of the Chinese marine sponge P. cfr. simplex, affording five new polyketides endoperoxides, four of which containing a 1,2-dioxene ring, never evaluated before for antimalarial activity.

Since the natural sources cannot be suitable to provide adequate amount of material for drug exploitation, we have designed and accomplished the total synthesis of endoperoxide derivatives inspired by the structure of plakortin compounds. A new series of simple endoperoxides, characterized by a 3-methoxy-1,2-dioxane scaffold, was thus designed and synthesized through a simplified and efficient one-pot three-component Mn(III)-mediated synthesis which utilizes cheap starting materials. In the first series we obtained 3,6,6-trisubstituted 3-methoxy-1,2-dioxanes with simple alkyl chains and bearing an ester, hydroxymethyl or methoxymethyl groups at position 4. (Persico et. al., 2011, J Med Chem, 54:8526–40; Persico et. al., Eur J Med Chem, 70:875-86). Most of compounds showed antimalarial activity in the low micromolar range and no cellular toxicity, both against chloroquine-resistant (CQ-R) and chloroquine sensitive (CQ-S) strains of Pf.

Keywords:
antimalarial, 1,2-dioxanes, 3D-pharmacophore

S2.9
BIOACTIVE MOLECULES OF MALARIAL PIGMENT HEMOZOIN AND THEIR PATHOMECHANISTIC ROLE IN MALARIA

Skorokhod O.[1], Uyoga S.[1], Marrocco T.[1], Aguilar R.[2], Gremo G.[1], Barrera V.[1], Gallo V.[1], Davalos D.[1], Schwarzer E.[1], **Arese P.*[1]**

[1]Dipartimento di Oncologia, University of Torino Medical School, Torino, Italy, [2]Centre de Recerca en Salut Internacional de Barcelona (CRESIB, Hospital Clínic-Universitat de Barcelona), Barcelona, Spain

1. Hemozoin (HZ) and malaria anemia
HZ generates lipoperoxides and sheds 4-hydroxynonenal (HNE) that reacts with amino-, thiol- and imidazole-groups in proteins. Parasite maturation was accompanied by increasing HNE-conjugation in parasitized-RBC (pRBC) membrane proteins. Trophozoite-derived HNE is transferred to adjacent non-pRBCs (npRBCs) in rosettes. Ex-vivo data from acute malaria patients suggest HNE-transfer from pRBC to npRBC may explain massive removal of npRBC in malaria anemia. Studies in children with acute malaria confirmed the in-vivo formation of HNE-conjugates that favours persistent anaemia.

2. HZ and residual bodies (RBs) as modulators of immune functions
HZ-containing RBs are released during schizogony. The released food-vacuole together with membrane remnants of parasite origin is an excellent source for ROS and HNE. Though well equipped with anti-oxidative enzymes, RBs were extensively modified by HNE mostly during schizogony. RB derived HNE and HETE impair the innate immune functions of monocyte, their differentiation to macrophages and dendritic cells.

Released RBs are non-living interfaces between parasite and host and bind several host proteins of mainly blood plasma origin. E.g. in blood to surfaces (like RBs) bound fibrinogen is a strong activator of immediate innate immune responses via TLRs.

3. HZ and cell motility
Phagocytosis of HZ by monocytes led to HNE-dependent cytoskeleton modifications with subsequent decrease of cell motility. Cells were no longer able to perform phagocytosis cycles, non directed migration and chemotaxis-attractants like fMLP, MCP-1 or TNF.

S2.10
AUTOIMMUNITY LOCI PLAY A ROLE IN THE SUSCEPTIBILITY TO PLASMODIUM FALCIPARUM INFECTION AND UNCOMPLICATED DISEASE

Mangano V.D.* [1-2], Bougouma E.C. [3], Verra F. [1], Sepulveda N. [4-5], Rockett K.A. [6-7], Kabore Y. [3], Diarra A. [3], Bisseye C. [1-3], Nebie I. [3], Kwiatkowski D.P. [6-7], Sirima B.S [3], Modiano D. [1-2] & The MalariaGEN Consortium [6-7]

[1]Dept. of Public Health and Infectious Diseases, Sapienza University of Rome, Italy, [2]Istituto Pasteur - Fondazione Cenci Bolognetti, Sapienza University of Rome, Italy, [3]Centre National de Recherche et Formation sur le Paludisme, Ouagadougou, Burkina Faso, [4] London School of Hygiene and Tropical Medicine, United Kingdom, [5]Center of Statistics and Applications of University of Lisbon, Lisbon, Portugal, [6]Wellcome Trust Centre for Human Genetics, University of Oxford, United Kingdom, [7]Wellcome Trust Sanger Institute, Hinxton, United Kingdom

INTRODUCTION:
Plasmodium falciparum malaria is considered a major selective factor in the recent history of the human genome but little is known on its effects on the evolution of the immune system. To investigate this aspect we conducted a large-scale epidemiological study in Fulani, Mossi and Rimaibé (Non-Fulani) populations from Burkina Faso.. The Fulani are known to be less susceptible to malaria and to mount stronger immune responses to P. falciparum than sympatric populations (Modiano et al., 1996, Proc. Natl. Acad. Sci. U. S. A., 93(23):13206-11). Key genes related to T reg cell function are down-regulated in the Fulani (Torcia et al., 2008, Proc. Natl. Acad. Sci. U. S. A., 105(2):646-51.). Furthermore, higher prevalence and titres of anti-nuclear antibodies, serological markers of Systemic Lupus Erythematosus (SLE), have been observed in this population (Mangano et al., in preparation). This disorder of immune homeostasis could be driven by genetic factors positively selected by P. falciparum and may underlie the Fulani higher reported susceptibility to autoimmune disease.
MATERIALS AND METHODS:
The study was conducted in four rural villages in 2007-8, and consisted of a combination of five cross-sectional parasitological surveys and two longitudinal surveys of malaria specific morbidity. We genotyped 370 SNPs on 2564 DNA samples using the Sequenom MassArray System. SNPs included polymorphisms involved in susceptibility and immune response to malaria, as well as SNPs at autoimmunity loci. We used Principal Component (PC) Analysis to investigate the genetic structure of our populations. We then compared allele frequencies between Fulani and Non-Fulani and calculated Fst, a measure of genetic differentiation among populations. Finally, we conducted association analysis with P. falciparum infection and with incidence of clinical malaria. We used a repeated measure model with logistic and poisson regression, respectively, adjusting for ethnicity, age, gender and village as well as for the first 3 PCs to account for genetic relatedness.
RESULTS:
PC Analysis using HapMap data confirmed that Mossi and Rimaibe are not genetically distinct among themselves, whereas the Fulani are a clearly distinct group, and the closest to the CEU population. Analysis of the distribution of Fst showed that the highest values are enriched in autoimmunity loci, suggesting that those genes are the main driver of the genetic differentiation between Fulani and Non-Fulani populations. Genetic association analysis of susceptibility to infection and uncomplicated disease revealed several significant results among autoimmunity loci. For instance, SNPs at the BLK gene show association with the prevalence of P. falciparum parasitaemia [strongest signal: p-value=0.004, OR(95%CI)=5.2(1.7-16.8)]. This gene encodes for B lymphoid Tyrosine Kinase, an enzyme involved in B cell receptor signalling and development. BLK SNPs have been previously reported to be associated with SLE (Hom et al., 2008, N Engl J Med, 358(9):900-9) and to affect gene expression (Ge et al., 2009, Nat Genet, 41(11):1216-22).
CONCLUSIONS:
Our results provide evidence that common gene regulatory networks could underlie susceptibility to malaria and to immunological disorders such as autoimmune diseases. Both haplotype and functional analysis are ongoing to unravel the nature of the observed association signals and to investigate whether they could explain the Fulani's lower susceptibility to malaria.

S2.11
ROLE OF MICROBIAL COMMUNITY OF BREEDING HABITATS IN LARVAL NICHE PARTITIONING OF ANOPHELES GAMBIAE MALARIA VECTORS IN BURKINA FASO

Pombi M.*[1], Totino V.[1], Iebba V.[1], Santangelo F.[1], Bassole I.H.N.[2], Guelbeogo W.M.[3], Sagnon N.[3], Della Torre A.[1], Schippa S.[1], Costantini C.[4]

[1]Dipartimento di Sanità Pubblica e Malattie Infettive, Università di Roma "Sapienza" ~ Roma ~ Italy, [2]Université de Ouagadougou, Laboratoire de Biochimie Alimentaire Enzymologie Biotechnologie Industrielle et Bioinformatique ~ Ouagadougou ~ Burkina Faso, [3]Centre National de Recherche et Formation sur le Paludisme ~ Ouagadougou ~ Burkina Faso, [4]Institut de Recherche pour le Développement, UMR MIVEGEC (UM1, UM2, CNRS 5290, IRD 224), Centre IRD France-Sud ~ Montpellier ~ France

INTRODUCTION:
The species complex Anopheles gambiae represents an unparalleled example of ecological speciation in which the sibling species An. coluzzii and An. gambiae sensu stricto are believed to undergo a process of ecological divergence acting predominantly at larval stage. The ecological factors involved in the partition of larval environment of these species are not well known. Differential oviposition behavior, possibly affected by microbial composition of water, could be among the factors driving this process. A study of the mechanisms of larval niche partitioning is crucial for predicting the distribution and niche expansion of these vector species in the face of changing environments or applied control measures.
MATERIALS AND METHODS:
To better understand how these three taxa share limiting resources such as larval breeding sites, we carried out a survey on 41 randomly chosen larval breeding sites in the village of Goundry (Burkina Faso) during rainy season (July-October 2003). For each site, we sampled An. gambiae sensu lato larvae by dipping, and then we assessed the relative frequency of the taxa by PCR-RFLP identification. In parallel, surface water samples were collected from the same breeding sites to obtain their microbiological profile by PCR amplification using consensus primers flanking 16S rDNA regions and subsequent separation by Temperature gradient gel electrophoresis (TTGE).
RESULTS:
Overall, 2,197 1st-2nd instar larval specimens were molecularly identified (42.3% An. coluzzii, 40.7% An. arabiensis, 17% An. gambiae s.s.), and 58 bacterial TTGE amplicons were detected from the water samples. Significant Pearson's positive correlations were observed between higher relative frequencies of An. arabiensis and 4 TTGE amplicons, while 9 positive and 1 negative correlations were observed for An. gambiae s.s. None of the TTGE amplicons significantly correlated with An. coluzzii relative frequency. Partial least squares discriminant analysis (PLS-DA) showed a weak but informative separation among TTGE amplicons for An. arabiensis and An. gambiae s.s. relative frequencies, with 66% correct discrimination of sites.
CONCLUSIONS:
These preliminary results support the hypothesis that the microbial component of the larval habitat could be a potential marker of abundance of sympatric species of An. gambiae complex in Burkina Faso. These observations lead us to plan deeper investigations about bacterial composition of breeding sites, in order to understand cause-effect relationships between microbial community and larval distribution of An. gambiae taxa, with the final aim to verify their role as ecological markers of larval niche partitioning.

Keywords:
speciation, Anopheles, microbial community

S2.12
ANOPHELES GAMBIAE SALIVARY PROTEINS AS A TOOL TO EVALUATE SPATIAL AND TEMPORAL VARIATION OF HUMAN EXPOSURE TO MALARIA VECTORS

Lombardo F.*[1], Ronca R.[2], Rizzo C.[1], Mangano V.[1], Sirima B.[3], Nebié I.[3], Bousema T.[4], Drakeley C.[4], Modiano D.[1], Arcà B.[1]

[1]Dept of Public Health and Infectious Diseases, Parasitology Section, Sapienza University, Rome, Italy, [2]Dept of Biology, Federico II University, Naples, Italy, [3]Centre National Recherche et Formation sur Le Paludisme, Ouagadougou, Burkina Faso, [4]Dept of Immunity and Infection, London School of Hygiene and Tropical Medicine, London, UK

Saliva of blood sucking arthropods, injected into host skin during feeding, evokes an antibody response that may be used as serological indicator of exposure to disease vectors. Studies on mosquito salivary repertoires highlighted that Anopheles saliva carries genus-specific proteins (i.e. not found in Aedes/Culex or in other blood feeders). These proteins, if immunogenic, may be ideal candidates as markers of exposure to anopheline vectors, a tool which would be very useful for malaria epidemiological studies.
Human plasma samples collected in an hyperendemic area of Burkina Faso (Ouagadougou) and in an area of moderate malaria endemicity in Tanzania (Korogwe) were used to evaluate by ELISA the IgG, IgG1 and IgG4 antibody responses to the anopheline-specific salivary proteins gSG6 and cE5 from the malaria vector Anopheles gambiae.
The anti-gSG6 IgG response varied according to transmission/rainy season and was positively associated with the level of Anopheles exposure. The cE5 protein was more immunogenic than gSG6, but no variation of the IgG response was found during the transmission season, most likely because of its longer lasting nature. Moreover, the anti-gSG6 IgG level decreased with age, suggesting the induction of tolerance mechanisms, which was not the case for the anti-cE5 IgG response. Finally, the analysis of IgG1 and IgG4 subclasses pointed out the differential responses evoked by these two salivary proteins in exposed individuals with (i) gSG6 triggering a short-lived response of the Th2-type, with high IgG4 levels and induction of tolerance and (ii) cE5 eliciting a longer-lived Th1-type response, with high IgG1 levels and no tolerance induction.
The Entomological Inoculation Rate, which measures the number of infectious bites/person/unit of time, is presently the gold standard for the assessment of malaria transmission intensity. However, its determination may be problematic or not fully reliable (logistic constraints, low vector density, micro heterogeneity, low infection level, sampling technique, etc.) and additional/alternative tools would be very helpful. Our studies provide clear evidence that the An. gambiae gSG6 salivary protein can be used in malaria transmission areas as a reliable marker to evaluate spatial and temporal variation of human exposure to Afrotropical vectors. The cE5 protein showed higher sensitivity and may be of use in conditions of low or transient exposure to Anopheles vectors (i.e. low vector density, travellers). In conclusion, since parasite antigens (AMA-1, MSP-1, CSP) are already in use for the serological assessment of malaria transmission, the addition of Anopheles salivary antigens for measuring vector exposure may allow for comprehensive epidemiological profiling using just serological techniques.

S2.13
SYMBIONTS AND MOSQUITO VECTORS: WORK IN PROGRESS AT UNICAM

Ricci I.* [1], Damiani C.[1], Rossi P.[1], Capone A. [1], Valzano M. [1], Cappelli A. [1], Bokzic J. [1], Mancini M.V. [1], Favia G.[1]

[1]School of Biosciences and Veterinary Medicine, University of Camerino, Italy

INTRODUCTION
The concept of holobiome proposes that a dynamic relationship exists between hosts and their microbial communities, by altering its composition, this 'holobiont' can adapt to changing of environmental conditions far more rapidly than by genetic mutation and selection alone. Thinking of the potential impact of the associated microbial community on (i) behavioural traits of insects as feeding, mating, breeding and (ii) vectorial competence (host immune modulation or antimicrobial molecules) we started working on the malaria vectors microbial communities, with the aim to identify *Plasmodium* antagonist microbes to be used in Symbiotic Control (SC) of malaria.

RATIONALE OF THE RESEARCH LINE

Laboratory experimentations	Semi-field trials	In-field studies



Laboratory experiments carried out at UNICAM are aimed at the development of efficient and safe *Plasmodium*-resistant mosquitoes carrying antagonist microbes expressing antiparasitic molecules. Semi-field and in-field activities, carried out at University of Perugia and IRSS (Bobo-Dioulasso, Burkina Faso) respectively, are focused on in-depth ecological studies to assess the release protocols.
RESULTS AND DISCUSSION
The breakthrough was the discovery of two candidates for malaria SC, the bacterium *Asaia* and the yeast *Wickerhamomyces anomalus*. We demonstrated that *Asaia* is stably associated to *Anopheles stephensi* and localise the midgut, gonads and salivary glands (Favia et al. 2007, Proc Natl Acad Sci USA, 104:9047-9051). *Asaia* shows both horizontal and vertical transmission routes within and across mosquito populations, including paternal transmission to offspring (Damiani et al. 2008, Current Biology 18: pR1087). *Asaia* showed similar traits in *A. gambiae* demonstrating that it is vertically transmitted by egg smearing (Damiani et al. 2010, Microb Ecol 2010, 60: 644-54). By genetic manipulation of the bacteria we proved the interaction between *Asaia*, *Plasmodium* and *Anopheles*, giving new insights into mosquito symbiosis and implications in malaria SC.
Regarding *W. anomalus*, we discovered that this yeast inhabits the midgut and gonads of *A. stephensi* (Ricci et al. 2011, Environ Microbiol, 13: 911-921). This yeast is an antagonist microbe able to produce a Killer Toxin with potential to interfere with the parasite development within the mosquito gut (Cappelli et al. 2014, Plos One, 9(5):e95988). The use of *W. anumalus* is already applied in food biotechnology for the bio-preservation and it is considered safe. This tool could avoid several objections and ethical issues related to the use of genetically modified microbes.
FROM BENCH TO FIELD
Currently we are performing semi-field trials for the release of recombinant *Asaia* strains in greenhouse to monitor the spread of the bacteria in the recipient populations, for the development of mathematical models. Additional semi-field studies have been planned in BF, using wild mosquitoes for the release simulations at environmental conditions. In-field studies will follow to set up the spreading stations for the development of release protocols.

ACKNOWLEDGEMENT
This research has received funding from the EU 7th FP grant agreement n. 281222 to IRENE RICCI

NOTES

SIMPOSIO 3

PARASSITOSI CARDIO-POLMONARI DEL CANE E DEL GATTO, UNO SCENARIO IN CONTINUA EVOLUZIONE

S3.1
EMERGING CARDIO-RESPIRATORY CANINE AND FELINE NEMATODES IN ITALY

Di Cesare A.*[1], Brianti E.[2], Traversa D.[1]

[1]Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy, [2]Department of Veterinary Sciences, University of Messina, Messina, Italy

The cardio-respiratory nematodes Aelurostrongylus abstrusus affecting cats, Angiostrongylus vasorum and Capillaria boehmi affecting dogs, Dirofilaria immitis and Capillaria aerophila affecting both dogs and cats, are presently spreading and emerging in Europe, including Italy (Traversa et al., 2010, Parasit. Vectors, 3:62; Colwell et al., 2011, Vet. Parasitol., 182:14-21). Moreover, the metastrongyloid Troglostrongylus brevior has been recently recorded in domestic cats from Spain and Italy (rev. in Traversa and Di Cesare, 2013, Trends Parasitol., 29:423-430; Brianti et al., 2014, Vet. Parasitol., in press). These parasites are of major relevance considering their pathogenic potential, the hindrances inherent in the diagnosis and the zoonotic role some of them play. The geographic range of these nematodes appears in expansion, although the reasons for this emergence are speculative. Most of these parasites require a biological vector for their development, i.e. A. abstrusus, T. brevior and A. vasorum are transmitted by gastropods, while D. immitis by mosquitoes. The life cycles of C. aerophila and C. boehmi are direct, although it has been hypothesized that earthworms could be involved as transporting hosts. Temperature, moisture and water availability influence development and survival of biological vectors, thus global warming is likely implicated into the geographic dispersion of these infections (Traversa et al., 2010, Parasit Vectors, 3:62; Colwell et al., 2011, Vet. Parasitol., 182:14-21). Also, changes in vector seasonal population dynamics might indeed nurture the current dispersal and spread of vector-borne nematodes (Traversa et al., 2010, Parasit. Vectors, 3:62; Otranto et al., 2013, Parasit. Vectors, 6:16). Other drivers, e.g. movements of animals from endemic to free regions, international trade of goods and animals, lack of harmonized control measures, and changes in wildlife habitat and populations can be involved in such emergence. Altogether, these drivers may significantly increase the occurrence of these parasites in endemic areas and the risk of introduction in free regions.

As a key example, the alternation of natural habitats may enforce wildlife (e.g. foxes) to move into peri-urban areas. Thus, the reservoir role of wild animals is a likely driver promoting bridging infections of both lungworms (e.g. C. aerophila, A. vasorum) and heartworms (e.g. D. immitis) between wild and domestic animals (Morgan et al., 2009, Parasitol. Int., 58:406-410; Traversa et al., 2010, Parasit. Vectors, 3:62). There is the evidence of both a rise in the number of cases by A. vasorum in known endemic foci and the appearance of new foci in free areas and/or regions. Interestingly, in Italy A. vasorum was first reported over twenty years ago in red foxes and for quite some time infection was likely confined to this host until recently, when angiostrongylosis has been reported with increasing frequency in dogs. The trend in higher incidence observed in other European countries and the concomitant expansion in wild animals, e.g. foxes (Eleni et al., 2014, Parasitol. Res., 113, 1247-1250.) and wolves (Eleni et al., 2014, Int. J. Parasitol. Parasites Wildl., in press), strongly indicate that the parasite is actually emerging in Italy. A similar expansion in the geographical range of A. abstrusus has been also suggested due to due to climatic and vector changes (Traversa et al., 2010, Parasit. Vectors, 3:62). Information on prevalence of lung capillarioses is poor, although there is the evidence that Capillaria spp. are distributed worldwide and apparently spreading in Italy in both pets and wildlife (Traversa et al., 2010, Parasit. Vectors, 3:62; Veronesi et al., 2014, 200:133-138).

The introduction of competent vectors may also favour the establishment and spread of parasites to new regions, as suggested for Aedes albopictus (the Asian tiger mosquito) and D. immitis (Colwell et al., 2011, Vet Parasitol., 182:14-21). Thus, it is noteworthy that D. immitis is indeed spreading southward in Italy (Giangaspero et al., 2013, Parasitol. Res., 112:1357-1361; Otranto et al., 2013, Parasit. Vectors, 6:16).

In the last few years T. brevior has been reported in different areas of Italy, with an apparent increasing trend (rev. in Traversa and Di Cesare, 2013, Trends Parasitol., 29:423-430; Brianti et al., 2014, Vet. Parasitol., in press). The role of wild animals (e.g. Felis silvestris silvestris) in this expansion remains to be elucidated.

Although in the last few years the current changes in the epidemiology of these cardio-respiratory nematodes have fascinated the scientific community, their prevalence in pets is likely underestimated mainly for diagnostic hindrances. Indeed, cardio-pulmonary infections of dogs and cats are often neglected by veterinarians and they cannot be easily diagnosed clinically or even via laboratoristic approaches. These gaps represent a major limit, as an accurate and definitive diagnosis of these infections is mandatory to ensure a pinpointed scenario on the spreading of cardio-respiratory nematodes. The Baermann method's is the gold standard to diagnose A. vasorum, A. abstrusus and T. brevior, despite this technique has inherent drawbacks. Canine and feline capillarioses and dirofilariosis are diagnosed by the microscopic identification of eggs and microfilariae in faeces and blood of the animals, respectively. However, a careful morphological and morphometric appraisal is always required for all these methods. Innovative molecular tests are available for diagnosing most of these infections and, interestingly, in-field serological kits are marketed to detect A. vasorum and D. immitis circulating antigens (Traversa et al., 2010, Parasit Vectors, 23:3:62; Di Cesare et al., 2012, J Clin Microbiol, 50:1958-1963; Brianti et al., 2014, Vet Parasitol., in press; Schnyder et al., 2014, Parasit Vectors, 7:72). These approaches indeed overcome the limits of the classical approaches and are powerful in both epidemiological and clinical settings. Given the impact that these nematodes may have on pet health, the zoonotic potential of some of them and the current trend in their geographic spread, new studies are warranted to understand on a wide level their current epidemiological impact and further modifications which will likely occur in the near future.

S3.2
LUNG AND NASAL CAPILLARIOSIS OF COMPANION ANIMALS AS EMERGING DISEASES

Veronesi F.*[1], Morganti G.[1]

[1]Department of Veterinary Medicine of Perugia, Italy

Introduction

Eucoleus aerophilus (syn. Capillaria aerophila) and Eucoleus boehmi (syn. Capillaria boehmi), two extra-intestinal trichurids affecting the airways of companion animals, have recently become the focus of increased attention from the Scientific Community as demonstrated by a number of recent studies. Several gaps on their morpho-biology, epidemiology, molecular diagnosis and genetic make-up have been recently filled.

E. boehmi inhabits the nasal cavities and sinuses of wild and domestic canids causing "nasal capillariosis", whereas E. aerophilus parasitizes the trachea, bronchi and bronchioles of domestic (e.g. cats and dogs) and wild mammals (e.g. wolf, fox, marten, etc) causing "lung capillariosis". Also worthy of mention is the zoonotic potential of E. aerophilus and its ability to mimic bronchial carcinoma in human beings (Lalosevic et al., 2008, Am J Trop Med Hyg, 78:14-16). For a long time these two trichurids have been regarded as a single species, but later they have been separated based on the different localization within the hosts and to key morphological features of adults and eggs. However, overlapping life cycle and transmission patterns are hypothesized, although not fully understood yet. A direct transmission was demonstrated but the role of earthworms as facultative intermediate or paratenic hosts has been also speculated.

Epidemiology

These diseases are underestimated and their distribution is difficult to assess, although they are widespread as showed by an increasing trend in the number of clinical cases in the last decade (Traversa et al., 2010, Parasites & Vectors, 3:62). Different factors might have a key role in this possible emergence, e.g. animal movement in absence of health controls and the increased number of synanthropic wildlife populations that may act as bridging hosts for companion animals. In Europe, E. aerophilus is commonly found in wildlife, but recently the nematode has also been identified in dogs in Germany (0.2%), Czech Republic (0.6%) and Spain (1.3%), and in cats in Romania (3.1%) (Traversa et al., 2009, Res Vet Sci, 87:270-272). In Italy data concerning infection by E. aerophilus is almost only limited to the finding of the parasite during studies on the helminthofauna of necropsied foxes and stone martens, however recently the presence of the parasite was reported in a survey (Traversa et al., 2009, Res Vet Sci, 87:270-272) showing an infection rate of 2.8% in canine and 5.5% in feline populations. The infection by E. boehmi is considered occasional and limited case reports or small case series have been described in the temperate regions of North America and Europe. Epidemiological information on nasal eucoleosis in dogs from Italy is scarce and almost supported only by copromicroscopic investigations (Di Cesare et al., 2012, Parasites & Vectors, 5:128; Magi et al., 2012, Parasite, 19:433-435). However, in a recent trial a prevalence rate of 6.6%, based on copromicroscopical analysis confirmed by rhinoscopy or species-specific PCR-coupled sequencing assays was recorded (Veronesi et al., 2013, Vet Parasitol, 200:133-138).

Clinical presentation

The majority of animals harboring E. aerophilus and E. boehmi remain sub clinically infected, although they may display respiratory symptoms with varying degrees of severity. Lung capillariosis is characterized by a chronic bronchitis with different respiratory signs, e.g. bronchovesicular sounds, sneezing, wheezing and chronic dry cough. The severity of symptoms varies in accordance with the parasite burden, host-related factors, e.g. age, immune response, presence of concomitant diseases and bacterial complications (Burgess et al., 2008, Can Vet J, 49, 389-392). Animals infected by E. boehmi may show sneezing, reverse sneezing, catarrhal blood-stained or muco-purulent nasal discharge, and impairment of scenting ability. Furthermore, E. boehmi has recently been recognised as a potential cause of meningoencephalitis in dog (Clark et al, 2013, J Small Anim Pract, 54:99-103).

Diagnosis

The diagnosis of respiratory capillariosis cannot be achieved by clinical examination due to the many other conditions with overlapping clinical pictures, and to the frequent occurrence of sub-clinical infections. A clinical suspicion need to be corroborated by the detection of eggs in stool samples through standard faecal flotation. However, this approach may be hampered by different drawbacks: (i) egg shedding is likely cyclic; (ii) morphological identification of eggs is challenging because their barrel-shape resembles those of other parasitic or pseudoparasitic trichurids; (iii) mixed infection with other trichurids can occur. Therefore, a careful appraisal of key features (e.g. shell wall surface pattern, size and plug morphology) of the eggs is required. Recently, PCR protocols specific for genetic markers within the mitochondrial DNA (cox1) and the small rRNA subunit (18S rRNA) of Eucoleus spp. have been developed to overcome the inherent limitations of conventional methods (Di Cesare et al., 2012, J Clin Microbiol, 50:1958-63; Guardone et al, 2013, Vet Parasitol, 197:364-369), but their use is currently limited to research studies. Collateral diagnostic tools as endoscopy (e.g. rhinoscopy and bronchoscopy) that allow the direct visualization of the parasites in situ as well as the collection of biological samples through biopsy or nasal/bronchial flushing, can be performed. Endoscopy represents a powerful and specific diagnostic approach but it is expensive and time consuming and may also show some limitations due to the inaccessible locations sometimes adopted by the worms or to the presence of abundant mucoid material which impairs an accurate visualization of the parasites.

Treatment

To date there are no drugs licensed for treating respiratory capillariosis of dogs and cats. The few therapeutic options available are based on benzimidazoles (BZs, febendazole), imidazothiazole derivatives (IMD, levamisole) and macrocyclic lactones (MLs, abamectin, ivermectin, milbemycin oxime) but are referred to single or few clinical cases and/or on off label protocols. Furthermore they sometimes show controversial degrees of efficacy thus making it difficult to infer any conclusion on the efficacy of these molecules.

However the most promising results were obtained by the use of moxidectin, in fact two recent field trials conducted on 36 E. aerophilus-infected cats (Traversa et al, 2012, Parasitol Res, 1793-1798) and on 16 dogs naturally infected by E. boehmi (Veronesi et al., 2013, Vet Parasitol, 200:133-138) respectively, proved that a spot-on formulation of imidacloprid /moxidectin (Advocate®, Bayer Animal Health) is safe and effective in the treatment of lung and nasal capillariosis.

S3.3
CLINICAL FEATURES OF CARDIOPULMONARY PARASITIC DISEASES IN DOGS AND CATS

Venco L.*[1]

[1]Veterinary Hospital Città di Pavia, Italy

The clinic feature of Heartworm disease in dogs and cats is well described. The disease in dogs leads to chronic pulmonary hypertension and congestive right heart failure while in cats (susceptible host but less tolerant) to an unpredictable course. From absence of symptoms to sudden death.
Less is described for other cardiopulmonary parasites.
Cardiopulmonary parasites are a frequent occurrence in dogs and cats (Traversa et al., 2010, Parasites & Vectors, 3:62). Diagnosis is based on microscopic examinations with concentration methods (Baermann technique). Symptoms are those typical for pulmonary diseases as dyspnoea and cough. Their severity is related to the immune status. The most severe forms are frequent in young or immunocompromised subjects. Haematology is characterized by eosinophilia, although not always present. Depending on the life cycle they may be divided in parasites with “direct” (C. aerophila or F.hirthi) and “indirect cycle” (A. vasorum in dogs and A. abstrusus). Infestation only occurs with the ingestion of an intermediate or paratenic host and then assumes a predatory attitude or curious attitude. For frequency and severity of symptoms in Italy special attention should be paid to A. abstrusus and A. vasorum.
Both have an indirect cycle with a host represented by snails ingested directly or via paratenic host (lizards, rodents, birds) as vectors of weed forms.
In cats, the respiratory parasite A. abstrusus is more important. It is located in the bronchioles as adult and larvae of the first generation are emitted into the environment with expectorate or swallowed and then excreted in the faeces (Biglietti et al., 2010, Veterinaria, 24: 1).The most frequently affected and/or who experience more severe symptoms are kitten or young cats. Typical symptoms are cough and dyspnoea, often accompanied by anorexia and almost always by eosinophilic leukocytosis. The radiographic feature is characterized by alterations as bronchial broncho-alveolar pattern and spotted areas of hyper inflated lung fields due to bronchial obstruction. Diagnosis is based on copromicroscopic examination. The Baermann technique has greater sensitivity, although it is common even to find viable larvae in fresh smears. Not rare the associated detection of other parasites with indirect cycle (Spirometra spp.).
Treatment includes symptomatic drugs: corticosteroids, antibiotics and oxygen therapy. For the elimination of the parasite is used Fenbendazole, oral route at the dose of 50 mg/kg s.i.d. for 15 days. The spot-on administration of Imidacloprid and Moxidectin has demonstrated high efficacy. The selamectin instead incomplete efficacy in the field, especially in severe forms.
A. vasorum is a parasite of the dog with the life cycle similar to that of A. abstrusus. The adult parasite is localized in the pulmonary arteries and eliminates the first generation larvae through the lung parenchyma. Larvae reach the bronchial tubes and, after swallowing, the environment by the faeces.
The clinical pictures are often serious with lung parenchymal abnormalities associated to gastrointestinal disorders and massive form of disseminated intravascular coagulation leading to death. (Hare et al., 2011, 109:505-8). Eosinophilic leukocytosis is often present. The best method for the diagnosis is the faecal examination with Baermann technique, even if first generation larvae can float in solutions of zinc sulphate. Recently an antigenic serological test is available on the market. This test showed sensitivity similar to that of faecal the examination and therefore may be useful for screening many subjects for his or as alternative to faecal examination.
Since both techniques are not normally used for routine, the presence of this parasite is undoubtedly underestimated in Italy despite the epidemiology studies have shown his presence with high values of prevalence (20%) in susceptible individuals. The elimination of the parasite can be obtained with the administration in “spot on” formulation of Moxidectin and Imidacloprid, and oral administration of milbemycin oxime at a dose of 0.5 mg/kg once a week for 4 weeks or Fenbendazole (25 mg kg orally once a day for 20 consecutive days).
Less frequently found parasite in cats and dogs is C. aerophila that is characterized by the opercolate eggs similar to those of T. vulpis with which (in the dog) is frequently confused (the eggs of Capillaria spp are asymmetric to their poles). This parasite has a direct cycle (although earthworms can act as paratenic host). The symptoms are mild and characterized by bronchial cough for the location of the parasite. The therapy is based on the administration of Fenbendazole (25-50 mg/kg per day orally for 2 weeks).
F. hirthi F. and F. osleri are parasites of the dog with direct transmission and tracheobronchial localization in the adult stage. Young dogs and puppies (transmission by milk in the litter) are more affected with symptoms such as cough and radiographs that may show granulomatous lesions of the trachea and main bronchi (“scallop shell “lesions). Diagnosis is based on founding the first generation larvae in faecal examination by flotation with zinc sulphate solutions (as well as on endoscopic examination). The therapy is based on the administration of Fenbendazole at a dose of 50 mg/kg day, orally for 2 weeks.
Crenosoma vulpis, often found in foxes, a nematode with indirect cycle and intermediate hosts (slugs and snails) can also infest the dog that may be asymptomatic or showing mild respiratory symptoms. The larvae need to be distinguished from those of A. vasorum. The therapy is based on the Fenbenzole, 50 mg / kg orally s.i.d. for 3 consecutive days.
There are only anecdotal reports in Europe but flukes of the genus Paragonimus, parasites of dogs and cats and even human it should be mentioned. Paragonimus causes cysts and sub pleural boils through which the adult eliminates embryonated eggs with expectorate or faeces. The cycle involves two intermediate hosts, a snail and a crustacean in which metacecaria develops. The parasites can be fatal, for the onset of acute pneumothorax. The therapy is based on high-dosage of Praziquantel (30 mg/kg) orally for 3 consecutive days.
Cardiopulmonary parasites in cats and dogs are numerous and cause of underestimated diseases. We need to take them into account, because for some of them the lack of a diagnosis can lead those affected to death or irreversible respiratory failure.

S3.4
NEW INSIGHTS ON THE BIOLOGY, EPIDEMIOLOGY AND DIAGNOSIS OF TROGLOSTRONGYLUS SPP. INFECTION IN CATS

Giannelli A.*[1], Brianti E.[2], Ramos R.A.N.[1], Giannetto S.[2], Dantas Torres F.[1][3], Otranto D.[1]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Italy, [2]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina, Polo Universitario Annunziata, Italy, [3]Department of Immunology, Aggeu Magalhães Research Institute, Brazil

The superfamily Metastrongyloidea comprises a number of roundworms, infecting the cardio-pulmonary system of vertebrate animals and primarily transmitted by gastropod molluscs. Among species infecting felids, Aelurostrongylus abstrusus (Strongylida, Angiostrongylidae) is commonly regarded as the most important cat lungworm, whereas other nematodes ranked into the genus Troglostrongylus (Strongylida, Crenosomatidae) have been for long time affiliated to wild felids only (Brianti et al., 2014, Vet. Parasitology, doi: 10.1016/j.vetpar.2014.01.019). Over the past few years, the increase of reports of Troglostrongylus spp. infections in domestic cats is probably a consequence of the renewed scientific interest towards these species. However, the identity of Troglostrongylus brevior and Troglostrongylus subcrenatus is still largely disputed, as well as their distribution among feline populations and their morphological differentiation from A. abstrusus (Traversa and Di Cesare, 2013, Trends Parasitology, 29:423-430; Otranto et al., 2013, Trends Parasitology, 29:517-518). The simultaneous occurrence of T. brevior and A. abstrusus has challenged the proper identification of these metastrongyloids, their diagnosis and therapy (Brianti et al., 2014, ibidem). Indeed, T. brevior and A. abstrusus share the same ecological niches, represented by terrestrial snails (e.g., Helix aspersa) (Giannelli et al., 2013, Parasitology, 5:1-7), and display a similar biology in their definitive felid hosts (Brianti et al., 2014, ibidem). However, while the species-specific identification of these lungworms may be tangled by the morphological similarities of their first-stage larvae (Figure 1), adult worms are either morphologically distinct and reside in specific respiratory tracts. Indeed, A. abstrusus affects the lung parenchyma, being confined in sub-pleural nodules (Traversa and Di Cesare, 2013, Trends Parasitology, 29:423-430), whereas T. brevior is easily detected during the necropsy in the upper respiratory airways (i.e., from trachea to the bronchi, Figure 2) (Brianti et al., 2014, ibidem). The pathogenic role of Troglostrongylus lungworms is still debated, probably due to the scant information available on the respiratory alterations in infected cats. Based on the current literature, feline troglostrongylosis may result in fatal outcomes especially in young animals, which usually display a severe respiratory syndrome, featured by dyspnoea, increasing respiratory rate, coughing, sneezing, often leading to respiratory failure (Brianti et al., 2014, Vet. Parasitology). Acute bronchitis is a common clinical sign during T. brevior infections, as well as the presence of an abundant eosinophilic infiltrate in lungs and catarrh draining from the trachea and bronchial tree. Nonetheless, during mixed infections by T. brevior and A. abstrusus, the histological panel may be even complicated by interstitial pneumonia (Traversa et al., 2014, Vet. Parasitology, 201: 158-162).
Despite the life-threatening potential of Troglostrongylus nematodes, the definitive understanding of their biology and ecology is still undefined. Accordingly, further studies are required to address several issues concerning the transmission route, distribution, diagnosis and treatment of feline troglostrongylosis. In particular, the recent finding of T. brevior infection in not-weaned kittens may be indicative of a direct transmission of this nematode, from the queen to kittens (Brianti et al., 2013, Parasitology, 140: 821-824). Infective larval stages of the parasite might cross the placenta and/or be transmitted via lactation. A similar pattern has been reported for other metastrongyloids infecting carnivores, such as Andersonstrongylus captivensis, Filaroides hirthi, and Oslerus osleri, which infect their definitive hosts through the first stage larvae (Brianti et al., 2013, Parasitology, 140: 821-824). Furthermore, additional diagnostic tools differentiating first-stage larvae of feline metastrongyloids are urgently required, in order to expedite the proper lungworm identification. From this viewpoint, molecular tools may be useful to detect the nematode DNA, also in case of co-infections (Annoscia et al., 2014, Vet. Parasitology, 199:172-178). Remarkably, neither pharmaceutical product nor therapeutic schedule is currently available for the prevention and treatment of Troglostrongylus infection in cats (Brianti et al., 2014, ibidem).
In conclusion, a rushed view may lead to considerer Troglostrongylus infestations as an anecdotal or unusual parasitosis. However, it should be considered that since mollusc-transmitted nematodes are at an increase, metastrongyloids originally associated to wild felids may also spread in association to climate changes (Patz et al., 2000, Int. J. Parasitology, 30:1395-1405.), thus finding new permissive hosts in domestic cats. In any case, the existence of Troglostrongylus feline lungworms cannot be ignored any more.

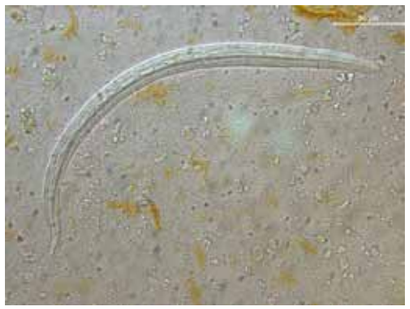


Figure1

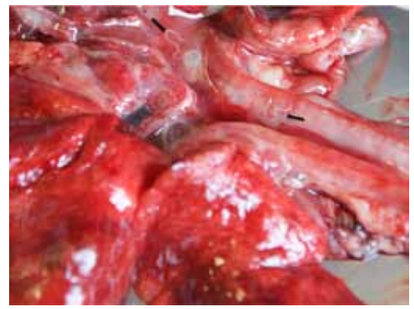


Figure2

Keywords: Troglostrongylus spp.; feline lungworms.

S3.5
ANIMAL DIROFILARIOSIS IN THE IBERIAN PENINSULA

Simón F.*[1], Simón L.[1], Morchón R.[1], González-Miguel J.[1]

[1]*Laboratory of Parasitology, Faculty of Pharmacy, University of Salamanca, Spain*

The dirofilarosis is a zoonotic disease caused by Dirofilaria species and transmitted by culicid mosquitoes of the genera Culex, Aedes and Anopheles, affecting canines, felines and humans all over the world. As a consequence, climate and its variations influence the distribution and incidence of the disease (Simón et al., 2012, Clin. Microbiology Rev., 25: 507-544). The Iberian Peninsula has a temperate and dry climate except in Northern and Western Atlantic regions where the climate is oceanic, while islands present an oceanic or semitropical dry climate, coming humidity from the dominant trade winds. Moreover, the existence of extensive irrigated crop areas in the continental part of both Portugal and Spain provides very favorable habitats for mosquito breeding which, "a priori", represents a risk factor for dirofilariosis transmission (Simón et al., 2014, Vet. Parasitology, 200: 257-264). Information on the epidemiology of animal dirofilariosis in the Iberian Peninsula is partial and mainly related to canine populations, being the data on feline dirofilariosis very limited. The objective of this review is to provide an updated picture of the distribution and prevalence of canine and feline dirofilariosis in Spain and Portugal.

A retrospective review of the data previously published on the distribution and prevalence of canine and feline dirofilariosis in Spain and Portugal has been performed using both internet data bases (PubMed and ScienceDirect) and local veterinary journals. Additionally, unpublished data from the authors obtained in recent epidemiological surveys were also used.

D. immitis is present in the whole territories of Spain and Portugal (Figure 1A) while, to the present, D. repens has been reported exclusively in regions of the Mediterranean coast (Spain). Recently, the first autochthonous human case of subconjunctival dirofilariosis by D. repens has been reported in Western Spain (unpublished own data) suggesting the presence of this species in canine populations outside the traditional Eastern endemic area.

In Spain there are very few epidemiological studies covering the whole territory or extensive areas and, in general, they show low prevalence of D. immitis in the Spanish canine population (< 4%) (Guerrero et al., 1989, Med. Veterinaria, 6: 217-220; Miró et al., 2013, Parasite & Vectors, 6: 117-125). At provincial level the prevalence of 58.9% from Gran Canaria (Canary Islands) is the highest prevalence recorded in Spain, although current prevalence is 19.2% as a consequence of the correct application of preventive therapy in the island (Montoya et al., 2011, Vet. Parasitology, 176:291-294). There is high prevalence also in Ibiza (Balearic Islands) and some Southern peninsular provinces (up to 39%), while in central and Northern provinces low/moderate prevalence (0.8-5%) has been observed (Montoya et al., 2007, in Dirofilaria immitis and D. repens in dog and cat and human infections. Rolando Editore, Naples, Italy, 175-180; Morchón et al., 2009, Epidemiology & Infection, 21: 1-4). Local studies performed in phisiographically and climatologically well defined areas associate high prevalence to irrigated crops and natural wetlands like river deltas (Figure 1B), while in neighboring dry areas dirofilariosis does not exist or is presents at low prevalence. Regarding wild reservoirs, D. immitis has been sporadically reported in wolves and foxes in different provinces. In an epidemiological survey conducted in Zaragoza (Northern Spain) prevalence of 32%, 1.7% and 0% was observed in foxes living in irrigated crops, dry lands and mountains, respectively (Gortázar et al., 1994, J. Wildl. Diseases, 30:545-547). Information is very limited regarding D. repens. Low prevalence (1%) has been observed in dogs from veterinary clinics of two Southern provinces (Rojo-Vázquez et al., 1990, Med. Veterinaria, 7: 297-305), while the prevalence in kennel dogs is much higher (>37%) (Cancrini et al., 2000, Veterinary Parasitology, 92: 81-86).

In Portugal, D. immitis is present also in the whole territory, having been reported the highest prevalence in the island of Madeira (30%). Moderate/high prevalence exist in some Atlantic coastal districts (6.7-27.3%) (Figure 1A) (Genchi, 2003, Helminthological Colloquium, Wien, Abstracts, 6; Vieira et al., 2014, Parasite, 21:5; own unpublished data).

Few epidemiological surveys have been conducted in cats. They revealed the presence of feline dirofilariosis in some areas of Spain (Gran Canaria Island and Barcelona province) and in the Northern half of Portugal (Montoya et al., 2011, Vet. Parasitology, 176:291-294; own unpublished data) with moderate/high seroprevalence, frequently higher than canine prevalence in the same areas.

Available data show a wider geographical distribution of D. immitis than D. repens in the Spanish and Portuguese canine populations. However, the recent report of the first autochthonous case of human subconjunctival dirofilariosis caused by D. repens in the Spanish Western side, near the Portuguese border, suggests that this species is undergoing a geographical spreading as in other European areas. Thus its status must be re-evaluated. The variety of local climatic, physiographic and anthropogenic characteristics could be the cause of the focal distribution of dirofilariosis in the Iberian Peninsula, changing prevalence among neighboring areas. Therefore, focused studies in well defined areas could be more suitable than studies covering all the territory or extensive areas, in which discrimination between areas with different environmental characteristics is more difficult.

Seroprevalence observed in cats, must be carefully analyzed. The usual absence of chemoprophylaxis in cats probably contributes to increasing seroprevalence. Moreover, the detection of anti-Dirofilaria and anti-Wolbachia antibodies, employed as diagnostic technique in these studies, could reveal not only active infections but also antibodies from past or abortive infections. Anyway, they clearly demonstrate the existence of feline Dirofilaria infections in Spain and Portugal.

There are still many areas in Spain and Portugal where the situation of canine and feline dirofilariosis has not been analyzed. Therefore, more epidemiological studies are needed for a fully and correct understand of its distribution and incidence in these two countries.

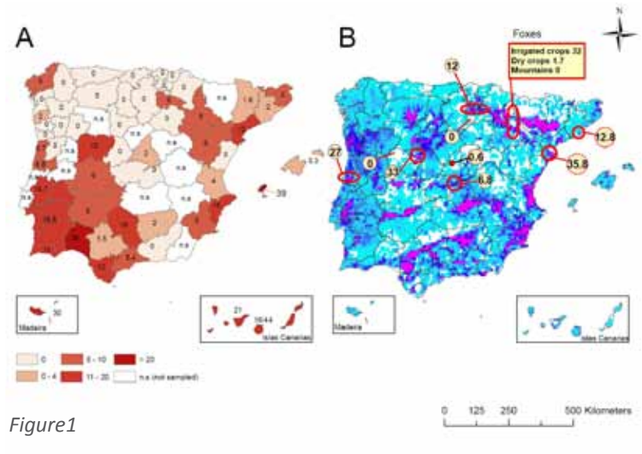


Figure1

Legend of the figure.

Current distribution and prevalence of canine dirofilariosis in Spain and Portugal. (A) Mean prevalence by provinces/districts. (B) Irrigated and wetlands areas of Spain and Portugal. Prevalence reported in some of these areas. Differences between prevalence of these selected areas and mean prevalence of the provinces/districts in which they can be seen.

NOTES

NOTES

SIMPOSIO 4

ZOONOSI DA ALIMENTI: UN APPROCCIO GLOBALE E NUOVE PROSPETTIVE

S4.1
TOXOPLASMOSIS IN A CHANGING WORLD: THE SOUTH-EAST EUROPEAN PERSPECTIVE

Djurković-Djaković O.*[1]

[1]National Reference Laboratory for Toxoplasmosis, Institute for Medical Research, University of Belgrade

An equal opportunity pathogen, the ubiquitous protozoan Toxoplasma gondii is the most successful parasite on Earth, infecting a broad range of mammals and birds which all act as intermediate hosts, whereas felids are the only definitive hosts. In intermediate hosts, following a brief acute stage characterized by circulating tachyzoites, parasites convert to tissue cysts localized mainly in the muscular and neural tissues. In felids, sexual reproduction of the parasite results in the production of oocysts shed with feces, thereby contaminating the environment. Infection with either form is mainly peroral, making toxoplasmosis an important foodborne infection. The disease burden of toxoplasmosis, estimated at 620 DALYs/year (Havelaar et al., 2007, Clin. Infect. Dis., 44:1467-74), is the greatest of all parasitic infections, similar to that of the major foodborne diseases, salmonellosis (670 DALYs/year) and campylobacteriosis (Kijlstra and Jongert, 2009, Trends Parasitol., 25:18-22). Consumption of meat containing tissue cysts and ingestion of oocysts from water and soil (through gardening and farming) have been shown to be major routes of human infection (Bobić et al., 1998, Eur. J Epidemiol., 14:605-10; Cook et al., 2000, Br. J. Med., 321:142-7).
Infection is generally benign, but may have serious consequences in the developing fetus and in immunosuppressed individuals. In animals, T. gondii infection can cause symptoms ranging from mild to serious infectious disease to reproductive issues, and even disseminated infection and death (Dubey, 2010, CRC Press, Boca Raton, FL).
For a parasite whose life cycle involves sexual reproduction, the T. gondii population structure is unexpectedly oligoclonal. Initially described as clonal, with three major lineages designated as types I, II and III, these are, with an emphasis on type II, only predominant in Europe and North America (Howe and Sibley, 1995, J. Infect. Dis., 172:1561-6; Ajzenberg et al., 2002, J. Infect. Dis., 186:684-9), whereas in South America and Africa, a higher frequency of non-clonal, highly polymorphic strains referred to as atypical has been revealed (Khan et al., 2006, Emerg. Infect. Dis., 12:942-9; Demar et al., 2012, Clin. Microbiol. Infect., 18:221-31). Moreover, specific African non-clonal genotypes, termed Africa 1, 2 and 3, have been identified (Ajzenberg et al., 2004, Int. J. Parasitol., 34:1185-96; Mercier et al., 2010, PLoS Negl. Trop. Dis.; 4:e876). But the picture is currently being complicated in the Western World as well, since a fourth clonal lineage (autochtonous haplogroup 12) has recently been described in North America (Khan et al., 2011, Int. J. Parasitol., 41:645-55).
Strain genotype has been associated with clinical severity of human toxoplasmosis. Type II strains have been shown to be most prevalent in congenital infection and AIDS patients in North America and Europe (Ajzenberg et al., 2002, J. Infect. Dis., 186:684-9; Ajzenberg et al., 2009, J. Infect. Dis., 199:1155-67). Atypical strains have been associated with severe toxoplasmosis in immunocompetent patients (Carme et al., 2002, J. Clin. Microbiol., 40:4037-44; Delhaes et al., 2010, Prenat. Diagn., 30:902-5), particularly in South America where they correlate with ocular toxoplasmosis (Khan et al., 2006, Emerg. Infect. Dis., 12:942-9), and in the setting of immunosuppression (Štajner et al., 2013, J. Clin. Microbiol., 51:2686-90). In animals, emerging information indicates severe histopathological lesions in sheep abortions caused by T. gondii of an atypical genotype (Edwards and Dubey, 2013, Vet. Parasitol., 192:129-36), but also the association of type II strains with fatal toxoplasmosis in wildlife (Jokelainen et al., 2011, J. Wildl. Dis., 47:154-63).
Clinically, the most important T. gondii-induced entity is congenital toxoplasmosis, which develops after vertical transmission of the parasite from the mother to the fetus. The conventional concept that transplacental transmission only occurs during primary infection in pregnancy has recently been challenged by reports of infected offspring of mothers traditionally considered immunized, where re-infection with a strain of a different genotype has been suggested (Elbez-Rubinstein et al., 2009, J. Infect. Dis., 199:280-5). Increased importation of fresh meats and produce, as well as increasing migration from and travel to areas with a high T. gondii genetic diversity, may increase the odds of such events.
The pattern of infection has been changing throughout Europe in the last decades, SE Europe included. A recent systematic review of T. gondii infection trends in SE Europe showed that the prevalence is currently below 50% across the region, after continuously decreasing in Slovenia, Serbia and Greece over the last 30 years, and in Montenegro and FYRoM during the past decade (Bobić et al., 2011, Wien. Klin. Wochenschr., 123, suppl.1:2-6). The decrease appears more pronounced going south. Seasonality of infection, with significantly more acute infections in the winter than in the summer, was observed in Slovenia and Serbia (Logar et al., 2005, Clin. Microbiol. Infect., 11:852-5; Bobić et al., 2009, Vector Borne Zoon. Dis., 10:465-9). Despite a common decreasing trend, different transmission risk factors seem predominant; while contact with cats was considered important in Slovenia, consumption of undercooked meat was shown to be the leading risk factor in Serbia (Bobić et al., 2007, Parassitologia, 49:227-30) and Albania (Maggi et al., 2009, New Microbiol., 32:89-92), and contact with soil in FYRoM (Cvetković et al., 2010, Parasite, 17:183-6) and in Greece (Diza et al., 2005, Clin Microbiol Infect., 11:719-23).
There is little data on the genetic structure of the T. gondii population in SE Europe. However, the predominance of type III human isolates in the islands of Crete and Cyprus (Messaritakis et al., 2008, Am. J. Trop. Med. Hyg., 79:205-9), and the presence of all clonal types along with an atypical one in humans, sheep and pigeons in Serbia (Marković et al., 2014, Comp. Immunol. Microbiol. Infect. Dis., doi: 10.1016/j.cimid.2014.03.001), suggest it may be more diverse than in the rest of Europe. The higher genetic diversity in this region reflects local environmental contamination but may also indicate phylogenetic ties among Asian, African and European T. gondii populations.
The changing pattern of infection, particularly the changing paradigm of congenital toxoplasmosis, along with the increase in global migrations and global climate changes that may favour parasite persistence, all call for novel strategies for the prevention of toxoplasmosis, preferably at the wildlife/livestock-human interface.

Keywords:
toxoplasmosis, South-East Europe, population structure

S4.2
SOLVED AND UNSOLVED PROBLEMS IN THE DIAGNOSIS OF TOXOPLASMOSIS

Meroni V.*[1], Meroni V.[2], Genco F.[2]

[1]Dipartimento di Clinica Medica e Medicina Interna Università degli Studi di Pavia, Pavia, [2]SC Microbiologia e Virologia Fondazione IRCCS Policlinico San Matteo, Pavia

Toxoplasma gondii is the most successful parasite in the world having a worldwide distribution, being capable of infecting all warm-blooded animals, and being highly transmissible as demonstrated by the fact that ¼ of the human population is chronically infected (10- 80% prevalence).
In Europe and North America it has been demonstrated, by means of interviews and questionnaires, that the main source of infection in humans are the cysts present in different type of undercooked meat, even if it was impossible to explain the route of infection in 14-49% of the cases (Cook et al., 2000, BMJ, 321:142-7; Jeffrey et al., 2009, Clin Infect Dis., 49:878-84). On the other hand in Brazil the main risk factors are: contact with soil and cats, low educational level, home-made water ice, residence in rural areas, vegetables washed with untreated water, all related to oocyst ingestion (Sroka et al., 2010, Am J Trop Med Hyg., 83:528-33). Therefore, even if the possibility of infection due to transplantation, transfusion and vertical transmission by an infected mother exists, the principal route of infection is the oral route through the ingestion of sporulated oocysts excreted in the soil and in the water with the cat faeces at the end of the sexual cycle, or through the ingestion of cysts present in the muscular and nervous tissues of infected farm animals, and rarely through milk (mainly goat milk) and eggs (Robert- Gangneux et al., 2012, Clin Microbiol Rev, 25:264-83).
The severity of infections depends on some characteristic of the host (weakness or immaturity of the immune response, susceptibility or resistance factors, HLA) but also on parasite characteristics (strain, inoculum size, parasitic stage) (Maubon et al., 2008, Trend Parasitol, 24:299-303). Experimental infections of mice, rats, and pigs with oocysts and cysts have assessed that infection with oocysts leads to an earlier death of experimental animals. An important factor that has effects on the severity of the diseases is the virulence of the Toxoplasma strain. In France almost all the strains (95%) isolated from domestic and wild animals and from congenitally infected babies are Type II (Ajzemberg et al., 2002, J Infect Dis., 186:684-9; Howe et al., 1995, J Infect Dis., 172: 1561-6). Only few (5%) are type III, confirming the low genetic diversity of Toxoplasma gondii strain in Europe and North America: 19% type I, 54% type II, 27% type III (Howe et al., 1995, J Infect Dis., 172: 1561-6; Khan et al., 2011, Int J Parasitol., 41:645-55). In South America strains are different from type I, II, and III, with high genetic diversity: atypical strains that derives from wild animals and are responsible for more severe infections. For example ocular lesion in congenital toxoplasmosis in Brazil are more frequent, more recurrent, more multiple, larger than in Europe (Gilbert et al., 2008, PLoS Negl Trop Dis., 2(8):e277). Furthermore, it has been demonstrated the possibility of reinfection with atypical strain in a patient with previous immunity to the European strain (Elbez Rubistein et al., 2009, J Infect Dis. 199:280-5). All these information may help in defining optimal preventive measures against toxoplasma infection in female at reproductive age and in immunocompromised patients.
This parasite, could cause 3 different diseases:

- Acquired toxoplasmosis in normoergic patients, which is mainly subclinical and only rarely could cause chorioretinitis;
- Congenital toxoplasmosis in foetus and newborns, transmitted by an infected mother, which could be subclinical but can also cause foetal loss, still birth, cerebral calcifications, hydrocephalus, and chorioretinitis and late ocular sequelae;
- Toxoplasmosis in immunocompromised patients, characterized by neurological, ocular, pulmonary localizations that could be life threatening.

However, the infection is usually asymptomatic and, when present, symptoms are mainly non-specific; therefore, diagnosis relies on laboratory tests. In the last years diagnostic tools have greatly improved, but some problems are still present.
In pregnant women with anti-toxoplasma negative IgG and positive IgM it is necessary to discriminate between false positive IgM and real seroconversion in order to treat the mother as soon as possible and, if necessary, counsel for prenatal diagnosis. In these cases IgM ISAGA test and IgG IgM Western Blot may be very useful.
When specific anti-toxoplasma IgG and IgM antibodies are present, the IgG Avidity test make possible to approximately date the infection. However, the therapy could delay avidity maturation, producing a not good agreement among the results of different commercial kits and the avidity maturation.
As far as prenatal diagnosis is concerned, an improvement came from the change of the technique employed (real time PCR instead of nested PCR), of the target Gene (ReP 529 instead of B1), which allowed us to reach a sensitivity of 92% and a specificity of 100% (Wallon et al., 2010, Obstet Gynecol., 115:727-33).
In newborns the traditional tests, even the more sensitive like IgM ISAGA, at birth allowed us to diagnose asymptomatic infection only in 70% of tested babies whose mothers were treated during pregnancy. Qualitative analysis of aspecific IgG and IgM now available by means of comparative IgG and IgM Western Blot has increased the sensitivity of the diagnosis up to 94% in the first 3 months of life. Moreover, the possibility of a serological follow-up at one year, monitoring IgG decrease in newborn saliva seems to be a not painful alternative to blood sampling.
Recently, a home-made miniaturized IFN- Gamma Release Assay opened a new complementary approach for the early diagnosis of congenital toxoplasmosis, useful also to confirm the infection both in IgG seronegative IgM seropositive pregnant women and in immunodeprimed patients (Robert-Gangneux et al., 2012, Clinical Microbiology Reviews, 25:264-96).
The need for a monthly serological screening of seronegative pregnant women, in Italy recommended by “Linee Guida sulla gravidanza fisiologica” (ISS 2010/2011), is underlined by Wallon et al. (Clin Infect Dis, 2013, 56: 1223-319) in a recent paper whose conclusion is that monthly prenatal screening decreases transmission rate and improves clinical outcome at the age of 3 years.

S4.3
ANISAKIS AND ALLERGY: A PHENOMENON OF GLOBALIZATION?

Daschner A.*[1]

[1]Servicio de Alergia. Instituto de Investigación Sanitaria- Hospital Universitario de la Princesa, Madrid, Spain.

Anisakis simplex is an ubiquitous fish-parasite, which is not adapted to humans. Gastric, intestinal or extra-intestinal diseases are well-known clinical syndromes and were first described in countries with raw fish-eating habits, such as the Netherlands or Japan. Whereas the third-stage larva it is not able to survive in the human host more than a few hours or days, it is able to produce not only acute but also chronic abdominal manifestations due to an exaggerated immune response.
The allergenicity of its excretory-secretory proteins has gained attention for the last 2o years and in the last decade Anisakis allergy has been a focus in international food security agencies as to the possible allergenic potential and risk for the consumer of fishery products and has been included as an aetiologic factor in the recent guidelines for the assessment of anaphylaxis. Acute allergic symptoms such as urticaria, angioedema or anaphylaxis are produced when the live larvae of Anisakis parasitizes the gastro-intestinal tract causing gastro-allergic anisakiasis (GAA). Chronic urticaria has also been associated with previous parasitic episodes.
It has to be emphasised that Anisakis (simplex & pegreffi) is the only known parasite in fishery products implicated in allergic reactions. It is clear that a marked increase in the reported prevalence of Anisakis allergy is not only due to an elevated awareness of clinicians as it parallels the prevalence of other Anisakis induced pathologies throughout the world. Thus Anisakiasis can be considered an emergent zoonosis.
There are several factors which are of interest when assessing Anisakis associated allergy and its distribution in different geographic areas: the presence of distinct Anisakidae species in a wide array of marketed fish across different world-wide regions, the association of Anisakis induced disease with fish-eating habits, fishing practice as well as probably genetic factors, all under study in different interdisciplinary fields comprising medicine, parasitology, marine biology, veterinary science, food science technology, etc. The allergenic properties of this peculiar nematode seem to be one of the major driving forces in such diverse research fields.
Globalization denotes to processes of international integration arising from the interchange of products, ideas or other aspects of culture. Likewise, disease and health or food preferences are also influenced by factors such as elevated transportation, trade or agriculture methods. Otherwise, diseases of civilization are also beginning to affect affluent countries, as are allergic diseases achieving high prevalence rates.
Knowing the possible health hazards of Anisakis, factors influencing the whole food chain are investigated. Mechanisms affecting the occurrence and distribution of Anisakis in fishing areas remain mainly unknown. Fishing practice during capture and handling influences the presence of Anisakis in different parts of marketed fish, but has also been proposed to contribute to ecologic disturbances in fishing grounds. There is a high research investment in determining prevalence and intensity of Anisakis infestation of fish in different geographical areas as well as in assessing risk of Anisakis in aquaculture fishery products. Finally, adequate preparation of potentially infected fish by the consumer is of importance for prevention of Anisakis induced disease as well as allergy.
However, within the fish parasite community, interest in Anisakis holds a special position due to the following considerations: Anisakis has a wide range of hosts throughout its life cycle. The third-stage larva causing disease in humans is found in a vast array of ocean-dwelling paratenic hosts. As humans are only accidental hosts, parasitism is of very short duration. Interestingly, Anisakis allergy should not be classified within food allergy, which is generally associated with atopic disease, but has been interpreted as an exaggerated immune phenomenon against a helminth parasite and epidemiologic studies do not show a typical atopy-associated age pattern distribution. Conversely, the main risk factor for Anisakis allergy has been shown to be raw fish-eating habits much more important than overall fish consumption or atopy status.
In this sense, Anisakis allergy has predominantly been reported from countries or regions with traditional raw fish eating habits, such as Spain and Italy, but as an example of globalization, sushi consumption, a classic responsible dish for the high prevalence of Anisakiasis in Japan, is increasing in Western countries and poses a universal emerging health hazard if not properly prepared.
Interestingly, there is a great variance in the reported description of Anisakiasis phenotypes, i.e. only gastrointestinal symptoms versus allergic or gastro-allergic symptoms induced by ingestion of live larvae of Anisakis. Thus, allergic symptoms account for less than 10% of cases in gastric Anisakiasis in Japanese reports, whereas the prevalence of gastro-allergic Anisakiasis episodes in Spain is by far higher than clinically evident gastrointestinal episodes without allergy. This gives rise to research comprising both genetic human factors and genetic differences in Anisakis species, for which molecular tools are now available, accounting for the observed differences. By characterization of Anisakis species it should now be possible to ascribe distinct Anisakis induced disease to different species in the Anisakis s.l. complex.
Several Anisakis allergens have been described in the last years and there is growing interest in searching for those allergens that can differentiate between subclinical sensitization or different Anisakis induced allergic phenotypes. One allergen in study is Anisakis haemoglobin recognized by Anisakis allergic patients but unexpectedly these antibodies do not recognize Ascaris haemoglobin. Analysing together with human and the only known allergenic haemogolobin of midge, bioinformatic studies have shown a different phylogenetic tree based on the fit of epitopic surface compared to the expected classic phylogenetic relationship between ascarid nematodes and puts forward the hypothesis that different evolution in host-parasite interactions have shaped haemoglobin proteins to display different IgE recognition.
Together, whereas prevention of parasitism and allergy by Anisakis can easily be achieved by proper cooking or deep-freezing procedures, there is sufficient rationale for a multi-disciplinary approach in research in the Anisakis field, comprising studies in the complete food chain from harvesting to post-processing by the consumer as well in all aspects of Anisakis biology and induced pathology.

S4.4
ALLERGY AND FOOD-BORNE PARASITES: NOT ONLY ANISAKIS

Bruschi F.*[1], D'elios M.M.[2]

[1]Department of Translational Research, N.T.M.S., Università di Pisa,Pisa, Italy, [2]Department of Experimental and Clnical Medicine, Università degli Studi di Firenze, Firenze, Italy

According to the hygiene hypothesis (Strachan, 1989) a higher exposition to a rural environment as well as to helminth burden, as it occurs in low-income countries, would prevent the development of allergic diseases which conversely have dramatically increased in industrialised Countries where such exposition has almost disappeared. This makes the relation between allergy and zoonoses, particularly the parasitic ones, an interesting issue. The most known relation of allergy to a parasitic zoonosis is certainly that with Anisakis spp. (Daschner et al., 2012, Trends in Parasitol., 28:9-15), but other food-borne parasitic infections may also be involved. Some examples will be discussed.
Toxoplasmosis which is estimated for a burden of 2-8x106 DALYs (disability adjusted life years) at global level (Torgenson and McPherson, 2011, Vet. Parasitol., 182:79–95), seems to underlie a progressive decline, especially in industrialised Countries, according to seroprevalence data (Pinto et al., 2011, Eur. J. Clin. Microbiol. Infect. Dis., 31:1151–6).
Animal stable contact during early life and raw milk consumption predicted Toxoplasma gondii seropositivity in young adulthood (Radon et al., 2004, Clin Exp Allergy, 34:1178–83). The lower concentration of proteolytic enzymes in the digestive tract of infants is responsible for a higher risk of transmission of T. gondii infection via raw farm milk (Tenter et al., 2000, Int. J. Parasitol., 30:1217–58).
The association between T. gondii seropositivity and atopy in a study carried out in the rural part of North Germany was not strong, however, the highest risk factor for developing atopy in rural subjects was represented by regular contact with farm animals. It has to clarify, possibly in studies involving a large number of individuals, whether T. gondii infection represent an intermediate factor in the association between farm contact and atopy (Radon et al., 2004, Clin Exp Allergy, 34:1178–83).
In the Baltic region no difference was observed in seroprevalence for toxoplasmosis between atopic and non-atopic individuals (Janson et al., 2007, J. Allergy Clin. Immunol., 120:673-9). On the contrary, in Brazil, a negative association between atopy and toxoplasmosis was demonstrated for the first time, focusing on the antibody and cytokine responses and suggesting that the immunomodulation induced by the parasite may play a protective role in the development of allergic diseases (Fernandes et al., 2010, Clin. Immunology, 136:148–56).
Helminths, particularly those localized at intestinal level, represent a major public health problem at global level, with an estimation of over 2 billions of infected individuals (Supali et al., 2010, Int. J. Parasitol., 40: 1171–6). Helminth infections are characterised usually by a persistent Th2 polarization, with eosinophilia and increased total IgE levels, and for this reason they represent ideal models to study allergy and other Th2-mediated pathological conditions (Bruschi et al., 2013, J. Parasitol. Res., 1-2). It is now well established that chronic helminth infections protect against allergic diseases through active regulatory processes (Smits et al., 2010, Curr. Allergy Asthma Rep., 10:3-12).
Echinococcosis is estimated for a burden of 2-5x107 DALYs (disability adjusted life years) at global level (Torgenson and McPherson, 2011, cited above). It is a neglected disease caused by the larva stage of Echinococcus granulosus or Echinococcus multilocularis, in most of cases. It still represents a major public health problem not only in developing countries. The infection is characterised by a fine interaction between host response, which is polarised in a Th2 direction, and immune response evasion strategy of the parasite (Siracusano et al., 2012, Endocr. Metab. Immune Disord. Drug Targets, 12:16-23). One of the most important complication of the disease is represented by the damage of tissue cyst wall with the following occurrence of type I hypersensitivity reactions such as urticaria, asthma up to anaphylaxis, especially in hydatidosis patients (Vuitton, 2004, Clin. Rev. Allergy Immunol., 26:93–104). Fatal reactions represent a negligible risk of all kinds of percutaneous treatment (Neumayr et al., 2011, Plos Negl. Trop. Dis., 5:1-7). To our knowledge, no study was carried out aiming to relate atopy to this disease, from an epidemiological point of view.
Trichinellosis is a widely spread infection caused by the parasitic nematode Trichinella spp. in mammals (included man), birds as well as in reptiles. Even in this infection the immune response is skewed to a Th2 type (Bruschi and Chiumiento, 2012, Endocr. Metab. Immune Disord. Drug Targets, 12:4-15).
Data are accumulating which show that infection with this helminth may affect the evolution of experimental models of human diseases such as diabetes, multiple sclerosis but also respiratory allergy or inflammation. These latter processes were ameliorated by infection, in fact the levels of macrophages and eosinophils in the bronchial alveolar lavage fluid in infected animals exposed to allergen were reduced compared to uninfected animal group and airway hyper-responsiveness completely disappeared. Furthermore, infection reduced the IL-5 levels, while stimulating those of IL-10 and TGFβ suggesting the activation of T regulatory cells, which in fact increased in lung draining lymph nodes (Park et al., 2011, Exp. Parasitol,. 127:539-44).
Experimental trichinellosis was used also to study the immune mechanisms of anaphylaxis in response to parasitic antigens. In infected IgE-deficient mice intravenous injection of Trichinella antigen caused a fatal anaphylactic shock in 100% of animals suggesting that this reaction can be also mediated by other isotypes such as, for example IgG1. Mice deficient for this isotype underwent fatal response, as well (Bruschi et al., 1999, Int. Arch. Allergy Appl. Immunol.. 119:291-6).
Studies in experimental trichinellosis, used as a model of Th2 polarization, allowed to show in vivo the down-regulating effect of the Helicobacter pylori neutrophil activating protein (atypical Th1 adjuvant) on the Th2 responses (Del Prete et al., 2008, Journal of Allergy Clin. Immunol., 122:908-13; Chiumiento et al., 2011, Int. J. Immunopathol. Pharmacol., 24:895-903).
Concluding remarks
A better understanding of the fine mechanisms exploited by food-borne parasites to modulate host immune response might be useful in the future to identify molecules capable to quench the allergic responses and hopefully to be tested as pharmaceutical drugs.

S4.5
FRESHWATER FISH-BORNE PARASITIC ZOONOSES IN ITALY

Fioravanti M.L.*[1], Gustinelli A.[1], Caffara M.[1], Menconi V.[1], Prearo M.[2]

[1]Department of Veterinary Medical Sciences, Alma Mater Studiorum University of Bologna, Italy, [2]State Veterinary Institute of Piedmont, Liguria and Valle d'Aosta, Italy

Fish-borne parasitic zoonoses have aroused an increasing interest in Italy over recent years for the changing of culinary habits towards an increasing consumption of raw/undercooked/marinated fish, in association with the expansion of international commercial trade in fishery products, globalization and migration flows across world, all factors which have driven to an increased risk of acquiring fish-borne zoonoses. Several zoonotic helminths can be transmitted through consumption of raw and/or undercooked fish products to humans, since fishes actively participate to biological cycles of heteroxenous parasites that involve man as definitive or accidental host, causing different degrees of pathology. Although currently WHO include, with regard to zoonotic fish helminthes, only Opisthorchiidae (Opisthorchis spp. and Clonorchis spp.) in the priority list of the foodborne parasites "that could produce a substantive burden of disease" (Torgerson et al., 2014, Trends Parasitol., 30: 20-26) it is undeniable that other fish parasites such as Anisakid nematodes and Diphyllobothriid cestodes should be taken into account when considering the zoonotic risks linked to European fish products consumption. In fact, according to EFSA Scientific Opinion on risk assessment of parasites in fishery products (European Food Safety Authority, 2010, EFSA J., 8, 1543) all wild fish should be considered at risk of containing any viable zoonotic parasites if these products are to be eaten raw or almost raw, pointing out the need to carry out epidemiological surveys on presence/diffusion of zoonotic parasites in all fishery grounds. Respect to infections due to Anisakid larvae in marine fish, until recently in Italy a lower attention has been devoted to the study of zoonotic helminths in freshwater fish populations, such as in primis the cestode Diphyllobothrium latum and the digenean Opisthorchis felineus. The recrudescence of cases of human Diphyllobothriasis registered in Switzerland, France and Italy during the last decades (Scholz et al., 2009, Clin.Microbiol.Rev., 22: 146-160) and the recent outbreaks of human Opisthorchiasis observed in Italy, with more than 200 autochthonous human cases reported from 2004 up to 2011 (Pozio et al., 2013, Acta Tropica, 126: 54-62), have determined a renewed interest in epidemiology of infections due to zoonotic parasites in freshwater fish. Concerning Diphyllobothriasis, recent surveys pointed out a widespread presence of D. latum plerocercoids in perch (P. fluviatilis) populations of Como Lake and, to a lesser extent, in perch from Iseo and Maggiore Lakes, confirming this species as elective second intermediate host of D. latum in Italy. The occurrence of D. latum plerocercoid larvae was also found in pike (Esox lucius) and burbot (Lota lota), main paratenic hosts of D. latum, coming from the same sub-alpine lakes, while fish from Garda lake did not show D. latum infections (Gustinelli et al., 2013, Atti XIX Conv. SIPI, Siracusa, 75). With regard to Opisthorchiasis, although several species of cyprinids have been described as suitable second intermediate hosts of O. felineus from different endemic countries, so far in Italy only tench (Tinca tinca) has been found positive from Bolsena, Bracciano and Vico lakes (De Liberato et al., 2011, Vet.Parasitol., 177: 67-71; Gustinelli et al., 2011, 15th Conf. EAFP, Split, HR, 424), always with high prevalence and intensities. The consumption of raw perch ("perch carpaccio") and marinated tench in the positive areas has been identified as the main food source of viable infective stages of D. latum and O. felineus respectively. In the case of O. felineus, it has to be pointed out that the microscopical size of metacercariae make their detection impossible through the visual inspection procedures commonly applied for the purpose of detecting parasites in fishery products, requiring more destructive and time consuming techniques. Anyway, both for Diphyllobothrium plerocercoids and Opisthorchiid metacercariae, morphology is not sufficient for identification at species level needing the support of molecular methods. The analysis of risk factors influencing the epidemiology of these zoonotic helminths indicate the lack of efficient urban wastewater depuration plants around lake shores as the main aspect influencing the maintenance of D. latum cycle, being humans the elective definitive hosts, together with the influence of abiotic parameters such as temperature and oxygen on development of Diphyllobothrium first stages (Wicht et al., 2009, Bull.Eur.Ass.Fish Pathol., 29: 58-65). Concerning O. felineus, the presence of parasitised stray cats, main reservoirs of the infection, and of Bythinia spp. gastropods, suitable first intermediate hosts, seem to be the key factors in the subsistence of this zoonotic helminth described in Italy since XIX century in dogs and cats (Rivolta, 1884, Giorn. Anat. Fisiol. Patol. An., 16: 20–28), although the role of fox and other wild animals as reservoirs and the possible involvement of cyprinids other than tench as second intermediate hosts have to be better studied. Since the maintenance of the complex biological cycles of these zoonotic helminths is influenced by several biotic and abiotic factors, investigating the interplay of these factors may be crucial in next future not only for a better understanding of parasite transmission patterns, but also for predictive studies on presence/maintenance of zoonotic parasites in different fish species in order to map the risks linked to consumption of freshwater fishery products. At this purpose, further surveys aimed at investigate the possible occurrence of larval stages of other zoonotic helminths such as the Opisthorchiid Metorchis sp. and the Heterophyid Metagonimus sp. in Italian freshwater fish populations should be carried out in order to increase the knowledge on epidemiology of fish-borne zoonotic parasites and to prevent outbreaks of new zoonoses. Furthermore, although so far in Italy the presence of zoonotic helminths has been documented only in wild fish populations, extensive epidemiological surveys should be conducted on parasites of public health importance in farmed fish species, as required by EFSA and by current EU food hygiene regulations, in order to confirm as null or negligible the zoonotic risk linked to consumption of national aquaculture products.

S4.6
THE ROLE OF ACCREDITATION FOR FOOD SAFETY

Tramontin S.*[1]

[1]Director of the Dept. of Testing Labs for Food Safety (ACCREDIA)

One of the basic conditions for the free movement of goods within the EU is that all products guarantee a high level of protection of public interests in terms of health and safety in general, in workplaces, and the protection of consumers and of the environment. It is therefore necessary that conformity assessment and market surveillance activities – undertaken in every Member State by the accreditation body and by the Public Administration authorities according to their competences – are based on trust and on the mutual recognition of certificates.
In order to avoid the adoption of different methods and systems by Member States, an EU regulation, issued in 2008, established a framework of rules and principles in the area of accreditation and market vigilance, recognizing accreditation as a suitable tool for demonstrating the technical competence of bodies performing conformity assessments. Regulation (CE) n. 765/2008 is the first legislative document which deals with accreditation.
To ensure equivalence in the level of competence of conformity assessment bodies accredited in the various Member States, a rigorous peer assessment system exists, managed by European co-operation for Accreditation (EA), recognized as the European infrastructure for accreditation in accordance with Regulation (CE) 765/2008. Thanks to the international agreements between accreditation bodies – EA MLA in Europe and IAF MLA and ILAC MRA worldwide – testing and inspection reports, certificates of conformity and calibration, issued by accredited bodies from different countries, enjoy mutual recognition, guaranteeing full validity in the world's main economies.
In 2004, by means of Regulation (CE) n. 882/2004 on official controls, accreditation was identified as a tool for demonstrating the competence of testing laboratories designated to conduct official controls. Article 12 states that "The competent authorities may designate only those laboratories which operate, are assessed and accredited, in conformity with the standard EN ISO/IEC 17025" for application also to EU and national reference laboratories, confirming the importance conferred upon accreditation.
Until 2009 the Italian accreditation system was undertaken by three different bodies: SINCERT (certification bodies of products, services, systems and persons, and inspection bodies), SINAL (testing laboratories), and SIT (calibration services), which operated mostly in a voluntary context. In order to comply with article 4 of Regulation (CE) n. 765/2008, SINCERT, SINAL, SIT and ISS-ORL merged their competences to create ACCREDIA, which was designated as the sole national accreditation body by the Inter-ministerial Decree of December 22, 2009. This recognized ACCREDIA's competence to attest the competence of bodies and laboratories, verifying their management system and their compliance with the internationally recognized standards. On an international market level the accreditation issued by ACCREDIA – signatory body to the agreements of mutual recognition, EA and IAF MLA and ILAC MRA – functions as a passport for the free movement of goods and services, combating systems of protection.
The benefits of accreditation can be summed up in the slogan "one test one stop". The positive effects of transparent and effective accreditation activity involve all operators of the socio-economic system: greater quality for the general public, international competitiveness for businesses and less institutional bureaucracy.

S4.7
VETERINARY PREVENTION AND PARASITIC ZOONOSES: AN EXAMPLE OF ONE-HEALTH MEDICINE

Grasselli A. **[1], Perrone V.*[1]**

[1]SIMeVeP ~ Roma ~ Italy

The American epidemiologist Calvin Schwabe was the first to introduce the One-Health concept as a unique vision of health and pathology of humans and animals. In Italy, this vision has been sustained firstly by Adriano Mantovani and more generally by the political and administrative unification of human and veterinary medicine. Together with the agrarian issue, health system found opposition of "moderate" and "radical socialist" cultures and after a harsh confrontation in parliament (20 march 1865), the administrative unification law was emanate, with Public Health rules in Section C about the role of vets in supervision and control of animal meet intended for human consumption. The regulatory path of the right to health care protection starts with the Law n. 2248 1888, issued by Luigi Pagliani (the first director of Health system) about the unity of medical and veterinary system, under the Ministry of Interior. The path goes on until 1978 when the Law n. 833 found the actual National Health Service, with Veterinary Public Health activity into the Prevention Department. This brief summary was possible thanks to numerous doctors and vets: among many, Ludovico A. Muratori and Bernardino Ramazzini, Antonio Alessandrini and Giovanni Battista Ercolani (doctors who founded the Veterinary school of Bologna), Sebastiano Rivolta focused on parasitic zoonoses and phytoparasitology. One important example is Edoardo Perroncito from Piedmont, an eminent pathologist and parasitologist: he identified Ancylostoma duodenale as the etiological agent of the disease of miners and he cured patients with fern extract. This medicine has led to the cure of many miners not only in Italy, but all around Europe allowing also to plan valid prevention system as Sempione tunnel demonstrated. The several works of Edoardo Perroncito were translated in all languaged and published in one volume "The miners disease: from S. Gottardo to Sempione. A settled issue".

Keywords:
parasitic zoonoses, Veterinary prevention, One Health

NOTES

TAVOLA ROTONDA 1

LA PARASSITOLOGIA ITALIANA NELLA COOPERAZIONE INTERNAZIONALE

(Modiano D., Cassini R., Bisoffi Z.)

Intervengono:

1) Gentile B. (MAE-DGCS)

2) Bartoloni A. (Università di Firenze - SIMET)

3) Esposito F. (Università di Camerino)

4) Maiori G. (ISS)

5) Crisanti A. (Università di Perugia)

6) Albonico M. (Fondazione De Carneri)

7) De Balogh K. (FAO)

8) De Meneghi D. (Università di Torino)

8) Corsi M. (Sigma-Tau)

9) Broglia A. (presidente VSF Italia e VSF International)

10) Ghirotti M. (MAE)

NOTES

TAVOLA ROTONDA 2

MIGRAZIONI E TUTELA SANITARIA

TV2.1
MIGRATION AND HEALTH IN THE PERSPECTIVE OF PUBLIC HEALTH

Marceca M.*[1]

[1]Dep. of Public Health and Infectious Diseases "Sapienza" University, Rome. Italian Society of Migration Medicine

The issue of "immigrants' health" has been the subject of increasing interest in recent years, both in scientific literature and in the declarations of international health institutions. Specifically, the Resolution of the 61st World Health Assembly [WHA. Resolution n. WHA61.17 of 24 may 2008], and the Report of the European Parliament on the reduction of the inequalities in health within EU [European Parliament. 2010/2089 (INI). Document A7-0032/2011 on Febr 8, 2011. Final Approval March 8, 2011] are worth highlighting. There is a clear convergence in the orientations recommended to local Governments regarding the health policies and interventions to be adopted in this sector [WHO. International Migration Health & Human Rights. 2003; UNFPA. International Migration and the Millennium Development Goals. 2005; UNDP. Human Development Report 2009. Overcoming barriers: Human mobility and development. 2009; Global Migration Group. International Migration and Human Rights. 2008; IOM. Migration Health. Better Health for All in Europe. Final Report. 2009; European Council. Final draft Lisbon Conference "Good practices on health and migration in the EU". 2007; WHO, Gobierno de Espana, IOM. Health of migrants - The way forward. Report on al global consultation, 2010].
We consider this issue a case study public health paradigm.
Universalistic health systems, as in the Italian case, tend to recognise the importance of adopting initiatives protecting the health of all immigrants, including those without regular documents, on the basis of the recognition of health as a fundamental, primary right of the individual and as a collective interest [The Italian Constitution, 1948 - 32nd Article).
Alongside ethical-legal recognition of the right to healthcare, the technical-scientific debate has also developed, especially following the publication of the WHO Report "Closing the gap in a generation" [WHO. Commission on Social determinants of Health, 2008]. This has enabled the acknowledgement of the relevant role played by the socio-economic conditions that distinguish the different groups of immigrants. Moreover, the proposal of inter-sectorial policies and of a approach aimed at the empowerment of the community has become increasingly significant.
The adoption of a similar "systemic" overview involves reference to models of intervention marked by complexity, characterized by multifaceted action, and permitting the integration of different professions and points of view, with flexible strategies, and close attention to context [The Lancet, 2006 (368) 23:1039; Materia et al., 2005, J Epidemiol Community Health, 59:534-5].
It may be stated that the health policies adopted in Italy in the 1980s - especially for the undocumented migrants - have been pioneering in both European and international contexts [Marceca et al., 2012, Italian J. Publ Health, 9:e7498-1-e7498-11]. Following the first exchanges of experience in the clinical-epidemiological sector, the various groups working in Italy on the "health of migrants" issue have also gradually managed to develop an approach to health policy planning. This has resulted in a grass-roots movement on the part of public sector health workers and civil society as a whole, strongly motivated and characterized by a high profile in specific skills. Typically of this form of group, the movement is distinguished by its focus on free contribution, commitment and liberty, and has ensured its credibility in terms of its analysis of the phenomenon and the proposals it offers. The creation of the Italian Society for the Medicine of Migration (Società Italiana di Medicina delle Migrazioni, SIMM) in 1990 should be viewed in this context. Since its founding it has influenced, through its constant role of advocacy, most national health care policy decisions in this sector, which has led to the enactment - not without controversy and difficulty - of "inclusive" laws (for documents, analyses and proposals produced by SIMM, see www.simmweb.it).
The "philosophy" of these deliberately "inclusive" health policies can be summarised in two major areas: 1) the complete equality of rights and obligations, regarding both health and rights to health care, between Italian citizens and foreigners legally present (with stay permit), with complete health care cover from the public health system; 2) the broad possibility of health protection and health assistance also for undocumented immigrants, especially for women and children, and in relation to infectious diseases [Geraci, 1998, Agenzia Sanitaria Italiana, 9:46-8; Marceca, 1999, L'Arco di Giano, 22:27-35].
However, an "inclusive" health policy needs to be accompanied by a real willingness to collaborate on the part of the local authorities, which over time have acquired a fundamental role in the provision of social and health services for foreigners, in terms of implementation and maintaining effectiveness.
Human migration has been rightly framed as a dynamic process that can be decomposed into distinct phases with specific critical aspects for health and different priorities for public health action. Specifically, a "migration phases framework" can be described in terms of: "pre-departure" phase (health conditions in the place of origin); "trave" phase (with different health risks on the basis of specific travel conditions); a possible phase of "interception" (particularly in case of a long-distance migration project); "destination" phase (this is the most interesting from a public health perspective); a possible "return" phase in the place of origin (especially in case of repatriation programs) [Zimmerman et al., 2011, PLoS Med., 8:e1001034. Epub 2011 May 24].
According to available data and to European medical literature, the area of infectious and parasitic diseases related to migrant health - even if worthy of attention - is not a public health priority [Rechel B, Mladovsky P, Devillè W, Rijks B, Petrova-Benedict R, McKee M. Migration and health in the European Union. European Observatory on Health Systems and Policies Series. McGraw-Hill, 2011]. This is confirmed by the results of the 13 epidemiologic surveillance programs on migrants who were arriving in Italy activated in 2011 by the Italian Ministry of Health [Marceca et al., 2013, Ann Ig., 25 (Suppl. 1):159-64].
The public health approach to the issue of migrant health seems currently unbalanced on a vision of emergency, excessively centred on the risks for infectious diseases; a change in the cultural and technical approach, moving to a broader view, is necessary. As the health of migrants is mainly influenced by social and economic daily living conditions, we need to adopt a modern public health approach based on the WHO theory on social determinants of health.

XXVIII Congresso Nazionale SoIPa, Roma 24-27 giugno 2014

MIGRAZIONI E TUTELA SANITARIA
TAVOLA ROTONDA 2

TV2.2
TUTELA DEI MIGRANTI PRESENTI IN ITALIA AFFETTI DALLA "MALATTIA INVISIBILE" DI CHAGAS

Angheben A.*[1]

[1]Società Italiana di Medicina Tropicale, Rome; Centre for Tropical Diseases, Hospital Sacro Cuore, Negrar)

Chagas disease (CD) is a complex zoonosis caused by the hemoflagellate protozoan Trypanosoma cruzi. Endemic to all continental Latin America, it affects around 7-8 millions of individuals worldwide (World Health Organization, 2013, Sustaining the drive to overcome the global impact of neglected tropical diseases: second WHO report on neglected diseases, WHO/HTM/NTD/2013.1). The disease can be transmitted through Triatominae insects bites ("kissing bugs") but also:
- parenterally through blood or organ donation and vertically from mother to child and
- enterally through ingestion of foods and beverages contaminated by Triatominae feaces.
Depending on this diverse modes of transmission, CD can be transmitted also in non endemic countries (Prata, 2001, Lancet Infect Dis, 1(2):92-100).,
Therefore, in the last years, CD has become a public health threat even out from continental Latin America, as a consequence of the dramatic increase of the migration flows at the beginning of the current century (Gascon et al. 2010, Acta tropica, 115(1-2):22-7).
In non endemic areas, autochtonous transmission of CD, cumulated to the number of imported chronic cases who began to show sequelae and complications. In many European countries the response to this challenge has been late and inappropriate. This fact caused the current worrisome scenario. However, in comparison with the last century, some European countries have set up programs at different level to control Chagas disease transmission within their borders and many efforts have been done to shed light on this emerging problem by different Institutions and specialized Centers. According to Eurostat, Italy is ranking second in Europe for number of Latin American (LA) immigrants with around 350000 documented resident immigrants (11% of the whole LA migrant population in European Union). Therefore number of cases of CD are estimated in Italy between 9200 and 17000 (Strasen et al. 2014, Clin res cardiol,103(1):1-10). Considering an average evolution of CD to chronic symptomatic disease of 30%, about 5000 cases of cardiac involvement can be estimated.
From a Public Health point of view, the issue of autochtonous transmission of CD in Italy needs to be seriously tackled. Italian directives on organ donation are effective in controlling CD transmission through this way (http://www.sanfilipponeri.roma.it/trapianti/file/140812_linee_guida_donatore.pdf, last accessed 20/03/2014), whereas a new directive regarding blood transfusion (mentioning CD not only as an exclusion criterion but also as need to test donors) is still awaited. To our knowledge, only few institutions up to now did follow a good strategy to avoid CD transmission through blood (and derivatives) donation (Blood Banks in Florence, Negrar, Bergamo and Rome). Regarding CD congenital transmission, control initiatives have been implemented in Italy at Regional level in Tuscany but also in some other institutions (Bergamo Province, Negrar "Sacro Cuore" Hospital, Rome).
World Health Organization (WHO) planned a strategy based on three principles (the so-called "tricycle strategy") to combat CD, based on "information and surveillance", "diagnosis and management of cases" and "transmission control". Regarding diagnosis, in Italy a very high under-diagnosis index has been calculated (98,3-99%) (Basile et al., 2011, Eurosurveill,16(37):pii=19968); therefore treated patients are less than 1% (such as worldwide, however).
This is favoured by the nature of the disease itself, that is often asymptomatic for decades, it affects vulnerable populations, it cannot be suspected through routinary blood or clinical controls. Efforts have been done to implement screening programmes in Italy by some Centres since 2009 (Angheben et al. 2011, Euro Surveill,16(37):pii=19969) but many efforts more are needed in the future to converge to common practices in the management of affected people and to offer access to diagnosis and care throughout the national territory.
From a report on the Italian situation presented at the Non-endemic Countries Initiative meeting, organized by WHO in Florence in 2013, in Italy a total of 568 cases have been diagnosed until the beginning of 2013. An ongoing screening set up in the Bergamo area by the Centre for Tropical Disease of Hospital "Sacro Cuore", Negrar, with the collaboration of OIKOS ONG and Medici Senza Frontiere - Italy (MSF - Italy), contributed identifing 509 cases among 2602 tested people (majority of Bolivian origin).
With the help of MSF - Italy other screening programmes of CD for LA population have recently began in Milan and Rome.
Many efforts have been done and are in on-going in Italy in order to improve access to diagnosis and care for CD at-risk population and to develop common strategies in case management.
An informal network of Centres has been informally set up and its actions already obtained to move consistent steps forward in the drugs procurement process, laboratory external quality control programmes, screening strategies and LA communities cooperation.

TV2.3
HEALTH PROTECTION, IN SPAIN, OF MIGRANTS AFFECTED BY CHAGAS DISEASE

Osuna A.*[1], Marti Gimenez M.[2], Munoz Calabuig E.[2], Parada C.[3], Rodrigues Garcia M.[4]

[1]Instituto de Biotecnología, Universidad de Granada, Spain, [2]Hospital La Fé. Valencia, Spain, [3]Centro de Transfusion de Comunidad Valenciana, Spain, [4]Hospital General Universitario Valencia, Spain

Currently, it is estimated that at least a billion people in the world suffer what are called neglected diseases, such as American trypanosomiasis or Chagas' disease, caused by Trypanosoma cruzi, a protozoan, endemic in America. Despite that some 8-10 million people are infected with this parasite in the Americas and an estimated 50,000-200,000 new cases are confirmed every year, it is considered among the neglected diseases (Tarleton et al. 2007, PLoS Med, 4: e332). The course of the disease is marked by two phases, an acute phase with a mortality rate of some 5%, especially in children and the chronic phase, approximately 70% of seropositive individuals are asymptomatic, whereas 30% develop serious cardiac/digestive pathologies several years or decades later, as well as necrotizing inflammatory injuries to the CNS (Carod-Artal & Gascon, 2010, Lancet Neurol, 9:533-42). One of the most serious complications is sudden death (Gascón et al., 2007, Rev Esp Cardiol, 60:285-93). Each year, 2-3% of symptomatic individuals start to present manifestations that can rapidly evolve to sudden death. It is important to take into account the possible episodes of reactivation of this disease in immunodepressed patients (Pérez-Molina et al., 2011, HIV Clin Trials, 2011,12:287-98). The determinants of this change are unknown and no prognostic markers are available to date. Of all infected individuals, 10% will die annually (Coura, Mem Inst Oswaldo Cruz, 2007, 102 (suppl. 1):113-22). Although the disease is transmitted through the dejections of triatome insects in endemic areas, other forms of transmission exist including oral transmission, transplacental transmission, blood transfusions, and organ transplants, and organ transplants. The disease afflicts the poor in 21 countries of South and Central America, where close to 10 million people are still infected today and 100 million are at risk. Over the last three decades, as human migration has intensified, this silent plague has extended to non-endemic countries, becoming a global concern. The WHO estimates that more than 300,000 infected people leave endemic areas in Mexico and Central America for the United States annually and close to 80,000 move to Europe every year. Unfortunately, 94-96 % of those emigrants carrying the disease remain undiagnosed and only around 4,000 laboratory-confirmed cases were diagnosed during the past decade: Spain, Belgium, France, Italy, Switzerland, and the United Kingdom. Sporadic cases have also in Denmark, Croatia, Germany, Luxembourg, Norway, Portugal, Romania and Sweden. In Europe, there are close 4 million immigrants from Latin America, of these more than and it is a safe estimate that more than 200,000 must carry Chagas disease (Schmuni, 2007, Mem Inst Oswaldo Cruz, 102:75-85). In 2009, 68,000-22,000 cases were diagnosed, of which 200 were babies with congenital Chagas disease. In 2010, it was estimated that there were 87,000 chronically infected individuals in Europe, only 2-10% of these had developed clinical cardiopathy and with 2000-8000 cardiomyopathies in Spain alone. In Europe, 40% of the immigrants from Latin America move to Spain, and 5% are infected with T. cruzi, with ~17,000 (> 20%) needing medical care (http://www.eurosurveillance.org/images/dynamic/ES/V14N01/V14N01.pdf). Recent studies have indicated that the seroprevalence of Chagas patients in Barcelona in 0.6% and in Valencia is 1.5%. With a seroprevalence of 11% in pregnant women. The estimated burden of the disease, as quantified by disability-adjusted life years (DALYs) is 590,000US$. Some figures estimate that for each 100,000 infected individuals the cost related to medical care, treatment, and in working days exceeds €40 million/year. Recent studies estimate that the global economic cost of Chagas disease in the world exceeds US $7 billion annually. This cost is 3.5-fold higher than that of rotavirus ($2 billion) and 1.48-fold higher than that cervical cancer ($4.7 billion). The total annual cost to society, health-care costs plus productivity losses, for an individual with chronic disease is €2941 in Latin American countries and €9840 in Europe.

Spanish legislation has adopted diverse measures to avoid the transmission of this disease by the forms described previously. In 2004, regulation was established concerning blood and organ donors from people originating in endemic zones. The first centre to do this was in Spain, the Centre of Blood-Transfusion and Organ Bank of the Region of Valencia, prior to the promulgation of the law (R. D.1088/2005, 16/9), establishing the technical requirements and minimal conditions of blood donation. In 2007, the Region of Valencia developed the first protocol in Spain to control congenital and perinatal infection in Spain 3/2007 DGSPG), which became compulsory in 2009. This protocol, including the routine determination of Chagas disease in all mothers from endemic areas, is the reference for most regions of Spain, where the measures have been adopted. At the moment in all of Europe, there are official programmes related to the vertical transmission of Chagas disease only in Valencia, Cataluña, Galicia and in Italy (Tuscany, Bergamo and Rome). The WHO recommends 2-3 simultaneous tests for accuracy, decreasing the inconclusive results to less than 2% (Research priorities on Chagas disease, TDR, 2012). Detection of parasite DNA by PCR during the chronic phase of the infection is less sensitive than serological tests (~60% sensitivity). Diagnostic approaches for the evaluation of post-treatment cure are urgently needed. In Spain, these recommendations are followed together with a PCR test, conducted in the centre where a confirmation diagnosis was also made in the National Microbiology Centre and, in the case of the Hospital la Fé, together with the CNM and another carry out in our laboratory. This PCR is based on the amplification with the 121F and 122R T. cruzi and the appearance of an amplicon of 330 pb (Zulantay et al., 2007, Ann Trop Med Parasitol., 101:673-9). All patients tested positive are treated with benzinidazol, repeating the laboratory diagnosis after one year, especially the PCR. This treatment is followed clinically with tests such as: ECG, X-ray, TBE, MRI, ECG-Holter, esophageal pH, manometry, ergometry and colonoscopy. In our specific case, based on a study in collaboration with the INGEBI of Argentina, we are trying to correlate symptomology with the DTUs parasite based on a PCR algorithm for DTU genotyping (Burgos et al. 2010, Clin. Infect. Dis., 51:485-95) and searching for antigens from a multigenic family of orthologues of the parasite.

XXVIII Congresso Nazionale SoIPa, Roma 24-27 giugno 2014

MIGRAZIONI E TUTELA SANITARIA
TAVOLA ROTONDA 2

TV2.4
PROSSIMITÀ, GRATUITÀ E RETI PER LA SALUTE DEGLI IMMIGRATI

Geraci S.*[1]

[1]Area sanitaria Caritas Roma, Società Italiana di Medicina delle Migrazioni

INTRODUCTION:
Uno dei protagonisti assoluti nella storia sociale di Roma tra il 1980 e la fine degli anni '90 è stato l'allora direttore della Caritas capitolina, mons. Luigi Di Liegro. Persona carismatica, cittadino impegnato, prete attento ai bisogni della gente e allemergere di nuove problematiche, ha saputo leggere i fenomeni sociali che hanno attraversato Roma e lintero paese, stimolando, prima ancora che risposte concrete di cui è stato maestro, l'attenzione culturale e politica ai temi della marginalità e della giustizia sociale. Un'intera generazione di cittadini sensibili, indipendentemente dell'appartenenza confessionale, culturale o politica, è maturata con quello stile che, partendo dai fatti, andava alle radici dei "mali di Roma" e ne tracciava soluzioni non delegandole ad altri ma che partivano dall'impegno di ognuno e si traducevano in input politici puntuali e concreti. In quel clima di fermento culturale e di impegno, alcuni medici ed operatori sanitari diedero vita a Roma ad un piccolo ambulatorio vicino la stazione Termini per assistere immigrati in condizione di marginalità sociale, veri "uomini ombra" in tema di diritti assistenziali.
MATERIALS AND METHODS:
La Società Italiana di Medicina delle Migrazioni
L'esperienza descritta non è stata isolata: dalla metà degli anni '80, in varie parti d'Italia, in modo spontaneo ed allora certamente non coordinato, vari gruppi di matrice confessionale o laica (oltre la Caritas a Roma, pensiamo al Naga a Milano, la Croce Rossa a Genova, il Biavati a Bologna, i salesiani di Santa Chiara e l'Università a Palermo) si sono organizzati per garantire il diritto all'assistenza sanitaria agli stranieri che ne erano esclusi.
Nel 1990 molte di queste organizzazioni si incontrano e dal confronto emerge la scoperta che quelle esperienze più o meno isolate possono confluire in una riflessione comune, si può passare dall'impegno personale ad una coscienza collettiva di una nuova realtà; dalla necessità di affrontare una emergenza per assenza di preparazione ed organizzazione pubblica, all'esigenza di capire, studiare, sperimentarsi nell'incontro con questi "nuovi cittadini"; da un diritto di fatto negato e/o nascosto alla volontà di affermare, anche sul piano giuridico, che la salute è un bene di tutti e per tutti. Da allora si comincia a parlare in Italia di medicina delle migrazioni, in un'ottica che oggi definiremmo di salute globale. Con queste premesse, proprio nel 1990 nasce la Società Italiana di Medicina delle Migrazioni - SIMM.
La costruzione di politiche e norme sanitarie "inclusive"
L'Italia, nell'ambito della tutela della salute degli immigrati, si è caratterizzata dalla costruzione nel tempo di norme esplicitamente inclusive che sono nate sotto l'azione di advocacy proprio di quei gruppi ed organizzazioni provenienti dal volontariato e dal privato sociale riunite nella SIMM, vera e propria rete tematica nazionale. Nel 1995 viene presentata una proposta di legge che è condivisa dalla grande maggioranza delle forze politiche ed ha avviato un processo normativo che attualmente garantisce a tutti gli immigrati presenti in Italia, anche coloro in condizioni di irregolarità, tutela sanitaria, accesso alle cure ed interventi di prevenzione.
Tra il 1998 ed il 2000 il corpo giuridico nazionale si rafforza e cominciano i primi studi di tipo epidemiologico di vasta scala e la riflessione su indicatori e politiche. Nello stesso periodo, con ala riforma del titolo V della Costituzione, si avvia un processo di decentramento amministrativo che da una parte mantiene alcune competenze giuridiche e politiche a livello statale (come l'immigrazione e l'asilo) ed altre sono cedute alle Regioni (come l'organizzazione sanitaria). Ciò produce a livello locale una frammentarietà di politiche specifiche per la tutela sanitaria degli immigrati e a volte una forte disomogeneità nella garanzia di accesso ai servizi.
L'importanza delle reti
In ambito scientifico si è però avviata un'alleanza culturale ed una collaborazione strutturata tra società civile, competenze professionali e mondo accademico e della ricerca, e ciò ha prodotto riflessioni e strumenti estremamente utili nella fase attuale di implementazione del sistema a livello locale. Al decentramento amministrativo si è risposto con la creazione di gruppi locali (Gruppi Immigrazione e Salute - GrIS, attivi nel Lazio, Trentino, Lombardia, Piemonte, Friuli Venezia Giulia, Veneto, Emilia-Romagna, Toscana, Liguria, Sardegna, Calabria, Campania, Sicilia, Puglia) che fanno della SIMM una "rete di reti" capace di dialogare con le diverse amministrazioni, "sorvegliare" una giusta applicazione e denunciare, quando necessario, una interpretazione discriminatoria delle norme. Ciò ha fatto emergere la necessità di uniformare a livello nazionale l'approccio normativo, e su iniziativa della Regione Marche è stato istituito, in sede di Commissione Salute della Conferenza delle Regioni, il Tavolo tecnico "Immigrati e servizi sanitari" dove partecipano tecnici degli assessorati alla salute delle Regioni, rappresentanti del Ministero della Salute ed esperti accreditati in materia di salute e immigrazione. Si concretizza una rete istituzionale in rete con altre reti e si rappresenta una opportunità unica per condividere competenze/proposte tecniche istituzionali e quelle maturate sul campo dagli operatori.
CONCLUSIONS:
La Caritas di Roma, agli inizi degli anni ottanta nella capitale istituì mense, ostelli, centri d'ascolto per dare risposte concrete ma anche per capire "dal di dentro" una problematica, per studiare un fenomeno, per stimolare le autorità a farsene carico. Questa spinta ha trasformato un piccolo Centro medico della periferia romana, nato per una risposta immediata a dei bisogni senza risposta, nell'attuale Area sanitaria, complessa ed efficace rete di servizi e progetti, laboratorio transculturale per una sanità centrata sulla persona, presente con la propria esperienza e passione nel processo che abbiamo brevemente descritto di emersione del diritto alla tutela sanitaria senza esclusioni. E questo con una specifica attenzione al lavoro di rete anche con le istituzioni pubbliche in termini di stimolo politico, proposte per la programmazione, consulenza tecnica, studio e ricerca. Nel 2013 ciò è stato riconosciuto dal Presidente della Repubblica che ha insignito l'Area sanitaria Caritas della medaglia d'oro al merito della sanità pubblica. Se un valore importante dell'impegno dei volontari della Caritas è certamente la gratuità e per questo non si ama ricevere premi e onorificenze, questo riconoscimento ha invece suscitato grande commozione e soddisfazione proprio perché inserito all'interno di un percorso di sanità pubblica, di lavoro per il bene comune dove giustizia e solidarietà si fondono per un sistema che deve essere equo e attento alle diversità e alle fragilità.

TV2.5
THE MSF EXPERIENCE AS PRIMARY HEALTH CARE PROVIDER FOR MIGRANTS IN ITALY: MAIN FINDINGS AND LESSONS LEARNT

Egidi S.*[1]

[1]Medici Senza Frontiere

Between 1999 and 2010 MSF (Medici Senza Frontiere) has been providing medical and humanitarian assistance to irregular migrants in Italy through activities implemented in different sectors:
-assistance at landing/reception centres, mainly in Sicily;
-assistance provided to seasonal workers in Southern regions;
-health care in outpatients' department devoted to foreigners without permit of stay (known as "ambulatori STP") fully integrated in the National Health System.
Despite the legal framework guaranteed by the Italian immigration law recognized the right to access the national health system for urgent and essential care, yet the application of legislation was in many cases insufficient and arbitrary, and a chronic deficiency in term of dedicated medical services was evident.
To ensure a more timely and efficient service, MSF applied a "general medicine" model, seen, like the general practitioner clinic, as the first link with the NHS and ruling the access to specialist inspections and instrumental analysis, also in order to reduce improper access to the emergency room. Essential component was linguistic and cultural mediation, able to ensure full and real access to available services; outreach activities performed in the community were also helpful to share information on health rights and facilities available according to law.
Extremely important, outpatients clinics worked in strict collaboration with secondary level of health care, to ensure prompt referral for cases in need of further and more specialist care. Direct links were established with specialist services, in order to ensure proper diagnosis and follow up for chronic communicable (HIV, Tb, chronic hepatitis) and non-communicable diseases (diabetes, renal impairment).
MSF has managed 37 clinics between 2003 and 2009 in 6 regions of Italy. The handover to the local health authority, including cultural mediation service, was gradually conducted and has been successfully completed for 35 outpatients' departments.
MSF doctors have performed about 42,000 consultations in total; mainly diagnosed illnesses were related to respiratory, musculoskeletal, digestive organs; gynecological diseases were common as well. Direct relation with extremely poor working and living conditions was evident in the majority of cases. Major infectious and tropical diseases were not surprisingly a minority of diagnosis, but for them fast track for referral to Infectious Diseases Departments was particularly important.
In this respect, it is important to say that basic knowledge on tropical diseases has been essential to allow health staff to suspect diagnosis and properly orient patients toward further investigations.

TV2.6
LABORATORY UNIT FOR THE DIAGNOSIS OF INTESTINAL PARASITIC INFECTIONS OF MIGRANTS IN CAMPANIA REGION

Galdiero M.*[1], Cringoli G.*[2]

[1]Department of Experimental Medicine of the Second University of Naples, [2]Department of Veterinary Medicine University of Naples "Federico II"

INTRODUCTION:
In the current era of globalization, the massive movement of populations causes the spread of parasitic infections to several developed countries and, therefore, an increased attention to Neglected Tropical Disease (NTD) has recently been registered in places where such diseases are generally considered unusual (Albonico et al., 2010, Parassitologia 52:29-32). For both geographical and historical reasons, the city of Naples in the Campania region is receiving an increasing number of immigrants and with 61,169 immigrants in 2008 is the first recipient city in southern Italy (Caritas, 2009, XIX Rapporto. Edizioni Idos, Italy:512).
Thanks to an active scientific collaboration between Prof. Cringoli of the Department of Veterinary Medicine University of Naples "Federico II", Dr. Gualdieri the "Centre for Health Protection of Immigrants" based at the Ascalesi Hospital and Prof. Galdiero of the Department of Experimental Medicine of the Second University of Naples, the "Research Unit for the Monitoring of Intestinal parasitosis of Migrants of the Mediterranean area" (URPIM) was set up in 2012.
URPIM conducts surveillance on the prevalence of intestinal parasites in communities exposed to risk of parasitic infection. In particular: migrants with temporary residence permit, populations still residing in endemic countries, volunteers, missionaries and tourists returning from endemic areas, adopted children, immunocompromised individuals and people living in rural areas. Moreover, monitoring and research are supported by testing of new drugs and by monitoring of resistance to drugs currently used.
The activities of the research unit are conducted in order to offer immigrants in Italy a proper health care for intestinal NTDs and to evaluate both the persistence of intestinal parasites in immigrants stably living in an urban context and the spread of infection within households with a lifestyle similar to that of the country of origin (occasional use of cutlery, eating from a common plate).
MATERIALS AND METHODS:
A parasitological monitoring of immigrants in Naples was conducted at the URPIM laboratories. The study population was represented by immigrants admitted to the Ascalesi Hospital for general health problems (Gualdieri et al., 2011, Acta Trop, 117:196-201).
Anamnestic information were used to evaluate the relationship between the persistence of parasitic elements and the length of permanence of the immigrants in Italy. The patients were asked to provide a single fecal sample; all the individuals resulted positive to copromicroscopic analysis received proper therapeutic regimens (Most, 1984, N. Engl. J. Med. 2, 310:298–304; Utzinger et al., 2012, Swiss. Med. Wkly. Nov 22:142). Moreover, fecal samples from individuals sharing residence with patients infected with at least one parasitic species were collected in order to gain a deeper insight into households modalities of transmission of parasitic infection in a western society setting.
The Laboratory is also involved in international studies of pediatric samples collected in primary schools in Cape Verde and Ivory Coast and by a survey on the prevalence of intestinal parasitic infections in individuals residing in Liberia.
The methodology used for the copromicroscopic analyses of samples collected in these epidemiological studies was the innovative FLOTAC dual technique (Cringoli et al., 2010, Nat. Protoc., 5:503–15), a new multivalent technique for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans, with its improvements represented by the use of Fill-FLOTAC and Mini-FLOTAC (Barda et al., 2013, PLoS. Negl. Trop. Dis., 7(8):e2344.). These tools, evolved from FLOTAC techniques, were adapted in order to perform the procedure without the necessity of a centrifugation step that render the system easily operative in resource-limited settings.
The results of the analyzes performed have been entered into a database and processed statistically through the use of Geographical Information Systems (GIS) in order to produce maps and models to verify and/or predict the risk of local epidemiological cycles for each parasitic infection.
RESULTS:
A total of 1822 subjects admitted at the Ascalesi Hospital were tested by copromicroscopic analyses. Among this population 348 subjects (19,1%) resulted positive for pathogenic intestinal parasite.
All the infected patients, who were retested after two weeks from the pharmacological treatment, resulted negative to copromicroscopic analyses; the absence of pathogenic parasites on the second analysis reveals the efficacy of the therapy administered.
As expected, the prevalence of intestinal parasites in the migrant population resulted lower than those observed for the samples analyzed in other epidemiological studies carried out by our research unit with samples shipped from developing countries. In particular, samples collected in primary schools in Cape Verde reported a prevalence of intestinal parasitic infections equal to 78,5%, while the study on the prevalence of intestinal NTDs carried out on stool samples collected in Liberia showed a rate of 44%. The study performed on pediatric samples shipped from the Ivory Coast revealed a prevalence equal to 99,5%.
CONCLUSIONS:
These data confirm the importance of a constant monitoring of intestinal parasitic infections for the populations coming from countries considered endemic for these diseases, in fact currently the persistence of intestinal parasites in the immigrant population having already resided in Italy for many years, appears to be a consequence of domestic transmission through contaminated food handling procedures or reinfection (Kaferstein, 2003, Geneva WHO:100-109). Nevertheless, our studies show that the availability of clean water, better socio-economic conditions, education, personal and public hygiene practices together with different weather conditions (temperature and humidity) lead to a reduced efficiency of transmission and/or reinfection in western countries.
In this context, URPIM is a one of the few laboratories present in the South of Italy for the epidemiological surveillance of intestinal parasites in populations at risk.

TV2.7
HEALTH CARE SERVICE FOR MIGRANTS IN POLICLINICO UMBERTO I HOSPITAL: EVOLUTION AND CURRENT ACTIVITY OF A DEDICATED SERVICE

Massetti A.P.*[1]

[1]Department of Public Health and Infectious Diseases, "Sapienza" University, Rome, Italy

The health care service for migrating people of Policlinico Umberto I Hospital (Sapienza University of Rome) was born in 2002 as a personal initiative of a few doctors and nurses of the hospital. Short after, a voluntary infectious disease service was instituted alongside. In December 2005 both structures were unified and the service transferred into the building of Infectious Disease Department. Since December 2008 the service has been officially recognized into the organization of Policlinico Umberto I Hospital and named Day Service for Migration Medicine.
Presently, the Day Service is open every afternoon from Monday to Friday and Tuesday and Thursday morning, too. People can access the Service directly, without previous appointment and visits take place upon presentation of STP or ENI card, which can be issued by the Office for Foreigners situated inside the Hospital or in different other Offices throughout the city. During the years the Service has established a network of partnerships with different other structures, both inside and outside the Hospital, all of which take care of migrant patients, such as Caritas, Rome government, local sanitary offices …
After an initial period in which the Service was little known and just a few patients were seen, the turnout increased in the following years and at present more than 450 visits take place every month. Since 2006, when the structure was transferred to Infectious Disease department, over 7000 patients have been visited in the Service, mostly men (61%) and young adults (66% of our patients are in the age range 25-44). Our patients arrive from all over the world, but the majority comes from Eastern Europe (38%) and Asia (36%); the five most frequently observed nationalities are Bangladesh, Romania, Moldova, Ukraine and Peru.
The service acts as a general practitioner office, paying specific attention to infectious diseases diagnosis and prevention. Apart from infectious diseases, the most frequently encountered pathologies are those concerning digestive and respiratory systems, shortly followed by bone and muscle problems and metabolic anomalies.
Among infectious diseases, the most frequent are viral hepatitis, followed by skin and urinary infections. After those, parasitic intestinal infections are seen with a reasonable high frequency.
In a recent survey a screening for different infectious diseases was offered to 232 subjects recently arrived to Italy and living in shelters for asylum seekers. For what parasitic diseases is concerned, out of 232 male persons visited, 198 (85,3%) underwent stool examination for research of intestinal parasites. The remaining 34 either had been tested earlier (6 subjects, 2.5%) or, even requested, did not bring the stool sample (28 subjects, 12%). Among the 198 stool samples examined, 12 (6%) revealed the presence of a pathogen parasite, as follows:

- Trichuris trichiura (4 samples, 33.3% of parasitic diseases, 2% of total samples)
- Giardia lamblia (3 samples, 25 and 1.5%)
- Entamoeba histolytica (3 samples, 25 and 1.5%)
- Ascaris lumbricoides (1 samples, 4 and 0.5%)
- Ancylostoma duodenale (1 samples, 4 and 0.5%)

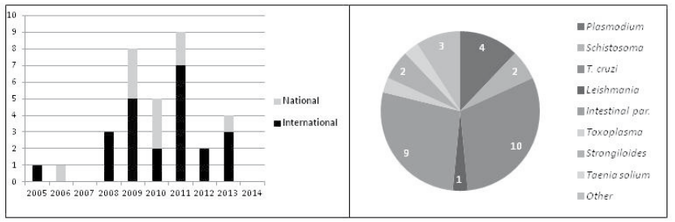
The 12 patients with parasitic infestations were 2 adults (16.7%) and 10 minors (83.3%). The adults arrived from Western Africa (Senegal and Guinea), while most of the younger subjects were from Bangladesh (9 patients), apart 1 patient arriving from Egypt. None of these patients reported symptoms related to parasitic intestinal disease.
In our analysis, we could therefore account for a relatively remarkable amount of parasites identified, particularly if referred to an otherwise healthy and asymptomatic population. Parasites could have been acquired either at their originating countries or during their journey to Italy. Our subjects, in effect, were all young people (mainly minors) just disembarked to Italy during the previous 3-4 months, after a long journey through different countries, often subjected to physical and psychological strains and who had been living in impoverished conditions.

TV2.8
PARASITES AND IMMIGRANTS: THE ROLE OF THE SCIENTIFIC COMMUNITY FROM THE ITALIAN PERSPECTIVE

Berrilli F.[1]

[1]Department of Experimental Medicine and Surgery, University of Rome Tor Vergata

The scientific contributions available in literature during the last ten years have been reviewed, targeting the health of immigrants in Italy from a parasitological point of view. The aim was to provide an extensive and homogeneous picture on the studies carried out in our country on the various aspects of parasites and parasitic diseases connected with immigrant people and, more generally, to disadvantaged communities (e.g. gypsies).
We started from some considerations: I) the awareness of the complex and constantly changing migration flows in Italy; II) the need to deeply recognize the local context of migrant communities at different geographical scale, which is crucial to organize a targeted parasitological monitoring and control (see the COHEMI project).
In order to collect papers on epidemiology, diagnosis and various aspects of parasitic infections in immigrants in Italy, papers were retrieved from various database (Medline, Google Scholar) using different combinations of selected keywords (e.g. im/migrant, [and] parasite, [and] Italy). Both national and international papers were reviewed.
In literature published over the 2005-2014 period, at least 33 publications have been found (23 on international journals, 10 on national ones) (Fig. 1). The majority were focused on Chagas diseases (10 papers), intestinal parasites (9 papers) and malaria (4 papers). Noteworthy, 1 paper has focused on intestinal parasites in a gypsy community and two were conducted in Lampedusa Island.
Most of the papers report data on diagnosis and screening, while only few more comprehensive articles take into account the survey of risk factors, diet composition and social determinants at local scale. However, it should be considered that many data have not been published.
The recent studies on the interactions between microrganisms, parasites and immune system advocate the need to a strict involvement and cooperation among parasitologists, microbiologists and tropical infectologists. From the available data, the presence of private and public healthcare centers for migrants appears to be sufficiently spread in the Italian territory, although a more efficient networking is needed, also to make the epidemiological data more robust than the actual fragmented scenario. Moreover, well targeted multidisciplinary projects should be encouraged (see the URPIM experience), aimed to involve multiple partners from different areas. This would provide a more detailed and complex picture of the parasitic diseases in migrant and marginal communities, not only devoted to the monitoring but especially to the deep understanding of the main determinants of these diseases. The involvement of local authorities appears also needed to establish effective control plans and to realize extensive improvements to the social and economic conditions of immigrants.



Figura

NOTES

SESSIONE PARALLELA O-01

INFESTAZIONI DA ELMINTI

O01.1
THE SEROINCIDENCE OF HYDATIDOSIS IN SOUTHEASTEN SERBIA: A LOT HAS BEEN DONE - A LOT OF WORK REMAINS

Otaševic S.*[1], Miladinovic Tasic N.[1], Ignjatović Ćupina A.[1]

[1]University of Niš ~ Niš ~ Serbia

INTRODUCTION:
Despite the fact that incidence and prevalence of echinococcosis-hydatidosis (E/H) decreased in last decades, this “old disease”, remains current health problem in many countries (Grosso et al., 2012, World J Gastroenterol, 18 (13): 1425-14371). Serbia, Southeastern Europe country in transition, in the post war period represents endemic area of E/H. In Serbia: i) the cumulative incidence (CI) of cases ranges from 0.46/105 to 39/105 inhabitants, ii) it was recorded continuous reduction of the incidence and prevalence of hydatidosis in children, no trends in adults and permanent decrease of this parasitosis in animals iii) genotyping confirme the presence of E. granilosus sensu stricto genotype G1 and E. canadensis G7 (Bobić et al., 2012, Foodborne pathog dis, 9 (11): 967-973) iv) the territory of the city of Niš (southeasten Serbia) is also endemic area of hydatidosis (Otašević et al., 2010, Acta Fac Med Naiss, 27(4):199-204). The goal of the study is to determine the seroprevalence and seroincidence of hydatidosis in the territory of our city, after legally prescribed measures and programs aimed at thesuppression of stray dogs and cats, and addition of confirmed Western-blot test in routine diagnosis of this neglected parasitosis.
MATERIALS AND METHODS:
In the last 4 years, sera of 339 patients were analyzed at the Public Health’s Institute of Niš Center of Microbiology and Parasitology, using three serological kits (Immunofuorescent test (IIFT-INEP-Zemun, Serbia); indirect haemogglutination assay (IHA-Siemens, Germany) indirect enzyme-immunoassay (ELISA-R-Biopharm, Germany) and confirmed by Western-blot test (LDBIO Diagnostics, Lyon, France). The results were elaborated using the statistical method SPSS 14.0 for Windows 2003. The congruence of WB with other applied tests was done by Cohen’s Kappa (K) that was interpreted according to Landis and Koch scale.
RESULTS:
Applying the confirmatory testing, the seroprevalence of 9.9% and the average seroincidence of 3.1/105 inhabitants (cumulative seroincidence 12.4/105 inhabitants) were confirmed. We’ve had an absolute coincidence of confirmatory testing in cases where all used serological tests were positive. The highest concordance of WB was found with IHA (k=0.854, p<0.001; taking into account the lower diagnostic insignificant titer).
CONCLUSIONS:
From year 2009, Serbia harmonized its laws with the EU regulation, and issued the act on animal welfare (Law 41/09) establishing the measures in order to reduce stray dogs and cats euthanasia procedure to preventive measures: catch-neuter- chip-release strategy. At the local level in the territory of the city of Niš, authorities have applied this law during 2010. However, the high seroprevalence and seroincidence that were determined in the territory of the city of Niš showed that region of our city is still endemic area despite the established preventive measures and needs continuation of great attention from public health authorities.

Keywords:
Echinococcosis/hydatidosis, seroprevalence, seroincidence

O01.2
UPDATE ON DIAGNOSTIC METHODS IN HUMAN PARASITOLOGY: WHEN TECHNOLOGY HELPS TRAINING

Barda B.*[1], Albonico M.[2], Levecke B.[3], Genchi M.[4], Vercruysse J.[3], Utzinger J.[5], Magnino S.[4], Cringoli G.[6], Montresor A.[7]

[1]San Raffaele Hospital ~ Milan ~ Italy, [2]Ivo de Carneri Foundation ~ Milan ~ Italy, [3]Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine ~ Merelbeke ~ Belgium, [4]Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia Romagna “B. Ubertini ~ Pavia ~ Italy, [5]Swiss Tropical and Public Health Institute ~ Basel ~ Switzerland, [6]Section of Veterinary Parasitology and Parasitic Diseases, University of Naples Federico II ~ Naples ~ Italy, [7]Department of Neglected Tropical Diseases, World Health Organization ~ Geneva ~ Switzerland

INTRODUCTION:
Control of neglected tropical disease is on the global public health agenda, therefore accurate diagnosis is a crucial basis for epidemiology and assessment of disease burden. The current diagnostic guidelines should be updated to include new tools for parasitology diagnosis and improved with the platform of digital technology. Transmission of knowledge should be enhanced by using informatics learning material and World Wide Web. One example of the importance of updating guidelines and teaching supports is the current manual on “Basic laboratory methods in human parasitology”, which was edited in 1991 by the World Health Organization (WHO). The aim of this project is to update the WHO manual by adding new techniques for faecal and urine examination, but also by including practical tutorials available as video clip on DVD and on the WHO You Tube website.
MATERIALS AND METHODS:
The manual is meant to be a multi-centre project, and it will implicate collaboration of many different groups. The partners involved have been chosen by the WHO, among the most competent and leading groups in the development and validation of parasitological diagnostic methods.
The manual will be divided in three main sections: general aspects, basic techniques and a new set of images of parasites that were formerly published in the WHO “Bench Aids for the Diagnosis of Intestinal Parasites”.
RESULTS:
The first part will deal with microscope care and calibration, safety of the operators and precautions for the safe collection, handling and disposal of specimens. A tutorial for the organization of stool and urine collections in a school setting will also be included in this section especially for Mass Drug Administration programmes. The second part will focus on laboratory work and specifically on the following techniques: Formol-ether concentration, Cellophane thick-smear (Kato-Katz thick smear), McMaster egg counting method, Mini-FLOTAC, Bearmann, Harada-Mori, Scotch test for pinworm, urine sedimentation, urine filtration and reagent strip for haematuria, Circulating Cathodic Antigen and Circulating Anodic Antigen test for Schistosoma. High-resolution images of parasites will be selected for the new edition of the Bench Aids. In the third part of the manual.
Under the leadership and coordination of WHO, video tutorials will focus on the processing of samples with different techniques. The tutorials include the process from sample collection to processing and reading in the laboratory. The video clips are shot on the field, in order to develop a training tool suitable for all kind of laboratories and technicians. Parasite images are being collected from the different collaborators and selected for the new electronic edition of the Bench Aids. The video tutorials will be made freely available on the WHO YouTube channel. Electronic versions of the manual will also be prepared.
CONCLUSIONS:
This project is meant to be an up-to-date reference guide for health workers and laboratory technicians who intend to apply updated parasitological diagnostic techniques based on standard operating procedures recommended by the WHO. This innovative project will aim to a broad dissemination of the manual, even in resource-limited settings. In addition, it will improve coordination and easiness of comparing data from surveys that will use standard diagnostic methods and protocols. A broad dissemination of the manual is envisaged, particularly in resource-limited settings.

Keywords:
Diagnostic guidelines, Human parasitology, video tutorial

O01.3
ACCURACY OF THE MINI-FLOTAC METHOD FOR THE DIAGNOSIS OF SOIL-TRANSMITTED HELMINTHS IN PRESERVED STOOL SAMPLES: HOW LONG DO THE EGGS RESIST?

Barda B.*[5], Albonico M.[1], Ianniello D.[4], Utzinger J.[2], Keiser J.[2], Speich B.[2], Rinaldi L.[4], Cringoli G.[4], Burioni R.[5], Montresor A.[3]

[1]Ivo de Carneri Foundation ~ Milan ~ Italy, [2]Swiss Tropical and Public Health Institute ~ Basel ~ Switzerland, [3]Department of Neglected Tropical Diseases, World Health Organization ~ Geneva ~ Switzerland, [4]Section of Veterinary Parasitology and Parasitic Diseases ~ Naples ~ Italy, [5]Laboratory of Microbiology, San Raffaele Hospital ~ Milan ~ Italy

INTRODUCTION:
Kato-Katz (KK) is the standard method recommended by World Health Organization (WHO) for the examination of stool specimen for the diagnosis of Schistosoma mansoni and soil transmitted helminths (STH) infection. This method requires processing the faecal specimens preferably within 24 hours from the collection. Mini-FLOTAC (MF) has been proposed as alternative to the KK method, allowing to analyse formalin-preserved faeces and maintaining the possibility to quantify faecal egg counts.
The aim of the study was to evaluate the performance of the MF and the possibility to store the samples for one month (from stool collection to microscope examination) maintaining good/optimal correlation in term of prevalence/intensity of infections, and a good/robust readability by which the different STH eggs can be recognized over time.
MATERIALS AND METHODS:
The study was carried out on Pemba Island, Tanzania, in October-November 2013. Forty-one school-aged children were enrolled, the inclusion criterion was to have a double or triple infection with Ascaris lumbricoides, Trichuris trichiura and/or hookworm. Samples were analysed with KK and MF on the day of collection and divided in 12 containers per sample each containing 2 gr of sample fixed with 5% formalin (ration 1:1) and analysed with MF over one month: from day 1 (D1) until day 15 (D15) every other day, and from day 16 (D16) until day 31 (D31) every three days.
RESULTS:
At D1 the combined prevalence calculated with KK and MF method was 36/41 (88%) for A. lumbricoides, 41/41 (100%) for T. trichiura and 22/41 (54%) for hookworm.
The prevalence of infections at D1 with a single KK was: 85% for A. lumbricoides, 98% for T. trichiura and 42% for hookworm; with MF method was 76% for A. lumbricoides, 100% for T. trichiura and 51% for hookworm.
KK was more sensitive for A. lumbricoides (87.5 vs 77.5), MF was more sensitive for T. trichiura (100 vs 97.5) and hookworm (65.6 vs 53.15). The mean eggs per gram (EPG) with KK and MF methods was 12075 and 11679 for A.lumbricoides, 1074 and 1592 for T.trichiura, 255 and 220 for hookworm, respectively. There was no statistical difference between the two methods in terms of EPG.
The mean prevalence with MF technique from D1 until D31 was 77.4% for A.lumbricoides, 99.8% for T.trichiura and 53.5% for hookworm. The accordance among the prevalence overtime was good for the three STH. The mean EPG from D1 until D31 was 10582 for A. lumbricoides, 1448 for T.trichiura and 144 for hookworm. There was no statistical difference among the EPG means of any STH infection from D1 until D31.
CONCLUSIONS:
In conclusion, the findings of our study suggest that for a qualitative diagnosis with MF method with 5% formalin, eggs can be preserved up to 30 days without impairing the quality of the data on prevalence of STH infections. For an accurate quantitative diagnosis for hookworm, however, we suggest a limit of 15 days of preservation; after this time hookworm eggs start to deteriorate and it might reduce the consistence of microscope reading, For the other STH, eggs remain stable over one month and therefore a longer preservation might still give accurate data on intensity of infections.

Keywords:
Soil-transmitted helminths, Mini-FLOTAC, Preserved stool

O01.4
INCREASED PREVALENCE OF TRICHINELLA PSEUDOSPIRALIS DUE TO THE MORE WIDESPREAD USE OF DIGESTION TESTS

Pozio E.*[1], Marucci G.[1], Interisano M.[1], Tonanzi D.[1], La Rosa G.[1]

[1]Istituto Superiore di Sanità ~ Rome ~ Italy

INTRODUCTION:
Trichinella pseudospiralis is the only Trichinella species infecting mammals and birds. This Trichinella species belongs to the clade of non-encapsulated species, i.e. the new born larva does not induce the development of a tick collagen capsule (the cyst) around the larva after its penetration in the striated muscle cell. The lack of a collagen capsule prevents the identification of the larvae in the striated muscles by a trichinoscopy since larvae are not coiled but elongated between the muscle fibers. It follows that non-encapsulated larvae can be detected only in highly infected muscles by trichinoscopy. In the last 15 years, the number of T. pseudospiralis reports in wild animals of many countries has increased. The aim of this work was to explore if this increasing number of T. pseudospiralis reports was due to an increased prevalence of this parasite in wildlife or to the more widespread use of digestion tests.
MATERIALS AND METHODS:
All literature reports on T. pseudospiralis since 1972, and all the T. pseudospiralis isolates identified at the International Trichinella Reference Center of Rome (www.iss.it/site/Trichinella/index.asp), have been recorded by year of isolation, country of origin, and host species.
RESULTS:
Trichinella pseudospiralis has been detected as single species in 14 animals and in one person from 1972 to 2001, in 33 animals from 2002 to 2011, and in 44 animals from 2012 to 2014. Furthermore, this species has been detected in association with T. spiralis in 7 animals in the last 9 years. The isolates identified as T. pseudospiralis originate from 85 mammals, namely, 38 from wild boar, 22 from mountain lions, 8 from raccoon dogs, 3 from domestic pigs, 3 from red foxes, 3 from brown rats, 1 each from a tiger cat, lynx, domestic cat, badger, corsac fox, Indian mole-rat, American mink, human; and from 7 birds (rock, tawny eagle, black vulture, tawny owl, little owl, swamp harrier, and Australian Masked Owl). Trichinella pseudospiralis has been isolated from animals of Asia (India, Kazakhstan, Thailand), Europe (Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, the Netherlands, Poland, Serbia, Slovak Republic, Sweden, United Kingdom), North America (Canada and USA), and Australia (Tasmania).
CONCLUSIONS:
The correlation among the year of T. pseudospiralis isolate detection in the countries, the type of investigation (e.g., routine test, surveillance program, scientific investigation), and the diagnostic test used (digestion test versus trichinoscopy) in each country and time period, shows that the increasing reports of this Trichinella species is related to the more widespread use of digestion tests instead of trichinoscopy to search Trichinella larvae in wild animals, the increased expertise of analysts performing the test, and the validation of the tests in the laboratories. In fact, most of the new reports originate from countries of the European Union where digestion tests have totally replaced the trichinoscopy test according to the Commission regulation 2075/2005 since 2010.

Keywords:
Trichinella, diagnosis, wildlife

O01.5
GENETIC CHARACTERIZATION OF HUMAN AND PIG ASCARIS SPP. FROM ITALY

Cavallero S.*[1], Gambetta B.[1], Gabrielli S.[1], Dutto M.[2], Perrone V.[2], D'Amelio S.[1]

[1]Department of Public Health and Infectious Diseases ~ Rome ~ Italy, [2]Department of Prevention Local Health Unit ASL CN1 ~ Cuneo ~ Italy, [3]Asl Roma B ~ Italy

INTRODUCTION:
Ascariasis is a Neglected Tropical Disease of major public health concern in many parts of the world, causing also economic losses. Humans and pigs can be infected by two of the most common soil-transmitted helmiths: Ascaris lumbricoides Linneaus, 1758 and A. suum Goeze, 1782. Morphological and genetic similarities bring ambiguity about their taxonomic status. Several evidences about hybridization, gene flow and cross-infection have been demonstrated (Anderson, 2001, Trends Parasitol, 17:183; Peng & Criscione, 2012, Infect Genet Evol, 12:227; Galvin, 1968, J Parasitol, 54:1085).
Taxonomic uncertainty may cause epidemiological ambiguity limiting species identification and diagnostic resolution, with direct implications for development of control programs.
A. lumbricoides is the main source of human infection in highly endemic regions (China, Guatemala, Africa), while a zoonotic acquisition seems to be significant in non-endemic regions (Europe), although few studies are available.
The presence of A. suum and of putative hybrids between the two species have been reported in human infections in Italy (Cavallero S et al., 2013, PLOS NTD 7:e2170; Dutto & Petrosillo, 2013, Cent Eur J Public Health 21:224).
The aim of the present study is to characterize specimens of Ascaris spp. collected in humans and pigs from Italy in order to identify the etiological agent of human cases and to compare results with genotypes circulating in pigs, to update knowledge on molecular epidemiology of Ascariasis in non-endemic regions.
MATERIALS AND METHODS:
A total of 130 Ascaris spp. worms (8 from humans and 122 from pigs) were examined using PCR-RFLP approach on nuclear rDNA ITS (Zhu et al., 1999, Int J Parasitol, 29:469) to differentiate A.summ, A. lumbricoides and hybrid genotype. Sequence analysis of mtDNA cox1 were performed on a subsample to define the cluster affiliation (Anderson & Jaenike, 1997, Parasitology, 115:325; Snabel et al., 2012, Jpn J Infect Dis, 65:179) using parsimony network and Bayesian phylogenetic analyses.
RESULTS:
A total of 120/130 positive amplicons were obtained (7 from humans and 113 from pigs).
PCR- RFLP analysis revealed 57% of the human nematodes displayed A. suum pattern and 43% showed A. lumbricoides pattern. Most of the pig samples showed the typical A. suum pattern (86,7%), about 2% showed A. lumbricoides pattern and 11,5% of specimens were hybrids.
Phylogenetic analyses revealed that both human and pig samples belong to clade A and C. Comparing to results from ITS genotyping, no clear association between genotype and clade were observed.
CONCLUSIONS:
Although the low number of human cases recovered does not allow to derive conclusions, this study confirmed the role of pig as the main source of human infections in Italy. All Italian patients were infected by A. suum, indeed. No data on nationality were available for the patients showing A. lumbricoides. Samples from pigs showed A. suum as the most common genotype, and both cross-infection and gene-flow were confirmed by the presence of A. lumbricoides and of hybrid genotypes, respectively. Phylogeny revealed the inclusion of two samples of human origin in clade C for the first time. Previous studies reported solely specimens of pig origin in this clade.
These results confirms the need to revise taxonomic status of Ascaris spp., since hybrid genotype were observed in pigs and no fixed polymorphism was clearly attributable to A. suum or A. lumbricoides.

Keywords:
Ascariasis, hybrid genotype, taxonomic uncertainty

O01.6
DUPLEX-PCR FOR THE SIMULTANEOUS DETECTION AND DIFFERENTIATION BETWEEN TROGLOSTRONGYLUS BREVIOR AND AELUROSTRONGYLUS ABSTRUSUS

Annoscia G.*[3], Latrofa M.S.[3], Campbell B.E.[3], Giannelli A.[3], Ramos R.A.N.[3], Brianti E.[1], Dantas Torres F.[2], Otranto D.[3]

[1]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina, Polo Universitario Annunziata ~ Messina ~ Italy, [2] Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães, Recife, Brazil; Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [3]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy

INTRODUCTION:
Metastrongyloids of cats include a heterogeneous group of nematodes responsible for respiratory infestations. In particular, besides to Aelurostrongylus abstrusus (Strongylida: Angiostrongylidae), Troglostrongylus brevior (Strongylida: Crenosomatidae) is an agent of emerging concern of cats (Jefferies et al., 2010, Vet. Parasitol., 173:344–348; Brianti et al., 2012, Parasit. Vectors, 5:178). The identification of the above species, based solely on the morphology of first-stage larvae (L1), is often challenging due to the similarities of morphological characters. To date, no molecular methods for the simultaneous detection and differentiation of the above species have been standardized. This study describes a new tool, based on a single-step duplex polymerase chain reaction (duplex-PCR), of the ribosomal internal transcribed spacer 2 (ITS-2) region for the molecular detection and delineation of T. brevior and A. abstrusus.
MATERIALS AND METHODS:
Larvae (L1) of T. brevior and A. abstrusus were isolated in faecal samples from a 50 day-old female kitten, referred to the Department of Veterinary Medicine of the University of Bari. Parasitological examination was performed using Baermanns' technique (MAFF, 1986, Manual of Veterinary Parasitological Laboratory Techniques, 3rd ed. HMSO). Individual larvae were collected and morphologically identified (Euzeby, 1981, Informations techniques des Services vétérinaires, pp. 303-307; Gerichter, 1949, Parasitology, 39:251-262). An aliquot of faeces was used as a test sample for a case of mixed natural infestation. All samples were subjected to molecular analysis by duplex-PCR using two species-specific forward primers coupled with one reverse primer targeting the ITS-2 region.
RESULTS:
The duplex-PCR produced species-specific bands of the expected sizes for single larvae (i.e., 220 bp, A. abstrusus; 370 bp, T. brevior), and two bands for the faecal sample containing both species. No non-specific bands, or amplification products from the negative control sample, were detected. The sensitivity of the method was confirmed by detecting small amounts of genomic DNA for A. abstrusus (i.e., 5.2×10−3 µg/µL), for T. brevior (i.e., 4.9×10−3 µg/µL), and for the co-infected fecal sample (i.e., 4.0×10−3 µg/µL).
CONCLUSIONS:
This molecular approach proved to be effective and highly sensitive for the simultaneous detection of the two lungworm species. Consequently, the tool should be considered and implemented toward enhancing current knowledge of the epidemiology and control of feline metastrongyloids infestations, mainly in areas where these species are endemic and/or occur in sympatry (Jefferies et al., 2010, Vet. Parasitol., 173:344–348; Brianti et al., 2012, Parasit. Vectors, 5:178).

Keywords:
Duplex-PCR , Troglostrongylus brevior , Aelurostrongylus abstrusus

O01.7
STUDY OF AELUROSTRONGYLUS ABSTRUSUS IN A SPONTANEOUS INFECTION IN CATS

Viglietti A.[1], Fonti P.[2], De Francesco I.[3], Genchi C.[3], Piazza C.[1], Ferrari N.[3], **Genchi M.*[4]**

[1]Veterinary Clinic ~ Veterinary Clinic, Carloforte, Carbonia-Iglesias ~ Italy, [2]Centro Veterinario Specialistico ~ Roma ~ Italy, [3] Dipartimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di Milano ~ Milano ~ Italy, [4]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Ubertini" ~ Pavia ~ Italy

INTRODUCTION:
Aelurostrongylus abstrusus is a lungworm of the domestic and wild felids distributed worldwide. Prevalence rates of A. abstrusus in cats are often variable. Global prevalence rate varies from 50% free roaming cats in Albania (Knaus et al., Wien. Klin. Wochenschr., 123:31-35) to 1% in a mixed cat population in Spain (Miró et al., 2004, Vet. Parasitol., 126: 249-55). The clinical manifestations are highly variable. Radiographic findings are dependent on the age of animal, on the period of illness and the chronicity of infection. The aim of this study was to define the effects of the number of larvae in faeces (LPG) on respiratory clinical signs and their relation with radiographic findings.
MATERIALS AND METHODS:
196 cats, referred to the veterinary clinic for routine examinations, vaccinations, or different conditions and or hospitalization were included in the study. All cats come from the island of San Pietro, Sardinia, Italy. The history of each cat and clinical symptoms were recorded. For each cats, fecal samples were collected on 3 consecutive days and examined by Bearmann technique and for quantifying larva (LPG) by FLOTAC. Four age groups and 4 groups of severity of symptoms were formed: 1) 2-6 months; 2) 7-11 months; 3) 1-5 years; 4) > 6 years; 0) asymptomatic; 1) mild symptoms; 2) moderate symptoms; 3) severe symptoms. Thorax radiographs in double orthogonal position were performed and radiographs, were evaluated to giving a score depending on the severity of the lesions. All radiographs were reviewed blinded by 3 coauthors one clinician, one expert radiologist and one veterinary radiology from academy. Positive cats were treated with emodepside 2.1%/praziquantel 8.6% spot-on (Profender®, Bayer). After 15 and 28 days a fecal examination with FLOTAC was performed to assess the treatment efficacy.
RESULTS:
52 out of the 196 cats (26.5%) were positive for A. abstrusus at fecal examination. The prevalence in lifestyle was: indoor 3.3%; outdoor 32.3% and mixed 25.6%. In age groups was: group 1) 28.1%; group 2) 23%; group 3) 24.3%; group 4) 23%. Asymptomatic cats were 27%, 33% presented mild symptoms, 29% moderate symptoms and 11% severe symptoms. The number of L1 ranged from a minimum of 25 LPG to a maximum of 144800 LPG. Increasing numbers of larvae were associate with higher probabilities to develop high symptoms scores while cats age, their Rx patterns and their interactions did not show significant effects. After 15 days and 28 days post-treatment with emodepside 2.1%/praziquantel 8.6% spot-on (Profender®, Bayer), 29.4% and 4.3% cats were still positive, respectively.
CONCLUSIONS:
Our data show that all age groups were infected equally and increasing of numbers of LPG were associated with higher probabilities to develop more severe symptoms. Moreover infestations by A. abstrusus are poorly defined and prevalences were high even in asymptomatic cats.

Keywords:
Aelurostrongylus abstrusus, cats, thorax radiographs

O01.8
PAST OCCURRENCE OF THE FELINE LUNGWORMS AELUROSTRONGYLUS ABSTRUSUS AND TROGLOSTRONGYLUS SPP. IN ENDEMIC AREAS

Di Cesare A.[1], Di Francesco G.[2], Frangipane Di Regalbono A.[3], Eleni C.[4], De Liberato C.[4], Marruchella G.[2], Iorio R.[1], Malatesta D.[1], Romanucci M.R.[1], Bongiovanni L.[1], Cassini R.[3], **Traversa D.*[1]**

[1]Faculty of Veterinary Medicine ~ Teramo ~ Italy, [2]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise ~ Teramo ~ Italy, [3] Department of Animal Medicine, Production and Health ~ Padua ~ Italy, [4]Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana ~ Roma ~ Italy

INTRODUCTION:
The lungworm Aelurostrongylus abstrusus infects the respiratory system of domestic cats worldwide. Troglostrongylus brevior and Troglostrongylus subcrenatus, two lungworms previously thought to infect occasionally wild felids, have been recently found in cats from Spain and Italy (Traversa and Di Cesare, 2013, Trends Parasitol, 29, 423-430). These findings have raised realistic doubts on the actual occurrence of Troglostrongylus spp., especially T. brevior, in cats and on cases of missed detection or misdiagnosis (Otranto et al., 2013, Trends Parasitol, 29:517-518; Traversa, 2014, Trends Parasitol, 30, 53-54). The present retrospective work evaluated the presence of lungworms in cats from central Italy with a past diagnosis of lungworm infection or with compatible lung lesions in 2002-2013.
MATERIALS AND METHODS:
Sixty-eight samples of DNA and larvae from cats with a diagnosis of aelurostrongylosis and 53 paraffin-embedded lung samples from necropsied cats were undertaken to two DNA-based assays specific for A. abstrusus or T. brevior (Di Cesare et al., 2014, Parasitol Res, 113:613-618). The sequences obtained were compared with those of other metastrongylids available in the GenBankTM. The report of each necropsied cat was retrospectively analysed and examined for compatibility with a lung parasitosis, and compared with the genetic results.
RESULTS:
All DNA and larval samples were positive for A. abstrusus and only 1 also for T. brevior. The majority of paraffin-embedded lung tissues was positive only to A. abstrusus, while 2 samples tested positive for both lungworms and 1 only for T. brevior. In almost all cases the molecular results were consistent with the descriptions in the necropsy reports and with the localization of parasitic stages. The bronchi of a cat which was PCR-positive only for A. abstrusus contained adult stages morphometrically consistent with Troglostrongylus spp., while the necropsy report of a PCR-negative cat indicated the presence of ~255 □m long larvae. Such evidence suggests the presence of T. subcrenatus in these 2 cats.
CONCLUSIONS:
This study supports the major role of A. abstrusus in causing cat respiratory parasitoses in endemic areas of central Italy. The almost null information on the occurrence of Troglostrongylus spp. in cats until 2010 and the present data suggest that likely these nematodes have not been with a high frequency misdiagnosed or confused for A. abstrusus in past cases of lungworm infections in cats. Further studies are warranted to elucidate the existence of wild reservoirs for Troglostrongylus spp. and the possibility of bridging infections with domestic cats populations. Also, it is necessary to study further if and how the geographic dispersion of Troglostrongylus spp. will change and to understand their impact in respiratory parasitoses of domestic cats.

Keywords:
Cat, Lungworm, Epidemiology

O01.9
CLINICAL ANGIOSTRONGYLOSIS: DIAGNOSIS AND CONTROL IN A TERRIER KENNEL

Di Cesare A.*[1], Miotti C.[2], Venco L.[3], Pampurini F.[4], Centaro E.[5], Traversa D.[1]

[1]Faculty of Veterinary Medicine ~ Teramo ~ Italy, [2]Veterinary Practice "Dr. Carlo Miotti, Dr. Marco Miotti" ~ Gallicano ~ Italy, [3] Veterinary Hospital "Città di Pavia" ~ Pavia ~ Italy, [4]Bayer Sanità Animale ~ Milano ~ Italy, [5]Idexx Laboratories ~ Milano ~ Italy

INTRODUCTION:
Angiostrongylus vasorum is a globally distributed nematode that causes severe clinical signs in dogs. Despite the widespread distribution of canine angiostrongylosis and its clinical importance, this disease is often underestimated and neglected by vet practitioners, likely for the lack of information on the epidemiology of the nematode and for the drawbacks inherent to the clinical and copro-microscopic diagnosis (Traversa et al., 2010, Parasit Vectors, 3:62). Indeed, clinical signs of dog angiostrongylosis are aspecific and the Baermann's method, i.e. the gold standard technique for the aetiological diagnosis, is not commonly performed in clinical practice. The present study aimed at describing cases of asymptomatic angiostrongylosis in a breeding kennel of Italy, alongside the evaluation of the efficiency of a newly marketed rapid kit (IDEXX Angio DetectTM Test) for the field diagnosis of the disease before and after an anthelmintic treatment with a parasiticide formulation licensed for the treatment of A. vasorum infection.
MATERIALS AND METHODS:
The study has been carried out in a kennel of Jack Russell Terrier located in central Italy with a recent history of angiostrongylosis. At Day -15 all fifteen dogs bred in the kennel were clinically examined and subjected to a copromicroscopic examination and to the rapid kit Angio DetectTM Test. Animals (n. 3) which scored positive at least at one diagnostic assay were again tested with the Baermann's and Angio DetectTM Test at Day 0 and then treated with a spot on formulation containing moxidectin 2.5%/imidacloprid10% (Advocate®, Bayer). Two and four weeks post treatment the treated dogs scored negative at both tests.
RESULTS:
At Days -15 and 0 three dogs scored positive for A. vasorum L1s and two of them also at the rapid kit. Two and four weeks after the treatment with Advocate®, they scored negative at both tests. The animals were healthy throughout the study period.
CONCLUSIONS:
The present study confirms that A. vasorum is present in certain areas of Italy, where it can cause both asymptomatic and symptomatic infections (Traversa et al., 2013, Parasitol Res, 112:2473-2480). The absence of symptoms in the herein examined dogs underlines the need to periodically perform diagnostic tests in dogs living in endemic areas, regardless the presence of clinical signs compatible with the infection. The Angio DetectTM Test is easy-to-use, quick and presents good sensitivity (i.e. ~85%) and high specificity (i.e. ~100%) (Schnyder et al., 2014, Parasit Vectors, 7:72). The negativity of one dog at the kit although positive at the Baermann's test may be accounted for an early stage of the infection, as supported by the lack of symptoms. Considering the low number of positive dogs in the present study, further studies are necessary to evaluate more in depth the concordance between the two methods in the field. Finally, this trial confirms that Advocate® spot on is highly effective in treating dog angiostrongylosis (Willesen et al., 2007, Vet Parasitol, 147:258-264).

Keywords:
Angiostrongylus vasorum, Diagnosis, Treatment

O01.10
ONCHOCERCA LUPI: CUTANEOUS DISTRIBUTION AND CIRCADIAN RHYTHM OF MICROFILARIAE IN DOGS

Giannelli A.*[1], Dantas Torres F.[2], Abramo F.[3], Ignjatović Ćupina A.[4], Petric D.[4], Cardoso L.[7], Mutafchiev Y.[5], Cortes H.[6], Otranto D.[8]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Ba ~ Italy, [2]Department of Immunology, Aggeu Magalhães Research Institute ~ Recife ~ Brazil, [3]Dipartimento di Scienze Veterinarie, Università di Pisa ~ Pisa ~ Italy, [4]University of Novi Sad, Faculty of Agriculture ~ Novi Sad ~ Serbia, [5]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences ~ Sofia ~ Bulgaria, [6]Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora ~ Evora ~ Portugal, [7]Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD) and Instituto de Biologia Molecular e Celular, Universidade do Porto ~ Villareal and Oporto ~ Portugal, [8]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy

INTRODUCTION:
Onchocerca lupi (Spirurida, Onchocercidae) is a neglected canine filarioid of increasing zoonotic concern, which has been sporadically reported in clinically affected animals (wolves, dogs and cats) from Europe (Hungary, Greece, Germany and Portugal) and from the United States (Otranto et al., 2013, Parasit. Vectors, 6:16) as well as in humans in Turkey, Tunisia, Iran and the United States. In dogs, this spirurid may cause ocular alterations, ranging from any overt clinical conditions up to blindness, following the onset of subconjunctival granulomas encysting the adult worms (Sréter and Széll, 2008, Vet. Parasitology, 151:1-13). In order to enhance the knowledge on the biology of this filarioid, the distribution and periodicity of O. lupi microfilariae were assessed in naturally infested dogs.
MATERIALS AND METHODS:
Skin biopsies were performed on five dogs, which scored positive for O. lupi microfilariae but did not show an overt clinical presentation. In particular, samples were collected from 18 anatomical regions of a necropsied animal (dog 1), while single biopsies were performed from the forehead, inter-scapular and lumbar regions of five dogs (dogs 2-6) at different time points (i.e., at 10:00, 18:00, and 23:00). For each sample, two aliquots (20 mL each) of the sediment were used to prepare temporary mounts, covered by an 18x18 mm coverslip, which were observed under a light microscope. Microfilariae were counted and their identity morphologically and molecularly confirmed through microscopy and sequencing, respectively.
RESULTS:
Most of microfilariae retrieved from dog 1 were observed in the head (i.e., ears, nose, and forehead) and inter-scapular regions. Accordingly, the overall mean number of microfilariae in dogs 2-6 was larger on the head (n=122.8), followed by the inter-scapular (n = 119.0) and lumbar (n=12.8) regions and increased in the afternoon sampling (n=153.4), followed by night (n=75.4) and morning (n=25.8).
CONCLUSIONS:
Results indicate that O. lupi microfilariae tend to concentrate in specific body regions, being featured by a moderate circadian periodicity. This distribution pattern may be useful for diagnostic purposes. Indeed, veterinary practitioners living in endemic areas should be aware about the best body sites and period of the day for performing the skin biopsy. In addition, the pattern of microfilariae of O. lupi and their concentration in specific body regions is most likely a result of the co-evolution with the vector, whose identity is still a mystery.

Keywords:
Onchocerca lupi, Periodicity, Cutaneous distribution

O01.11
ABERRANT OCULAR INFECTION BY ANGIOSTRONGYLUS VASORUM IN A DOG

Lia R.P.*[1], Traversa D.[2], D'Anna N.[3], Giannelli A.[1], Dantas Torres F.[4], Otranto D.[1]

[1]Dipartimento di Medicina Veterinaria Università degli Studi di Bari ~ Bari ~ Italy, [2]Facoltà di Medicina Veterinaria Università degli Studi di Teramo ~ Teramo ~ Italy, [3]Clinica Veterinaria Roma Sud ~ Roma ~ Italy, [4]Department of Immunology, Aggeu Magalhães Research Centre ~ Recife ~ Brazil

INTRODUCTION:
Canine angiostrongylosis caused by Angiostrongylus vasorum (Strongylida: Angiostrongylidae) is an emerging mollusc-borne parasitosis causing cardio-respiratory signs and coagulopathies. The nematode resides in the pulmonary arteries and in the right side of the heart of dogs that become infected by the ingestion of gastropod intermediate hosts (i.e., slugs and snails). This helminthic disease is an emerging issue in several European countries, including Italy (Traversa et al., 2013, Parasitol Res, 112:2473-2480). Although A. vasorum may localize in other districts (i.e., spleen, pancreas, kidneys, liver, skeletal muscles, brain), aberrant ocular migrations have been reported only sporadically (Raillet & Henry, 1913, Bullettin de la Societe Centrale de Medicine Veterinarie, 67:209-215; Henry & Lesbouyries, 1927, Bullettin de la Societe Centrale de Medicine Veterinarie, 80:263-265; Rosenlund et al., 1993, Eur J. Compan Anim. Pract., 3:31-33; King et al., 1994, J. Small Anim. Pract., 35:326-328; Parry et al., 1994, Can. Vet. J., 32:430-431; Payen, 2004, International Veterinary Ophthalmology Meeting; Munich. p. 125; Manning, 2007, Vet Rec., 160:625-627). The present study describes an unusual case of ocular angiostrongylosis in a dog living in central Italy.
MATERIALS AND METHODS:
A 5 month-old mixed-breed dog was referred to a private veterinary clinic in Roma for an ophthalmological examination, during which corneal oedema and episcleral hyperaemia in the right eye was observed. After a diagnosis of anterior uveitis, the ultrasound examination of the eye showed a blood clot in the anterior chamber. Fifteen days after the visit, the hyphema disappeared but a free-swimming nematode was retrieved in the anterior chamber. The nematode was extracted surgically and morphologically and morphometrically identified (Costa et al., 2003, Revue Med. Vet., 154:9-16). Topical and systemic antibiotics and steroids were administered for the following 3 weeks and dog recovered without any complication.
RESULTS:
The nematode (105 mm in length and 0.29 mm in width at the middle portion; anterior and posterior ends of 0.109 mm and 0.176 mm in length, respectively) was identified as a male A. vasorum. Briefly, the nematode presented a smooth cuticle and a slender body, attenuated at the end and enlarged anteriorly where a small buccal aperture was observed. The posterior end was ventrally curved, with a short copulatory bursa. Spicules measured 0.404 mm and 0.388 mm in length.
CONCLUSIONS:
Several clinical signs have been associated to A. vasorum, but the presence of larvae and pre-adult stages within the eyes are an occasional finding. Ocular angiostrongylosis has been sporadically described in dogs from France, United Kingdom, Denmark and Canada. This is the first report of A. vasorum in the eye of a dog from Italy. Practitioners working in areas where A. vasorum is endemic should include canine angiostrongylosis not only in the differential diagnosis of canine cardio-respiratory diseases but also of ocular diseases.

Keywords:
Angiostrongylus vasorum, dog, ocular infection

O01.12
ASSESSMENT OF SOME POTENTIAL RISK FACTORS FOR FASCIOLA HEPATICA INFECTION IN GOATS BY USING A CAPTURE ELISA

Pérez A.[1], Díaz P.[1], López C.[1], Martínez Sernández V.[2], Panadero R.[1], Ubeira F.[2], Morrondo P.[1], **Díez Baños P.*[1]**

[1]University of Santiago de Compostela ~ Lugo ~ Spain, [2]University of Santiago de Compostela ~ Santiago de Compostela ~ Spain

INTRODUCTION:
Fasciolosis is one of the most important pathogenic helminth diseases worldwide, affecting all important ruminant livestock species. F. hepatica is responsible for important production losses, shown mainly as decreases in milk and meat production and reduced fertility. The main objective of the present work is to evaluate both the seroprevalence of F. hepatica in goats from Galicia (N.W. Spain) and the effect of some potential risk factors.
MATERIALS AND METHODS:
Blood samples were collected from 556 goats in 47 Galician (NW Spain) flocks. Serum samples were analyzed for antibodies against the trematode using a capture ELISA (MM3-SERO), based on the use of the monoclonal antibody MM3 (Mezo et al., 2007, J. Parasitol., 93:65-72). The effect of some extrinsic (climatic areas) and intrinsic (sex, breed and age) factors on the infection by F. hepatica was assessed by means of Chi-square and classification tree (exhaustive CHAID) tests.
RESULTS:
A high presence of F. hepatica was detected in goats from Galicia, since 19.4% of animals and 53.2% of the sampled flocks were positive. Animals from the coast-central area showed a higher seroprevalence (22.2%) than those from the mountain area (12.8%); these differences were significant (X2= 6.560; p= 0.038). The warmer and more humid conditions of the coast-centre area seem to be more suitable for both the development of the amphibian snails acting as intermediate hosts (Galba truncatula) and the survival of the external stages of the trematode.
Cross-breed goats (25.2%) showed a significantly higher seroprevalence (X2=24.162; p< 0.001) than "Cabra Galega" breed goats (7.7%). Several studies have reported some level of resistance to Fasciola in indigenous ruminant breeds, often seen by reduced worm burdens or reductions in egg output following challenge (Roberts et al., 1997, Vet. Parasitol., 68: 69–78; Wiedosari and Copeman, 2006, Trop. Anim. Health Prod., 38: 43–53).
The lowest seroprevalence was detected in young goats (14.5%) whereas higher percentages of infection were detected in those animals between 2-5 years (22.4%) and older than 5 years (20.3%), probably due to their more extensive contact with infective metacercariae; nevertheless, these differences were not significant (p> 0.05). Sex was also not a risk factor for acquiring the infection by F. hepatica, since the seroprevalences were very similar in both males (18.8%) and females (19.5%).
The CHAID algorithm indicated that the breed was the most important determining factor for seroprevalence at individual level.
CONCLUSIONS:
1. F. hepatica is a very prevalent parasite in goats from Galicia.
2. "Cabra Galega" goat breed seem to be more resistant to F. hepatica infection than cross-breed.
3. Sex and age are not potential risk factors for acquiring the infection with F. hepatica.

Acknowledgements: This work was supported by a grant for Consolidating and Structuring Competitive Research Groups (CN2012/326, Xunta de Galicia) and grant AGL2011-30563-C03 (Ministerio de Ciencia e Innovación, Spain).

Keywords:
Fasciola hepatica, goats, NW Spain

O01.13
POOLING FAECAL SAMPLES IN SHEEP FOR THE ASSESSMENT OF GASTRO-INTESTINAL STRONGYLE INFECTION INTENSITY AND ANTHELMINTIC DRUG EFFICACY USING MCMASTER AND MINI-FLOTAC

Rinaldi L.*[1], Vercruysse J.[2], Bosco A.[3], Levecke B.[2], Ianniello D.[3], Pepe P.[3], Charlier J.[2], Cringoli G.[1]

[1]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania); Inter-University Center for Research in Parasitology (CIRPAR) ~ Naples ~ Italy, [2]Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University ~ Merelbeke ~ Belgium, [3]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania) ~ Naples ~ Italy

INTRODUCTION:
A field study was conducted to validate pooled faecal samples in sheep for the assessment of gastro-intestinal (GI) strongyle infection intensity (faecal egg count - FEC) and anthelmintic drug efficacy (FEC reduction - FECR).
MATERIALS AND METHODS:
Ten sheep farms located in the Campania region of southern Italy were selected for the study. In each farm, individual faecal samples from 20 adult sheep (when possible) were collected, before (D0) and after (D14) an anthelmintic treatment with albendazole. For each farm and at each time point (D0 and D14) the faecal samples were examined individually and as pools. Specifically, three different pool sizes (5, 10 and 20 individual sheep samples) and three different analytic sensitivities (namely 10 eggs per gram of faeces (EPG) using Mini-FLOTAC; 15 EPG and 50 EPG using the two variants of McMaster - McM15 and McM50) were compared for FEC and FECR using individual and pooled faecal samples.
RESULTS:
GI strongyle intensity (EPG) of pooled samples correlated positively with mean EPG of individual samples, with very high correlation coefficients (ranging from 0.94 to 0.99) across the 3 different pool sizes and analytic sensitivities. Mini-FLOTAC was more sensitive compared to the two variants of McMaster (McM15 and McM50) for the diagnosis of GI strongyles in sheep (100% vs 88.5% vs 75.9%) and resulted in significant higher FEC compared to both McM15 and McM50, with a mean difference in egg counts of approximately 90 EPG ($p<0.001$). The drug efficacy results showed that FECR was higher than 98% at most farms independently of the pool size and analytic sensitivity. With the exception of two farms, FECR was 100% when calculated for individual animals and across the different pool size and analytic sensitivities.
CONCLUSIONS:
In conclusion, the present study highlighted that pooling ovine faecal samples is a rapid procedure that holds promise as a valid strategy for assessing GI strongyles FEC and FECR in sheep as already proposed in simulation-based studies (e.g. Morgan et al., 2005, Vet. Parasitol., 131: 79-87).

Acknowledgements: The European Union Seventh Framework Programme FP7 –KBBE-2011-5 GLOWORM (no. 288975) are sincerely thanked.

Keywords:
pool, Mini-FLOTAC, McMaster

O01.14
PREVALENCE AND RISK FACTORS OF GASTROINTESTINAL PARASITES IN CALVES FROM CATTLE HERDS OF TUSCANY

Matia M.[1], Stefanelli S.[3], Banchi A.[2], **Perrucci S.*[1]**

[1]Dipartimento di Scienze Veterinarie-University of Pisa ~ Pisa ~ Italy, [2]Freelance Sociologist ~ Pisa ~ Italy, [3]Istituto Zooprofilattico delle Regioni Lazio e Toscana ~ Pisa ~ Italy

INTRODUCTION:
Gastrointestinal parasites are prevalent among calves and may be responsible for diarrhoea, growth reductions and death. The main objectives of the present study were to evaluate the prevalence of gastrointestinal parasitic infections in calves from cattle herds of Tuscany and to identify possible risk factors. The evaluation of possible relationships between diarrhoea/parasites/identified risk factors represented a further important objective of the study.
MATERIALS AND METHODS:
In the period between January 2010 and May 2012, 157 calves aged between 1 day and about 6 months and reared in beef (n=7) and dairy (n=4) cattle herds, including conventional and organic farms, were examined for gastrointestinal parasites. From all examined calves individual fecal samples were collected, scored as diarrhoeal (faeces of decreased consistency or liquid) or normal, stored at 4°C and quali-quantitatively analysed within 24 hours by flotation test, a McMaster method and by the examination of modified Ziehl-Neelsen stained faecal smears. For the detection of Cryptosporidium spp. and Giardia duodenalis faecal antigens, a commercial rapid immunoassay was used. For the identification of isolated Eimeria at the species level, positive faecal samples were suspended in 2.5% potassium dichromate ($K2Cr2O7$) in Petri dishes at 20°C ± 1°C until the sporulation of the oocysts that were morphometrically evaluated. Obtained data were statistically analysed by univariate analysis using SPSS 16.0 (SPSS © inc.1989-2007) and by the logistic regression analysis (multivariate analysis) by using the program STATA version 12.0 (StataCorp © LP 1996-2012). The following five categories of risk factors were investigated: I) age (in days, calves were divided into four age classes: 0-30, 31-90, 91-180, or >181 days); II) breed (Italian Friesian, Chianina, Limousine, Romagnola, Pisana); III) farm management (indoor, outdoor), IV) type of farms (conventional, organic); V) season (winter, spring, summer, autumn).
RESULTS:
Overall, a prevalence of 58.6% was found in examined calves. Gastrointestinal strongyles (28.7%), including Nematodirus spp. (8.9%), Strongyloides papillosus (10.8%), Eimeria spp. (30.6%), Cryptosporidium spp. (8.3%), Giardia duodenalis (2.5%), Balantidium coli (1.9%), Buxtonella sulcata (0.6%) and Moniezia benedeni (0.6%), were isolated. Among coccidia, Eimeria bovis was present in all coccidia-positive herds. Eimeria zuerni, Eimeria ellipsoidalis and Eimeria auburnensis, were also isolated. From univariate statistical analysis, calves from 0 to 30 days and from 91 to 180 days resulted more frequently infected ($p<0.05$). In particular, Cryptosporidium and Giardia were prevalent in 0-30 days age group ($p<0.01$), while coccidia and gastrointestinal strongyles were prevalent in 91-180 days age group ($p<0.01$; $p<0.05$). Coccidia were significantly more prevalent among diarrhoeic calves ($p<0.05$). Among the most significant results of multivariate analysis, in organic farms a higher risk of Eimeria and gastrointestinal strongyles infections and of diarrhoea resulted.
CONCLUSIONS:
Results obtained in this study show that gastrointestinal parasitic infections are prevalent in examined calves and that coccidiosis may represent a significant cause of diarrhoea in animals between approximately 3 and 6 months of age. These findings indicate the need for the control of gastrointestinal parasites in calves from Tuscany, while identified risk factors may be useful to formulate appropriate control strategies.

Keywords:
calf, gastrointestinal parasites, prevalence

O01.15
ARE SMALL STRONGYLES (CYATHOSTOMINAE) INVOLVED IN HORSE COLIC OCCURRENCE?

Stancampiano L.*[1], Marigo A.[1], Spadari A.[1], Rinnovati R.[1]
[1]Dipartimento di Scienze Mediche Veterinarie - Università di Bologna ~ Ozzano dell'Emilia (Bologna) ~ Italy

INTRODUCTION:
Intestinal parasites have been traditionally regarded as a possible cause of horse colic (Love, 1992, Equine Vet. J., 24:5-9); however, the evidence of small strongyles causing intestinal disorders has never been clearly demonstrated and the evidence of parasites causing colic in horse was recently questioned (Uhlinger, 2007, Vet. Clin. North Am. Equine Pract., 23:509-517).
Aim of the study was the parasitological comparison between subjects with or without colic syndrome, with particular regard to small strongyles infections.
MATERIALS AND METHODS:
Quali-quantitative coprological analysis were performed on 86 horses referred to the Department of Veterinary Medical Sciences: 43 horses with medical or surgical colic (22 and 21 respectively) and 43 horses referred for different reasons (controls). Coprocolture was performed on positive samples to differentiate small strongyles and S. vulgaris infections. Data were analysed using STATA 11.2 using negative binomial regression.
RESULTS:
Strongyle egg prevalence was 39.5%, the mean number of eggs per gram of faeces (epg) was 145.34. Four horses (4.6%) had S. vulgaris infection.
Negative binomial multiple regression highlighted no influence of horse sex on epg, while there was a negative relationship between age (in month) and epg; the same analysis revealed a significant difference of epg ($p<0.05$) between control horses (mean epg=178.1) and horses with surgical colic (mean epg=68.6) when controlling for S. vulgaris prevalence including it in the model. Medical colic (mean epg=154.5) did not significantly differ from controls.
CONCLUSIONS:
The unexpected negative relationship between cyathostomine infection and the severity of colic syndrome raises questions about the role of these parasites in intestinal damage and occurrence of colic in horses. It is possible that the presence of adult luminal parasites, inhibiting mucosal larvae (Eysker et al., 1989, Vet. Parasitol., 34:87-93), would have a protective effect against the pathogenic action exerted by the development and emergence of small strongyles larvae from intestinal mucosa.

Keywords:
horse, Cyathostominae, colic

NOTES

NOTES

SESSIONE PARALLELA O-02

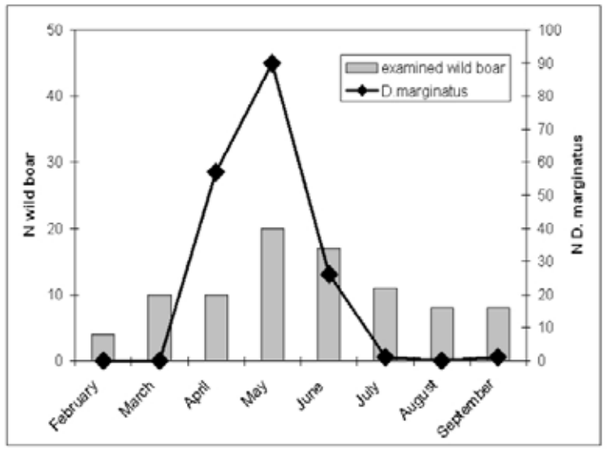
PARASSITOSI TRASMESSE DA ARTROPODI

O02.1
DERMACENTOR MARGINATUS TICKS AND ASSOCIATED PATHOGENS IN WILD BOAR POPULATION OF COLLI EUGANEI AREA, NORTHERN ITALY

Cassini R.*[1], Drigo M.[1], Signorini M.[1], Mondin A.[1], Tessarin C.[1], Da Rold G.[2], Pizzocaro M.L.[3], Gallo M.[3], Martini M.[1]

[1]Dipartimento MAPS - Università di Padova ~ Legnaro (PD) ~ Italy, [2]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [3]Parco Regionale dei Colli Euganei ~ Este (PD) ~ Italy

INTRODUCTION:
In the Colli Euganei regional park (NE Italy), an allochthonous population of wild boars ranges over the whole hilly territory, despite the on-going campaign to reduce its size. A potential role of wild boar as reservoir of tick-borne pathogens and of the tick Dermacentor marginatus as vector of tick-borne lymphadenopathy (TIBOLA), an emerging zoonosis, has been suggested by several Authors, based on surveys conducted in Italy and Spain. The aim of this study was to investigate the tick species associated with the wild-boar population of the study area and their capacity to carry pathogens potentially dangerous for humans and domestic animals.
MATERIALS AND METHODS:
Adult ticks attached to wild boars caught from February to September 2010 were collected, identified morphologically and stored in 70% ethanol until DNA extraction. Sex and estimated age of all sampled animals were recorded.
Differences in tick prevalence and mean infestation intensity (N ticks/N positive hosts) according to sex and age class (0-12 months; >12 months) of wild boars were assessed using Pearson's chi-square test, and Mann-Whitney U test, respectively.
DNA samples extracted from ticks using NucleoSpin® Tissue (Macheray-Nagel, Düren, Germany) were screened by conventional PCR for the presence of piroplasms (Cassini et al., 2012, Vet Par 184: 77-82) and Rickettsia spp. (Regnery et al., 1991, J Bacteriol., 173: 1576-89). A multiplex realtime PCR was used to detect A. phagocytophilum and B. burgdorferi s.l. (Courtney et al., 2004, J Clin Microbiol, 42: 3164-8). Both strands of amplicons obtained from positive samples were sequenced using Big Dye Cycle Sequencing kit on 3100 Genetic Analyzer (Applied Biosystems). The consensus sequences were obtained using ChromasPro (Version: 1.42).
RESULTS:
Out of 88 sampled wild boars, adult Dermacentor marginatum ticks (n=175; 77 females and 98 males) were collected from 31 (35.2%) animals, and Ixodes ricinus ticks (n=9; all females) from 7 (8.0%). The burden of D. marginatum ranged from 1 to 23 ticks, with an overall mean intensity of 5.6. The occurence of this tick species was concentrated from April to June, with a peak in May (Fig. 1). Young animals show higher prevalence (48.1%) than older animals (29.5%), whereas intensity is higher in males (7.3), than in females (3.8), although differences were not statistically significant. Totally, 169 D. marginatus and all nine I. ricinus ticks were individually analysed by PCR. Forty-two D. marginatus ticks (24.9%) resulted positive to R. slovaca, and four (2.4%) to Hepatozoon spp.. Among I. ricinus, one tick (11.1%) was positive to Rickettsia monacensis, and one to R. slovaca. No ticks resulted positive to B. burgdorferi s.l. or A. phagocytophilum.



CONCLUSIONS:
The main finding of the study is the unexpected high prevalence of R. slovaca, the aetiological agent of TIBOLA, especially in D. marginatus ticks, suggesting to clinicians to be aware of the possible presence of this emerging pathogen. D. marginatus, which is clearly dominant among adult ticks feeding on the wild boar population, shows a clear seasonality and infests the whole host population, with slight differences according to age and gender. At present, no information is available on seasonality and abundance of larval stages, which they may play an important role in pathogens transmission to humans.

Keywords:
ticks, wild boar, tick-borne pathogens

O02.2
RHIPICEPHALUS SANGUINEUS GROUP AS VECTOR OF PATHOGENS THROUGHOUT THE WORLD

Latrofa M.S.*[2], Dantas Torres F.[1], Giannelli A.[2], Otranto D.[2]

[1]Department of Immunology, Centro de Pesquisas Aggeu Magalhães, Recife, Brazil; Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [2]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy

INTRODUCTION:
The Rhipicephalus sanguineus group comprises a large number of tick species, some of which act as vectors of several pathogens of medical and veterinary relevance (Dantas-Torres et al., 2012, Trends Parasitol., 28:437–446). Due to their morphological similarities, the identification of species within this group may be a difficult task, as recently suggested by the morphological and molecular identification of Rhipicephalus sanguineus sensu lato (s.l.) and three different operational taxonomic units (OTUs), defined Rhipicephalus sp. I-III (Dantas-Torres et al., 2013, Parasit. Vectors, 6:213). Interestingly, no investigation has yet been conducted to assess the role of the above OTUs in transmitting pathogens. Thus, this study aimed to detect and molecularly characterize some CVBD causing pathogens (i.e., Anaplasma platys, Cercopithifilaria spp., Ehrlichia canis and Hepatozoon canis) in ticks belonging to the R. sanguineus group.
MATERIALS AND METHODS:
A total of 206 tick specimens were collected from dogs in different geographical areas of Europe, Asia, Australia, Africa and South America. Some of them (n = 138) were morphologically and molecularly identified in a previous study (Dantas-Torres et al., 2013, Parasit. Vectors, 6:213), whereas, additional tick specimens (n = 68) were subjected to molecular characterization by the amplification and sequencing of partial mitochondrial 16S rDNA (~300 bp) and cytochrome c oxidase subunit 1 (cox1; ~600 bp) genes, using protocols previously described (Dantas-Torres et al., 2013, Parasit. Vectors, 6:213). Specific amplification was performed to detect the DNA of pathogens using primer sets targeting on partial cox1 (300 bp) and 18S rDNA (670 bp) of Cercopithifilaria spp. and H. canis, respectively, and 16S rDNA gene of both A. platys (380 bp) and E. canis (390 bp) (Inokuma et al., 2002, Vet. Parasitol., 106:265–271; Otranto et al., Parasit. Vectors, 5:1; Santos et al., 2009, Vet. J., 179:145–148).
RESULTS:
Ticks were molecularly identified as R. sanguineus s.l. (n=9), R. turanicus (n=3), Rhipicephalus sp. I (n=42) and Rhipicephalus sp. II (n = 14). Out of 206 specimens tested, 5 (2.4%), 15 (7.2%) and 44 (21.3%) scored positive for A. platys (i.e., R. sanguineus s.l., Rhipicephalus sp. I), H. canis (i.e., Rhipicephalus sp. I-III, R. turanicus) and Cercopithifilaria spp., respectively. Co-infections with two pathogens (i.e., Cercopithifilaria bainae and H. canis or A. platys) were detected in four specimens.
CONCLUSIONS:
The potential vector role of R. sanguineus group ticks in the transmission of A. platys, H. canis, and Cercopithifilaria spp. is herein assessed in relation to their geographical provenance. In the same way our results confirm the global distribution of the pathogens examined, with Cercopithifilaria spp. being the most widespread, followed by H. canis and A. platys.

Keywords:
Rhipicephalus sanguineus group, phatogens, molecular identification

O02.3
DEVELOPMENT OF A SURVEILLANCE SYSTEM ON TICKS AND TICK-BORNE ZOONOSES IN PIEDMONT

Pintore M.D.*[1], Tomassone L.[2], Ceballos L.[2], Pautasso A.[1], Francese D.R.[1], Bardelli M.[3], Rizzo F.[1], Mandola M.L.[1], Peletto S.[1], Torina A.[4], Casalone C.[1], Mannelli A.[2], Iulini B.[1]

[1]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta ~ Turin ~ Italy, [2]Department of Veterinary Sciencies, University of Turin ~ Turin ~ Italy, [3]ASL VCO ~ Verbania ~ Italy, [4]Istituto Zooprofilattico Sperimentale della Sicilia ~ Palermo ~ Italy

INTRODUCTION:
Ticks can transmit infections involving both humans and animals. Ixodes is the largest genus of hard ticks with 241 species (Jongejan et al., 2004, Parasitology, 129: S3-S14.). It comprises three-host ticks that are widely distributed in Europe. The most important species is Ixodes ricinus. This tick feeds on a great variety of hosts and is an important vector of tick-borne zoonoses (TBZ) (Rizzoli et al., 2004, Parassitologia, 46: 119-122). TBZ have increased in Europe over the last decade and human risk is linked to the density of questing ticks, in particular to the nymphs density. Twelve clinical cases of borreliosis were officially diagnosed since 2007 in Verbano-Cusio-Ossola (VCO) Province. The following study was performed to evaluate the tick abundance in VCO province and regional Parks of Mandria and Stupinigi (Torino province), and to investigate pathogen prevalence in questing ticks and ticks from bitten patients.
MATERIALS AND METHODS:
In the VCO province, 24 sites located at different altitude were selected and questing ticks were monthly collected by dragging from April to September 2011-2013. Sites were selected in the territory of the municipalities of Cannobio, Calasca-Castiglione, Cavandone, Beura-Cardezza, Crodo and Varzo. Study sites were chosen based on previous reports of human cases of borreliosis in the province. In addition, 15 sites were selected in the Mandria and Stupinigi regional Parks. Ticks collected from bitten humans in different areas in Piedmont were also studied. Ticks were identified and a sample was subjected to molecular analysis for the detection of pathogens. A PCR protocol targeting for the 5S-23S intergenic spacer region of Borrelia burgdorferi sl was applied; for Rickettsia spp., a PCR specific for the genes gtlA and OmpA and for Anaplasma spp an assay targeting the 16S rRNA gene. For the search of Tick Borne Encephalitis virus (TBEV) RNA, a Real time PCR specific for a portion of the 3' NC region was carried out.
RESULTS:
Preliminary results show a total of 4434 collected ticks (3192 in VCO province, 908 in the Regional Parks and 334 from man). In VCO all ticks were identified as I. ricinus, and most of them were found at an altitude between 1000 and 1200 m above sea level. Three genera of ticks were collected in the regional Parks (75.6% Ixodes, 24% Haemaphysalis and 0.4% Dermacentor) and from humans (78.8% Ixodes, 0.9% Rhipicephalus and 0.3% Dermacentor). Preliminary tests targeting Borrelia burgdorferi sl, Rickettsia spp, Anaplasma spp show an infection prevalence in questing ticks of 10.4%, 4.3% and 1.5% respectively, and of 4.5%, 16% and 3.4% respectively in ticks from humans. No TBEV was found. Borrelia positive samples were sequenced, and four genospecies were found: B. afzelii, B. garinii, B. valaisiana and B. lusitaniae. Finally, phylogenetic analysis based on the OspC gene showed that most of the Borrelia strains from pathogenic genospecies might have the potential for human infection and for secondary invasion.
CONCLUSIONS:
TBZ in humans are correlated with local tick abundance, density of vertebrate reservoir hosts, climate changes and ticks infection prevalence. Analyses of these factors can help assess risks and guide the implementation of public health policies against TBZ.

Keywords:
Piedmont, ticks, zoonoses

O02.4
DEVELOPMENT OF NOVEL SEROLOGICAL TOOLS FOR DIAGNOSIS OF IXODES RICINUS BITE

Sassera D.*[1], Plantard O.[2], Bardoni A.[1], Fumagalli M.[1], Epis S.[3], Mariconti M.[4], Salvini R.[1], Viglio S.[1], Bazzocchi C.[3], Bandi C.[3]

[1]Universita' degli Studi di Pavia ~ Pavia ~ Italy, [2]UMR INRA-Oniris 1300 Biologie, Epidémiologie, Analyse de Risque en santé animale ~ Nantes ~ France, [3]Universita' degli Studi di Milano ~ Milano ~ Italy, [4]Fondazione IRCCS Policlinico S. Matteo ~ Pavia ~ Italy

INTRODUCTION:
Ixodes ricinus is the most widespread tick in Europe, responsible, through the bite, of the transmission of various pathogens. Tick bites often go unnoticed due to the absence of immediate symptoms. A quick and reliable method to detect tick bites would be an invaluable tool to help understanding the epidemiology of the tick bite, as well as to investigate potential correlations between tick bite and insurgence of chronic pathologies. I. ricinus harbors the symbiont Midichloria mitochondrii, present in 100% females, both in the ovaries and in the salivary glands. Our results indicate transmission of M. mitochondrii to the mammalian hosts of the ticks. Identification of I. ricinus and M. mitochondrii antigens would allow to devise novel serological tests to detect tick bites.
MATERIALS AND METHODS:
By sequencing the genome of M. mitochondrii we were able to identify a protein with potential antigenic activity, the flagellar FLID. We expressed FLID in recombinant form and obtained polyclonal antibodies against it. We are currently performing immunoproteomic experiments to detect additional I. ricinus and M. mitochondrii antigens. After 2D-electrophoresis of Midichloria-infected and uninfected ticks we will perform immunoblots with sera of experimental hosts infested with Midichloria-infected and uninfected ticks. In parallel, we are collecting sera from tick-bitten animals and humans, as well as negative controls and sera from human patients affected by chronic pathologies. After recombinant proteins expression, ELISA tests will be used to screen for the presence of specific antibodies in the sera of mammalians potentially exposed to tick bite.
RESULTS:
The work is in progress but we already achieved various results: i) expression of a recombinant protein of Midichloria; ii) development of an ELISA to screen mammalian sera for the presence of anti-rFLID antibodies; iii) screening of a set of sera from tick exposed humans and other animals.
Additional expected results, that will be obtained during the course of the project are: i) proteomic characterization of I. ricinus salivary glands and ovaries, in presence and absence of Midichloria ii) identification and expression of additional antigens of I. ricinus and Midichloria, iii) collection of sera from tick-bitten animals and human, controls and patients with chronic degenerative pathologies; iv) evaluation of the developed antigenic markers to obtain the best test for tick bite; v) serological screening of the collected sera, to evaluate the epidemiological importance of tick bite and the possible correlation between tick bite and insurgence of chronic degenerative pathologies.
CONCLUSIONS:
The final goal of our project is to obtain a serological test for the detection of the tick bite. In order to do so we are implementing a multidisciplinary approach to identify antigens from the tick and the symbiont, to use them as serological markers and to test them on various sera samples, from experimental hosts to human patients. The fulfillment of our objectives will allow to obtain a test useful for the epidemiology of tick bite and to evaluate potential correlations between tick bite and chronic pathologies.

Keywords:
Ixodes ricinus, symbiosis, tick bite diagnosis

O02.5
FIRST DETECTION OF BABESIA MICROTI IN PEOPLE LIVING IN THE PLURINATIONAL STATE OF BOLIVIA

Gabrielli S.*[1], Macchioni F.[2], Totino V.[1], Suniga F.[3], Rojas P.[3], Lara Y.[4], Roselli M.[5], Bartoloni A.[5], Cancrini G.[1]

[1]Università Sapienza ~ Roma ~ Italy, [2]Università di Pisa ~ Pisa ~ Italy, [3]Distrito de Salud Cordillera ~ Santa Cruz ~ Bolivia, Plurinational State of, [4]Hospital S. Antonio de Los Sauces ~ Monteagudo ~ Bolivia, Plurinational State of, [5]Dipartimento di Medicina Sperimentale e Clinica ~ Firenze ~ Italy

INTRODUCTION:
Tick-transmitted hemoparasites of the genus Babesia (phylum Apicomplexa) show a worldwide distribution and have a wide spectrum of vertebrate hosts that includes, occasionally, humans (Gray et al, Ticks Tick Borne Dis, 2010, 1:3–10). Indeed, the cattle species B. divergens (and B. venatorum) in Europe and the rodent species B. microti (and B. duncani) in North America have been shown as responsible for most human infections. Babesiosis is a malaria-like illness that ranges from asymptomatic infection to an even fatal disease, associated to hemolytic anemia and severe systemic complications (Vannier et al., N Engl J Med, 2012, 366:2397–407). B. microti infection is generally mild, self-limiting or asymptomatic, but a fatal outcome may be occasionally observed. Deer, heavily infested by vector ticks that -at larval stage- feed on rodents, are important in the epidemiology of the infection (Telford et al., J Med Entomol, 1993, 30:223-7). In Bolivia, only B bovis and B. bigemina have been investigated in cattle and seem to be stably endemic in the Eastern part of the country (Carrique et al., Veterinary J, 2000, 160:162–4). No data are available about the presence of B. microti.
MATERIALS AND METHODS:
Research aims to investigate on human babesiosis in two rural communities of Southeastern Bolivia (Ivamirapinta and Bartolo), as part of a screening on hematic parasites carried out in 2013. In the study area, economy is based on agriculture and animal farming, and people live in close contact with pets and wild animals (deer included).
Blood samples were taken from 273 healthy subjects (1-83 years-old) and used to arrange thick and thin smears, to extract DNAs, and to obtain sera. Smears were Giemsa stained and examined by microscopy. DNAs were amplified by PCR using a pair of generic apicomplexan 18S rRNA-specific primers. Positive amplicons were confirmed by sequencing. Several sera (from infected people and from older components of some families) were submitted to an IFI test available to detect B. microti infection in humans (B. microti-IFA IgG Antibody kit, Fuller Laboratories, Fullerton, CA).
RESULTS:
A total of 10 (3.7%) thin smears were positive to B. microti. Morphological diagnosis was confirmed by sequencing. As expected, preliminary serological results evidenced reactivity to B. microti antigens not only in all positive subjects (at >1:128 dilutions) but also in further 4/43 subjects negative to microscopy and PCR (at dilutions 1:64 or 1:128).
CONCLUSIONS:
This is the first record of human babesiosis in the Plurinational State of Bolivia, in areas where people is exposed to many other parasites that can induce severe anemia. More extensive studies are needed to improve knowledge about all zoonotic babesial species, and people should be alerted about sanitary risks originated by ticks. Finally, physicians should be aware of the presence of B. microti, and well trained on clinical features of babesiosis in order to intervene, when needed, with appropriate treatment.

Keywords:
Babesia microti, Humans, Bolivia

O02.6
MOLECULAR AND SEROLOGICAL DETECTION OF TICK-BORNE PATHOGENS IN DONKEYS (EQUUS ASINUS) IN CENTRAL ITALY

Morganti G.*[1], Ravagnan S.[2], Veronesi F.[1], Capelli G.[2], Giusepponi V.[3], Diaferia M.[1], Moretti A.[1], Piergili Fioretti D.[1]

[1]Department of Veterinary Medicine ~ Perugia ~ Italy, [2]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [3]DVM ~ Perugia ~ Italy

INTRODUCTION:
Donkeys, due to the frequent outdoor activity, are exposed to high risk of tick bites and, consequently, of infection with tick borne pathogens (TBPs). The present work is aimed to detect infection or exposure of donkeys reared in Central Italy to Theileria equi, Babesia caballi, Anaplasma phagocytophilum and Borrelia burgdorferi using molecular and serological approaches.
MATERIALS AND METHODS:
From April to September 2013, 122 adult donkeys were selected to participate to the cross-sectional survey. The animals originated from 9 herds located in areas covering parts of four Central Italy provinces, in which heavy tick infestations were frequently reported by veterinary practitioners. The sampling population was based on statistical criteria and stratified according to the proportion of donkeys in each herd. Blood samples were collected from the selected animals; IgG antibodies to T. equi, B. caballi and A. phagocytophilum were detected by IFAT, using commercial kits (MegaScreen®) whereas for the B. burgdorferi antibody detection a rapid ELISA (SNAP® 4Dx test kit) was performed. DNA was extracted from EDTA-blood samples using the QIAamp DNA Blood Mini kit (QIAGEN) and two conventional PCRs targeting the gene MSP2 (334bp) and the flagellin gene (482bp) were used for the detection of A. phagocytophilum (Massung RF & Slater KG, 2003. J Clin Microbiol, 41: 717-722) and B. burgdorferi s.l. (Skotarczak B et al., 2002, Ann Agric Environ Med, 9: 25-28) respectively. A Real Time PCR Sybr Green was used to detect Babesia/Theileria spp. using the primer BJ1 and BN2 (Casati S et al., 2006. Ann Agric Environ Med, 13: 65-70) and the species identity was determined by subsequent amplicons sequencing. All sequences generated were compared to sequences available in GenBank using Basic Local Alignment Search Tool (BLAST).
RESULTS:
Forty eight (39.34%) and 58 (47.54%) animals tested positive for T. equi and B. caballi antibodies, respectively, 9 animals (7.38%) were found positives for antibodies against A. phagocytophilum whereas negative results were obtained for B. burgdorferi. Twenty-six (21.31%) animals showed antibodies for both T. equi and B. caballi. Neither A. phagocytophilum nor B. burgdorferi PCR results were positive. Twenty-three (18.85%) donkeys were positive to Babesia/Theileria spp. PCR assay; out of 21 sequenced amplicons obtained, 20 (16.39%) were identified as T. equi, belonging to three main groups designated A, B and C (GenBank a.n AY150062 ID 99%, AB515310 ID 99-100% and AB515311 ID 99%, respectively) and 1 (0.82%) as B. caballi, belonging to group A (EU642512 ID 100%).
CONCLUSIONS:
To the authors' knowledge, this represents the first description of a serologic and molecular survey on TBDs occurring in donkeys from Central Italy. The results indicated that T. equi and B. caballi are widespread among donkey populations in the studied with high prevalence rates, whereas a lower circulation of A. phagocytophilum was detected. Moreover the results obtained provide information on the genetic identity of the T. equi strains circulating in central Italy.

Keywords:
Donkey, Tick borne pathogens, Central Italy

O02.7
MOLECULAR DETECTION OF TOSCANA VIRUS (BUNYAVIRIDAE: PHLEBOVIRUS) IN SANDFLIES (DIPTERA: PSYCODIDAE) FROM REGGIO EMILIA PROVINCE

Maioli G.[1], Calzolari M.[1], Defilippo F.[1], Pinna M.[1], Massirio I.[2], **Dottori M.*[1]**, Bonilauri P.[1]

[1]IZSLER ~ Reggio Emilia ~ Italy, [2]Ausl ~ Reggio Emilia ~ Italy

INTRODUCTION:
Toscana virus (Bunyaviridae: Phlebovirus) (TOSV) is recognized as one of first cause of summer meningitis in Mediterranean countries, particularly in Italy. This virus is transmitted by phlebotomine sandflies and the vertical transmission in vectors, reported at high rate in experimental studies, has been suggested as a possible mechanism of environmental persistence (Maroli et al.,1993, Med Vet Entomol.,7(3):283-6.), but its ecology is still largely unknown.
MATERIALS AND METHODS:
The entomological surveillance was conducted in hilly areas (between 65 and 500 a.s.l.) of Reggio Emilia province. We placed carbon dioxide baited traps, working overnight, in 21 georeferenced sites; 13 sites were sampled for two years, while, in 2013, 8 more sites were sampled in different locations.
Sandflies male and female were separated. Males were identified at optic microscope after chlorolactophenol clarification by morphology keys, particularly by shape of aedeagus (Romi e tal., 1994, Quad ISS: 114 p). Females were divided in groups with a maximum of 200 individuals, and ground in 1.5 ml eppendorf vial with 500 ul of PBS by pellet pestle, and then submitted to biomolecular analysis.
Phlebotomus RNAs were extracted using TrizolHLS Reagent (Invitrogen, Carlsbad, CA); cDNA synthesis was achieved using random hexamers (Roche Diagnostics, Mannheim, D) and SuperScriptH II Reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, and then submitted to specific Real Time PCR for TOSV detection (Pérez-Ruiz et al. ,2007, J Clin Virol, 39(4), 276-281)
RESULTS:
A total of 3388 sandflies were collected in 2012 and 10164 sandflies in 2013. Most of sandflies male identified belong to Ph. perfiliewi species (n= 3784; 97%), only 3% of males belong to Ph. perniciosus species (n=109). Females represented 71% of sandflies catched by our traps (n=9656).
We examined by Real-time PCR 78 pools in 2012 and 91 pools in 2013.
Sandflies abundance was similar in the two years of sampling, with one peak of activity (almost 1000 sandflies per trap/night) in the second half of July both in 2012 and 2013. For sites monitored only in 2013 there was a peak in one trap at the end of August, catching about 3000 exemplars; in this site we found TOSV RNA in five pools; other one pool resulted positive in the same week but in other site.
CONCLUSIONS:
Two species of sandflies were collected during the survey, Ph. perfiliewi and Ph. pernicious. Both species are considered efficient vector of TOSV (Charrel et al., 2005, Emerg Infect Dis., 11(11):1657-63), but due to the overwhelming abundance Ph. perfiliewi seems to be the main vector of TOSV in Emilia-Romagna. The different detection of TOSV between years may be due to different population dynamics of the vector: TOSV RNA was found the in the most abundant samples obtained during the survey, that group over than 3000 specimens. These results seem to imply a strong correlation between the presence of the virus and sandfly density, according to previous field evidence that link the peak in human cases with periods of highest density of the vector (Nicoletti et al., 1991, Am J Trop Med Hyg. Oct; 45(4):429-34). If confirmed, this strong correlation would be a very useful tool for assessing the risk of infection of TOSV. Further studies are needed to identify possible TOSV reservoirs, particularly in study areas in which positive pools were sampled, characterized by agricultural fields, woods and hedges.

Keywords:
Toscana virus, Phlebotomus, Emilia-Romagna

XXVIII Congresso Nazionale SoIPa, Roma 24-27 giugno 2014

PARASSITOSI TRASMESSE DA ARTROPODI
SESSIONE PARALLELA O-02

O02.8
INCIDENCE OF CERCOPITHIFILARIA BAINAE IN DOGS AND PROBABILITY OF CO-INFECTION WITH OTHER TICK-BORNE PATHOGENS

Ramos R.A.N.*[1], Giannelli A.[1], Lia R.P.[1], Brianti E.[2], Tarallo V.D.[1], Stanneck D.[3], Breitschwerdt E.[4], Dantas Torres F.[5], Otranto D.[1]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [2]Dipartimento di Sanità Pubblica Veterinaria, Università degli Studi di Messina ~ Messina ~ Italy, [3]Bayer Animal Health GmbH ~ Leverkusen ~ Germany, [4]Intracellular Pathogens Research Laboratory, North Carolina State University ~ Raleigh ~ United States, [5]Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães ~ Recife ~ Brazil

INTRODUCTION:
Cercopithifilaria bainae is a filarioid parasite that infects dogs, being transmitted by Rhipicephalus sanguineus group ticks in many countries of the Mediterranean basin (Otranto et al., 2012, Parasit. Vectors, 5:1). The pathogenic role of this filarioid has not been clearly defined. Histological findings suggest that microfilariae may cause mild focal epidermal and subepidermal edematous changes and a perivascular interstitial dermatitis (Otranto et al., 2012, Vet. Parasitology, 190:143-150). It is not clear whether the infection by C. bainae affects the dog's immune response to other tick-borne pathogens or vice versa, facilitating their acquisition during the tick bite. Given the paucity of data available about the biological behavior of this nematode, this study aimed to assess the incidence of C. bainae in dogs and the probability of co-infections with other tick-borne pathogens.
MATERIALS AND METHODS:
This study was carried out in a private shelter in the municipality of Putignano, province of Bari, Apulia region. This area has a high prevalence of canine tick-borne pathogens and dogs (n=58) maintained in this shelter were frequently exposed to ticks (Lorusso et al., 2010, Med. Vet. Entomology, 24:309-315). Skin and blood samples obtained from each dog six times over an 18-month period were tested for C. bainae by parasite detection within skin snip sediments, with subsequent confirmation through PCR amplification and DNA sequencing. Infections with other tick-borne pathogens (i.e., Anaplasma platys, Babesia vogeli and Hepatozoon canis) were confirmed by cytological examination of stained blood smears or by PCR amplification and DNA sequencing of selected amplicons. The incidence density rate (IDR) of infection was calculated based on the number of dogs infected with Cercopithifilaria spp. larvae at each sampling time point divided by the number of dog-months of follow-up. In addition, the conditional probability of co-infections was calculated by assessing the absolute frequency of positive animals, based on the Bayes' theorem.
RESULTS:
The IDR for positive animals per month at risk was 3.8% and 1.7%%. All 58 C. bainae-infected dogs were simultaneously infected with at least one other tick-borne pathogen. After the first summer season (assessment in November 2011), a C. bainae-infected dog had a 33% probability of being infected with H. canis or A. platys, whereas after the second tick season (assessment in October 2012) the probability of co-infection was 78%, 22% and 11% for H. canis, A. platys and B. vogeli, respectively.
CONCLUSIONS:
Our data indicate that tick-infested dogs are at risk of acquiring infection by C. bainae. In addition, the detection of C. bainae microfilariae indicates a prior tick exposure, stimulating testing for other tick-borne pathogens.

Keywords:
Cercopithifilaria, co-infection, incidence

O02.9
TEMPORAL AND SPATIAL PATTERNS OF DIFFERENT LINEAGES AND STRAINS OF WEST NILE VIRUSES IN NORTH-EASTERN ITALY IN THE MOSQUITO VECTOR CULEX PIPIENS

Ravagnan S.*[1], Cazzin S.[1], Da Rold G.[1], Salviato A.[1], Palei M.[2], Russo F.[3], Montarsi F.[1], Marangon S.[1], Capelli G.[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [2]Veterinary Public Health Service Friuli Venezia Giulia region ~ Udine ~ Italy, [3]Promotion and Development Hygiene and Public Health Service, Veneto Region ~ Venezia ~ Italy

INTRODUCTION:
West Nile virus (WNV) is an arthropod-borne Flavivirus representing a serious public health concern for Europe, where several outbreaks of neuroinvasive disease occurred in the past 20 years. In Italy from 2008 to 2013 human and veterinary cases caused by Lineage 1 (WNV-1) and Lineage 2 (WNV-2) were reported. Our aim was to describe the temporal and spatial patterns of lineages and strains in the mosquito vector Culex pipiens.
MATERIALS AND METHODS:
From 2010 to 2013, 13,002 pooled mosquitoes, collected during the entomological surveillance in Veneto and Friuli Venezia Giulia Regions, were screened for WNV by rRT-PCR. Positive samples were characterized by partial sequencing of NS5 and NS3 genes (Bakonyi et al., 2006, Emerg Infect Dis, 12(4):618-23; Savini et al., 2010, Vet Microbiol, 158(3-4):267-273). Phylogenetic analyses were carried out using the neighbour-joining (N-J) method with 1000 bootstrap replicates implemented in the MEGA 4 programme.
RESULTS:
Overall, 27 pools of Cx. pipiens were positive for WNV-1 and 59 for WNV-2.
WNV-1 (strain Italy08) emerged in 2008 in the Po delta in animals and humans (Macini et al., 2008, Euro Surveill, 13(39)). In 2010, this strain was still circulating in mosquitoes in Rovigo (RO) and Treviso (TV) areas (Gobbi et al., 2014, BMC Infect Dis, 14:60). Simultaneously, in the Venice (VE) area, a WNV-1 belonging to a separate cluster (Livenza strain) emerged in humans and mosquitoes (Barzon et al., 2012, Euro Surveill, 17(31)). The Livenza strain continued to circulate through 2012 in VE, TV and Udine (UD), while the original WNV-1 Italy08 apparently disappeared. The strain Italy08 re-emerged in 2013 in mosquitoes in the Po delta again (RO), slightly different but closely related to the original once.
WNV-2 was detected for the first time in North-Eastern Italy in the province of UD in 2011 (Mulatti et al., 2013, Zoonoses Public Health, 60(5):375-82). Based on NS3 and NS5 partial sequence alignments including all recent European and Italian WNV-2 strains, the new circulating strain shared high similarity (100-99%, respectively) with a Hungarian isolate. In 2012 another WNV-2 was found in RO, with 99-100% identity with the 2010 Greek isolate (Capelli et al., 2013, Vet Ital, 49(3):263-8). The protein NS3 analysis of this strain showed proline instead of histidine at the 249 site (H249P). This substitution has been suggested as a possible cause of higher pathogenicity for humans (Papa et al., 2011, Emerg Infect Dis, 17(5):920-2). The 2013 was featured by a large increase of WNV-2 (55 samples). NS5 analysis showed six different variants all belonging to a new cluster (99% identity with both the Greek and Hungarian isolates). Only one of these variants showed the H249P replacement.
CONCLUSIONS:
The phylogenetic analyses demonstrated the high complexity of the WNV circulation in North-Eastern Italy. The findings highlight the fact that new introductions, likely through migratory birds from Africa and within Europe, have occurred in few years (WNV-1 and WNV-2) and indicate the capability of these viruses to become endemic and to rapidly evolve and emerge in different sites.
Our results also confirm North-Eastern Italy as a high-risk area for WNV emergence and outbreaks, and strongly call for the need of a continuous surveillance in the future.
This work was supported by the Veneto and Friuli Venezia Giulia Regions.

Keywords:
West Nile virus , mosquito vector Culex pipiens, phylogenetic analyses

O02.10
CAN IXODES RICINUS AND DERMACENTOR RETICULATUS BE A RESERVOIR FOR FRANCISELLA TULARENSIS?

Genchi M.*[1], Prati P.[1], Manfredini A.[1], Vicari N.[1], Bragoni R.[1], Sacchi L.[2], Epis S.[3], Fabbi M.[1]

[1]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "B. Ubertini" ~ Pavia ~ Italy, [2]Dipartimento di Biologia Animale, Università degli Studi di Pavia ~ Pavia ~ Italy, [3]Diaprtimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di Milano ~ Milano ~ Italy

INTRODUCTION:
Ixodes ricinus and Dermacentor reticulatus (Acari: Ixodidae) are small hard ticks able to transmit a large variety of pathogens to humans and animals. These ticks have been described as potential vectors of Francisella tularensis, a highly contagious zoonosis for a wide number of mammals, reptiles and birds. Transtadial transmission of this bacterium from larva to adult has been demonstrated under laboratory conditions. However, transovarial transmission is still debated. The aim of this study was to evaluate the possible transovarial transmission of F. tularensis in these two ticks.
MATERIALS AND METHODS:
four guinea pigs were infected with 0.5 ml of F. tularensis type B (1000 cfu/ml). After 2 days, 25 females and 35 males of I. ricinus and 25 female and 35 males of D. reticulatus were placed on 4 guinea pigs. The experiment was done in six replicates for a total of 150 I. ricinus females and 150 females of D. reticulatus. After 6 days from the infection, 24 engorged females of both species were dissected and salivary glands, midgut and ovary were collected and analyzed by real-time PCR target 23 kDa, transmission electron microscopy (TEM) and fluorescence in situ hybridization (FISH). The remaining females were maintained in incubator at 26°C and 90 RU to allow oviposition. Eggs were recovered and the ticks analyzed by PCR, TEM and FISH. Pool of 50 eggs were analyzed by PCR, culture and bioassay. Approximately 600 eggs were homogenized, suspended in saline solution and injected sc in 2 mice. Other groups of eggs were used to obtain larvae and nymphs. Three hundred larvae and 300 nymphs were used to infect 4 mice. A similar quantity of larvae and nymphs was tested by culture and PCR.
RESULTS:
All ticks were positive by PCR (midgut), TEM (ovary), FISH (ovary, salivary glands) and culture (midgut, ovary). The PCR carried out on eggs was positive but the culture and bioassay were negative. The same result was obtained on larvae: PCR positive and culture and bioassay negative. The nymphs after blood meal on mice were negative by PCR and culture.
CONCLUSIONS:
TEM and FISH showed the successful infection of the ovary by F. tularensis and PCR showed the presence of bacterial DNA in eggs larvae. However, the negative results obtained by culture of eggs, larvae and nymphs and especially the negativity of bioassay, indicate the passage of non-viable DNA from ticks to eggs, larvae and nymphs. In conclusion, the data suggest the failure of transovarial transmission of F. tularensis in eggs of I. ricinus and D. reticulatus.

Keywords:
Ixodes ricinus, transovarial transmission, Francisella tularensis

O02.11
ON A CASE OF HUMAN VISCERAL LEISHMANIASIS IN UMBRIA

Di Giuli C.[1], **Piergili Fioretti D.*[2]**, Tozzi S.[1], Palumbo M.[1], Frongillo F.R.[1]

[1]Clinic of Infectious Diseases, Terni Hospital, University of Perugia ~ Terni ~ Italy, [2]Department of Veterinary Medicine, University of Perugia ~ Perugia ~ Italy

INTRODUCTION:
Visceral leishmaniasis (VL) caused by Leishmania infantum is an endemic zoonosis in Mediterranean Europe transmitted by phlebotomine sandfly species of the genus Phlebotomus.
Domestic dogs are the main reservoir hosts. A retrospective analysis (Gramiccia M. et al., 2013, Euro Surveill, 18: 29) showed a generalized increase of VL in immunocompetent individuals and patients with secondary immunodeficiency due to immunosuppressive treatment. These last patients may represent management difficulties due to misleading or delayed clinical signs with a consequent misdiagnosis. Moreover, opportunistic or superimposed infection can modify the clinical picture. Here, we present a case of VL in a corticosteroid treated patient with an initial clinical presentation of oesophagitis by Candida spp..
MATERIALS AND METHODS:
In November 2013 a 76 years-old Italian woman, living in Terni (Umbria), was hospitalised at the Clinic of Infectious Diseases of Terni Hospital, University of Perugia, with a diagnosis of oesophagitis by Candida spp., Fluconazolo resistant, and gastric polyposis that was cause of odynophagia and dysphagia to solid food from 3 months. The patient, undergone treatment with Echinocandine showed, after two days, high hyperthermia preceded by shiver; anamnestic episodic feverish states were referred. On admission the patient showed anorexia, asthenia, epatho-splenomegaly proved by ultrasonography and referred of a steroid therapy for arthralgia from 2 years. Biochemical and haematological tests, including serum protein electrophoresis and blood culture, were requested together with serological test for HIV, autoimmune diseases, and a bone marrow aspirate for cytological exams owing to suspected haemolymphopathia.
In addition, being Umbria region highly endemic for canine leishmaniasis, a serological test by IFAT was requested for a possible differential diagnosis with VL.
RESULTS:
Results of laboratory examination showed a picture of pancytopenia (severe hypochromatic microcytic anemia, leukopenia with neutropenia, thrombocytopenia and increase of reticulocytes), electrophoretic hypergammaglobulinemia with a suspected monoclonal peak, reduction of albumin and increase of systemic inflammation indices. The patient resulted negative for HIV and autoimmune diseases except a positivity for Ab-antinucleus with liposomal pattern. Blood culture for bacteria and mycetes were negative. A slight proteinuria of Bence-Jones was found. The bone marrow aspirate revealed Leishmania amastigotes inside and outside the hystiocytes. L. infantum IgG antibodies on IFAT found at high titres (1/1280) confirmed the diagnosis of VL. A PCR lately carried out on bone marrow aspirate gave a positive result (van Eys GJJM et al., 1992. Mol. Biochem. Parasitol., 51: 133-142).
The clinical picture showed a complete resolution after therapy with Liposomal Amphotericin B (3 mg/kg IV q24h for 14 days repeated for two days after one week).
After one month a control bone marrow aspirate resulted negative for amastigotes and IgG anti-Leishmania were detectable at low titre (1/160).
CONCLUSIONS:
This case emphasizes the importance, in endemic areas, of including VL in the differential diagnosis of haemolymphopathies.

Keywords:
Visceral Leishmaniasis, Human, Umbria region

O02.12
DEVELOPMENT OF ACANTHOCHEILONEMA RECONDITUM (SPIRURIDA, ONCHOCERCIDAE) IN THE CAT FLEA CTENOCEPHALIDES FELIS (SIPHONAPTERA, PULICIDAE)

Napoli E.*[2], Brianti E.[2], Falsone L.[2], Gaglio G.[2], Radicchi G.[5], Abramo F.[4], Annoscia G.[1], Dantas Torres F.[3], Giannetto S.[2], Otranto D.[1]

[1]Dipartimento di Medicina Veterinaria, Università di Bari ~ Bari ~ Italy, [2]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina ~ Messina ~ Italy, [3]Department of Immunology, Aggeu Magalhães Research Centre, Oswaldo Cruz Foundation ~ Recife ~ Brazil, [4]Dipartimento di Scienze Veterinarie, Università di Pisa ~ Pisa ~ Italy, [5]AbLab Laboratorio di analisi istopatologiche veterinarie ~ La Spezia ~ Italy

INTRODUCTION:
Acanthocheilonema reconditum has a global distribution and in many geographical areas is the sole or the most prevalent filarioid infesting dogs (Brianti et al., 2012, Parasitology, 139:530-536). Despite its large frequency and distribution information on its biology and life history is meagre. Our research aimed at studying the development of Acanthocheilonema reconditum (Spirurida, Onchocercidae) inside the cat flea, Ctenocephalides felis instrumentally to elucidate: i) the correlation between the concentration of microfilariae in the blood and the rate of flea infection; ii) the development time span from microfilariae to infective third stage larvae; iii) the anatomical localization of the developing larvae inside the flea's body.
MATERIALS AND METHODS:
Ctenocephalides felis fleas were fed through an artificial feeding system, named "artificial dog" (Wade and Georgi, 1988, J Med Entomol, 25:186-190), with A. reconditum infected blood at different concentrations of microfilariae (i.e., low, group L=250; medium, group M=500; high number, group H=1,500 microfilariae/ml) or with uninfected dog blood (group C). Fleas were sampled in 12 different time-points throughout the study period (i.e., D1-D28) and A. reconditum was detected by dissection, PCR and histology.
RESULTS:
Of 2,105 fleas fed with infected dog blood, 891 (38.7%) died during the study before being sampled and the remaining (n = 1,214) were examined for A. reconditum. No statistically significant differences in the mortality rate were detected among infected groups and between these and the control group at any time-point. At the dissection first-stage larvae (L1) were identified after 2 days post infection (D2), the second stage (L2) at D13, and infective third-stage larvae (L3) were found at D15 (Figure 1). Eighteen (30%) flea pools of the 60 molecularly examined tested positive. Although not statistically significant, positive pools were higher in groups M (8/19; 42.1%) and H (7/20; 35.0%) than in group L (3/21; 14.3%). At the histology, L2 were detected at D13 in the sub-cuticle region of one female flea embedded in the back muscle (Fig. 2).

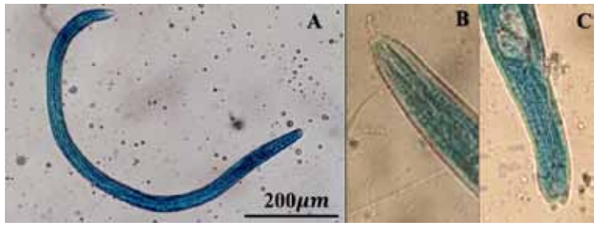


Figure 1: Developing stages of Acanthocheilonema reconditum inside the flea. A) L2 found at D13; B) Anterior and C) caudal ends of a L3 stage retrieved at D15.

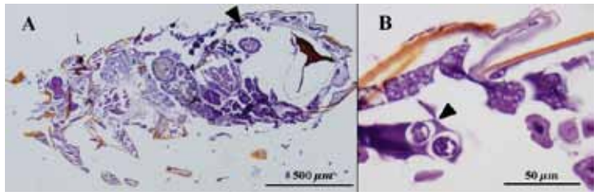


Figure 2: A) Histology (50X) of flea at D13, arrowhead indicates the location in which the larva of Acanthocheilonema reconditum was evidenced. B) Magnification of A, note cross-sections of the larval body (arrowhead).

CONCLUSIONS:
This study demonstrates that the "artificial dog" may be successfully used to infect the cat flea with A. reconditum larvae, reducing the use of live animals in the experimentation. Although arthropod-transmitted pathogens may cause alterations in the vector's fitness, playing an important role in the dynamics of transmission (Hurd, 2003, An. Rev. Entomol., 48:141–161), findings herein presented suggest that the development of A. reconditum larvae do not impair the survival of C. felis. In artificially infected fleas, the development of A. reconditum to L3 infective stage takes 15 days being one of the shorter times among filarioids of dogs. Although further confirmatory studies are needed, results of the present study confirm that fleas act as an intermediate host of A. reconditum and suggest that the transmission to dogs may occur via the ingestion of infected fleas.

Keywords:
Acanthocheilonema reconditum, Ctenocephalides felis, biology

NOTES

SESSIONE PARALLELA O-03

FARMACORESISTENZA

O03.1
DRUGS FOR NEGLECTED TROPICAL DISEASES: THE CHALLENGE OF IMPROVING ACCESS WHILE SUSTAINING EFFICACY

Albonico M.*[1], Gabrielli A.F.[2], Montresor A.[2], Savioli L.[2]

[1]Fondazione Ivo de Carneri ~ Milan ~ Italy, [2]World Health Organization ~ Geneva ~ Switzerland

INTRODUCTION:
More than 700 million treatments are being delivered annually for the control/elimination of neglected tropical diseases (NTD) due to helminths and amenable to preventive chemotherapy, i.e. lymphatic filariasis, onchocerciasis, schistosomiasis and soil-transmitted helminthiasis. Yet, if the ambitious 2020 goals set in the NTD Roadmap are to be met, it is necessary to scale up preventive chemotherapy interventions and reach approximately 1.2 billion more people than those targeted in 2013. While commitment by partners as recently reaffirmed through the London Declaration has enabled large-scale donations of benzimidazoles (200 million tablets of mebendazole/year, 400 million tablets of albendazole/year), ivermectin (as long as needed to eliminate onchocerciasis and lymphatic filariasis), DEC (2.2 billion tablets), and praziquantel (up to 250 million tablets/year), the priorities are to increase the current capacity within national NTD programmes to deliver the treatments as well as monitoring that efficacy of anthelminthic drugs is maintained.
MATERIALS AND METHODS:
The risk of drug resistance is a potential threat for any of the mentioned drugs, and is already a reality in animals, as such drugs are also used in veterinary practice. Consequently, WHO has set up a working group of experts dedicated to develop policies and practices to monitor drug efficacy in order to be ready to face this challenge should it appear in humans as well. One of the key measures taken has been the implementation of multicentric trials assessing efficacy of single-dose treatments through standard operating procedures, with the aim of establishing reference efficacy thresholds. This step has enabled the development of guidelines that provide all the necessary practical information to programme managers. Guidelines are adaptable to different public health contexts, as they factor in differences in intensity of infection before treatment, sensitivity of diagnostic methods used, and timing of follow up of patients.
RESULTS:
Mathematical modelling are being developed to assist programme managers to predict the expected impact of treatment, thus setting an alarm bell that will ring if such impact is not observed. Treatment failures due to resistance may be one possible cause but thorough investigations should be made to exclude other factors.
Whilst few new drugs are in the pipeline, the best way forward for preventing/delaying the occurrence of resistance is the use of drugs in combination, and recent trials have gone towards this direction. The challenge in the control/elimination of helminth diseases is to scale-up treatment coverage to reach as many people in need, and, at the same time, to safeguard the efficacy of the few good drugs which are able to do the job.
CONCLUSIONS:
Preventive chemotherapy aims at decreasing morbidity and reducing transmission of target helminth infections, towards the progressively more ambitious goals of control, elimination as a public health problem, and elimination (the latter term meaning interruption of transmission). Even though evidence shows that preventive chemotherapy alone can be sufficient to interrupt transmission, the implementation of complementary interventions, including health education, provision of safe water and sanitation not only can accelerate the accomplishment of this objective , but by limiting the dependency of the programmes on the exclusive use of medicines, it reduce drug pressure on parasites and therefore the risk of developing resistance.

Keywords:
drug efficacy, preventive chemotherapy, monitoring

O03.2
GENETIC AND MOLECULAR MECHANISMS OF OXAMNIQUINE RESISTANCE IN SCHISTOSOMES

Cioli D.*[1], Pica Mattoccia L.[1], Guidi A.[1], Basso A.[1]

[1]CNR, Institute of Cell Biology and Neurobiology ~ Roma ~ Italy

INTRODUCTION:
Oxamniquine (OXA) was used before the introduction of praziquantel (PZQ) to treat many million people that were infected with S. mansoni. The main limitation of OXA is that it is not active against S. haematobium or S. japonicum, a fact that discouraged its use outside of South America, where only S. mansoni exists. The restricted market of OXA prevented its competitive production and the expected price reduction, so that today PZQ is cheaper than OXA and has replaced it even in countries, like Brazil, where OXA has been for many years the successful cornerstone of control programs. With respect to both safety and efficacy against S. mansoni, OXA has proved to be at least as good as PZQ, sharing its advantages of single oral administration and mild side effects.
MATERIALS AND METHODS:
Sporadic instances of OXA resistance observed in Brazil and the duplication of the phenomenon in the laboratory permitted the isolation and the maintenance of S. mansoni strains that were highly refractory to the drug, surviving doses 500-fold higher than those that are lethal to sensitive parasites. Genetic crosses between sensitive and resistant schistosomes were performed by the surgical introduction of one male and one female schistosome into the mesenteric veins of mice.
RESULTS:
The results of genetic crosses led to the conclusion that OXA resistance is a recessive trait controlled by a single autosomal gene. This suggested the existence of a schistosome 'factor' that is essential to convert the prodrug OXA into the active compound. A series of further biochemical data narrowed down the hypothesis and predicted that a parasite sulfotransferase is the activating enzyme and that a loss of its function is at the basis of OXA resistance.
CONCLUSIONS:
Our predictions were recently confirmed (Valentim et al., 2013, Science 342: 1385-1389) using a linkage mapping approach that unambiguously identified the S. mansoni sulfotransferase gene and permitted the crystallographic analysis of the enzyme and of its interaction with the drug. This represents the first complete elucidation of an anthelmintic drug's mechanism of action, and -most importantly- opens the way to a structure-based redesign of OXA to extend its activity to the S. haematobium sulfotransferase analog. Thus, there is now a realistic hope that a new broad-spectrum OXA may represent the long sought-after partner/substitute of PZQ.

Keywords:
Schistosoma, Oxamniquine, resistance

O03.3
WHY DID THE VETS SHOULD NOT USE THE ANTI-LEISHMANIA DRUGS CURRENTLY USED FOR HUMANS?

Oliva G.*[1], Gradoni L.[2]

[1]Department of Veterinary Medicine and Animal Production, University Federico II ~ Naples ~ Italy, [2]MIPI Department, Istituto Superiore di Sanità ~ Rome ~ Italy

INTRODUCTION:
The use in canine leishmaniasis (CanL) of drugs that are employed for human leishmaniasis treatment is widespread and unlikely to be stopped, being alternative drugs unavailable. Even though there is no evidence that treatment failures in CanL are due to parasite drug resistance, knowledge on drug susceptibility of clinical L.infantum isolates is very limited. Development of practical in vitro assays and increased surveillance are needed. These concepts were debated at the 5th World Congress on Leishmaniasis held in May 2013 in Brazil, a country where drug treatment of CanL is prohibited, and also emphasized in a Drugs for Neglected Diseases initiative meeting held in November 2013 in Geneva. It was noted that CanL treatment in Europe appears to be the only option to manage the problem, because culling is considered both ethically unacceptable and unfeasible owing to the number of infected dogs estimated to be millions.
MATERIALS AND METHODS:
CanL consists of a broad spectrum of Leishmania infections, with low consensus about 'parasite exposure', 'established infection' or 'asymptomatic' definitions. In our model, infection conditions in dogs are categorized as i) subpatent, an asymptomatic infection although non-infectious, dogs being usually seronegative and parasite DNA only detected by sensitive molecular methods. Subpatent dogs can remain as such for life, or convert to negative or progress to be actively infected; ii) active infection in asymptomatic but infectious condition, dogs being seropositive at high titers and invariably progressing to clinical disease; iii) active infection associated to disease, a highly infectious condition accounting for 1/3 of infected dogs in cross-sectional surveys. Dogs actively infected show positive parasite cultures and should be treated. A limited range of drugs with a broad range of regimens adopted in practice without base of evidence, is in use for dogs. Reported radical cure is extremely rare and very few studies have addressed the problem of reducing the reservoir potential of permanently infected dogs by means of drugs.
RESULTS:
There are 4 clinical stages of CanL ranging from mild with good prognosis – no/few relapses or acceptable life expectancy - to very severe with bad prognosis. In Europe, mild, moderate and severe disease cases are treated with meglumine antimoniate or miltefosine both combined with allopurinol, whereas treatment of very severe disease is with allopurinol alone. Due to several reasons, dog treatment is often incomplete, underdosed or intermittent, which could promote Leishmania resistance. Strains with reduced susceptibility to antimony, miltefosine and allopurinol have been recorded in dogs following therapy.
CONCLUSIONS:
Treatment guidelines for vet practitioners should be recommended, along with the use of topical insecticides for actively infected/sick dogs, euthanasia of dogs with bad prognosis, and a common strategy to deal with stray dogs. Severe gaps on pharmacodynamic/pharmacokinetic of drugs used to treat different canine infection categories and stages of disease may contribute to the establishment of drug regimens that are definitively useless, and also potentially selective for resistant Leishmania strains.

Keywords:
Canine leishmaniasis, drugs, drug resistance

O03.4
EQUINE STRONGYLID EGG RE-APPEARANCE PERIOD AFTER IVERMECTIN OR MOXIDECTIN TREATMENT IN ITALY

Traversa D.*[1], Frangipane Di Regalbono A.[2], Di Cesare A.[1], Otranto D.[3], Lia R.P.[3], Beraldo P.[4], Besognet B.[5], Geurden T.[6]

[1]Faculty of Veterinary Medicine ~ Teramo ~ Italy, [2]Department of Veterinary Medicine, Production and Health ~ Padua ~ Italy, [3] Department of Veterinary Medicine ~ Bari ~ Italy, [4]Department of Food Science ~ Udine ~ Italy, [5]Zoetis ~ Paris ~ France, [6]Zoetis ~ Zaventem ~ Belgium

INTRODUCTION:
Drug resistance in cyathostomins is a potential threat for horse health, welfare and production. While resistance to benzimidazoles (BZs) is worldwide recognized and to tetrahydropirimidines (THPs) is increasing, it is up until recently that the first evidence of reduced efficacy of macrocyclic lactones (MLs) has been reported. Recent studies indicated a reduction in egg reappearance period (ERP) after treatment with ivermectin (IVM) in different countries, while moxidectin (MOX) is still highly effective in Europe (von Samson-Himmelstjerna et al., 2007, Vet. Parasitol. 144,74-80; Lyons et al., 2011, Parasitol. Res. 108,1315-1319; Traversa et al., 2009, Parasit. Vectors 2:S2; Traversa et al., 2012, Vet. Parasitol., 188, 294-300). This study investigated the ERP after treatment with IVM or MOX in horses infected with cyathostomins in Italy.
MATERIALS AND METHODS:
Ten farms have been selected in Italy on the basis of a history either of <100% efficacy of MLs or of frequent use of IVM or MOX. In each farm ten horses have been selected on the basis of a minimum pre-treatment faecal egg count (FEC) of 50 (range 50 -2425) per gram of faeces (EPG). Horses were randomly allocated to one of two treatment groups based on pre-treatment FEC and treated either with IVM or MOX on Day 0. Individual faecal samples were taken from all animals on Day 14, 28, 42 and 56 for IVM and MOX, and 70 and 84 for MOX. All samples were examined using a modified McMaster technique with a sensitivity of 25 EPG and the efficacy for each treatment was calculated based on the arithmetic mean (AM) FEC before and at each time point after treatment with the following formula: %reduction= 100x (AMpre-treatment - AMpost-treatment)/AMpre-treatment.
RESULTS:
Efficacy at Day 14 was high for both IVM and MOX in all sites. The ERP was as expected for MOX except on one farm, in which decreased ERP was found for both MLs. Decreased ERP and efficacy was found in another farm on Day 56 for IVM, while decreased ERP and efficacy was detected in tail (i.e. on Day 84) for MOX on a third farm.
CONCLUSIONS:
The reduced cyathostomin ERP was found for IVM and/or MOX in 3 farms of this study, consistently with the results of a similar study carried out in Belgium and the Netherlands which demonstrated reduced ERP for IVM and MOX (Geurden et al. 2014, Vet. Parasitol., submitted). The duration of the ERP varies with anthelmintic class and worm populations, thus effective molecules must be evaluated separately in each herd to characterize local properties. Given that a reduction in the ERP is possibly indicative of early anthelmintic resistance, appropriate use of anthelmintics in worm control strategies, including reduced treatment frequency or selective treatments, along with maintenance of refugia, should be implemented to delay the occurrence of drug resistance in horse cyathostomins.

Keywords:
Horse, Cyathostomins, drug-resistance

O03.5
EVALUATION OF THE DISTRIBUTION OF PLASMODIUM FALCIPARUM N86Y POLYMORPHISM IN PFMDR1 GENE IN UGANDA

Cosentino V.*[1], Paganotti G.M.[1], Tabacchi F.[1], Russo G.[1], Coluzzi M.[1], Romano R.[1]

[1]Department of Public Health and Infectious Diseases, "Sapienza" University of Rome ~ Italy

INTRODUCTION:
Increasing resistance to antimalarial drugs is a leading problem in fighting against malaria. Nowadays, since in most malaria endemic countries the antimalarial regimens are based on artemisin combination therapies (ACT), monitoring Plasmodium falciparum resistance is the most important issue and an emerging global problem in public health. There are now many evidences that link drug resistance for different compounds, included artemisinin-derivatives to parasite pfmdr1 polymorphism. Here we test and discuss the spread of the resistant pfmdr1 86Y allele in Kampala and Karamoja regions in Uganda, where different transmission intensity and drug usage are present.
MATERIALS AND METHODS:
We analysed 261 DNA specimens from young subjects. Blood collection was performed in two different zones of Uganda: in the Kampala suburbs (Central-Southern Uganda) and in rural sites of Karamoja region (North-Eastern Uganda), where the available antimalarial therapy was mainly a combination of artemether and lumefantrine (AL) and, as an alternative, artesunate (AS) plus and amodiaquine (AQ). P. falciparum DNA was amplified by nested PCR-RFLP technique to identify N86Y polymorphism in pfmdr1 gene.
RESULTS:
The overall parasite rate in the samples was 36.78 % (25.6% and 54.5% respectively, in urban and rural zone; P<<0.001). Moreover, the overall rate of drug resistant infections was 81.25 % (higher in the urban than in the rural zone, being 87.8% and 74.5%, respectively; P = 0.0002)
CONCLUSIONS:
The results show an inverse correlation between P. falciparum parasite rate and drug resistance spread in the two different zones, this being associated to two different transmission patterns, drug usage and immunity between rural and urban contexts. There is evidence that selection and exposure to drug resistant malaria parasites is higher in a urban context than in the rural one.

Keywords:
P. falciparum, drug resistance, Uganda

O03.6
MOLECULAR SURVEILLANCE OF PLASMODIUM VIVAX DHFR AND DHPS MUTATIONS IN ISOLATES FROM IRAN

Fuda I.[1], Menegon M.[1], Rezanezhad H.[2], **Severini C.*[3]**

[1]Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità ~ Rome ~ Italy, [2]Department of Microbiology, Jahrom University of Medical Sciences ~ Jahrom ~ Iran, Islamic Republic of, [3]Department of Infectious, Parasitic and Immunomediated Diseases and WHO Collaborating Centre for Research and Training in Tropical Diseases Control, Istituto Superiore di Sanità ~ Rome ~ Italy

INTRODUCTION:
Plasmodium vivax is the most prevalent human Plasmodium outside Africa and the emergence of drug resistance in P. vivax isolates is a major concern for the current strategies of malaria control in endemic countries (Baird, 2004, Antimicrob. Agent Chemother, 48:4075–4083). Although sulfadoxine/pyrimethamine (SP) is not used for treatment of P. vivax, SP resistance in this species was reported previously (Imwong et al., 2001, Antimicrob. Agents Chemother, 45, 3122-3127; Korsinczky et al., 2004, Antimicrob. Agents Chemother, 48, 2214-2222). This phenomenon is due to the exposition of vivax parasites to SP drugs used for treatment of uncomplicated P. falciparum infections in the endemic regions where both species co-exist and co-infections of these two species are common. In Iran, where Plasmodium vivax is the predominant species with about 90% of the total annual reported cases of malaria (WHO, 2010), SP/artesunate is the first-line treatment for falciparum malaria, and recently the frequency of parasites carrying mutations related to SP resistance has increased in some provinces of the country, indicating the emergence of drug-tolerant/resistant P. vivax. (Afsharpad et al., 2012, Inf. Gen. Evol, 12:38-44). In the present study, in order to assess antimalarial drug resistance in Iranian patients from Hormozgan province (Southern Iran), we investigated the frequency distribution of mutations in dihydrofolate reductase (pvdhfr) and dihydropteroate synthetase (pvdhps) genes conferring resistance to SP. Size polymorphisms, due to the presence of a single tandem repeat region in both investigated genes, was also evaluated in all Iranian isolates.
MATERIALS AND METHODS:
A molecular screening on pvdhfr and pvdhps genes was carried out on 29 samples obtained from malaria-infected patients attending the Port of Jask health center (Hormozgan province). Total DNA was extracted by using PureLink Genomic DNA Kit (Invitrogen), following the manufacturer's instructions. Pvdhfr and pvdhps mutations and size polymorphisms were detected by PCR (de Pécoulas et al., 1998, Mol. Biochem. Parasitol, 92:265-273 ; Menegon et al., 2006 Acta Trop. 98:196-199) and sequencing. All the PCR products were sequenced at the MWG Biotech (Germany) and the results were analyzed by means of DS gene 2.5 (ACCELRYS, Cambridge).
RESULTS:
The results of molecular screening on pvdhfr/pvdhps mutations showed the high prevalence of the wild type codon profile in both genes characterized in the plasmodial isolates from the study areas.
We detected double mutated pvdhfr 58R/117T codons in 3,5% of isolates whereas only three isolates showed mutations in pvdhps codons (S382A in 2 isolates; V585F in 1 isolates). In addition, among the analyzed isolates, two and four different allele sizes were distinguished in pvdhfr and pvdhps gene, respectively.
CONCLUSIONS:
The results of this preliminary investigation showed that wild-type pvdhfr and pvdhps alleles are still prevalent in this area. This finding suggests that SP is probably still effective in the treatment of vivax malaria in Hormozgan province. However, the increased frequency of mutant haplotypes in the neighbouring province of Sistan and Belucistan since 2006 should lead the local health authorities to carry out a surveillance program, aimed at preventing the spread across the whole country of vivax isolates SP-resistant.

Keywords:
Plasmodium vivax, Drug resistance, Iran

O03.7
THE MAINTENANCE OF ANTHELMINTIC EFFICACY IN SHEEP IN THE CAMPANIA REGION (SOUTHERN ITALY)

Rinaldi L.*[1], Bosco A.[2], Coles G.[3], Morgan E.[3], Parrilla M.[2], Cringoli G.[1]

[1]1Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania); Inter-University Center for Research in Parasitology (CIRPAR) ~ Naples ~ Italy, [2] Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania) ~ Naples ~ Italy, [3]School of Veterinary Sciences, University of Bristol, Langford House ~ Bristol ~ United Kingdom

INTRODUCTION:
The aim of the present study was to determine whether management practices of sheep farms in the Campania region (southern Italy) have allowed the maintenance of anthelmintic efficacy against gastrointestinal (GI) strongyles.
MATERIALS AND METHODS:
Trials were conducted on 54 sheep farms. Tests were run with groups of sheep (12–20 animals per group) using six anthelmintics administered orally, namely: levamisole in 8 farms, ivermectin in 8 farms, moxidectin in 3 farms, monepantel in 8 farms, netobimin in 22 farms and albendazole in 5 farms (Table 1). Faecal egg counts were determined using the FLOTAC dual technique (Cringoli et al., 2010, Nat. Prot., 5: 503–515) with a sensitivity of 2 eggs per gram (EPG) of faeces. In addition, on each sampling day a composite faecal culture was conducted and third stage larvae identified. Different formulae and statistics were used to calculate FEC reductions and confidence intervals: FECR1 (Coles et al., 1992, Vet. Parasitol., 44: 35–44), FECR2 (Dobson et al., 2009, Vet. Parasitol., 161: 162–167; Dash et al, 1988, Aust Vet J, 65: 66–68; Pook et al., 2002, Vet. Parasitol., 106: 331–343), FECR3 (McKenna, 2006, N. Z. Vet. J., 54: 365–366) and FECR4 (Dobson et al., 2012, Vet. Parasitol., 186: 79–92).
RESULTS: The genera of GI strongyles at the time of treatment were (minimum and maximum percentages in each treatment group): Trichostrongylus (68.9–80.4%); Teladorsagia (11.6–16.3%); Oesophagostomum/Chabertia (2.7–11.2%); Haemonchus (1.9–7.4%); Cooperia (0.6–2.6%) and Bunostomum (0–0.2%). The efficacies of the anthelmintic treatments are given in Table 1.

Anthelmintics			The activities calculated by the different methods: min and max FECR (min and max lower confidence limits)			
Class [a]	Molecule	Dosage of drug (mg/kg)	FECR1	FECR2	FECR3	FECR4
BZ/PBZ	Netobimin	7.5	-	-	92.0-100	92.0-100 (85.9-99.9)
	Albendazole	3.8	-	-	100	100 (94.4-98.3)
LV	Levamisole	7.5	98.3-100 (92.2-98.8)	98.3-100 (92.0-99.3)	-	98.5-100 (97.4-99.9)
ML	Ivermectin	0.2	99.5-100 (95.5-98.8)	99.6-100 (97.0-99.6)	-	99.7-100 (98.6-99.9)
	Ivermectin	0.1	98.8-100 (90.2-98.8)	98.0-100 (90.4-99.6)	-	96.4-100 (93.4-99.9
	Moxidectin	0.2	99.9-100 (99.1-99.4)	99.9-100 (99.5-99.6)	-	99.9-100 (99.8-99.9)
AAD	Monepantel	2.5	97.3-100 (94.9-99.4)	97.8-100 (95.7-98.5)	-	97.0-100 (96.1-99.8)

[a] BZ/PBZ = Benzimidazoles/Probenzimidazoles; LV=Imidazothiazoles/Tetrahydropyrimidines, ML =Macrocycliclactones; AAD = Amino AcetonitrileDerivates

Table 1. The anthelmintics used on sheep farms and activity of drugs calculated by the four methods (FECR1, FECR2, FECR3, FECR4).

CONCLUSIONS:
The present data suggest that the development of anthelmintic resistance on sheep farms could be limited in regions with a Mediterranean type of climate provided that refugia of the nematode populations are maintained, anthelmintic use is restricted and movement of animals is not permitted to spread resistance.

Acknowledgements: The European Union Seventh Framework Programme FP7 –KBBE-2011-5 GLOWORM (no. 288975) are sincerely thanked.

Keywords:
anthelmintic efficacy, sheep, Campania region

O03.8
GASTROINTESTINAL NEMATODES OF DAIRY GOATS IN NORTHERN ITALY: ANTHELMINTIC RESISTANCE AND PRACTICES OF PARASITE CONTROL

Zanzani S.A.[1], Gazzonis A.L.[1], Di Cerbo A.R.[1], **Manfredi M.T.*[1]**

[1]Department of Veterinary Science and Public Health, University of Milan ~ Milano ~ Italy

INTRODUCTION:
Gastrointestinal nematodes (GINs) are one of the main constraints to ruminant production worldwide. GINs infection is of economic importance also in goat farming and the peculiarities of goat-nematode interactions could affect the control strategies of GINs. Anthelmintic resistance (AR) has been reported in Europe and some data showed that GINs develop anthelmintic resistance more rapidly in goats than in sheep (Domke et al., 2012, Parasitol Res, 111:185-193).Few data are available on AR in Italian goat farms; resistance to benzimidazoles (BZs) in Trichostrongylus colubriformis was recently demonstrated in southern Italy (Cringoli et al., 2007, Parasitol Res 101:577-581) and a generic resistance to BZs was reported by Genchi et al. (1993). Further, AR has been reported in sheep farms (Ambrosini 2000, JAEID, 94:105-126K; Traversa et al., 2007, Parasitol Res, 101:1713-1716). The aims of the study were: i) determine the frequency of AR in GINs in goat flocks in Lombardy; ii) survey goat farmers on the current practices of parasite control, iii) update the species composition of the gastrointestinal helminthofauna.
MATERIALS AND METHODS:
1288 individual fecal samples from 33 goat flocks were collected. Based on the egg per gram (EPG), 15 flocks were selected to evaluate the presence of AR to BZs and macrocyclic lactones (MLs) in GINs with the Fecal Egg Count Reduction Test (FECRT). A questionnaire on farm management and drenching practices against GINs was submitted to 110 dairy goat farmers. Further, the gastrointestinal tracts of 42 goats were analyzed for the isolation and classification of helminths.
RESULTS:
The FECRs indicated that five of the 15 flocks had problems of AR, both in BZs and MLs. Resistance and suspected resistance was found in 40% of ten flocks treated with BZs (albendazole resistant flock: FECR=81.11%, lower 95% CI limit=69%; oxfendazole suspected resistant flock: FECR=95.17%, lower 95% CI limit=88%; febantel resistant flocks: FECRs=40.52% and 76.01% respectively, lower 95% CI limits= 58% and -3%, respectively). One out of five flocks treated with eprinomectin had resistant GINs, (FECR=87.43%, lower 95% CI limit=76%). Teladorsagia/Trichostrongylus L3s were isolated from the post-treatment coprocultures of all flocks with resistance except for the flock with suspected oxfendazole resistance. Treatments against helminths were performed once a year in 73.63% of the flocks, although 20% of farmers declared not regular treatments. Annual treatments usually occurred in autumn or winter at sheep dose rate. Anthelmintics were not rotated on any of the farms. T. circumcincta, H. contortus, T. colubriformis, Skrjabinema caprae and Oesophagostomum venulosum were the most abundant and prevalent species of the gastrointestinal tract.
CONCLUSIONS:
The presence of AR in 30% of the tested flocks suggests that strategies to prevent the development of AR should be widely adopted in Lombardy. Further, farmers and practitioners should be educated about the importance of using the correct dose rates in goats and about the rotation of different anthelmintic classes, because AR can develop even at low frequencies of treatment. In addition, the presence of highly pathogenic GINs with high worm burden in all gastrointestinal tract suggests that improved diagnosis and active surveillance of GIN infection are needed. Furthermore, the knowledge of gastrointestinal helminthofauna and epidemiological parameters is also essential to reach this important objective and control the AR.

Keywords:
Anthelmintic resistance, goats, gastrointestinal nematodes

NOTES

SESSIONE PARALLELA O-04

CONTROLLO DELLE PARASSITOSI

O04.1

A FIELD CLINICAL STUDY EVALUATING THE EFFICACY AND SAFETY OF MILBEMAX® (NOVARTIS ANIMAL HEALTH) IN THE CHEMOPREVENTION OF DOG SUBCUTANEOUS FILARIOSIS

Di Cesare A.[1], **Paoletti B.*[1],** Bartolini R.[1], Aquilino V.[1], La Torre F.[2], Drake J.[3], Braun G.[4], Traversa D.[1]

[1]Faculty of Veterinary Medicine ~ Teramo ~ Italy, [2]Novartis Animal Health S.p.A ~ Origgio (VA) ~ Italy, [3]Novartis Animal Health S.p.A ~ Greensboro (NC) ~ United States, [4]Klifovet ~ Munchen ~ Germany

INTRODUCTION:
The mosquito-transmitted nematode Dirofilaria repens is the causative agent of subcutaneous filariosis in dogs, other animals and humans. Adults and circulating microfilariae may cause different forms of skin conditions, and various allergic reactions (McCall et al., 2008, Adv. Parasitol., 66:193-285; Traversa et al., 2010, Vet Parasitol, 169:128-132). The infection is distributed throughout Europe and apparently spreading in several areas. The control of D. repens is pivotal to reduce the transmission in dogs and to minimize the risk of infection in humans. Given that the most reliable approach to control canine dirofilarioses is the chemoprevention, this clinical field study evaluated the efficacy and safety of a monthly administration of an oral formulation containing 0.5 mg/kg milbemycin oxime and 5 mg/kg praziquantel (Milbemax®, Novartis Animal Health) in the chemoprevention of D. repens infection in dogs.
MATERIALS AND METHODS:
The study was carried out from June 2012 to June 2013 in a canine shelter (Site A) and a private practice (Site B) in Abruzzo region, central Italy. It was a GCP study carried out according to the European and national regulatory requirements, which was approved and authorized by the Italian Ministry of Health and local veterinary services. In May 2012, 249 dogs microscopically and molecularly negative for D. repens were allocated in two groups, Treated-T1 and Untreated-T2 (T1: no.124; T2: no.125), and subjected to monthly clinical examinations. Animals belonging to T1 group received monthly administration of Milbemax®, while those of group T2 were untreated. Blood was collected from each dog at days 28±2, 56±2, 84±2, 168±2, 196±2, 224±2, 252±2, 280±2, 308±2 and 336±2, and microscopically (Knott's method) and genetically (PCRs) examined. Any adverse event observation (AE) was recorded.
RESULTS:
The study was completed by 111 and 108 dogs in groups T1 and T2, respectively. No treated animals became positive for D. repens while 6 control dogs (5.3%, based on the population excluding animals withdrawn due to reasons other than D. repens) became positive upon at least one diagnostic test. The positive animals received a rescue treatment which showed a 100% efficacy. None of the AEs observed during the trial were determined to be related to the study medication and were typical of group-housed dogs in a shelter situation.
CONCLUSIONS:
This study demonstrates that a monthly administration of Milbemax® (Novartis Animal Health) is effective (100%) in preventing D. repens infection in dogs living in an endemic area. The product was well tolerated in all study animals. This study adds another piece in the complex mosaic of the chemoprevention of canine dirofilarioses, given that only few macrocyclic lactones molecules are licensed for its prevention. Hence, a regular monthly treatment with Milbemax® has the potential to interrupt the life cycle of D. repens, with important implications under epidemiological and clinical standpoints.

Keywords:
Dirofilaria repens, dog, Milbemax®

O04.2

SUSTAINABLE AND LONG-TERM CONTROL OF TICK AND FLEA INFESTATIONS IN DOGS THROUGH THE USE OF SLOW RELEASE COLLAR CONTAINING IMIDACLOPRID (10%) AND FLUMETHRIN (4.5%)

Brianti E.*[1], Falsone L.[1], Napoli E.[1], Gaglio G.[1], Prudente C.[1], Aprile S.[2], Giannetto S.[1]

[1]Dipartimento di Scienze Veterinarie, Università di Messina ~ Messina ~ Italy, [2]Medico Veterinario libero professionista ~ Siracusa ~ Italy

INTRODUCTION:
Ectoparasites of dogs represent a year-round hazard and their control may be challenging and expensive especially in highly populated dog communities. This study reports on the efficacy of a slow release collar containing 10% imidacloprid and 4.5% flumethrin (Seresto®, Bayer Animal Health) to cure dogs highly infested by ectoparasites and to prevent new infestations for 8 months.
MATERIALS AND METHODS:
After a series of unsuccessful individual and environmental treatments against ectoparasites all dogs hosted in a shelter were treated with slow-release imidacloprid/flumethrin collars and any other anti-parasitic action was interrupted. Ectoparasites on dogs were not removed and the magnitude of infestation was estimated in each dog at the time of treatment. In particular, dogs were inspected by checking the most common predilection sites of attachment for ticks and by combing for fleas. Dogs were followed-up for ectoparasites presence after 2, 7 and 14 days and thereafter biweekly up to 90 days. Furthermore, dogs were again examined for ectoparasites whilst replacing collars 8 months after their application.
RESULTS: At the time of treatment 79 (96.3%) out of 82 included dogs were heavily infested by ticks and 53 (68.8%) out of the 77 combed dogs were infested by fleas. Tick infested dogs had an estimated mean intensity of 46.9 (±65.7), while flea infested animals had a load between 20 and 50 fleas. In addition, some of the flea infested dogs (18.9%) were presenting signs of flea allergic dermatitis (FAD). Two days after treatment, 49 (60.5%) and 9 (11.7%) dogs were still infested by live ticks and fleas, respectively. However, the mean intensity of ticks decreased to 3.5 (±4.3) with a reduction of 92.5%. Except for sporadic cases, no attached ectoparasites were found on dogs from the day 14 visit until the end of the investigation (Figure 1). Cases of FAD resolved without any other treatment within 30 days.



Resolution of a severe tick infestation in a dog enrolled in the trial: Day 0 (A), Day 2 follow-up (B) and Day 7 follow-up (C).

CONCLUSIONS:
Based on the observed evidence of efficacy, long-term duration and safety, the imidacloprid/flumethrin slow release collar can be regarded as an efficacious and sustainable means for ectoparasite control and for treatment of FAD in dogs.

Acknowledgements: Bayer Animal Health is thanked for the supply of products.

Keywords:
Dog, Ectoparasites, Control

O04.3
A PILOT TRIAL EVALUATING THE EFFICACY OF A 10% IMIDACLOPRID/2.5% MOXIDECTIN SPOT-ON FORMULATION IN THE TREATMENT OF NATURAL NASAL EUCOLEOSIS IN DOGS

Veronesi F.*[1], Morganti G.[1], Diaferia M.[1], Di Cesare A.[2], Schaper R.[3], Traversa D.[2]

[1]Department of Veterinary Medicine, University of Perugia ~ Perugia ~ Italy, [2]Faculty of Veterinary Medicine, University of Teramo ~ Teramo ~ Italy, [3]Bayer Animal Health GmbH ~ Leverkusen ~ Germany

INTRODUCTION:
No drug is presently licensed for the treatment of Eucoleus boehmi (syn. Capillaria boehmi) infection in dogs, thus the therapeutic regimens attempted so far have been derived empirically. With the aim to fill these gaps on canine nasal eucoleosis, a pilot trial was performed to investigate the efficacy and safety of a spot-on formulation containing 10% imidacloprid and 2.5% moxidectin (Advocate®, Bayer Animal Health GmbH, Leverkusen, Germany) in the treatment of the infection by E. boehmi in dogs.
MATERIALS AND METHODS:
N. 287 dogs kept in kennels or referred to practitioners for varying upper respiratory disorders tract were submitted to a pre-inclusion copromicroscopic screening for E. boehmi eggs using a flotation procedure with a sugar solution (1.200 specific gravity). Eggs of E. boehmi were identified on the basis of morphometric features (Di Cesare A et al, 2012, Parasit and Vectors, 5: 128). The 16 animals copromicroscopically positives were confirmed as being affected by nasal eucoleosis, either by rhinoscopy or by species-specific PCR-coupled sequencing assays on faeces (Di Cesare A et al, 2012, J Clin Microbiol, 50: 1958-1963) and included in the trial. The animals were randomly allocated, in a ratio of 1:1, to 2 different groups: Group T, treated topically on Day 0 with a single dose of Advocate® and Group C, left untreated. All dogs underwent clinical examination and quantitative copromicroscopy using a McMaster technique on Days -6 and -2 (baseline) and Day 28±2 (post-baseline). On Day 28±2 the efficacy of the treatment or the persistence of the infection were confirmed by rhinoscopy or, alternatively, by molecular procedures. Treated dogs still infected at Day 28±2 received a second dose of Advocate® and were re-examined at Day 56±2. Control dogs which were still positive on Day 28±2 received a rescue dose of Advocate® and were re-examined at Day 56±2.
RESULTS:
No side effects were recorded in any of the treated dogs. Seven of the 8 dogs in Group T were negative on Day 28±2 (reduction of baseline faecal egg counts by 99.14%), while one dog resulted negative at Day 56±2 after a second treatment on Day 28±2 (reduction of baseline faecal egg counts by 100% in Group T). Seven control animals received a rescue dose of Advocate® on Day 28±2 and scored copromicroscopically and molecularly negative on Day 56±2, thus increasing the reduction of post-baseline egg counts to 99.57% after a single administration. A complete recovery of the clinical signs present in seven of the eight symptomatic animals of the Group T and in five animals that received the rescue treatment was obtained 4 weeks after the first treatment.
CONCLUSIONS:
The results of the present pilot study showed that a single administration of a spot-on moxidectin formulation is a safe and effective option for treating clinical signs, stopping egg shedding and eliminating the worms in dogs with a natural infection by E. boehmi. GCP studies are warranted to confirm these promising results.

Keywords:
Eucoleus boehmi, Dog, Treatment

O04.4
PRELIMINARY ASSESSMENT OF THE EFFICACY OF PARASITICIDES IN THE TREATMENT OF TROGLOSTRONGYLUS BREVIOR INFECTION IN CATS

Crisi P.E.*[1], Boari A.[1], Di Cesare A.[1], Iorio R.[1], Santori D.[1], Seghetti M.[2], Di Costanzo R.[3], Dimitri C.F.[4], Traversa D.[1]

[1]Faculty of Veterinary Medicine ~ Teramo ~ Italy, [2]Ambulatorio Veterinario Riviera ~ Grottammare ~ Italy, [3]Clinica Veterinaria L'Arca ~ Teramo ~ Italy, [4]Ambulatorio Veterinario "Carmelo Flavio Dimitri" ~ Isola del Gran Sasso, Teramo ~ Italy

INTRODUCTION:
Troglostrongylus brevior, a sporadic lungworm infecting wild felids, has been recently described in domestic cats. Despite it may induce severe clinical signs especially in kittens (Traversa and Di Cesare, 2013, Trends Parasitol, 29:423-430), information on treatment of the infection is almost null. This report describes a promising, although preliminary, efficacy of three anthelmintics in treating troglostrongylosis. Pre- and post-treatment examinations were performed with Baermann's method and PCRs (Di Cesare et al., 2014, Parasitol Res, 113:613-618).
MATERIALS AND METHODS:
Cases 1 and 2: Two 2,5-month old kittens belonging to the same litter were positive in July 2013 for Aelurostrongylus abstrusus and T. brevior. The first (female, Case 1) showed severe cough and dyspnea while the other (male, Case 2) was asymptomatic. Both kittens were treated (Day 0) with a single dose of 2 mg/kg of an oral formulation containing milbemycin oxime (Milbemax®, Novartis Animal Health).
Case 3: A ~7 week-old male kitten referred in November 2013 with intense sneezing, coughing, hypertermia and deprexion, and positive for A. abstrusus and T. brevior, received (Day 0) a spot-on containing emodepside (Profender®, Bayer).
Case 4: A symptomatic (i.e. severe respiratory distresses, cough and dyspnea) ~8 week-old female kitten infected by T. brevior and Capillaria aerophila was treated (Day 0) in December 2013 with Profender®.
Case 5: A ~4 months old male kitten was referred in January 2014 with cough, dyspnea, open-mouthed and abdominal breathing. The kitten, positive for T. brevior, received (Day 0) a spot-on containing moxidectin (Advocate®, Bayer).
RESULTS:
One week post treatment (p.t.) Case 1, still symptomatic and positive for T. brevior, died for respiratory failure. Case 2 was asymptomatic and negative at daily examinations performed until Day +10. Case 3 showed no symptoms within a week p.t. but he was positive for T. brevior until Day +14, when he received a second dose of Profender® and scored negative at Days +21 and + 28. Case 4 was healthy and negative for T. brevior (and C. aerophila) at Days +14, +21 and +28. At two weeks p.t. Case 5 showed only a moderate cough and he resulted negative for T. brevior.
CONCLUSIONS:
Milbemycin oxime was effective against A. abstrusus in both Cases 1 and 2, and T. brevior in one of them (Case 2). The efficacy of a single administration of emodepside against A. abstrusus is shown by Case 3, and a second treatment succeeded in treating also T. brevior. In Case 4 a single treatment with emodepside guaranteed a cessation in the shedding of C. aerophila eggs and T. brevior L1s after a week and a clinical recovery at Day +14. In Case 5 a single administration of moxidectin was effective in treating troglostrongylosis. Larger studies are warranted to confirm the efficacy of these compounds in treating cat troglostrongylosis, even in co-infections with more common lungworms

Keywords:
Cat, Lungworms, Treatment

O04.5
EFFICACY OF ORAL SPINOSAD (COMFORTIS®) TO CONTROL CAT FLEAS IN A FELINE SHELTER

Longhini A.[1], Colnago G.[2], Manuguerra B.[1], **Kramer L.H.*[1]**

[1]Dipt. Scienze medico Veterinarie ~ Parma ~ Italy, [2]Elanco Animal Health Italy ~ Sesto Fiorentino ~ Italy

INTRODUCTION:
Comfortis® (spinosad) is an oral product for the treatment and control of flea infestation in dogs and cats.
Spinosad is a mixture of two natural macrocyclic lactones (spinosyn A and spinosyn D) isolated from a soil bacterium. It acts primarily by binding to nAChRs, leading to hyperexcitation of the insect CNS and, in turn, paralysis and death (Paarlberg et al., 2013, JAVMA, 242:1092-98). The present study was aimed at evaluating the effect of Comfortis® in outdoor cats housed in a cat shelter in Parma Italy.
MATERIALS AND METHODS:
A total of 62 cats were enrolled in the study, from June – Sept. 2013. Inclusion criteria included: age ≥14 weeks; weight >1.2 kgs; no concurrent disease. Flea infestation status was not an inclusion criteria: cats with fleas (see below) were evaluated for treatment and preventive efficacy while cats without fleas were evaluated for preventive efficacy. Each cat was visually evaluated for signs of flea infestation: generalized scratching and hair thinning or loss in the lombar region was noted. Every cat was combed with a flea comb for 5 minutes. All fur and attached material collected with the comb was placed in a zip-lock plastic bag that was labelled with the cat's i.d. number and study day. All combings were examined under a stereo microscope (Leica M80) for the presence/absence of fleas and/or debris within 2 hours from collection. Comfortis was administered at a dose of 50-75mg/kg, monthly for three months.
RESULTS:
On day of enrolment, 13 cats showed positive combings for the presence of fleas and/or flea dirt. Of these, 6 showed signs of pruritus and thinning or loss of hair in the lombar region.
Twenty-four hours following the first administration of Comfortis, only 8 cats were positive on combing, and only debris and dead fleas were observed. Clinical signs of pruritus were no longer observed from Day 15 onward and no subsequent combings were positive: all cats remained flea –free for the remainder of the observation period. Nine cats found Comfortis palatable enough to assume it without food at the first administration. This decreased to 8 (D28) and 7 (D60) cats on subsequent administrations.
The only apparent side effect noted was vomiting: 7 cats vomited following the first administration (of these, only one vomited within the first hour, but re-ate its own vomit so it was not necessary to re-administer the drug). All other cats vomited several hours following administration. The number of cats who vomited decreased on subsequent administrations, to 3 (D28) and 3 (D60).
CONCLUSIONS:
In the particular setting of a humane shelter, where many cats may refuse handling, thus making the use of topical treatment difficult, if not impossible, the ease of oral administration of Comfortis is especially interesting.
In the present field experience, palatability was not high for spontaneous administration, but when mixed with food, it was nearly always acceptable.
The only side effect observed was vomiting. The prevalence, however, was lower than that indicated on the product label.

Keywords:
Spinosad, cat, flea

O04.6
PILOT STUDY ON EFFICACY AND SAFETY OF TINIDAZOLE AGAINST GIARDIA LAMBLIA IN NATURALLY INFECTED CATS

Napoli E.*[1], Gaglio G.[1], Gallo D.[2], Falsone L.[1], Brianti E.[1], Giannetto S.[1], Pennisi M.[1]

[1]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina, ~ Messina ~ Italy, [2]Medico Veterinario libero professionista ~ Messina ~ Italy

INTRODUCTION:
Giardia lamblia is a protozoan parasite of the small intestine causing disease in animals and humans (Olson et al., 2000, Parasitol. Today, 16: 213-217). Metronidazole and fenbendazole are the most common drugs employed for the treatment of giardiasis in pets. However, treatment failure and suspected resistance have been reported for both the molecules. Therefore, new agents and effective treatment protocols are evocated. In this study we evaluated the efficacy and safety of tinidazole, a nitroimidazole derivate, against G. lamblia infection in cats.
MATERIALS AND METHODS:
Study population was composed by 10 cats with a history of chronic diarrhoea. All animals recruited in the study tested positive for G. lamblia either by copromicroscopy (Decok et al., 2003, Parasite, 10:69-72) and PCR. The cats were housed in individual cages for all the study period, fed by a commercial feline food and the faecal score was daily assessed in a scale ranging from 1 to 7 (Purina faecal scoring system). Tinidazole was given at 30 mg/kg PO, q24 for 3 days in form of gastro-resistant capsules coated by guar gum. After the treatment cats were observed for 48 hours. Faecal samples were collected at 4, 5 and 6 days after the end of treatment. The presence of G. lamblia in these samples was assessed either by copromicroscopy and PCR analyses. Cats were further follow-up 15 and 30 days after treatment and faecal samples were collected and examined as above.
RESULTS:
All cats enrolled in the study were adult non-pedigree cats (6 males and 4 females). The mean value of faecal score at the time of recruitment was 5. Drug administration was successful in enrolled animals and no adverse events or side effects were recorded. Faeces scored ≤ 3 and tested negative for Giardia at both diagnostic techniques in all the follow-up (i.e., D4, D5, D6, D15 and D30) thus resulting in 100% of efficacy.
CONCLUSIONS:
Results of this pilot study suggest as tinidazole may be safe and highly efficient against G. lamblia infection in cats. Three doses of tinidazole were effective to cure the infection in all the enrolled cats making this protocol shorter compared to those using metronidazole and fenbendazole (Gruffydd-Jones et al., 2013, J. Feline Med. Sur., 15:650-652). In addition, the formulation in gastro-resistant coated capsules increased gastric-tolerance and allowed drug absorption in the small intestine. Based on the observed evidences tinidazole in form of gastro-resistant capsules could represent an efficient candidate drug for the treatment of cat giardiasis.

Keywords:
Giardia lamblia, cat giardiasis, tinidazole

O04.7
DIMINISHING ROLE OF TRADITIONAL HEALERS IN THE MANAGEMENT OF MALARIA IN MALINDI, KENYA, IN THE CONTEXT OF DECLINING MALARIA

Kibe L.[2], **Habluetzel A.*[2]**, Kamau A.[1], Jones C.[1], Mbogo C.[1]

[1]Kenya Medical Research Institute, Centre for Geographic Medicine Research-Coast ~ Malindi ~ Kenya, [2]School of Pharmacy, University of Camerino ~ Camerino ~ Italy

INTRODUCTION:
Traditional Healers (THs) form an important community institution to which people resort for the treatment and management of various health problems including malaria. This study aimed to explore community perceptions on the importance and role of THs in the context of declining malaria incidences in Malindi, an area that benefited from massive and successful scaling up of national malaria control efforts realized over the last decade in Kenya.
MATERIALS AND METHODS:
A cross sectional study was carried out in Malindi and Magarini Sub Counties in the coastal region of Kenya. In-depth qualitative research methods were employed to collect data in four selected locations (Malimo, Jilore, Garashi and Marekebuni). First, data was collected using a variety of participatory rural appraisal tools, which included Community River of life, Trend lines, Community Resource Maps, photo voice and Focus Group Discussions. Secondly, Key Informant interviews were conducted with health personnel, THs, Community Health Workers (CHWs), youth group leaders, women group leaders and area chiefs.
RESULTS:
The study revealed a diminished role of THs with the decline in number of malaria cases and improved health care services provided through the modern health care structures. The factors associated with the reduced role and importance of THs include increased knowledge and awareness among community members about malaria. This knowledge is acquired from CHWs' during home visits, by listening to radio and/or from informative posters illustrating the symptoms of malaria, its causes and management of clinical signs and symptoms among children. Community members' awareness on availability and accessibility of dispensaries, private clinics and pharmacies, and the presence of CHWs had further reduced the need to seek the THs services. Religious leaders also tend to demonize the activities of THs saying their activities are ungodly and this has also contributed to the reduced role and importance of THs. Some community representatives noted, however, that "there is a confusion that a mganga (traditional healer) and a mchawi (witchdoctor) are one and the same". This belief and lack of differentiation has resulted into many THs being beheaded especially by the younger generation who think that the practices of THs inhibit societal progress and that the THs are exploiting the poor. On their part, the THs interviewed noted that the profession was slowly dying out because of the lack of interest by youth to become traditional healers.
CONCLUSIONS:
The importance of THs has evidently declined in the past decade in Malindi. As a result, the once treasured community institution is slowly dying and the indigenous technical health care knowledge is at risk of getting lost. There is need for identification and preservation of relevant knowledge and practices that can benefit the population. Thus THs should be offered a complementary role and a collaborative effort should be initiated and promoted between modern and traditional health care providers.

Keywords:
traditional healer, health care, malaria

O04.8
MAPPING INFECTION PROBABILITIES FOR PARASITOLOGICAL SURVEILLANCE IN LIVESTOCK USING A BAYESIAN KRIGING MODEL

Musella V.*[1], Rinaldi L.[2], Catelan D.[3], Biggeri A.[3], Cringoli G.[2]

[1]Department of Health Sciences, University of Catanzaro Magna Graecia ~ Catanzaro ~ Italy, [2]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania); Inter-University Center for Research in Parasitology (CIRPAR) ~ Naples ~ Italy, [3]Department of Statistics, Informatics and Applications "G. Parenti" University of Florence ~ Florence ~ Italy

INTRODUCTION:
Model-based geostatistics is appropriate on point data collected by valid study design (Cressie, 1991, Statistics for spatial data, New York, John Wliley & sons). These approaches are used to explain the spatial pattern of the phenomenon under study, to investigate spatial autocorrelation and, conditioning on the observed data, to predict a continuous risk surface on the whole study area. The aim is to predict a continuous infection risk surface. Little work has been done on predicting infection probabilities at unit level. In this work we developed a Bayesian kriging model to obtain an estimate of infection probability and related uncertainty for each one of the 8794 geo-referenced sheep farms of the Campania region (southern Italy).
MATERIALS AND METHODS:
The survey was conducted in the Campania region (southern Italy). A Geographical Information System (GIS) was developed and all the sheep farms present in the region were geo-referenced. In order to uniformly sample the farms throughout the entire region, a systematic grid sampling approach was used (Rinaldi et al., 2006, BMC Vet. Res., 2:29). Specifically, a grid representing quadrants of 10 km x 10 km was overlaid on the region map within a GIS. Parasitological data come from a cross-sectional survey carried out to study the spatial distribution of selected helminths in sheep farms. A grid sampling was performed to select the farms for coprological examinations. Faecal samples were collected for 121 sheep farms and the presence of 21 different helminths were investigated using the FLOTAC technique.
RESULTS:
The 21 responses were very different in terms of geographical distribution and prevalence of infection. The observed prevalence range was from 0.83% to 96.69%. The distributions of the posterior predictive probabilities for all the 21 parasites were very heterogeneous.
CONCLUSIONS:
We exemplify how the results of the Bayesian kriging model can be used to plan a second wave survey based on posterior predictive probabilities, their uncertainty or combining both information. The proposed model is simple, represents a useful tool to communicate with field researchers and to address targeted infection control treatments and sampling strategies. It is easily extendable to other fields of research.

Acknowledgements: The European Union Seventh Framework Programme FP7 –KBBE-2011-5 GLOWORM (no. 288975) are sincerely thanked.

Keywords:
Parasitological surveillance, Bayesian Kriging model, sampling strategies

O04.9
EVALUATION OF THE EFFICACY OF ANTHELMINTHIC TREATMENTS IN SHEEP AND HORSES OF SARDINIA, ITALY

Scala A.*[1], Varcasia A.[1], Pipia A.P.[1], Tamponi C.[1], Muntoni S.[1], Dore F.[1], Sanna G.[1]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari ~ Sassari ~ Italy

INTRODUCTION:
In the present abstract authors present the results of different field trials on the efficacy of anthelmintic treatments respectively in sheep and horses, in order to highlight if anthelmintic resistance (AR) is present in animals bred in Sardinia.
MATERIALS AND METHODS:
The efficacy of seven anthelmintic treatments have been assessed in sheep breedings of Sardinia, 5 treatments with moxidectin (Cydectin® 0.1%: 0.2 mg/kg bw) against Gastro-Intestinal Nematodes (GIN) with EPG >100 and 2 against dicrocoeliosis with albendazole in sheep with EPG >70 in a breeding with suspected AR for Dicrocoelium [Valbazen 1.9% - Zoetis: 10.45 mg/kg bw repeated after a week (a) and then with Albendazole at 20,9 mg/kg bw in a single administration (b)]. Five anthelmintic products were evaluated in 74 horses with EPG >150. Animals were divided into five treatment homogeneous groups, according to EPG, breeding, age: IVM 1: n°15 (Eqvalan®: ivermectin 1.87% 0.2 mg/kg PV); IVM 2: n° 17 (Eraquell®: ivermectin 1.87% 0.2 mg/kg PV); MOX: n°10 (Equest®: moxidectin 1.89% 0.4 mg/kg PV); PYR: n° 19 (Strike®: pyrantel pamoate 38% 6.6 mg/kg PV) and FBZ: n°13 (Panacur®: fenbendazole 1.87% 7.5 mg/kg PV). Individual faecal samples were taken directly from rectum at Day -7 (enrolment), D0 (day of treatment) and Day 14. Quali-quantitative analysis for GIN were performed using McMaster technique, using supersaturated NaCl solution (PS 1150) for flotation and for dicrocoeliosis with Flotac® method with ZnS (ps 1350). The effectiveness of the treatments was carried out according to Coles et al., 1992 (Vet. Parasitol., 44: 35-44).
RESULTS:
The efficacy of the five treatments with moxidectin in sheep with GIN ranged from 97.1% (CI 95%: 91.1 – 103.1) to 100%. The two treatments performed with albendazole against sheep dicrocoeliosis have highlighted an efficacy respectively of 62% (CI 95%: 49.05 – 74.94) and 58.8% (CI 95%: 37.22 – 80.37).
The FECR values obtained in horses at D14 in each trial group has always highlighted a ≥ 95% reduction with C.I. 95% > 90% (IVM 1-100%; IVM 2- 99,4%; MOX – 100%; PYR 98.9%; FBZ – 99%).
CONCLUSIONS:
The trial carried out against GIN in sheep with moxidectin did not show any AR, in agreement with what reported by other authors in Italian sheep where AR against febantel, levamisole and ivermectin have been found (Traversa et al., 2007, Parasitol. Res., 101: 1713-1716; Geurden et al., 2014, Vet. Parasitol., 201: 59-66).
An episode of AR was highlighted in a sheep breeding with dicrocoeliosis against albendazole. This data could confirm the low efficacy of current posology of this drug suggested by pharmaceutical brands against this trematode in sheep (Manga-Gonzales et al., 2010, Veterinarni Medicina, 55: 19-29). All the drugs used in the horses trial have shown percentages of efficacy at D14 greater than 95% with the 95% confidence level is >90% in all groups. Our data indicate that AR is not yet present in Sardinian horses Intestinal Strongyles population, in spite of what observed in other Italian regions.

Acknowledgements: Regional Government of Sardinia, CRP-60126 (L.R. 7, 2012) and Mr. F. Salis, Technician, Parasitology Lab., University of Sassari.

Keywords:
anthelminthic treatments, sheep, horses

O04.10
COMPARATIVE EFFICACY OF TWO PYRANTEL ORAL FORMULATIONS (PASTE AND GRANULATE) IN DONKEYS NATURALLY INFECTED WITH INTESTINAL STRONGYLIDAE

Veneziano V.*[1], Smaldone G.[1], Mariani U.[2], Neola B.[1], Roncoroni C.[3], Gokbulut C.[4]

[1]Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Italy ~ Napoli ~ Italy, [2]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy ~ Portici ~ Italy, [3]Istituto Zooprofilattico Sperimentale Lazio e Toscana,Italy ~ Roma ~ Italy, [4]dDepartment of Medical Pharmacology, Balikesir University Turkey ~ Balikesir ~ Turkey

INTRODUCTION:
Donkeys and horses share many internal parasite including the nematodes Strongylidae. Pyrantel (PYR) is an imidazothiazole derivative, highly effective (95%-97%) against small strongyles, Parascaris equorum and Strongylus vulgaris, and has moderate active against Strongylus edentatus (70%) and Oxyuris equi (65%) (Gokbulut et al., 2001, J.Vet. Pharmacol. Therap., 24:77-79). There is a paucity of data available on the efficacy of anthelmintics used in donkeys. Therapeutics, such as antiparasitic compounds, are often administered to donkeys based on dosage and intervals recommended for horses and cattle, because very few drugs have donkey-specific label indications (Grosenbaugh et al. 2011, Equine Vet Educ, 23:523-530). PYR pamoate oral formulations currently available in Italy for horses are paste 38% and granulate 20%. In the present study the comparative anthelmintic efficacy of 2 different oral formulations (paste and granulate) of PYR are reported in donkeys.
MATERIALS AND METHODS:
The trial was conducted on a donkey farm in southern Italy. Faecal examinations (individual Faecal Egg Counts and pooled coprocolture) performed before the beginning of the study showed high prevalence of intestinal nematodes (Cyathostomum spp., Strongylus vulgaris) in all donkeys. Twenty-one female crossbreed donkeys were selected on the basis of faecal egg counts (FEC). The animals were allocated to 3 groups (n: 7 donkeys). One group was untreated control (C-group) and the PYR P-group and PYR G-group were treated with Strike paste 38% and Strike granulate 20%, both administered orally at the manufacturer's recommended horse dose of 6.94 mg/kg bodyweight. FECs were performed on each study animal before the start of the trial (day -3), at days 7, 14, 21, 28, 35, 42, 56 after treatment. FECs were determined using a modified McMaster technique (sensitivity 10 eggs per gram - EPG). On each sampling day, faecal samples were incubated at 27 °C for 7-10 days for larval identification. To determine the efficacy of PYR against intestinal strongyles following the WAAVP guidelines (Duncan et al., 2002, Vet Parasitol, 103:1-18) at each faecal sampling time, arithmetic mean of EPG was calculated and the percent efficacy (%) of each animal was calculated in terms of FEC Reduction (FECR) according to the formula:

$$FECR\ (\%) = \frac{\text{MeanEPG Control Group} - \text{MeanEPG Treated Group}}{\text{Mean EPG Control Group}} \times 100$$

RESULTS:
No adverse reactions to treatments were detected. The results of FECs in the C-group and PYR groups are shown in the following table.

Day	C group		PYR Paste group		FECR (%)	P value	PYR Granulate group		FECR (%)	P value
	EPG AM	EPG Range	EPG AM	EPG Range			EPG AM	EPG Range		
-3	1,104	450-1,470	1,061	160-1,770	-	0.8636	1,139	170-2,440	-	0.9220
7	849	450-1,170	14	0-30	**98.3**	0.0000	24	0-80	**97,1**	0.0000
14	936	500-1,340	14	0-30	**98.5**	0.0000	26	0-10	**97,3**	0.0000
21	1,036	140-1,450	19	0-30	**98.2**	0.0001	39	0-110	**96,3**	0.0001
28	1,334	170-2,030	46	0-110	**96.6**	0.0002	49	20-80	**96,4**	0.0002
35	1,163	610-2,000	213	20-740	81.7	0.0003	124	40-290	89,3	0.0001
42	854	90-1,370	520	40-1,790	39.1	0.2657	303	70-540	64,5	0.0095
49	823	90-1,680	616	10-1,950	25.2	0.5055	533	150-1,090	35,2	0.1925
56	663	210-1,130	737	80-1,410	-11.2	0.7371	651	240-1,410	1,7	0.9536

* Parametric *t*-test (PYR-groups vs C-group) P < 0.001

Pretreatment EPG were not significantly different (P>0.1) between groups. Post-treatment EPG for both PYR treatment groups were significantly different (P<0.001) from the control group until day 35. Following treatments the PYR formulations were efficient (>90% efficacy) until day 28.
In all studied donkeys, coprocoltures performed at day -3 revealed the presence of Cyathostomum spp., S.vulgaris. Faecal coltures performed at different days from C-group confirmed the presence of the same genera. Coprocoltures from treated animals revealed the presence of few larvae of Cyathostomum spp..

CONCLUSIONS:
This trial demonstrates that both PYR oral formulations at the manufacturer's recommended horse dose are effective (96–98%) against intestinal strongyles on donkeys. Therapeutic equivalence was demonstrated for PYR oral formulations paste and granulate based on FECR. Therefore similar dosage regimens of PYR could be used for horses or donkeys.

Keywords:
Donkey, Pyrantel, efficacy

O04.11
METAPHYLACTIC TREATMENT WITH TOLTRAZURIL AND DICLAZURIL FOR THE CONTROL OF COCCIDIOSIS IN BUFFALO CALVES

Bosco A.*[1], Rinaldi L.[2], Cappelli G.[1], Santaniello M.[1], Morgoglione M.E.[1], Guariglia I.[1], Cringoli G.[2]

[1]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania) ~ Naples ~ Italy, [2]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania); Inter-University Center for Research in Parasitology (CIRPAR) ~ Naples ~ Italy

INTRODUCTION:
The aim of the present study was to evaluate the parasitological and performance effect of metaphylactic treatments with toltrazuril and diclazuril for the control of coccidiosis by Eimeria spp. in naturally infected buffalo calves.
MATERIALS AND METHODS:
A controlled field trial was conducted in 5 buffalo farms located in the Salerno province of southern Italy; these farms were divided into two typologies based on management system. In 3 farms, the buffalo calves were bred in individual boxes from the birth to the seventh/eighth week of age and then transferred to the ground in multiple boxes (Typology A); in the other 2 farms, the calves were bred on the ground in multiple boxes from the birth (Typology B). In each farm, 36 calves were divided at random into three groups of 12 calves each. One group was treated with toltrazuril (group TOL), the second group was treated with diclazuril (group DIC), and the third group was left as untreated control group (group CONT). In the buffalo farms of Typology A, the calves of the groups TOL and DIC were treated only once, with the respective drug, at the seventh/eighth week of age. In the buffalo farms of typology B, the calves of the groups TOL and DIC were treated twice, once at the fifth and once at the seventh/eighth week of age. Parasitological analyses were performed weekly on each buffalo calves using the FLOTAC double technique (Cringoli et al., 2010, Nat. Prot., 5: 503–515), with a sensitivity of 2 oocysts per gram (OPG). The body weight was recorded fortnightly, starting from the day of treatment and continued until the twentieth week of age.
RESULTS: In the 5 buffalo farms the average oocyst excretion decreased significantly in both the treated groups (TOL and DIC groups) compared to the CONT group; however the TOL groups showed intensities (OPG) significantly lower than the DIC groups. The species of Eimeria found in the studied animals are reported in Table 1 and the body-weight gains recorded fortnightly are reported in Table 2.

FARM	TYPOLOGY	*E. ellipsoidalis*	*E. subspherica*	*E. bovis*	*E. zuernii*	*E. barellivi*
1	A	70%	10%	8%	2%	10%
2	A	11%	28%	25%	36%	0%
3	A	5%	22%	36%	13%	24%
4	B	45%	16%	20%	11%	8%
5	B	27%	13%	14%	31%	15%

Table 1. Species of Eimeria in the 5 experimental buffalo farms.

FARM	TYPOLOGY	Body-weight gains	
		GROUP TOL	GROUP DIC
1	A	+5.8 kg	+1.7 kg
2	A	+8.1 kg	+2 kg
3	A	+6.4 kg	+3.1 kg
4	B	+6.6 kg	+3 kg
5	B	+5.4 kg	+2.3 kg

Table 2. The body-weight gains of buffalo calves belonging the groups treated (Group TOL and Group DIC) compared with the control group.

CONCLUSIONS:
The field trials demonstrate the high efficacy of toltrazuril administered orally to buffalo calves in subclinical natural Eimeria infections in farms of southern Italy.
Furthermore, the study showed that subclinical coccidiosis may impair growth performance in buffalo calves reared either in individual boxes or in multiple boxes. The reproductive cycle of Eimeria in calves naturally exposed to infection was susceptible to metaphylactic treatment with toltrazuril and diclazuril, exhibiting a growth response of treated calves.

Keywords:
Metaphylactic treatment, toltrazuril, diclazuril

O.04.12
THE SUPPLY OF DRUGS FOR NEGLECTED TROPICAL DISEASES IN ITALY: CRITICAL ISSUES AND POSSIBLE SOLUTIONS

Angheben A.*[1], Bisoffi Z.[1]

[1]Ospedale Sacro Cuore - Don Calabria, Centro per le Malattie Tropicali ~ Negrar ~ Italy

INTRODUCTION:
Neglected tropical diseases (NTDs) are a clinically various group of infections caused by a different pathogens such as viruses, bacteria, protozoa and helminths. The World Health Organization (WHO) prioritized 17 neglected tropical diseases, which are responsible of more than 1 billion sick people worldwide and are endemic in 149 countries. NTDs cause immense human suffering and disability, and death. They cause a devastating obstacle to health status and mantain a biunivocal relationship with poverty, being an impediment to poverty reduction and being generated from poverty. In particular, they are silent diseases, with chronic long-term sequelae, potentially importable also to non endemic countries. In the last decades in fact, the growing migration flows around the world, made possible the appearance of NTDs in non endemic countries where they are consequently under-diagnosed or mis-diagnosed, often in the late phase of their natural history or in the phase of organ damage, when treatments are generally less effective.
Non endemic countries are generally unprepared to offer the right management to this group of diseases. In Italy for instance, for many NTDs treatments are unavailable, even though the drugs are part of the list of essential drugs of the WHO. The lack of drugs is a boost to under-diagnosis and under- or mis-treatments.
The Ministry Decree of Febrary 11th, 1997 governs the procedures for the supply of drugs available abroad but not in the national drugs formulary. A drug registered abrod can be imported in Italy under medical responsibility for identified patients (or storage) through motivated request, custom and Ministry of Health controls. The procedure is often slow and requires a proactive attitude in this sense, not common to all nosocomial institutions.
MATERIALS AND METHODS:
We organized a meeting of clinicians and pharmaceutical officers of Centres from four Italian Regions (Veneto, Emilia Romagna, Toscana, Lombardia) doing an audit in order to find a possible way to deal with difficulties in NTDs drug procurement.
RESULTS:
The need to interface with the board of representatives of the regional pharmacological services at the State-Regions Conference, without fear of submitting this issue, has been stressed. It has been planned to create of a list of drugs for NTDs to be proposed to AIFA under the Italian Law 648/96.
This law establishs that:
- a list of innovative medicines authorized in other states, but not in Italy, or investigational medicinal products not yet authorized in Italy or medicines that can be used in the absence of a viable alternative for not authorized indications can be created;
- for all drugs on the list a second-phase study should be available, in order to take the drug in consideration;
It was finally decided to proceed on this line preparing files of the various drugs for NTDs in accordance with the current regulations, asking for their inclusion in the list of drug products granted under the National Health System.
CONCLUSIONS:
After the meeting, participants were convinced that the inclusion in the list of drugs of Law 648/96 of a package “drugs for NTDs” represents a huge step forward for the improvement of health in global prospective and could have an important reflection in improving diagnosis and care of NTDs in Italy.

Keywords:
neglected tropical diseases, drugs, procurement

NOTES

SESSIONE PARALLELA O-05

ENTOMOLOGIA MEDICA E VETERINARIA

O05.1
CULICOIDES INDOOR WINTER ACTIVITY IN CENTRAL ITALY

Magliano A.[1], Scaramozzino P.[1], Cincinelli G.[2], Moni A.[3], Silvestri P.[4], **De Liberato C.*[1]**

[1]Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana ~ Roma ~ Italy, [2]ASL8 Arezzo, Zona Valdarno ~ Arezzo ~ Italy, [3]ASL 1, Massa Carrara ~ Massa ~ Italy, [4]ASL Rieti Distretto Mirtense ~ Poggio Mirteto (Ri) ~ Italy

INTRODUCTION:
Bluetongue virus (BTV) is an arbovirus affecting domestic and wild ruminants, endemic in tropical latitudes worldwide. In the last decades the virus spread northward, causing outbreaks first in Mediterranean countries and then in Central Europe. BTV is transmitted by Diptera of the genus Culicoides and in Europe C. obsoletus is considered its main vector. Following the Central-European epidemic started in 2006, questions arose regarding the overwintering means of the virus. One of the hypotheses is the continuous viral circulation through adult C. obsoletus populations remaining active in cattle sheds during the winter. In this context, a research project granted by the Italian Ministry of Health is underway, aimed at studying Culicoides indoor winter activity in stables. Preliminary results of this research are here reported.
MATERIALS AND METHODS:
Three study farms, one for each of the Massa (M), Arezzo (A) and Rieti (R) provinces, were selected according to the following criteria: winter temperatures (mild (M), cold (A) and intermediate (R)), presence of cattle both indoor and outdoor and no use of insecticides during the sampling period. Catches were performed weekly from November to March for 3 seasons (2010-2013) using Onderstepoort black-light traps. In each farm 2 traps worked simultaneously, one inside and one outside the stable, to monitor Culicoides spp. indoor and outdoor activity. Culicoides spp. specimens were identified according to the specific wing patterns and males genital morphology (Delecolle, 1985). Female specimens were classified, according to their physiological stage, as nulliparous, parous and engorged.
RESULTS:
In total 336 collections were performed and 17.487 Culicoides were caught: 11.586 outdoor and 5.901 indoor. C. obsoletus group accounted for 98,2% of sampled specimens. Results are summarised in Tab.1.

Tab.1 – *Culicoides* spp. females mean catches: tot = total; par = parous; nul = nulliparous; eng = engorged; obs = *C. obsoletus* group; pul = *C. pulicaris* group; imi = *C. imicola*; O = outdoor; I = indoor.

	Culicoides tot		obs tot		obs par		obs eng		obs nul		pul tot		imi tot	
Site	O	I	O	I	O	I	O	I	O	I	O	I	O	I
M	114,4	52,8	133,9	52,7	24,1	10,7	10,3	13,5	77,5	27,5	0,8	0,1	0,1	0,03
A	15,0	30,8	14,4	30,1	4,7	16,6	0,5	5,8	9,2	7,7	0,3	0,3	0,0	0,0
R	73,9	21,1	72,1	19,5	19,9	5,9	1,1	0,1	51,0	13,1	1,3	0,3	0,0	0,0

CONCLUSIONS:
The first noteworthy finding of this study is the record indoor of all the considered species and species groups, despite the presumed esophily of Culicoides biting midges. Relevant is also the record indoor of females in all the physiological stages and of C. imicola, the main BTV vector worldwide, active in winter and inside stables. The results regarding engorged specimens in site A and M could be explained with a propensity of C. obsoletus females to feed indoor or to take shelter indoor after feeding outdoor. Finally, as in the two warmer sites (M and R) outdoor catches always outnumbered indoor ones while in the coldest one (A) indoor catches were higher than outdoor ones, it could be postulated that low outdoor temperatures would drive Culicoides midges indoors.

Keywords:
Culicoides, indoor, Italy

O05.2
ECOLOGICAL NICHE MODEL OF P. PERNICIOSUS (DIPTERA: PSYCHODIDAE) IN NORTH-EASTERN ITALY

Signorini M.*[1], Cassini R.[1], Frangipane Di Regalbono A.[1], Pietrobelli M.[1], Babiker A.M.A.[2], Stensgaard A.S.[3], Stansgaard A.S.[4]

[1]1Dipartimento di Medicina Animale, Produzioni e Salute, Università degli Studi di Padova ~ Legnaro, Padova ~ Italy, [2]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro, Padova ~ Italy, [3]3Center for Macroecology, Evolution and Climate, Department of Biology, University of Copenhagen, Universitetsparken 15 ~ Copenhagen ~ Denmark, [4]Section for Parasitology and for Aquatic Diseases, University of Copenhagen ~ Copenhagen ~ Denmark

INTRODUCTION:
In northeastern Italy the recent spread of Canine Leishmaniosis (CanL) and the presence of two species of vector sandflies (Phlebotomus perniciosus and P. neglectus) is well documented. Understanding the ecoclimatic and environmental characteristics that could influence the distribution patterns of vector species and consequently the epidemiology of the disease is crucial, also considering the possible impact of on-going climate changes on the emergence of Leishmaniosis. A retrospective database, describing the results of 12-years entomological survey conducted in northeastern Italy, has been analyzed through the use of GIS tools and Ecological Niche Models approach (ENM), based on remote sensing environmental data, in order to map the potential distribution of P. perniciosus, the main vector species in northeastern Italy.
MATERIALS AND METHODS:
Sandfly trapping was conducted from 2001 to 2012 in 175 sites, located in Veneto, Friuli Venezia Giulia and Trentino Alto Adige Regions. At all trap sites, GPS coordinates were acquired and a GIS-platform created, including the entomological data and several environmental variables. The presence/absence of P. perniciosus was explored in regard to the environmental characteristics considered, such as seasonal and annual MODIS data (Normalized Difference Vegetation Index, [NDVI] and daily and nightly Land Surface Temperature [LST]), a land cover map (Corine Land Cover 2006 [CLC]), a Digital Elevation Model (GTOPO30) and climate layers obtained from the WorldClim database. Initial exploratory analysis of the environmental data revealed high levels of colinearity, hence only a subset of the most promising features were used to develop a predictive model of potential distribution of P. perniciosus in northeastern Italy, using the software MaxEnt (Maximum Entropy Modeling approach).
RESULTS: Overall, 6.144 sandflies were collected and identified, and P. perniciosus was the most abundant species (3.797, 61,8%). Out of 175 sites surveyed, 60 resulted positive to P. perniciosus and were used in model building (75% presence-only records randomly selected as training points, 25% selected as test points, used for model validation). The variables resulted more relevant to the presence of the vector species were NDVI winter, LST day summer, LST night winter, altitude, CLC, and precipitation during the Warmest Quarter. The analyses of ecoclimatic features showed that P. perniciosus prefers hilly areas, characterized by temperate climate, high vegetation cover and moderate rainfall. The model showed a high predictive power (AUC 0,933; st. dev. 0,030) and demonstrated to be realistic, since areas highly suitable for sandfly overlap with CanL foci in the study area (fig.1).

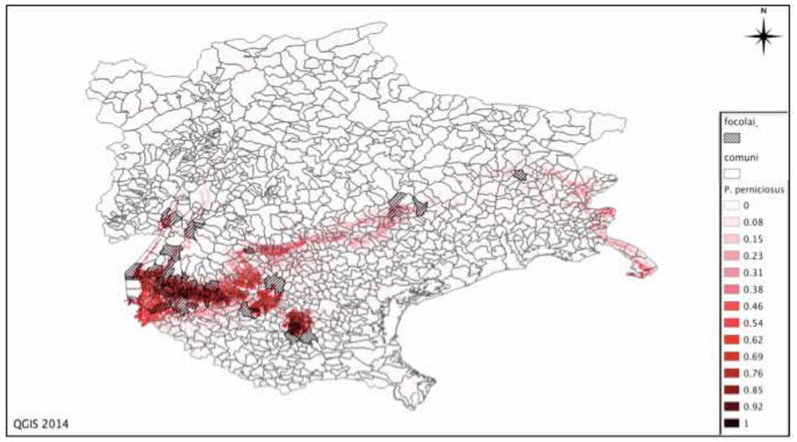


Figure 1: potential distribution of P. perniciosus in northeastern Italy and municipality with CanL foci

CONCLUSIONS:
This study represents the first attempt to develop a risk map of potential distribution of P. perniciosus in northeastern Italy. The map highlights heterogeneity in the spatial distribution of the vector sandfly, clustered in some areas, with specific features, consistent with the biological and ecological requirements of the species and with the known distribution. Visualisation of distribution patterns of vectors species in environmental space, using ENM and Remote Sensing data, seems to be a useful tool for the proper identification of the surveillance actions, mostly in the framework of wide programs for the control of CanL.

Keywords:
Ecological Niche Models, MaxEnt, Phlebotomus perniciosus

O05.3
DIVERSITY AND SEASONALITY OF PHLEBOTOMINE SAND FLIES IN A MILITARY TRAINING AREA IN NORTH-EASTERN BRAZIL

Moura A.C.D.A.[1], Da Silva K.G.[1], Miranda D.E.D.O.[1], Ramalho M.[1], De Moura M.R.A.[1], Da Silva F.J.[1], Brandão Filho S.P.[1], Otranto D.[2], **Dantas Torres F.*[1]**

[1]Aggeu Magalhães Research Centre ~ Recife ~ Brazil, [2]University of Bari ~ Valenzano ~ Italy

INTRODUCTION:
Sand flies (Phlebotominae) are vectors of several viral, bacterial, and protozoal pathogens, including those of the genus Leishmania, the causative agents of leishmaniasis. The ecology of sand flies is of interest because it may provide background information for a better understanding of the epidemiology of leishmaniasis and to predict the spatial and temporal variations in the risk of leishmaniasis in a given area. The objective of this study was to identify the diversity of species that make up the sand fly fauna in a remnant of Atlantic Forest located in the Campo de Instrução Marechal Newton Cavalcanti (CIMNC), Paudalho municipality, Pernambuco state, north-eastern Brazil.
MATERIALS AND METHODS:
From July 2012 to July 2013, light traps were placed (ca. 1.5 m above the ground) in 10 sampling sites (P1-P10) near the burrows of wild animals, trunks and roots of large trees in shady and humid environments. Light traps worked from 18:00 until 6:00 am, for 4 consecutive days each month. The insects captured were initially examined under a stereomicroscope, separated according to sex and kept in 70% ethanol. The specimens were mounted on slides and identified morphologically.
RESULTS:
A total of 14,403 specimens belonging to 23 species were identified, as follows: Lutzomyia choti (n=10,968), L. longispina (n=1,482), L. sordellii (n=657), L. complexa (n=650), L. walkeri (n=182), L. amazonensis (n=113), L. wellcomei (n=84), L. evandroi (n=54), L. quinquefer (n=52), L. ayrosai (n=48), L. naftalekatzi (n=23), L. barrettoi barrettoi (n=20), L. claustrei (n=20), L. umbratilis (n=16), L. schreiberi (n=11), L. capixaba (n=7), L. brasiliensis (n= n=4), L. viannamartinsi (n=3), L. whitmani (n=3), L. shannoni (n=3), L. migonei (n=1), L. yuilli pajoti (n=1) and L. oswaldoi (n=1). The number of males (n=7,278) and females (n=7,125) was close to unity. The overall monthly average number of sand flies captured per trap was 110.8, with peaks in March (169.1 sand flies per trap) and April 2013 (332.4 sand flies per trap).
CONCLUSIONS:
This study indicates a high level of sand fly diversity, revealing the occurrence of eight potential vectors of Leishmania spp. in the examined area, including L. complexa, L. amazonensis, L. wellcomei, L. ayrosai, L. umbratilis, L. whitmani, L. shannoni and L. migonei. It also shows a high abundance of sand flies in the study area, with peaks after raining periods. These data will help us to predict the period in which there may be an increased risk of Leishmania transmission in remnants of Atlantic Forest in north-eastern Brazil.

Keywords:
sand flies, diversity, seasonality

O05.4
A REAL-TIME PCR FOR THE CHARACTERIZATION OF MULTIPLE BLOOD SOURCES IN FIELD-COLLECTED SAND FLIES

Da Silva K.G.[1], De Morais R.C.S.[1], Miranda D.E.D.O.[1], Moura A.C.D.A.[1], Brandão Filho S.P.[1], Otranto D.[2], De Paiva Cavalcanti M.[1], **Dantas Torres F.*[1]**

[1]Aggeu Magalhães Research Centre ~ Recife ~ Brazil, [2]University of Bari ~ Valenzano ~ Italy

INTRODUCTION:
Sand flies (Phlebotomine) are vectors of protozoa of the genus Leishmania, the causative agents of leishmaniasis. The female sand flies are haematophagous and feed on various vertebrate species, including humans (Maroli et al., 2013, Med. Vet. Entomol., 27:123-q47). Different methodologies have been applied to assess the dietary habits of phlebotomine sand flies, but in most cases, they are laborious or lack sensitivity. In this context, the objective of this study was to standardize and evaluate the usefulness of a quantitative PCR (qPCR) assay to characterize the blood sources of field-collected female sand flies.
MATERIALS AND METHODS:
Seven pairs of primers were designed based on multiple alignments of cytochrome b gene sequences available in Genbak of the following potential hosts: Canis lupus familiaris (CLF), Felis catus (FC), Sus scrofa domesticus (SSD), Rattus rattus (RR), Equus caballus (EC), Gallus gallus (GG) and Homo sapiens (HS). Firstly, a SYBR® Green based qPCR was conducted using a standard curve with eight different concentrations (i.e., 10 ng/µL, 1 ng/µL, 100 pg/µL, 10 pg/µL, 1 pg/µL, 100 fg/µL, 10 fg/µL and 1 fg/µL) of DNA samples extracted from EDTA blood samples from each animal species. Then, DNA extracted from 100 field-collected engorged female sand flies belonging to three species (i.e., 92 Lutzomyia longipalpis, 7 L. migonei and 1 L. lenti) were tested by the protocols standardized herein.
RESULTS:
Both in silico and cross tests confirmed the specificity of the primers designed. The protocols worked efficiently with detection limits of 1 pg for RR, 100 fg for CLF, CE, FC and GG, and 1 fg for HS and SSD. Field-collected sand flies females fed on blood from humans (85%), chickens (23%), dogs (22%), horses (17%), rats (11%) and cats (2%). Interestingly, 88% of the L. longipalpis females were positive for human blood and 85% of the L. migonei females were dog blood-positive, whereas L. lenti was positive for both human and dog blood. In total, 39% of the tested females were fed on single sources, 41% on two and 13% on three or more.
CONCLUSIONS:
In conclusion, the currently developed protocols allowed the detection of multiple blood sources in field-collected sand flies, with high sensitivity and specificity. Knowledge of the dietary pattern of sand flies is of great importance, because it may provide information on putative reservoirs of Leishmania parasites. The protocols developed herein will be useful for epidemiological studies and contribute to the designing of specific control measures directed to reduce the availability of potential blood sources for sand fly vectors in endemic areas.
Financial support: CNPq, CAPES and FACEPE.

Keywords:
sand flies, ecology, Leishmania

O05.5
MATING BEHAVIOR OF ANOPHELES GAMBIAE IN ENCLOSED ENVIRONMENT: CHALLENGES AND OPPORTUNITIES

Facchinelli L.*[1], Valerio L.[1], Oliva C.[2], Lees R.S.[2], Persampieri T.[1], Collins M.C.[3], Crisanti A.[1], Benedict M.Q.[1]

[1]Department of Experimental Medicine, University of Perugia ~ Perugia ~ Italy, [2]Polo d'Innovazione Genomica, Genetica e Biologia S.C.a.R.L. ~ Perugia ~ Italy, [3]Department of Life Sciences, Imperial College London ~ London ~ United Kingdom

INTRODUCTION:
Anopheles gambiae is the most important mosquito responsible for malaria transmission in sub-Saharan Africa. Long-term control of A. gambiae has been difficult to achieve and maintain because the abundance of this species is increased by human activities, adults usually form large populations, and it has developed insecticide resistance in many areas of Africa. Genetically Modified (GM) A. gambiae strains that bear sterility genes are potentially powerful new tools to control the disease (Scott et al., 2002, Science, 298:117-9; Windbichler et al., 2007, Nucl. Acids Res., 17: 5922-33). A key component of GM mosquito control strategies requires natural male mating behavior in order to spread transgenes into the wild type population. Mating behavior involves the ability of males to form swarms, which is crucial to inseminate females in the wild. In spite of its biological importance and relevance for vector control, swarming is a poorly understood process because it is hard to study in the wild and is very difficult to stimulate under laboratory conditions (Knols et al., 2002, Malar. J. 18:1-19).
MATERIALS AND METHODS:
Here we describe features that promote male A. gambiae s.s. swarming in large enclosures. Numerous visual characteristics features were tested to determine which were important for swarm formation. We asked whether swarming behavior would affect mating performance of wild-type (WT) G3, β2-Ppo1 and β2-Ppo2 transgenic A. gambiae sexually sterile males competing for G3 females. We performed mating competition experiments and recorded female insemination rate and proportion of matings by WT and GM males.
RESULTS:
We were able to produce visual stimuli that promote A. gambiae males swarming in large insectary cages. In 15.6 m^3 cages, a dark foreground and contrasting illuminated background with a contrasting mark on the ground stimulated and localized swarm formation during artificial twilight. The presence of swarming stimuli was associated with an increase in mating frequency from 77.4 to 97.4 % at the end of a one-week mating period. Testing competitiveness revealed no decrease in the proportion of matings by transgenic males as a function of swarming stimuli.
CONCLUSIONS:
The increase of mating frequency in the presence of swarming stimuli highlights the importance of swarming in A. gambiae mating behavior. Reproducing A. gambiae swarms in controlled conditions gives the possibility to dissect the mating behavior of this species and explain the mechanisms controlling it, which is innovative in mosquito research. We will discuss the results and the possible applications of our findings to investigation of A. gambiae biology and to support vector control strategies.

Keywords:
Anopheles gambiae, swarms, transgenic mosquitoes

O05.6
IMPACT OF REPEATED NEEMAZAL® TREATED BLOOD MEALS ON THE FITNESS OF ANOPHELES STEPHENSI MOSQUITOES

Dembo E.[1], Abay S.M.[2], Lupidi G.[2], Lucantoni L.[2], Ogboi J.S.[2], Dahiya N.[2], Esposito F.[2], **Habluetzel A.*[2]**

[1]Department of Pharmacy, College of Medicine, University of Malawi ~ Blantyre ~ Malawi, [2]School of Pharmacy, University of Camerino ~ Camerino ~ Italy

INTRODUCTION:
Homemade remedies and herbal standardized medicines are widely used as complementary tools for malaria management in endemic countries. Based on the existing knowledge on anti-malarial plants secondary metabolites and their biological properties, innovative herbal products may be designed and developed for prevention of the disease and for reducing its transmission and this may benefit both the individual consumer and the community at large. Neem represents a good candidate for the development of innovative herbal products due to its potential to inhibit the reproduction of parasite stages that cause clinical manifestation of malaria, suppress parasite stages responsible for malaria transmission (Lucantoni et al., 2010, Malaria J. 2:9-66) and disrupt reproductive processes in insect vectors (Lucantoni et al., 2006, Tissue Cell. 38:361-71). On the basis of these facts, we undertook a study to determine the effects of the standardized neem extract NeemAzal® (Trifolio-M GmbH) on the malaria vector Anopheles stephensi following repeated exposure to treated blood meals.
MATERIALS AND METHODS:
Female BALB/c mice (20-25g) were treated with 3 doses of NeemAzal® containing 40, 70 and 100 mg/kg of azadirachtin A. Adult An. stephensi mosquitoes (5-7 days old) were blood fed on treated mice. Mosquito treatments were repeated every fourth day up to the fifth blood meal. Blood meal capacity of An. stephensi was estimated by measuring the hematin content of the rectal fluid excreted by the mosquitoes during feeding. The number of eggs laid in treated and control groups was determined and the eggs were incubated in well water to assess hatchability.
RESULTS:
There was a dose and frequency dependent impact of NeemAzal® treatment on the quantity of blood taken up per mosquito. At the 5th treated blood meal feeding capacity was reduced by about 60% , 79% and 85% in the 40, 70 and 100 mg/kg treatment group, respectively. At the highest dose of 100 mg/kg the mosquito feeding capacity was reduced by 50% or more from the 2nd blood meal onwards. NeemAzal® treatment did not influence the proportion of fed females, the percent of fed mosquitoes was very similar in treatment and control mosquitoes ranging between 87% and 91%.
A dose dependent impact was also revealed on the femalesì reproductive capacity. The number of eggs laid per mosquito after 5 treated blood meals was reduced by about 52%, 54% and 63% in the 40, 70 and 100 mg/kg treatment group, respectively. From hatchability experiments with these eggs a pronounced impact of NeemAzal® treatment emerged, resulting in a 54%, 62%, 70% in the 3 treatment groups.
CONCLUSIONS:
The findings from this study and previous work highlight the challenging perspective of designing formulations on the basis of azadirachtin A rich neem plant parts as multi-target herbal medicines exhibiting preventive, transmission-blocking and anti-vectorial properties.

Keywords:
azadirachtin A, Anopheles, neem

O05.7
BITING RATE AND HOST PREFERENCE OF CULEX PIPIENS IN AN AREA REPEATEDLY AFFECTED BY WEST NILE VIRUS

Montarsi F.*[1], Danesi P.[1], Martini S.[2], Drago A.[2], Cazzin S.[1], Frangipane Di Regalbono A.[3], Capelli G.[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [2]Entostudio snc ~ Brugine (PD) ~ Italy, [3]University of Padua ~ Padua ~ Italy

INTRODUCTION:
West Nile Virus (WNV) is an arthropod borne virus causing neuroinvasive disease (WNND) in animals and in humans. In the Veneto region, repeatedly affected by WNV since 2008, the only recognized vector is Culex pipiens (Gobbi et al., 2014, BMC Infect Dis, 14:60; Mulatti et al., 2014, Parasit Vectors, 7:26). The risk of WNV transmission to humans depends on species and density of mosquitoes, infected mosquito rate, mosquito host preference, biting rate, timing of biting activity and time of human exposition to their bites (Farajollahi et al., 2011, Infect Genet Evol, 11:1577-85). In this study, the Cx pipiens density, human biting rate and host preference were assessed by means of CDC-CO2 baited traps, human landing collections (HLC) and molecular blood host identification, respectively, in five sites located in the area of viral circulation of north-eastern Italy.
MATERIALS AND METHODS:
The sites were selected in rural areas where human cases of WNND usually occur. The collections were carried out from July to September 2013 during three-hour periods (21:00, 22:00 and 23:00) for 20 minutes each one. This sampling time represents the favorite time of human outdoor activities in summer and the time of higher biting activity of Cx. pipiens (Montarsi et al, 2014, J Med Entomol, submitted). HLC was compared to simultaneous CDC-CO_2 collections. The host preference of Cx. pipiens collected in 2012 in the study sites was evaluated in fed females using PCR (Alcaide et al., 2009, Plos One, 21:4-9) and sequencing. The correlation between the mosquitoes collected by CDC traps and HLC was tested using the Pearson correlation. The study was approved by the Ethical Committee of the Istituto Zooprofilattico Sperimentale delle Venezie (no CE.IZSVE.17/2013).
RESULTS:
Overall 435 specimens belonging to nine species were collected through HLC. Aedes caspius was the most abundant species (61%). Culex pipiens represented the 26% out of total mosquitoes, however most of them were collected in a single sampling (n=68, 61% out of total Cx. pipiens, on July 23); the following mean biting rates were calculated excluding this extraordinary sampling. Culex pipiens was caught on humans mainly at 21:00 h (n=25, mean 1.47), then at 23:00 h and 22.00 h (both n=9, mean 0.53). The Cx. pipiens biting rate per evening ranged from 0 to 68. The CDC-CO_2 traps collected in total 2.932 Cx. pipiens (97%) respect to 111 (26%) collected by HLC at the same time. The two collections were not correlated (r=0,1). An average of 9.8 fed Cx. pipiens/site (range 5-19) were found; the favorite hosts were mainly birds (61%) and no blood of humans was found.
CONCLUSIONS:
Culex pipiens was the most abundant species according to CDC-CO_2 collection. However, the low biting rates and the absence of detection of human blood into mosquitoes in the sites examined confirm that Cx. pipiens is scarcely attracted by humans. Likely in the rural area were the sites are located Cx. pipiens is mainly represented by the form Cx.pipiens pipiens which is well known to be ornithophilic. The results of this study could explain the relative low incidence of WNV clinical cases in horses and humans compared to other infected areas of eastern Europe and USA. The data provided are also of importance for WNV transmission risk models.

Keywords:
biting activity, Culex pipiens , West Nile Virus

O05.8
DISTRIBUTION AND ABUNDANCE OF A POTENTIAL RIFT VALLEY FEVER VIRUS VECTOR, AEDES VEXANS, IN CENTRAL AND NORTHERN ITALY

Magliano A.[1], Montarsi F.[2], Di Domenico M.[1], **De Liberato C.*[1]**

[1]Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana ~ Roma ~ Italy, [2]Entomologist Consulting ~ Padova ~ Italy

INTRODUCTION:
In the context of the EMIDA-ERA-NET research programme “Vector competence of European mosquitoes to Rift Valley fever virus (RVFV)”, a field entomological activity was carried out to get data on Aedes vexans distribution and abundance in Italy. In case of virus introduction, due to its ability to transmit transovarially the RVFV, at our latitudes this species could be the overwintering means of the virus.
MATERIALS AND METHODS:
The survey was carried out in Central Italy (CI) (Lazio and Toscana Regions) and Northern Italy (NI) (Veneto and Friuli Venezia Giulia Regions) in 2012 and 2013. In NI areas with known presence of Ae. vexans were sampled to define its abundance and seasonality. In CI, areas with no historical data were sampled to check the presence of the species. In NI adult collections were made by CO_2 baited CDC traps operated fortnightly from May to October. In CI a preliminary GIS study was carried out to locate areas theoretically suitable for Ae. vexans, selecting those with high probability of flooded sites by considering steepness, water-ground level and presence of clay in soil. Both larval and adult collections were performed.
RESULTS:
NI: 1050 collections were made. Ae. vexans was recorded in 46/54 sites; in 7 sites mean catches exceeded 10 specimens, in 3 sites maximum catches exceeded 100 specimens. The species was recorded in 3 habitat types: agricultural areas (mean catch<13 specimens); urban and suburban areas (mean catch between 13 and 19 specimens); forest, woods or semi-natural areas (mean catch >19 specimens). Overall seasonal activity was bimodal with peaks in June and late August. CI: 150 collections were made in 18 selected sites. Ae. vexans was recorded in four sampling sites in natural or agricultural/semi-natural areas: 1) Circeo National Park, area with a network of irrigation ditches and buffalo farms (larvae in June and September-October, adults in July and September-October); 2) larval breeding site 10 km north of Rome, in a temporary puddle due to meteoric waters in a deciduous wood (larvae in April); 3) Conca Reatina, alluvial plain, area with a network of irrigation ditches (adults in July, August and October); 4) Val di Merse (Siena Province), rice fields alluvial plain rich in canals and ditches along the Merse River (adults in September-October).
CONCLUSIONS:
In NI Ae. vexans is widespread almost on the whole territory, wherever the ecological conditions are suitable. Although only rarely dominant, this species is locally very abundant, constituting a nuisance to human population. In CI Ae. vexans seems to be rare, with a patchy distribution, present only in few, very localized sites and with low numbers. Ae. vexans needs a relatively warm climate following eggs flooding; as larval breeding sites are natural/semi-natural quite large water bodies, heavy rains are needed. In CI heavy rains are rare during the warm season and those occurring in autumn are followed by the cold season, hence not suitable for larval development. On the other hand, spring and late summer rains only rarely provoke water level rising in natural/semi-natural water bodies. In CI these conditions are often linked to artificial water supply due to agricultural activities.

Keywords:
Aedes vexans, Italy, vector

O05.9
HOST PREFERENCE OF THE INVASIVE MOSQUITO AEDES KOREICUS [HULECOETEOMYIA KOREICA]

Cazzin S.*[2], Ciocchetta S.[1], Ravagnan S.[2], Drago A.[3], Carlin S.[2], Capelli G.[2], Montarsi F.[2]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy; QIMR Berghofer Medical Research Institute ~ Brisbane ~ Australia, [2]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [3]Entostudio snc ~ Brugine (PD) ~ Italy

INTRODUCTION:
Aedes koreicus is an invasive mosquito species detected in the north part of Veneto Region in 2011 (Capelli et al., 2011, Parasit Vectors 4:188). During the last three years data have been collected in order to study the biology, the distribution and the characteristic of breeding sites of this species (Montarsi et al., 2013, Parasit Vectors, 6:292). Little information on the feeding behaviour and the related vector competence status of Ae. koreicus is available. Some authors report humans, domestic mammals and sea birds as possible feeding target of this species (Tanaka et al., 1979, Contrib A. Entomol Inst, 16:1-987). The aim of this survey was to assess the feeding preference and the host range of Ae. koreicus in field and laboratory conditions.
MATERIALS AND METHODS:
The samplings were carried out from June to September 2013 in urban and periurban sites located in Belluno and Treviso provinces. The sites selected were mainly catch basins located in public streets, squares, cemeteries near private and public buildings; garden centre and one poultry house were also monitored. The mosquitoes were mainly collected by manual aspiration on the vegetation and into the catch basins, but also captured by CO2-baited BG-Sentinel traps and sticky traps.
All fed mosquitoes were tested singularly using only the abdomen for the extraction of the DNA of the blood meal. All the samples were analysed with a specific set of primers for the COI vertebrate region (Alcaide et al., 2009, PLos One, 21:4-9). Positive results were confirmed by direct amplicons sequencing.
For the Ae. koreicus reared in laboratory artificial feeding was performed using the Hemotek feeding system (Discovery Workshops, Lancashire, United Kingdom) and different blood types were used (human, chicken, turkey, dog and sheep).
RESULTS:
Overall, 234 mosquitoes were collected from 13 sites. Twenty-three samples (9.8 %) were found positive to blood meal analysis. Sequencing of the PCR products identified human (Homo sapiens) blood in 22 samples and cattle (Bos taurus) blood in one sample. In the laboratory tests engorgement was successfully achieved with dog (83%), human (76.5%) and chicken (65.4%) blood.
CONCLUSIONS:
In the laboratory studies Ae. koreicus indicated a preference for dog, human and chicken blood, while in fields conditions clearly showed a preference for humans. However, these latter results could be biased by the position and characteristics of the sites selected (urban and periurban areas). However, the antropophilic behaviour of this species was confirmed by the resident people in areas where only Ae. koreicus was present (Montarsi et al., 2013, Parasit Vectors, 6:292). Further studies are needed to assess the opportunistic or anthropophilic nature of this species.
The knowledge of the host preferences of this mosquito is important to evaluate the risk of transmission of eventual animal and zoonotic vector-borne diseases.
Funding: Veneto Region
The presentation of this work is sponsored by Bayer Animal Health

Keywords:
Aedes koreicus, Blood meal, Host preference

O05.10
STUDIES OF VECTOR COMPETENCE OF ITALIAN AEDES ALBOPICTUS POPULATIONS BY EXPERIMENTAL INFECTIONS WITH CHIKUNGUNYA VIRUS

Fortuna C.*[1], Remoli M.E.[1], Di Luca M.[2], Severini F.[2], Toma L.[2], Benedetti E.[1], Bucci P.[1], Ciufolini M.G.[1], Boccolini D.[2], Romi R.[2]

[1]Unit of Viral diseases and attenuated vaccine, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy, [2]Unit of Vector-borne Diseases and International Health, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy

INTRODUCTION:
Chikungunya virus (CHIKV), belonging to the Togaviridae family, Alphavirus genus is endemic in Africa, India and South-East Asia. Aedes aegypti and Aedes albopictus, both vectors of CHIKV, are two mosquito species co-existing in the Indian Ocean region. In the Reunion Island, a single mutation in the viral envelope has been selected leading to high levels of replication and a short extrinsic incubation period in Ae. albopictus (Estelle et al., 2010, BMC Ecol., 10:8). In 2005 the mutated CHIKV spread from the Indian Ocean producing large epidemics in India, Southeast Asia and Italy (Rezza G. et al., 2007, The Lancet 370: 1840–1846).
The purpose of this study is to evaluate the vector competence of several Italian populations of Ae. albopictus by experimental infections with CHIKV using a membrane feeding apparatus.
MATERIALS AND METHODS:
Experimental infections were performed using CHIKV GB M2V2 seed, isolated from serum of a patient during the Italian outbreak (2007).
Nine laboratory colonies were tested: two from Sardinia region (Cagliari, Sant'Antioco), two from Lazio region (Roma, Borgo Vodice), two from Calabria region (M. di Zambrone, Rende), one from Molise region (Termoli), one from Puglia region (Vieste) and one from Veneto region (Padova). Females 8-12 days old were fed and monitored for 3 weeks after the infected meal to determine the length of viral extrinsic incubation period. CHIKV titre of infected mosquitoes was evaluated both by quantitative Real Time PCR and titration by Plaque Forming Units/ml in VERO cells. Infection, Dissemination and Transmission Rates (IR, DR, TR) were assessed by detection of the virus in abdomen, legs plus wings and saliva respectively.
RESULTS:
The six populations analyzed up to now (Cagliari, Borgo Vodice, Termoli, Padova, Sant'Antioco and Volgograd) resulted susceptible to the CHIKV infection with similar trend of the viral titre in the abdomen of tested mosquitoes. All the specimens showed 100% positivity on day 0 post infection (p.i.). We detected high values of IR and DR in all tested populations (ranging from 82%-100% and 67%-100%, respectively). The virus presence in legs and wings was detected since day 3 p.i. The viral titre increased, both in abdomen and legs plus wings of tested infected females, reaching the higher value on day 7 p.i. The viral titration on VERO cells showed that the viral particles in the infected mosquitoes, collected at different time points, were also live.
CONCLUSIONS:
Our preliminary results demonstrated the susceptibility of all tested populations to CHIKV infection and the virus ability to quickly disseminate in the processed specimens. The analysis of the TR, to determine the capacity of the considered populations to transmit the virus, is still in progress. These findings confirm that Ae. albopictus could represent a potential threat in Italy playing an important role as CHIKV vector, in particular where high densities of the species are seasonally recorded, as occurred in Emilia Romagna in 2007.

This research was founded by EU Grant CAPACITIES-228421 INFRAVEC

Keywords:
CHIKV, Italy, Ae. albopictus

O05.11

STUDIES OF VECTOR COMPETENCE OF CULEX PIPIENS POPULATIONS BY EXPERIMENTAL INFECTIONS WITH WEST NILE VIRUS

Fortuna C.*[1], Remoli M.E.[1], Di Luca M.[2], Severini F.[2], Toma L.[2], Benedetti E.[1], Bucci P.[1], Platonov A.E.[3], Capelli G.[4], Nicoletti L.[1], Boccolini D.[2], Romi R.[2], Ciufolini M.G.[1], Fedorova M.V.[3]

[1]Unit of Viral diseases and attenuated vaccine, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy, [2]Unit of Vector-borne Diseases and International Health, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy, [3]Central Institute of Epidemiology ~ Moscow ~ Russian Federation, [4]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy

INTRODUCTION:
West Nile virus (WNV, Flaviviridae, Flavivirus) is an emerging zoonotic arbovirus widely distributed throughout the world and with considerable impact both on human and animal health (Beck C. et al., 2013, Int. J. Environ. Res. Public Health, 10:6049–6083).
WNV is maintained in nature in an enzootic cycle involving ornithophilic mosquitoes as transmission vectors and birds as reservoir hosts. Culex spp. mosquitoes, in particular Cx. pipiens, play a primary role in enzootic maintenance and transmission of WNV. The objective of this study is to evaluate the potential competence of different geographic populations of Cx. pipiens to transmit WNV. Moreover one population of Aedes albopictus coming from Cagliari (Sardinia) was also tested.
MATERIALS AND METHODS: Four Italian populations of Cx. pipiens were tested, two laboratory colonies, Frascati and Caffarella (Latium) and two wild populations, Zafferana (Sicily) and Legnaro (coming from a WNV endemic area in Veneto Region). Two Russian laboratory colonies, Sochi and Volgograd (from a WNV endemic area), were also included in the study. Mosquito females were infected using a membrane feeding apparatus. A viral seed of WN lineage 1 strain, isolated from a patient from 2011 Sardinia outbreak, was used for the infected blood meal. Fed females were monitored for 4 weeks to determine the length of viral extrinsic incubation period (Fig. 1). WNV titre of infected mosquitoes was evaluated both by quantitative Real Time PCR and titration in VERO cells by Plaque Forming Units/ml (PFU/ml). Infection (IR), dissemination (DR) and transmission (TR) rates of the Cx. pipiens populations were assessed by detection of the virus in abdomen, legs plus wings and saliva respectively.

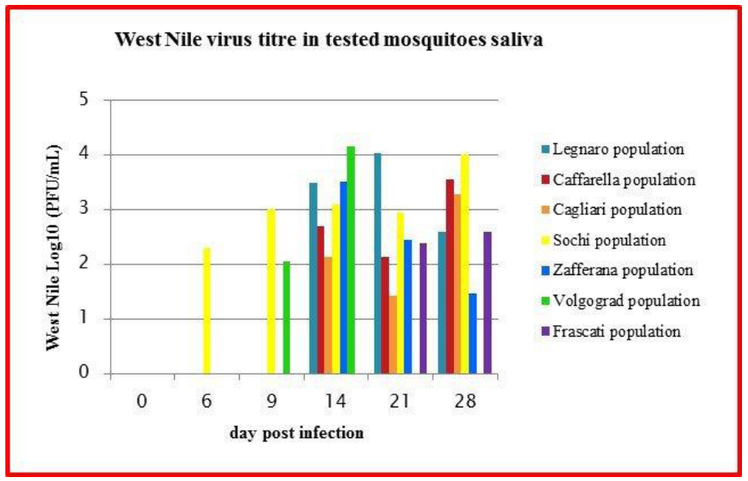


Figure 1

RESULTS:
All populations showed a similar trend of the viral titres at different post infection days. The highest values of IR and DR were found in the populations of Legnaro (89% and 100% respectively) and Sochi (73% and 75%). The WNV presence was revealed in the saliva of all populations analysed: high percentages of TR were found for the Russian populations (Sochi 69% and Volgograd 60%) while among the Italian populations Legnaro showed a good level of transmission (38%).
CONCLUSIONS:
All tested Cx. pipiens populations resulted susceptible to the WNV infection. The two Russian populations showed a shorter extrinsic incubation period with respect to the Italian ones. Sochi, Volgograd and Legnaro showed the highest values of TR suggesting a better vector competence. Regarding, Ae. albopictus, although the IR, DR and TR values were lower compared to the Cx. pipiens populations, the species is confirmed able to transmit WNV, representing a potential threat in particular in Italy. Ae. albopictus could in fact play a role of additional vector in the urban areas, where high densities of the species are seasonally recorded.
This research was founded by EU Grant HEALTH-F3-2011-261391 EuroWestNile

Keywords:
WNV, Italy, Cx. pipiens

NOTES

NOTES

SESSIONE PARALLELA O-06

PARASSITI DELLA FAUNA ACQUATICA

NOTES

O06.1

HISTOLOGICAL EXAMINATION, BIO-MOLECULAR ANALYSIS AND ULTRASTRUCTURAL OBSERVATIONS OF MICROSPORIDIA IN WHITE-CLAWED CRAYFISH AUSTROPOTAMOBIUS PALLIPES (LEREBOULLET, 1858) IN LOMBARDY (ITALY)

Berton V.*[1], Tosi F.[1], Montesi F.[1], Manfrin A.[1], Terregino C.[1], Pretto T.[1]

[1]Istituto Zooprofilattico sperimentale delle Venezie ~ Legnaro (PD) ~ Italy

INTRODUCTION:
The white-clawed crayfish Austropotamobius pallipes is an endangered European species. Considering its key role in the biodiversity of freshwater ecosystems, its health monitoring is essential to keep up with conservation initiatives.
A chronic yet lethal infection called porcelain disease is caused by the microsporidian Thelohania contejeani and has been implicated in mass mortalities of the indigenous European crayfish populations (Henneguy and Thelohan, 1892, Annuales Micrographie, 4, 617-641).
The aim of this study was to identify the microsporidia in muscle tissues of macroscopically affected A. pallipes, collected from two creeks in the province of Brescia.
MATERIALS AND METHODS:
Heavily infected crayfish with a whitish appearance of the ventral abdominal musculature were collected from the Agna (5 specimens) and Toscolano creeks (3 specimens). The cephalothorax and the abdomen, from each specimen, were fixed in Davidson's for 24 h and processed following standard histological techniques. Sections were stained with H-E and Giemsa.
Muscle samples for bio-molecular analyses were taken from each specimen (50 mg) and total DNA was extracted with a commercial kit. Two PCR end-point were carried out as described by Imhoff et al., (2010, Freshwater Crayfish 17:145-150) one with a primer set for generic microsporidian SSU rDNA and the other species-specific for T. contejeani. PCR products were visualized on agarose gel and then sequenced.
For the ultrastructural examination, the samples of the affected muscles were fixed in 2.5% gluteraldehyde in 0.1M cacodilate buffer, processed following standard techniques and embedded in Durcupan ACM resin. Ultrathin sections were examined with a Philips 208S transmission electron microscope.
RESULTS: All specimens collected from the Agna creek and two from the Toscolano showed proliferation of microsporidia, at various developmental stages, only within striated muscle fibers with absence of any inflammatory response. However, in one specimen from the Toscolano creek, microsporidia were evident in the heart, intestinal and skeletal muscle fibers with a slight haemocytic infiltration and melanisation.
Amplification with generic primers was obtained from all the specimens analyzed, while amplification with T. contejeani specific primers was positive only in one specimen from the Toscolano creek.
In the ultrathin sections, obtained from T. contejeani positive sample, the microsporidia presented two different kind of mature spores: aploid spores with 9-10 turns of polar tube and diplokaryotic spores with the polar tube coiled 5-6 times (Fig. A, 8900X).
Instead, the spores detected in the other samples were aploid and the polar tube was coiled 12-13 times (Fig. B, 8900X).

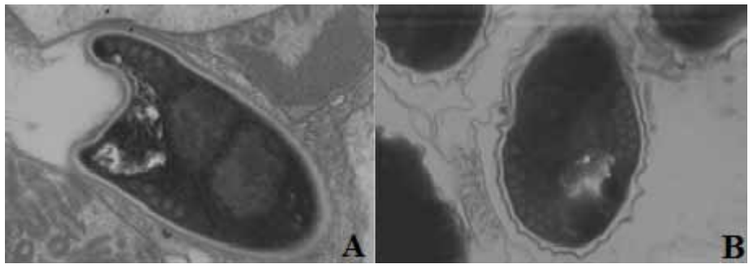


Fig. A) Ultrathin section of microsporidia T. contejeani: diplokaryotic spores with 5-6 coils of the polar tube (8900X); B) Ultrathin section of another microsporidia: aploid spore containing 12-13 polar tube coils (8900X).

CONCLUSIONS:
The ultrastructural features of the spores, identified by molecular methods as T. contejeani (identity of 99% with published sequences), were similar to those already described by Lom et al. (2001, Parasitol Res, 87: 860-872) for this species.
The bio-molecular and ultrastructural analysis of the other samples demonstrated the presence of a different microsporidia, which may be related to the family Nosematidae.

Keywords:
Austropotamobius pallipes, Microsporidia, Transmission Electron Microscopy

O06.2
PARASITE FAUNA OF THE HEAD OF JUVENILE THUNNUS THYNNUS (OSTEICHTHYES: SCOMBRIDAE) FROM THE WESTERN MEDITERRANEAN SEA

Mele S.*[1], Culurgioni J.[2], Rodríguez Llanos J.[3], Palacio Abella J.[3], Mazzotta P.[1], Garau S.[2], Sanna N.[2], Macías D.[4], Addis P.[2], Garibaldi F.[5], Garippa G.[1], Merella P.[1], Montero F.E.[3]

[1]Parassitologia e Malattie Parassitarie, Dipartimento di Medicina Veterinaria, Università di Sassari ~ Sassari ~ Italy, [2]Dipartimento di Scienze della Vita e dell'Ambiente, Università di Cagliari ~ Cagliari ~ Italy, [3]Unidad de Zoología Marina, Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València ~ Valencia ~ Spain, [4]Centro Oceanográfico de Málaga, Instituto Español de Oceanografía ~ Malaga ~ Spain, [5]Laboratorio di Biologia Marina, DIP. TE. RIS., Università di Genova ~ Genova ~ Italy

INTRODUCTION:
The Atlantic bluefin tuna, Thunnus thynnus (BFT), is a pelagic fish inhabiting the Atlantic Ocean and the Mediterranean Sea. Several studies deal with the parasite fauna of wild and the cage-reared BFT (see Mladineo et al., 2011, Parasitol. Int., 60:25-33; Culurgioni et al., 2014, Folia Parasitol., 61: in press), whereas the parasite fauna of young-of-the-year (YOY) tuna is unknown. The current study describes the metazoan parasite assemblages of the head of YOY BFT from the western Mediterranean Sea.
MATERIALS AND METHODS:
Parasites were studied in 142 YOY BFT (20-61 cm fork length) caught in the western Mediterranean Sea from 2011 to 2013: 91 specimens from the Balearic Sea, 15 from the Ligurian Sea, 6 from the Strait of Sicily, and 30 from the Tyrrhenian Sea. Prevalence and mean intensity of infection of each parasite species were calculated, and the differences between localities evaluated (Reiczigel and Rózsa, 2005, Quantitative Parasitology Web1.0, Budapest).
RESULTS:
Nine parasite species/taxa were found: Capsala sp., Hexostoma thynni, Cardicola sp., Didymocystis reniformis, Didymocystis sp. 2 (sensu Rodríguez-Marín et al., 2008, Aquatic Living Resour, 21:365-371), Didymosulcus sp. 2 (sensu Rodríguez-Marín et al., 2008), Didymosulcus wedli, Didymozoon pretiosus, and Nematobothriinae gen. sp. The parasite richness ranged from four species in the Sicilian BFT assemblage (Capsala sp., H. thynni, D. pretiosus, Nematobothriinae gen. sp.) to nine species (all) in the Balearic one. Cardicola sp., D. reniformis, and D. wedli were not recorded in the Sicilian and Tyrrhenian BFT; Didymocystis sp. 2 was not found in the Ligurian and Sicilian BFT; Didymosulcus sp. 2 and D. pretiosus were not recorded in the Ligurian and Sicilian ones, respectively. Comparing localities, the prevalence of four parasite species showed significant differences between localities (Fig. 1, p≤0.05). Hexostoma thynni was the most prevalent species in the Balearic, Ligurian and Sicilian BFT (60%, 67%, 50%, respectively), and D. pretiosus the most prevalent parasite in the Tyrrhenian BFT (63%).

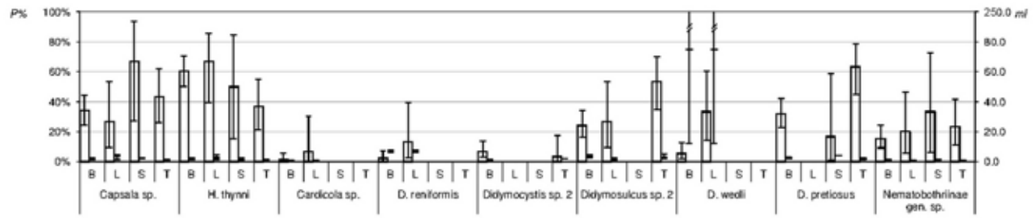


Fig.1. Prevalence (P%) and mean intensity (ml) of the parasites of young-of-the-year Thunnus thynnus from the western Mediterranean Sea. Localities: B, Balearic Sea; L, Ligurian Sea; S, Strait of Sicily; T, Tyrrhenian Sea.

CONCLUSIONS:
The results showed that the parasite fauna of YOY BFT is not homogenously distributed in the western Mediterranean Sea. The environmental conditions of each sampling area could influence the occurrence of parasites, and for trematodes also the distribution of intermediate hosts could affect (Culurgioni et al., 2014). According to Williams et al. (1992, Rev. Fish Biol. Fish., 2:144-176), parasites can be useful to separate host populations and used as biological tags. The prevalence of Didymosulcus sp. 2, D. wedli and D. pretiosus differed between BFT from Balearic, Ligurian, and Tyrrhenian Seas, three nursery areas of the Mediterranean BFT (Rooker et al., 2003, Fish. Oceanogr., 12:75-84), suggesting these parasites as possible tags to discriminate the tuna populations from such areas.

Research supported by the Spanish Government project PARATUN AGL2010-20892 and the Master and Back 2012-2013 project of the Regione Autonoma della Sardegna.

Keywords:
Atlantic bluefin tuna, Western Mediterranean Sea, Parasite assemblage

O06.3
GASTROINTESTINAL HELMINTHS INFECTION AND DIGESTIVE HORMONES IN THE FISH-PARASITE SYSTEMS

Bosi G.[1], Giari L.[2], **Sayyaf Dezfuli B.*[2]**

[1]Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, St. Trentacoste 2, 20134 Milan ~ Milan ~ Italy, [2]Department of Life Sciences and Biotechnology, University of Ferrara ~ Ferrara ~ Italy

INTRODUCTION:
Parasitic helminths represent an extreme in the spectrum of pathogens, as large multicellular animals. Helminths do not simply ward off immune attack; rather, they influence and direct immune responses away from the modes most damaging to them, regulating the host immune response to create niches that optimize successful feeding and reproduction. Intestinal helminths often in turn induce changes in the morphology of the host tissues, which can provoke alterations in the digestive physiology of the host. Almost all the activities involved in the physiological control of vertebrate gut function during fasting or feeding are mediated by the neuroendocrine system (NS), which consists of the nervous cells in the myenteric plexus, and the intestinal epithelial endocrine cells belonging to the diffuse endocrine system (DES). The components of the gut NS and central nervous system communicate one each other by a wide number of neuromodulators (peptides, amines, and chemical substances). Among them, Cholecystokinin-8 (CCK-8) and Bombesin are considered negative feedback signals that stop eating behaviour, while Gastrin is a satiety hormone that stimulates pepsinogen production, increases gastric motility and induces pancreatic enzyme secretion. Glucagon rises hematic glucose level, and Secretin counteracts this effect increasing insulin secretion. The balance between anorexigenic (CCK-8, Bombesin, Glucagon) and orexigenic (Secretin) factors, as well as the control of the secretion level of satiety or fasting peptides in gastrointestinal regions appears to play an important role in short-term regulation of food intake. A local damage to the gastrointestinal DES could induce an impairment of this balance, which may result in disorders of feeding behaviour. There is a considerable body of information regarding the effects of helminths infection in animals and several well-documented papers were published on the influence of enteric worms on the fish gut DES.
MATERIALS AND METHODS:
Rainbow trout (Oncorhynchus mykiss) and brown trout (Salmo trutta) collected from tributaries of the River Brenta (Italy), resulted to be infected with the cestode Cyathocephalus truncatus or with the acanthocephalan Pomphorhynchus laevis. Rainbow trout from Loch Awe (Scotland) harboured the tapeworm Eubothrium crassum.
RESULTS:
In this investigation immunohistochemical detections of a panel of digestive hormones showed in above fish-parasites systems significant changes in the number of intestinal endocrine cells, especially near the point of worms attachment, in comparison to uninfected conspecifics.
CONCLUSIONS:
The aim of the current study was to assess the impact of different taxa of intestinal worms on the presence, distribution and role of specific neuromodulators of the food intake in different species of fish and to compare the profiles obtained with uninfected conspecifics.

Keywords:
Intestinal fish, endocrine cells, helminth infection

O06.4

METACERCARIAE OF ASCOCOTYLE SP. AND HETEROPHYES SP. (TREMATODA: HETEROPHYIDAE) IN GREY MULLETS (OSTEICHTHYES: MUGILIDAE) FROM SARDINIA BRACKISH WATERS (WESTERN MEDITERRANEAN SEA)

Masala S.*, Garippa G., Piras M.C., Merella P.

Parassitologia e Malattie Parassitarie, Dipartimento di Medicina Veterinaria, Università di Sassari ~ Sassari ~ Italy, [2]Institute of Parasitology Academy of Sciences of the Czech Republic ~ Ceske Budejovice ~ Czech Republic

INTRODUCTION:
The metacercariae (mtc) of heterophyids (Trematoda: Heterophyidae) are usually encysted in fish tissues, with adults living in the gut of fish-eating birds, mammals and humans. Several heterophyid genera are agents of fish-borne zoonosis, transmitted by the consumption of raw or undercooked fish. Grey mullets (Osteichthyes: Mugilidae) are among the most important second intermediate hosts of these parasites (Scholz, 1999, Syst. Parasitol., 43:147–158). The aim of this study is to give a contribution to the morphological and molecular identification, and to the epidemiology of heterophyid mtc in grey mullets from a Sardinian brackish water pond (western Mediterranean Sea).
MATERIALS AND METHODS:
From March 2012 to January 2013, 60 specimens of mugilids (13 Chelon labrosus, 18 Liza aurata, 6 Liza ramada, 8 Liza saliens, 15 Mugil cephalus) were sampled from the Mistras Lagoon. Metacercariae were isolated using pepsin digestion of a 50 g of muscle subsample (Thu et al., 2007, Kor. J. Parasitol., 45:45-54). Metacercariae were observed with a light microscope, both in vivo and mounted in ammonium picrate, and morphologically identified at the genus level. Adult heterophyids were obtained with an experimental infection of a hamster (Mesocricetus auratus) with 300 mtc morphologically identified as Heterophyes sp. The molecular analysis of mtc and adults was made by PCR and sequence analysis. The ITS2 portion of rDNA was amplified with the primers 3S forward and BD2 reverse. A maximum likelihood rooted tree was drawn using Haplorchis taichui (GB# AB517578) as outgroup. The sequences of Metagonimus yokogawai (GB# B470525), Ascocotyle (Phagicola) longa (GB#AY245703), Ascocotyle (Phagicola) nunezae, and Timoniella sp. were also included in the dataset.
RESULTS:
A total of 17899 mtc were recovered from the muscle of the examined fish, with 86% of hosts infected. After microscopical examination four morphological types/genera of mtc were identified: Heterophyes sp. (n=14113), Heterophyes sp. - type small (1225), Stictodora sp. (1606), Phagicola (Ascocotyle) sp. (955). The experimental infection allowed to retrieve eight adult flukes, six identified as Heterophyes heterophyes and two as Heterophyes cf. nocens. The PCR of mtc and adults produced a fragment 450 bp long. The sequence analysis confirmed the morphological identification, highlighting four clusters corresponding to the four genera previously identified. Metacercariae of Heterophyes sp. and Heterophyes sp. - type small clustered closely, and all the adults grouped together with the Heterophyes sp. mtc.
The presence of mtc was high in all host species, mainly those of Heterophyes sp. (prevalence≥78%; mean intensity≥135 mtc/100 g muscle), and chiefly in M. cephalus (prevalence = 100%; mean intensity 841 mtc/100 g muscle).
CONCLUSIONS:
The results confirmed the presence of the zoonotic tramatodes Heterophyes sp. and Phagicola (Ascocotyle) sp. in grey mullets from Sardinian brackish waters, and provided for the first time the molecular description of these parasites from the Mediterranean Sea. The unusual finding of the Indo-Pacific H. cf. nocens needs to be confirmed. The most important method to prevent heterophyidosis is cooking fish, and a real risk could arise with the consumption of raw (carpaccio, sushi) or inadequately processed (cold smoked, superficial grilled) fish. Further studies are necessary to estimate the risk related to this fish-borne zoonosis in Sardinia.

Keywords:
Heterophyidae, Sardinia, Zoonosis

O06.5

EUSTRONGYLIDES SP. LARVAE IN MUSCLE OF EUROPEAN PERCH, PERCA FLUVIATILIS: HISTOPATHOLOGY AND RISK OF ZOONOTIC DISEASES

Sayyaf Dezfuli B.*[1], Pironi F.[1], Lorenzoni M.[2], Giari L.[1]

[1]Department of Life Sciences and Biotechnology, University of Ferrara ~ Ferrara ~ Italy, [2]Department of Cellular and Environmental Biology, University of Perugia ~ Perugia ~ Italy

INTRODUCTION:
Nematode species of the genus Eustrongylides have been reported from birds throughout much of the world. They have complex life cycles involving a definitive host (aquatic birds), and their first intermediate hosts are aquatic oligochaetes and second intermediate host are different species of fish of which some act as paratenic hosts. In latter, larval Eustrongylides sp. observed encysted in the mesenteries, spleen, and gonads and mainly in body musculature of fish. This study set out to determine by histochemical methods how Eustrongylides sp., larvae affect the health of its paratenic host population, the European perch (Perca fluviatilis) collected from Lake Trasimeno in Central Italy. Perca fluviatilis is widely distributed throughout the Palaearctic region and serves as a host to numerous endoparasitic helminths; its parasite fauna is relatively well known. As a common fishery species, it is of great economic importance in many lake systems throughout Europe.
MATERIALS AND METHODS:
A total of 54 perch were examined, and 15 specimens (27.8 %) were infected with Eustrongylides sp., larvae (1-2 worms fish-1).
RESULTS:
Pathological alterations were more marked in perch with 2 parasites. In the muscle, larvae were encysted, surrounded by a capsule of host tissue. The occurrence of macrophage aggregates (MAs) distributed among the mast cells (MCs) was observed around the encysted larvae. Most authors ascribe a role to MAs in the destruction, detoxification and recycling of endogenous and exogenous materials. It appears that MAs are involved with late stages of a chronic inflammatory response to severe tissue damage and have roles in host innate and adaptive immunity. Mast cells in fish are motile and differentiate in the haematopoietic organs, reaching their target tissues via the circulatory system as immature cells. Mast cells were irregular in shape with a cytoplasm filled by numerous electron-dense, membrane-bound granules. In infected European perch muscle, numerous MCs were seen within the capsule, both on the outside and within capillaries. The parasites may induce recruitment of MCs to the site(s) of infection and stimulate their proliferation in situ. Many types of tissue injury, including pathogens, can activate MCs.
CONCLUSIONS:
In the United States cases of known human infection with Eustrongylides since 1980 are several. Humans who have consumed raw or undercooked infected fish have experienced gastritis of inflammation of the stomach and intestinal perforation requiring surgical removal of worms. Increase of raw fish consumption in North America alerted the clinician and public health authority and induced a continue monitoring of fish. The current study represents the first record of Eustrongylides sp. within the muscle of perch from Italy. Emphasis will be placed on the role of MCs and MAs as important components of the host's innate immune system. Indeed, zoonotics cases of the Eustrongylides in the USA should alter also Italian European Public Heath Authority specially in lakes where raw perch consumption is a local tradition.

Keywords:
raw fish, nematode larvae, food-borne zoonosis

O06.6
PRESENCE OF ANISAKIS SPP. (NEMATODA: ANISAKIDAE) IN LOCAL AND ATLANTIC FISH SOLD IN SARDINIAN MARKET

Piras M.C.*[1], Garippa G.[1], Masala S.[1], Muglia S.[2], Frau F.[2], Spanu V.[2], Merella P.[1]

[1]Parassitologia e Malattie Parassitarie, Dipartimento di Medicina Veterinaria, Università di Sassari ~ Sassari ~ Italy, [2]Ispezione degli Alimenti di Origine Animale, Dipartimento di Medicina Veterinaria, Università di Sassari ~ Sassari ~ Italy

INTRODUCTION:
Third stage larvae of Anisakis sp. (Nematoda: Anisakidae) are commonly found in the body cavity and muscle of many fish species. Through the consumption of raw or undercooked fish, these nematodes can cause a zoonotic disease called anisakiasis, responsible for digestive disorders and/or allergies (Chai et al., 2005, Int. J. Parasitol., 35:1233-1254). The aim of this study is to describe the presence of Anisakis larvae in local and Atlantic fish sold in Sardinian market.
MATERIALS AND METHODS:
The study was carried out from January to December 2013, on four fish species: Engraulis encrasicolus (600 specimens), Scomber colias (42), Trachurus mediterraneus (100), Merluccius merluccius (200). Of these, the first three species were local fish from the Gulf of Asinara (North Sardinia, western Mediterranean Sea), while 95 specimens of M. merluccius were fish imported from the northeastern Atlantic Ocean, and the rest (105) was local fish. Anisakis larvae were detected and isolated by both visual inspection and pepsin digestion. A subsample of larvae was identified by PCR-RFLP of the ITS region of nuclear rDNA using restriction enzymes HinfI and HhaI (D'Amelio et al., 2000, Int. J. Parasitol., 30:223-226; Pontes et al., 2005, J. Parasitol., 91:1430-1434).
RESULTS:
Of the 942 fish examined, 374 specimens (total prevalence 40%) were infected by 5623 Anisakis sp. larvae, all morphologically identified as Type I (sensu Berland, 1961, Sarsia, 2:1-50). Scomber colias showed the highest prevalence (100%), followed by M. merluccius (Atlantic 92%, local 69%), T. mediterraneus (29%) and E. encrasicolus (24%). The highest mean intensity was recorded in the Atlantic M. merluccius (54.3), followed by S. colias (10.0), T. mediterraneus (3.2), local M. merluccius (2.4) and E. encrasicolus (1.5). The mean intensity in the Atlantic M. merluccius was significantly higher than in the local one (and in all other host species), but also must be considered that the mean total length of M. merluccius differed significantly between localities (Atlantic 34 cm (24-43) vs local 25 cm (18-37)). To confirm this, abundance was significantly correlated with host size in all fish species, apart from S. colias. A relevant number of larvae (39%) was found in the muscle of all species, particularly in the Atlantic M. merluccius (45%). In this latter, the muscular prevalence was 77% and the mean intensity 29.1. In the other hosts the muscular infection was significantly lower, with prevalence ranging between 3% (E. encrasicolus) and 13% (local M. merluccius), and mean intensity between 1.0 (E. encrasicolus) and 2.0 (S. colias). The molecular analysis allowed to identify the larvae found in the Mediterranean hosts as Anisakis pegreffii and those from the Atlantic M. merluccius as Anisakis simplex s.s.
CONCLUSIONS:
The present study confirms the widespread distribution of A. pegreffii in the Mediterranean Sea. The high prevalence found in E. encrasicolus confirms previous data from the same area (Rello et al., 2009, Int. J. Food Microbiol., 129:277-281; Angelucci et al., 2011, J. Food protect., 74:1769-1775; Piras et al., 2014, Vet. Parasitol., in press). Considering that this fish is associated with human anisakiasis in Italy, the results suggest a potential Anisakis risk in anchovies caught off northern Sardinia.

Keywords:
Anisakis, Sardinia, Zoonosis

O06.7
DISTRIBUTION AND INFECTION LEVELS BY ANISAKIS PEGREFFII AND A. SIMPLEX (S. S.) LARVAE (NEMATODA : ANISAKIDAE) IN FISH TISSUES OF MERLUCCIUS MERLUCCIUS FROM TYRRHENIAN SEA AND NE ATLANTIC WATERS (ATLANTIC COAST OF SPAIN): IMPLICATIONS FOR FOOD SAFETY

Cipriani P.*[1], Acerra V.[2], Smaldone G.[3], Bellisario B.[2], Anastasi A.[3], Palma G.[4], Nardi V.[2], Nascetti G.[2], Mattiucci S.[1]

[1]Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, Italy ~ Roma ~ Italy, [2] Department of Ecology and Biology (DEB), Tuscia University, Viterbo, Italy ~ Viterbo ~ Italy, [3]Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Napoli ~ Napoli ~ Italy, [4]Federazione Nazionale delle Impresa di Pesca - Federpesca ~ Roma ~ Italy

INTRODUCTION:
Human consumption of Merluccius merluccius is widespread in European countries, where the fish species reaches high commercial value. To date, different larval species of Anisakis have been identified at larval stage in M. merluccius; A. pegreffii and A. simplex (s. s.) are the two most common parasites of hakes in European waters (Mattiucci et al., 2004, J Fish Biol, 65: 495-510). The distribution patterns of Anisakis spp. larvae, genetically identified, have been used also as biomarkers in stocks characterization of demersal fish species, including hake, in European waters (Mattiucci et al., 2014, Parasitology, in press). Aim of the study is to give data on the occurrence of Anisakis spp. larvae in viscera and flesh of M. merluccius.
MATERIALS AND METHODS:
The distribution and infection rate by different species of Anisakis, in different host sites (viscera, dorsal and ventral fillets) was investigated in samples fished from the Central Tyrrhenian Sea (FAO 37.1.3) and Atlantic coast of Spain (FAO 27 IXa). A sample of 65 individuals (total length>26 cm), was examined parasitologically, from each fishing grounds. The fillets were examined by pepsin digestion (Llarena et al., 2013, Vet Parasitol, 191:276-83). To date 1310 specimens were identified by multilocus allozyme electrophoresis (MAE), and mtDNA cox2 sequences analysis. The parasitic infection levels by Anisakis spp. and the statistical significance of the differences observed were assessed by the Fisher's exact test, Bootstrap t-test, Kruskall-Wallis one-way ANOVA.
RESULTS:
814 larvae of A. simplex (s. s.) and 476 of A. pegreffii have been identified. They infect both viscera and flesh of M. merluccius. The two species co-infected the same individual fish (both in viscera and fillets) from the FAO27 area, while only A. pegreffii was found in hakes from Tyrrhenian Sea. The average amount of parasitic burden of A. pegreffii in hakes from Tyrrhenian Sea resulted significantly lower to that observed by the same species from Atlantic coast of Spain.
While no significant difference in the overall prevalence values was recorded between the two Anisakis species in the viscera of the FAO27 fish sample, significant differences were found in the abundance levels between the two Anisakis species in the flesh of the hakes, with A. simplex (s. s.) showing significantly higher level than that observed by A. pegreffii.
CONCLUSIONS:
Similar findings were observed in Scomber japonicus from the Pacific coast of Japan, where the average number of A. simplex (s. s.) per fish fillet was significantly higher than that for A. pegreffii (Suzuki et al., 2010, Int J Food Microbiol, 137, 88-93). The same Authors observed higher penetration rate by A. simplex (s. s.) than A. pegreffii (Suzuki et al., 2010; Quiazon et al., 2011, Parasitol Int 60: 126-31). Given the pathogenic role to humans by the two parasite species (Umehara et al., 2009, Parasitol Int, 56: 211-215; Mattiucci et al., 2013, Emerg Infect Dis, 19: 496-499) fish fillets inspection and their infection rates by different Anisakis species, acquires particular importance for seafood safety.

Keywords:
Anisakis, Merluccius merluccius, food safety

O06.8

REAL TIME PCR USING DNA PROBES, BASED ON MITOCHONDRIAL COX2 GENE, FOR THE SPECIFIC IDENTIFICATION OF LARVAL ANISAKIDS OF THE GENERA ANISAKIS AND PSEUDOTERRANOVA

Paoletti M.*[1], Nascetti G.[1], Mattiucci S.[2]

[1]Department of Ecology and Biology (DEB), Tuscia University, Viterbo, Italy ~ Viterbo ~ Italy, [2]Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, Italy ~ Roma ~ Italy

INTRODUCTION:
Larval stages of anisakid nematodes of the genera Anisakis and Pseudoterranova have been reported as responsible of human infections (anisakiasis or anisakidosis) caused by the consumption of raw, undercooked seafoods harbouring larvae in their fillets. The larvae do not further develop in humans; however, they can penetrate the gastrointestinal tract and form eosinophilic granulomas, and they could cause Gastro-Allergic Anisakiasis (Daschner et al., 2011, Trends in Parasitol, 28:9-15; Mattiucci et al., 2013, Emerg Infect Dis 19: 496-450). In the last years, the anisakiasis has become a public health and it has increased the need for more information about it (EFSA Journal, 2010, 8:1543). Anisakid larvae have been detected in numerous fish species of commercial value. Genetic/molecular analysis of different nuclear and mitochondrial genes have been used to identify larval anisakids occurring in fish. RT-PCR using specific DNA primers/probe based on ITS rDNA to detect DNA of A. pegreffii and A. simplex s. s. in fillets was performed by Mossali et al. (2010, Foodborne Pathog Dis, 7:391–397). Aim of the present work was to develop new primers/probes based on the mitochondrial cox2 gene, to be used in RT-PCR for the DNA detection of the most frequently anisakids of the genera Anisakis and Pseudoterranova, occurring in European fish.
MATERIALS AND METHODS:
DNA was extracted using the automated DNA purification instrument Maxwell-16 (by Promega) or the Wizard Genomic DNA Purification Kit (Promega). Starting from the original sequences of mtDNA cox2 previously carried out on those anisakid species (Mattiucci et al., 2014, J Parasitol,100:199-214;; Timi et al., 2013, Vet Parasitol, 199: 59–72), specific internal regions were selected for each species of Anisakis and Pseudoterranova; new couple of primers were designed, so far, for six species of the genera Anisakis (A. pegreffii, A. simplex (s. s.), A. physeteris) and Pseudoterranova (P. decipiens (s. s.), P. bulbosa and P. krabbei), often occurring in syntopy in the flesh of the same individual fish host, of commercial value. Further, species-specific probes were designed, able to detect and identify those anisakid species, infecting the fillets. The LC480-Roche Instrument were used to validate the primers/probe for each species considered in the study, combined in a multiplex RT-PCR assay.
RESULTS:
By using the new primes, DNA fragments between 200 and 279 bp of mtDNA cox2, were obtained in the studied species. Accordingly, specific probes for each species of the genera Anisakis and Pseudoterranova were drawn and labeled with different fluorescent colors; PCR condition were standardized. The assay was performed as single primers/probe combination for each species considered in order to discriminate between them. DNA serial dilutions for anisakids samples, to test the detection limit of the minimum amount of parasite DNA, were performed. The primers/probe combinations were tested also in a multiplex reaction; in case of positive signal for samples labeled with the same flourophore, a successive single test, using different specific probe, was performed in order to discriminate between the two species.
CONCLUSIONS:
The Real Time PCR using DNA probes, based on mitochondrial cox2 gene, could represent a rapid and sensitive molecular test for the specific identification of larval anisakids of the genera Anisakis and Pseudoterranova, in the fish flesh.

Keywords:
Real Time PCR, hydrolysis probe, Anisakis

O06.9

PUTATIVE HYBRIDS OF TWO ANISAKIS CRYPTIC SPECIES IN STENELLA COERULEOALBA FROM THE MEDITERRANEAN SEA

Cavallero S.*[1], Costa A.[2], Caracappa G.[2], Currò V.[2], Gambetta B.[1], Amprimo V.[1], D'Amelio S.[1]

[1]Department of Public Health and Infectious Diseases ~ Rome ~ Italy, [2]Istituto Zooprofilattico Sperimentale della Sicilia ~ Palermo ~ Italy

INTRODUCTION:
Anisakid nematodes are parasites linked to aquatic ecosystem: larval stage is found in crustaceans, fishes and cephalopods; adult stage parasitizes marine mammals. The genus Anisakis includes nine recognized species and in particular the complex of cryptic species Anisakis simplex s. l. (Mattiucci et al., 1997, J Parasitol, 86: 401-16; 2013, J Parasitol, 100:199-214) is often associated with the disease known as anisakiasis (D'Amelio et al., 1999, Parassitologia, 41: 591; Mattiucci et al., 2011, BMC Infect Dis, 31: 11). During the last decades the use of nuclear ribosomal ITS allowed the identification and description of numerous anisakid nematodes (among others: Zhu XQ et al, 1998, Int J Parasitol, 28: 1911; D'Amelio S et al, 2000, Int J Parasitol, 30: 223) and favored the discovery of recombinant genotype or putative hybrids, as between A. simplex sensu stricto and A. pegreffii (Abollo et al., 2003, Infect Genet Evol, 3: 175), even in other parasitic helminths (Peng et al., 2003, Electrophoresis, 24:2308; Cavallero et al., 2013, PLOS NTD 7:e2170). The existence of pure hybrids of the two sibling species has been long debated due to the large recovery of larval forms from sympatric areas and the rare observation of adult hybrids, as solely Umehara et al. (2006 Parasitol Int, 55: 267) described in Balenoptera acutorostrata from the Japanese waters.
The aim of the present report is to identify anisakid nematodes collected from 5 individuals of Stenella coeruleoalba found stranded on the Sicilian coast using PCR-RFLP of ITS and to focus the interest on hybrid forms, since the new record of putative hybrid at adult stage.
MATERIALS AND METHODS:
A total of 77 nematodes collected were subjected to microscopy observation. PCR-RFLP analysis of the ITS was carried out using endonucleases HinfI and HhaI for species identification (D'Amelio et al., 2000, Int J Parasitol, 30: 223). Positive amplicons of hybrid forms were purified and sequences were carefully analysed using Trace implemented in MEGA v.6 (Tamura et al., 2013, Mol Biol Evol, 30:2725) to find diagnostic polymorphic sites.
RESULTS:
The morphological observation combined with PCR-RFLP analysis enabled to identify 62/77 anisakids specimens. The digestion of ITS amplicons with Hinf I showed 3 known patterns: A. simplex s.s. (1,5%), A. pegreffii (87%) and the heterozygous genotype (11,5%).
The morphological observation allowed the detection of eggs in uterus in two hybrid nematodes, revealing the presence of sexually mature females of Anisakis simplex s.l. in S. coeruleoalba.
The ITS sequences of putative hybrid individuals shows the diagnostic polymorphisms described firstly by Abollo et al. (2003) in addition to alternative pictures in which only one position of the two polymorphic sites showed the heterozygous status (C/T), except for one individual showing A. pegreffii polymorphism (CC).
CONCLUSIONS:
The new record of adult hybrids between the two cryptic species A. simplex s.s. and A. pegreffii in the definitive host Stenella coeruleoalba rekindle the long debate about the existence and the evolutive meaning of putative hybrids. Particularly, the observation of hybrid females sexually mature requires to clarify the genetic identity of putative hybrids, since the reproductive isolation between A. simplex s.s. and A. pegreffii is the assumption for their existence as sibling species (Mattiucci et al., 1997, J Parasitol, 86: 401). In this perspective, the use of alternative molecular markers and population genetic studies on adult anisakids are recommended.

Keywords:
Anisakis simplex complex, putative hybrid, cryptic species

O06.10
PROGENESIS IN TREMATODES

Di Cave D.*[1], Coiante V.[2], Ronci L.[2], Berrilli F.[1], Gustinelli A.[3], Pintus D.[2], Setini A.[2]

[1]University of Rome "Tor Vergata", Department of Experimental Medicine and Surgery, [2]University of Rome "La Sapienza", Department of Biology and Biotechnology "Charles Darwin", [3]University of Bologna , Department of Veterinary Medicine

INTRODUCTION:
The concept of progenesis commonly employed in paleontology and botany is widely reported in many animal taxa (Anura, Urodela, Polychaeta). The phenomenon is wellknown also in helminth parasites. In some trematode taxa, selection has even favoured the truncation of the life-cycle: progenetic larval stages (metacercariae) are capable of producing eggs inside their usual intermediate hosts, eliminating the need to be transmitted by predation to a definitive host.
MATERIALS AND METHODS:
From 2011 to 2014, specimens of amphipod Echinogammarus veneris (Heller, 1865) from the Natural Monument "Oasi di Ninfa" (central Italy) were collected and observed. During amphipods observation and dissection, were found larval stages of trematodes. A morphological and morphometric study has been performed on larvae fixed in alcool (70%), stained by boracic carminium and observed by optical microscope (40X).
RESULTS:
Total amphipods, both males and females, were infected with larval stages of trematodes mainly located within haemocoel, in thoracic-dorsal position. The mean number of metacercariae per amphipod was 12.4 ± 7.9. Rounded cysts (256.7 ± 24.7 µm) with a double thick wall composed by an outer layer 5.3 ± 1.4 µm and an inner layer 6.9 ± 2.4 µm. Occurrence of eggs has been observed inside. The present study confirms the precocious egg production in trematodes adding a new record to the already 79 described species (Lefevre and Poulin, 2005) .
CONCLUSIONS:
Numerous parasite species have evolved complex life cycles with multiple, subsequent hosts. In trematodes, each transmission event in multi-host life cycles selects for various adaptations, one of which is facultative life cycle abbreviation. This typically occurs through progenesis, precocious maturity and reproduction via self-fertilization within the second intermediate host. Progenesis eliminates the need for the definitive host and facilitates life cycle completion.

Keywords:
trematodes, life-cycle, progenesis

O06.11
SPIRORCHIIDIASIS IN STRANDED LOGGERHEAD CARETTA CARETTA IN NORTHERN ADRIATIC SEA (ITALY)

Marcer F.*[1], Marchiori E.[2], Tessarin C.[1], Danesi P.[3], Poppi L.[2]

[1]Dipartimento di Medicina Animale Produzioni e Salute - Università di Padova ~ Legnaro (PD) ~ Italy, [2]Dipartimento di Biomedicina Comparata e Alimentazione - Univerisità di Padova ~ Legnaro (PD) ~ Italy, [3]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy

INTRODUCTION:
The loggerhead-turtle Caretta caretta is one of three sea turtle species regularly found in Mediterranean basin (Lucchetti and Sala, 2010, Rev. Fish. Biol. Fisheries, 20: 141-161). The North Adriatic Sea represents an important foraging area for subadult and adult loggerhead turtles (Zbinden et al., 2008, Mar. Biol., 153: 896-906). Cardiovascular flukes (Spirorchiidae) are worldwide considered an important cause of stranding and mortality in sea turtles, including loggerheads (Stacy et al., 2010, Dis. Aquat. Org., 89: 237-259). No data on spirorchiid infections in Mediterranean Sea are available, although e.g. the blood fluke Hapalotrema mistroides (Monticelli, 1896) was described for the first time in C. caretta from Naples (Italy). The aim of this study was to assess prevalence, pathology and identity of spirorchiid species isolated from C. caretta, stranded dead along northwestern Adriatic coast.
MATERIALS AND METHODS:
During five years (October 2009-2013) ninety loggerheads were analyzed. Age classes and preservation status of the animals were valued. Necropsies were performed according to Flint et al. (2009, J. Vet. Diagn. Invest., 21: 733-759). Heart chambers, major vessels and blood clots were observed with dissecting microscope to detect adult parasites. Flukes' morphology was compared with descriptions given by Platt and Blair (1998, J. Parasitol., 84: 594-600). Stool specimens were partially frozen (-20°C) and partially analyzed by a routine sedimentation/flotation method. Egg types were morphologically identified according to the classification suggested by Wolke et al. (1982, J. Wild. Dis., 18: 175-185). A nested-PCR (Stacy et al., 2010, J. Parasitol., 96: 752-757) was performed on adult flukes and positive faecal samples. The amplicons were sequenced and compared with sequences available in GenBankTM. Tissue samples, depending on the decay status of the carcasses, were fixed with 4% formaldehyde and routinely processed for histological examinations.
RESULTS:
Spirorchiidiasis was recorded in 8 turtles (8.9%). The prevalence in the three observed age classes was 3.2% in juveniles (1/31), 11.5% in subadults (6/52) and 14.3% in adults (1/7). No important gross lesions associated to spirorchiid infection were observed. Adult flukes were recovered in the heart of one animal. Spirorchiid eggs, all identified as type I, were revealed by copromicroscopic examination in eight animals. All positive samples (adults and eggs) were molecularly identified as H. mistroides (accession number GU937893.1). Histological examinations could be performed in 7 out of 8 positive turtles; in all cases migrating eggs were detected in tissues.
CONCLUSIONS:
Spirorchiid prevalence in sea turtles from Adriatic Sea appears to be lower than in other areas in the world (Chen et al., 2012, J. Parasitol., 98: 437-439) and the unique isolated species was previously described in Mediterranean waters. Spirorchiidiasis, in these animals, was never directly related to mortality. Our study provides a measure of the prevalence and status of this infection in loggerheads from North Adriatic. Further studies are needed to assess the epidemiological aspects of spirorchiidiasis in other Mediterranean areas.

Keywords:
Caretta caretta, Spirorchiidae, Italy

NOTES

SESSIONE PARALLELA O-07

BIOLOGIA E FILOGENESI MOLECOLARE APPLICATE A PARASSITI E VETTORI

O07.1
ISOLATION AND CHARACTERIZATION OF PARAMYOSIN FROM ONCHOCERCA LUPI, A NEGLECTED ZOONOTIC NEMATODE

Campbell B.E.*[1], Giannelli A.[1], Annoscia G.[1], Dantas Torres F.[2], Cortes H.[3], Cardoso L.[4], Otranto D.[1]

[1]Universita di Bari Aldo Moro ~ Bari ~ Italy, [2]Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães (Fiocruz-PE) ~ Recife ~ Brazil, [3]Victor Caeiro Laboratory of Parasitology, Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Evora ~ Evora ~ Portugal, [4]Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real ~ Vila Real ~ Portugal

INTRODUCTION:
Paramyosin, an invertebrate muscle protein, is a well-recognized allergen, which has also been widely studied as a potential vaccine candidate against parasites (Gobert and McManus 2005, Parasitology International, 54:101–107). Fort example, antibodies against this molecule form the basis of a commercial kit for the serological detection of the human filarial nematode Wuchereria bancrofti (Langy et al., 1998, Infection and Immunity, 66:2854–2858). The antibodies also recognize antigens from Dirofilaria immitis and other filarial nematodes. The aim of this study was the isolation and characterisation of paramyosin from Onchocerca lupi, an increasingly important filarial nematode that causes acute and chronic ocular disease in dogs, cats and also humans. Current methods to detect microfilariae of O. lupi include skin snips, whereas adults may be removed surgically from ocular nodules in infected hosts. Both procedures are invasive, time consuming and laborious. In addition, no chemotherapeutics are available. Therefore, a more rapid, less invasive technique is required to detect the parasite. Development of a serological diagnostic test, based on antibodies to O. lupi paramyosin, would assist in diagnosis of infestation and in the analysis of the prevalence, distribution and potential vectors of this zoonotic parasite.
MATERIALS AND METHODS:
Adult and microfilarial nematodes were isolated from the eye and skin, respectively, of a dog from Portugal. RNA from one adult female was reverse transcribed into single stranded cDNA and paramyosin PCR amplified from this material. The paramyosin cDNA was cloned into a sequencing vector and sequenced in both directions. Bioinformatic analysis included BLAST, phylogenetic trees, protein translations and analysis and also epitope mapping (including epitopes published in the literature).
RESULTS:
A 2.5kb paramyosin cDNA was isolated from O. lupi, BLASTn and BLASTx analysis indicating 98% nucleotide and 99% amino acid identity to paramyosin from Onchocerca volvulus. Phylogenetic analysis showed that O. lupi paramyosin molecule clustered with O. volvulus and other filarial nematode paramyosin proteins. The predicted protein is 101kDa, has a conserved nematode-specific proton donor site, no signal peptide and no transmembrane spanning domains. Epitope mapping suggested the presence of potential antigenic sites.
CONCLUSIONS:
The paramyosin molecule is a suitable candidate for the development and testing of a serological diagnostic tool to detect the presence of O. lupi.

Keywords:
Onchocerca, zoonotic, paramyosin

O07.2
ANALYSIS OF RIBOSOMAL DNA INTERNAL TRANSCRIBED SPACER SEQUENCES OF LEISHMANIA DONOVANI ISOLATES FROM SUDANESE PATIENTS

Babiker A.M.A.*[3], Ravagnan S.[3], Porcellato E.[3], Signorini M.[1], Hassan M.M.[2], Cassini R.[1], Cattoli G.[3], Capelli G.[3]

[1]Università degli Studi di Padova ~ Padova ~ Italy, [2]Tropical Medicine Research Institute ~ Khartoum ~ Sudan, [3]istituto zooprofilattico sperimentale delle venezie ~ Padova ~ Italy

INTRODUCTION:
Over the last decade human leishmaniasis became an increasingly significant problem associated with poverty and HIV/AIDS in developing countries and is considered as imported or re-emerging diseases in Europe. Leishmania donovani complex (L.donovani, L.infantum and L.archibaldi) cause very different clinical forms ranging from disfiguring cutaneous and muco-cutaneous lesions to fatal visceral disease. Mixed infections with different Leishmania strains may explain the differences in the clinical course of the disease and may be the reason for treatment failures (Antoniou et al, 2004, Am J Trop Med Hyg., 71(1):71-2).
MATERIALS AND METHODS:
Ten bone marrow samples obtained from three visceral leishmaniasis patients (VL), one Muco-cutaneous (ML), one Post Kalazar Dermal leishmaniasis (PKDL) and five Cutaneous Leishmaniasis (CL) were amplified using specific primers targeting the ITS gene and generating 1044bp (El tai et al., 2000, Exp Parasitol, 97:35–44; Babiker et al., 2014, J Trop Med, ID 170859). The ten amplificates were cloned separately, DNA of at least 20 colonies from each sample were extracted and sequenced. Polymorphisms based on different microsatellite repeats were detected and compared to those identified by other Authors (Kuhls et al., 2005, Microbes Infect., 7:1224-34).
RESULTS:
Three out of the five CL samples showed mixed infection with L.donovani and L.major. This is the first report of mixed infection by these two Leishmania species from a single cutaneous ulcer in Sudan (Babiker et al., 2014; J Trop Med, ID 170859). One colony of one of these samples showed a hybrid L.donovani/L.major sequence. The other two CL samples clustered with the L.donovani genetic group. The PKDL and ML samples showed six and four different sequences patterns, respectively. The three VL samples showed five and four different sequence patterns. One of the VL samples showed two dominant types: type D and type E (Type D attributed to L. archibaldi MON 82, L. archibaldi MON 258, L. archibaldi MON 257 and L. donovani MON 274; type E attributed to L. infantum MON 30, L. infantum MON 81 and L. archibaldi MON 82) while in the other two VL samples type D was absent and the two samples showed dominant type F (L. infantum MON 30, L. infantum MON 267 and L. donovani MON 18). Type C (L.donovani MON 35) was found in some polymorphic sites in the PKDL sample as well as in some sites of one VL samples. In this latter sample, type A and type B sites (attributed to different zymodems of L.infantum) were also found.
CONCLUSIONS:
The results showed the presence of mixed infection with different strains of L. donovani in VL, ML, PKDL and CL patients in the same geographical area. The results also suggest the presence of L. infantum hybrid strains as an etiological agent of VL in Sudan, however, this hypothesis needs to be confirmed. The detection of different strains of L. donovani in the same patient is likely due to the fact that samples were directly extracted from biological materials and not from cultured parasites. Previously, it has been demonstrated that in co-cultivation of different Leishmania the dominant ones tend to inhibit the growth of the others; as a consequence, the degree of laboratory detection of such phenomenon remains unclear and likely underestimated (Pacheco et al., 1987, Mem Inst Oswaldo Cruz, 82:537-542; Ibrahim et al, 1994, Acta Trop, 57= PAG138:327-332).

Keywords:
Leishmania, L.donovani, mixed infection

O07.3

CHARACTERIZATION OF THE GLYCEROL-3-PHOSPHATE DEHYDROGENASE (GG3PD) OF GIARDIA DUODENALIS AND ITS INTERACTION WITH THE MULTIFUNCTIONAL G14-3-3 PROTEIN

Finelli R.[1], Cecchetti S.[1], Pozio E.[1], **Lalle M.*[1]**

[1]Istituto Superiore di Sanità ~ Roma ~ Italy

INTRODUCTION:
The flagellated protozoan Giardia duodenalis (syn. lamblia or intestinalis) is a worldwide parasite causing giardiasis, a diarrheal disease. Giardia has a limited metabolic diversity and few metabolic pathways (i.e, carbohydrate pathways) have been described so far. Understanding of its metabolism is than important to select new targets for drug development. We have recently identified a putative giardial glycerol-3-phosphate dehydrogenase (gG3PD) as a putative binding partner of the single g14-3-3 protein. The 14-3-3s are a family of eukaryotic pSer/pThr binding proteins that play a regulatory role in multiple cellular processes, including primary metabolism. G3PDs are involved in glycolisis, electron transport chains, hyperosmotic stress response and, moreover, they are emerging as promising drug target against Trypanosomatidae, we decided to analyze the gG3PD and its interaction with g14-3-3.
MATERIALS AND METHODS:
The coding gene for the gG3PD was cloned and expressed as His-tagged recombinant protein in E. coli or as FLAG-HA-tagged protein in Giardia WBC6. A polyclonal antibody against the recombinant His-gG3PD was produced in mouse and used for immunofluorescence and western blot analysis. A glycerol-3 phosphate oxidation assay was set up to evaluate the gG3PD enzymatic activity.
RESULTS:
Here we demonstrate that the gG3PD and g14-3-3 co-immunoprecipitate at the trophozoite stage but not during encystation and the localization of gG3PD is stage dependent, with the formation of cytosolic aggregates during encystation. We proved that gG3PD is a functional enzyme able to convert glycerol-3-phosphate into DHAP. Furthermore, during encystation, the enzymatic activity of gG3PD increases together with the intracellular glycerol level.
CONCLUSIONS:
Altogether these data point out on the involvement of gG3PD in: i) an alternative respiratory pathway and ii) the differentiation of Giardia into cyst, possibly via an hyperosmotic stress response signalling. The suggested relevance of gG3PD and of its interaction with the g14-3-3 make both proteins ideal targets for antigiardial drug development.

Keywords:
Giardia duodenalis, Encystation, Drug target

O07.4

DISTINCT PROPERTIES OF THE EGRESS-RELATED OSMIOPHILIC BODIES IN SEXUAL STAGES OF THE RODENT MALARIA PARASITE PLASMODIUM BERGHEI

Olivieri A.*[1], Bertuccini L.[2], Deligianni E.[3], Franke Fayard B.[4], Currà C.[1], Siden Kiamos I.[3], Hanssen E.[5], Superti F.[2], Janse C.[4], Ponzi M.[1]

[1]Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Parassitarie ed Immunomediate ~ Roma ~ Italy, [2]Istituto Superiore di Sanità, Dipartimento di Tecnologia e Salute ~ Roma ~ Italy, [3]Institute of Molecular Biology and Biotechnology, FORTH ~ Heraklion ~ Greece, [4]Leiden Malaria Research Group, Department of Parasitology, Centre for Infectious Diseases, Leids Universitair Medisch Centrum (LUMC) ~ Leiden ~ Netherlands, [5]Bio21 Molecular Science and Biotechnology Institute, Electron Microscopy Unit and Department of Biochemistry and Molecular Biology, University of Melbourne ~ Melbourne ~ Australia

INTRODUCTION:
During its complex life cycle, malaria parasites alternate between a vertebrate host and an Anopheles mosquito vector. Gametogenesis is the earliest event after uptake of malaria parasites by the mosquito, with a decisive impact on colonization of the mosquito midgut. In a few minutes male and female gamete precursors (the gametocytes) escape from the host erythrocyte by rupturing, in rapid succession, the parasitophorous vacuole and erythrocyte membranes. The female gametocyte forms a single macrogamete, while the male gametocyte undergoes DNA replication and axoneme formation, finally resulting in the release of eight flagellated microgametes. Evidences presented in this study indicate that molecular events leading to gametocyte egress differ between female and male gametocytes and involve gender-specific secretory organelles.
MATERIALS AND METHODS:
Early events of gametogenesis were investigated in the rodent malaria model Plasmodium berghei. Electron microscopy and electron tomography analyses combined with immuno-localization experiments were used to define morphological features distinctive of male and female secretory organelles. In addition, we investigated the functional role of Pbg377, a female-specific protein localizing to electron-dense secretory organelles, the osmiophilic bodies (OB), by generating two independent P. berghei knockn-out lines.
RESULTS:
Comparative ultrastructural analyses on activated gametocytes revealed distinctive morphological features of gender-specific, electron dense vesicles. Female OB are oval-shaped vesicles with a volume approximately three times greater than the one of club-shaped male osmiophilic bodies (MOB). MOB are distributed throughout the cytoplasm of non-activated male gametocytes, while these punctuate structures progressively coalesce in few focal points within minutes after activation of gametogenesis. Using specific antibodies, we confirm that P. berghei g377, like the P. falciparum ortholog, localizes to female OB. In P. berghei mutants lacking g377, female gametocytes develop normally but display a dramatic reduction in number and size of OB, that lack their typical oval-shaped morphology. This structural change is accompanied by a significant delay in egress efficiency but not in female gamete fertility.
CONCLUSIONS:
Evidences presented in this paper indicate that molecular events leading to gametocyte egress differ between female and male gametocytes of P. berghei. A further dissection of this process, essential for fertilization and development within the mosquito, may provide knowledge for designing strategies that can effectively block transmission.

Keywords:
Malaria, gametocytes, Plasmodium berghei

O07.5
POSITIVE SELECTION DRIVES ACCELERATED EVOLUTION OF MOSQUITO SALIVARY GENES ASSOCIATED WITH BLOOD FEEDING

Arcà B.*[1], Struchiner C.[2], Pham V.[3], Sferra G.[1], Lombardo F.[1], Pombi M.[1], Ribeiro J.[3]

[1]Dipartimento di Sanità Pubblica e Malattie Infettive, Sezione di Parassitologia, Sapienza Università di Roma ~ Roma ~ Italy, [2]Escola Nacional de Saude Publica, Fundaçao Oswaldo Cruz ~ Rio de Janeiro ~ Brazil, [3]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases ~ Rockville, MD ~ United States

INTRODUCTION:
The saliva of bloodsucking animals carries dozens to hundreds of proteins whose main role is to counteract their hosts' haemostasis, inflammation and immunity. Previous studies on both anopheline and culicine mosquitoes highlighted the high rate of interspecific diversity of salivary (S) proteins as compared to housekeeping (H) ones. In An. gambiae-An. stephensi [Ae. aegypti-Ae. albopictus] S proteins are 62.4% [71.5%] identical whereas H proteins share 93.1% [94.0%] amino acid residues. This pattern may be explained by relaxed selection or random drift. Alternatively, may be the result of positive selection driven by the intense pressure of the host immune system on proteins/genes that are essential for blood feeding and, therefore, strongly affect mosquito reproduction/fitness. In such a scenario, host antibody response to salivary proteins may favour the selection of protein variants with conserved biological functions and different antigenic properties.
MATERIALS AND METHODS:
To test our working hypothesis we looked for selection signatures in five salivary genes involved in blood feeding (Apy, D7r2, gSG6, gSG7, gVAG). Genes were amplified from individual An. gambiae s.s. mosquitoes (n=63-81) collected in October 2008 in the village of Soumousso (Bobo-Dioulasso, Burkina Faso). For each gene equimolar amounts of the amplified products were mixed and used to construct plasmid libraries. Recombinant clones (192/library) were randomly picked and sequenced. The rpS7 ribosomal protein gene was used as quality control. Sequences were aligned by the cap3 assembler, edited by BioEdit and analyzed using DnaSP, MEGA and HyPhy.
RESULTS:
Overall, 873 SNPs (395 coding, 478 noncoding) were identified, which corresponds to ~11.1 SNPs/kb; in comparison 9.2 and 8.9 SNPs/kb were found in a previous study for 72 immune and 37 non-immune An. gambiae genes. gSG6 was under strong purifying selection, whereas gSG7 appeared to evolve at a very fast rate. For all genes analyzed dN/dS<1 which indicates overall negative selection acting on the salivary genes. However, when positive selection was tested on individual codons by the HyPhy program using both the FEL (fixed-effects likelihood) and the MEME (mixed-effects model of evolution) models, nearly all salivary genes showed one or more codons with significant signatures of positive selection.
CONCLUSIONS:
According to the neutral theory most of the variability/change within a species is caused by random drift of selectively neutral mutants (non affecting fitness). Against this pervasive random drift selection may occur, with negative selection removing deleterious mutations and positive (Darwinian) selection promoting beneficial ones. Positive selection is rare and most of the selection signatures deviating from neutral expectations are found for negative selection. Exceptionally, cases for positive selection are found in genes associated with immunity, mate- and self-recognition, or with genes associated with virulence in pathogen recognition. Mosquito salivary genes associated with blood-feeding are shown here to be under strong positive selection, which explains their fast rate of divergence. We believe that our study provides evidence for a new class of positively selected genes, those coding for salivary proteins involved in bloodsucking by arthropods, and, specifically, the salivary proteins of the malaria vector An. gambiae.

Keywords:
positive selection, salivary genes, Anopheles gambiae

O07.6
PHYLOGENETIC EVIDENCE FOR THE ORIGINS OF WOHLFAHRTIA MAGNIFICA ISOLATES FROM SHEEP IN ITALY

Marangi M.*[1], Giangaspero A.[1], Gaglio G.[2], Paul D R.[3], Hall M.J.R.[3]

[1]University of Foggia ~ Foggia ~ Italy, [2]University of Messina ~ Messina ~ Italy, [3]Natural History Museum ~ London ~ United Kingdom

INTRODUCTION:
Wohlfahrtia magnifica (Diptera: Sarcophagidae) is an important obligate parasite whose larvae cause a serious traumatic myiasis. Sheep are the major hosts but other animals and even humans can be affected (Hall & Wall 1995, Adv. Parasitol, 35:257-334). W. magnifica occurs along the so called 'Eurasian ruminant road' (Slingenbergh et al., 2004, RSTOIE, 23:467-484) i.e. from Europe through Turkey, Iraq and Iran and eastwards into China. The presence of W. magnifica in Italy has been hypothesised since 1994. However, its presence as a livestock pest was confirmed in 2005 in a Central Italian region (Giangaspero et al., 2011, Vet. Parasitol, 175:109-112) where an increasing incidence and spread was demonstrated (Giangaspero et al., 2014, Med. Vet. Entomol, in print). Previous molecular studies reported the presence of two lineages of W. magnifica: western (Spain/Morocco) and eastern (Hungary/Greece) (Hall et al., 2009, Med. Vet. Entomol, 23:72-79). The presence of the eastern lineage in an isolate from Italy was previously identified (Hall et al., 2009, Med. Vet. Entomol, 23:59-71).
In order to evaluate the geographical origin of the species affecting sheep in Italy, the aims of the present study were to genetically characterize specimens of W. magnifica from Italy and to compare the phylogenetic relationships between Italian isolates and homologous sequences from other countries.
MATERIALS AND METHODS:
Twenty 2nd and 3rd stage larvae of W. magnifica -collected during epidemiological studies on wohlfahrtiosis in sheep farms- from Sicily, Lazio, Molise (Giangaspero et al., 2011 o.c.) regions and from a recently recorded infested sheep in Apulia region were individually subjected to DNA extraction. The 3' terminal 715 bp of the cyt b gene was amplified following the protocol described in Ready et al. (1997, Bull. Entomol. Research, 87:187-195), purified, sequenced and aligned with twelve homologous published sequences (Hall et al., 2009 o.c.). Each alignment was subjected to phylogenetic analysis by Neighbour Joining (NJ) methods using the MEGA v5.1 software (Tamura et al., 2011, Mol. Biol. Evol, 28:2731-2739) with Oestrus ovis as outgroup.
RESULTS:
The phylogenetic tree showed three genetically distinct groups, named 1, 2 and 3. The mean genetic distance between groups 1 and 2 was 0.036%, while the mean genetic distances were 0.017% and 0.023% between group 1-3 and group 2-3, respectively. Phylogenetic analyses including the haplotypes from the other countries showed that 4 isolates from Lazio and Molise regions belong to the 'eastern lineage' and 16 from Lazio, Molise, Sicily and Apulia regions belong to the 'western lineage'.
CONCLUSIONS:
The presence of the eastern lineage supports the data previously obtained (Hall et al., 2009 o.c.) while the finding of the western lineage suggests that this lineage was previously overlooked or is a more recent introduction, either by natural movement of flies and/or as larvae in infested sheep from Spain and/or North Africa. The importation of sheep to Italy from Spain and PECO EU-countries is documented (e.g. http://www.assonapa.it). It is possible that animal/s with a covert infestation might have been shipped from those countries and then mature larvae left the wounds, pupated and emerged as adults in Italy.
A broader geographical survey and more in depth genetic studies are needed throughout the range of W. magnifica to fully explore its genetic diversity and the factors that contribute to its distribution and dispersal.

Keywords:
Wohlfahrtia magnifica, cyt b, phylogeny

O07.7
EMERGING PATHOGENS IN VERTEBRATES: BIOLOGY, GENOMICS AND INFECTIVITY OF BACTERIA ASCRIBED TO THE FAMILY MIDICHLORIACEAE

Bazzocchi C.*[1], Cafiso A.[1], Petroni G.[2], Lanzoni O.[2], Sassera D.[3], Epis S.[1], Mariconti M.[4], Bandi C.[1]

[1]DIVET, University of Milan ~ Milan ~ Italy, [2]Department of biology, University of Pisa ~ Pisa ~ Italy, [3]DBB, University of Pavia ~ Pavia ~ Italy, [4]IRCCS; Policlinico S. Matteo ~ Pavia ~ Italy

INTRODUCTION:
The order Rickettsiales encompasses a variety of bacterial species, many of which are etiological agents of important diseases in humans and animals. Recently, a new family has been proposed within this order, the Midichloriaceae. In ecological terms, this new family is even more complex than Rickettsiaceae and Anaplasmataceae, and its hosts range from parasitic arthropods (ticks, fleas, bedbugs) to marine organisms (sponges and corals) and protists, including pathogenic amoebae (genus Achantamoeba). In addition, several lines of evidence suggest that some Midichloriaceae are infectious to vertebrates (Mariconti et al., 2012, Pathog. Glob. Health. 106:391-96; Bazzocchi et al., 2013, Parasit Vectors. 6:3503) and that they could be responsible for disorders such as the strawberry disease in salmonids (Metselaar et al., 2010, J Fish Dis. 33:849-58) However, the infectivity and pathogenicity of Midichloriaceae for humans/mammals has yet to be demonstrated. The objectives of this work are to 1) produce reagents for the detection of these bacteria (antigens, antibodies, primers); 2) investigate the infectivity for salmonid fishes and mammals.
MATERIALS AND METHODS:
The infectivity of Midichloriaceae could be studied using different experimental procedures: a) bacteria detection (PCR; immunostaining) in skin lesions of salmonids and in corneal tissue of human patients infected by Achantamoeba; b) serological analysis in humans infected by Achantamoeba and in fishes positive for the presence of this bacterium.
A quantitative PCR based on the 16S gene of the uncultured Midichloriacea bacterium present in the lesions of samonid affected by Strawberry disease was developed.
Starting from the alignment of the gene coding for a surface protein of phylogenetic distant Midichloria spp. bacteria detected in ticks, we designed conserved primers for the amplification of this gene from the bacterium present in the salmonid lesions or in corneal tissues. The most conserved portion of this gene among bacteria belonging to the family Midichloriaceae will be cloned for the expression of the protein in recombinant form. The corresponding polyclonal antibodies will be produced.
RESULTS:
The specificity of the Real time PCR developed permits a rapid identification of salmonid positive for the presence of this bacterium and its quantification in the skin lesion (compared to control skin).
The surface protein, conserved between different bacteria ascribed to the family Midichloriaceae, will be expressed in recombinant form and purified end the corresponding polyclonal antibodies will be produced. ELISA assays specific for the detection of circulating antibodies against bacterial proteins in vertebrates will be developed using sera of patients infected by Achantamoeba and sera of fishes affected by Strawberry disease. Furthermore, antibodies could be used for the detection of bacteria in damaged tissues using an immunostaining assay.
CONCLUSIONS:
Results of this work will be useful to study whether bacteria belonging to the family Midichloriaceae could be considered as potential pathogens. The evidence of infectivity of Midichloriaceae bacteria for vertebrates could lead to a better understanding of the pathological changes showing clinical conditions that are difficult to interpret.

Keywords:
symbiosis, Midichloriaceae family, vertebrate infectivity

O07.8
BACTERIAL SYMBIOTIC CONTROL OF MOSQUITO VECTORS: FROM BENCH TO FIELD

Mancini M.V.*[1], Bozic J.[1], Capone A.[1], Cappelli A.[1], Damiani C.[1], Epis S.[2], Rossi P.[1], Valzano M.[1], Bandi C.[2], Ricci I.[1], Favia G.[1]

[1]Scuola di Bioscienze e Medicina Veterinaria, Università degli Studi di Camerino ~ Camerino ~ Italy, [2]Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria, Università degli Studi di Milano ~ Milano ~ Italy

INTRODUCTION:
Vector-borne diseases are a worldwide concern and a significant cause of human morbidity and mortality accountable for nearly half of the world's population.The scientific effort was propelling forward the development of new plans focusing on control of vector capacity. In light of this, the impact of mosquito role is becoming increasingly apparent.Likewise, recent years have seen growing interest in developing understanding of vector microbiota, the complement of microorganisms living in symbiosis with a host. The current state of the art opens encouraging possibilities for paratransgenic strategies, showing that the 'symbiotic control' seems particularly promising. Our idea of paratransgenesis was matured along with the discovery of a mosquito-stably-associated bacterium of the genus Asaia (Favia et al., 2007, Proc Natl Acad Sci USA, 104:9047-9051).Asaia's intrinsic characteristics, together with its colonization throughout the mosquito life and its co-localization in malaria parasite invasion hot-spots, as well as in reproductive organs of both sexes, indicate it as an interesting tool for diseases management.
MATERIALS AND METHODS: Our work aims to develop a method for an effective symbiotic control of insect pests, first of all malaria, focusing on two main lines of research:1)development of paratransgenic mosquitoes with reduced ability to transmit malaria;2)release experiments of paratransgenic mosquitoes in "semi-field" conditions to determine the rates of transmission in recipient populations and in subsequent generations. The first working line is based on colonization of the mosquito species Anopheles stephensi (major vectors of malaria in Asia) with recombinant Asaia sp., specifically Asaia borogensis, able to express a surface protein of Wolbachia (WSP): these bacteria were showed to infect the reproductive organs of many species of mosquitoes, with the exception of malaria vectors. Its ability to counteract pathogens, through molecular interactions or by enhancing host immune responses, has been widely described (Kambris et al., 2010; PLoS Pathog 6(10):e1001143).The second project supposed the release of paratransgenic mosquitoes in a recipient population in contained semi-field facilities. It has been possible by the application of modified strains of A. borogensis expressing fluorescent proteins, allowing their detection across recipient populations and successive generations.



Particular of the semi-field facility in Perugia, PG.

RESULTS:
Successfully established Asaia-WSP has been tested for transmission blocking trials:preliminary outcomes suggest possible effects on transformed mosquitoes. However, additional aspects, especially those related to mosquito immune system, deserve further investigations. Results from the second project followed the transmission dynamics of the engineered bacteria in semi-field conditions, showing that recipient mosquitoes emerged to be consistently infected by recombinant Asaia: spread can be ascribed to either horizontal or vertical route.
CONCLUSIONS:
Toward exploiting the potential of mosquito microbiota to reduce vector competence, research is currently at its infancy, but some bacteria like Asaia already emerge as promising candidates of paratransgenesis. These outcomes provide decisive insights for a detailed determination of the real potential of Asaia, in terms of symbiont diffusion rate and anti-pathogen effectiveness, in paratransgenesis.

Keywords:
Symbiosis, Mosquito, Asaia

O07.9

USING SYMBIOTIC YEASTS ASSOCIATED TO MOSQUITOES TO PREVENT PLASMODIAL INFECTION IN MALARIA VECTORS: CURRENT STATUS AND FUTURE STRATEGIES FOR SYMBIOTIC CONTROL OF MOSQUITO BORN DISEASES

Bozic J.*[1], Capone A.[1], Valzano M.[1], Cappelli A.[1], Damiani C.[1], Rossi P.[1], Mancini M.V.[1], Favia G.[1], Ricci I.[1]

[1]Scuola di Bioscenze e Medicina Veterinaria, Università degli studi di Camerino ~ Camerino ~ Italy

INTRODUCTION:
Owing new advances in molecular biotechnology, genetic manipulation of the microbial society associated with mosquitoes has been achievable. Paratransgenic approach has conceded straightforward premises for mosquito-gut-residing-symbionts to be exploited and produce anti-parasite molecules. Among bacteria, one of the best paratransgenic candidate is Asaia, an alfa-proteobacterim (aceto bacteriacaea) inhabiting the mosquito midgut and gonads (Favia et al., 2007, Pnas, 104:9047–9051). Further analysis of the mosquito's microbiota led to the discovery and identification of yeasts' species Wicheramomyces anomalus, which has been detected in the same organs of An. stephensi, and moreover, it is already used for biotechnological applications in the food industry (Ricci et al., 2011, A. V. Leeuwenhoek, 99:43–50, Ricci et al., 2011, Environ Microbiol, 13:911-921). W. anomalus produces killer toxins (KTs) which have been confirmed against various microorganisms, including bacteria, viruses and parasites (Bevan EA, Makover M 1963, XI International Congress on Genetics. Oxford Pergamon Press, Oxford. Vol 1, pp. 53-58). Discovery of mosquito-yeast association yielded innovative strategies for a potential use of wild W. anomalus killer strains in the symbiotic control of mosquito-borne diseases, since they can express natural antimicrobial activity. Furthermore as eukaryotic microorganisms for paratrangenesis, they can be genetically manipulated expressing more than one effector molecule, using their own delivery mechanism.
MATERIALS AND METHODS:
Mosquito colony of A. stephensi, W. anomalus (Wa) strain F17.12 isolated from A. stephensi, target yeasts strains for antimicrobioal activity of WaKT test (WaATCC 96603, WaUM3, Candida albicans ATCC 24433, Saccharomyces cerevisiae ScATCC 2601), monoclonal antibody mAbKT4 targeted against WaKT. Experimentation has been encompassed using different techniques and assets like TEM, PCR, FISH; production of crude WaKT extracts, western blot analysis, immunofluorescence assays (IFA), cloning and sequencing genes of interest; transformation by electroporation) and KT characterization.
RESULTS:
By Western blot and IFA in vitro analyses using mAbKT4, we demonstrated that WaF17.12 isolated from A. stephensi expresses at the cell surface and secretes a soluble KT in cultural medium. By IFA in vivo we've observed that cultivated yeasts added to the mosquito diet were able to colonise gut and gonads and actively release WaKT during the whole mosquito life span and progeny as well. Tests on KT activity have demonstrated that WaF17.12 secretes a toxin expressing antimicrobial activity against sensitive yeast strains.
CONCLUSIONS:
Our study points out the potential of mosquito-yeast association, to contribute the effectiveness of symbiotic control strategies. Furthermore, Wa strains effective against malaria parasite could become promising biopesticide candidate, in addition to its advantage to be supposed as non-threatening to humans.

Acknowledgements:
This research project has received funding from the European Union Seventh Framework Programme under grant agreement n.281222.

Keywords:
Symbiotic control, Mosquito borne disease, Yeasts

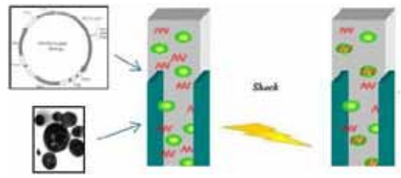
O07.10

ENGINEERING OF THE YEAST WICKERHAMOMYCES ANOMALUS, SYMBIONT OF MOSQUITO SPECIES RELEVANT TO PUBLIC HEALTH, FOR PARATRANSGENIC CONTROL STRATEGIES

Capone A.*[1], Bozic J.[1], Cappelli A.[1], Damiani C.[1], Rossi P.[1], Valzano M.[1], Epis S.[2], Favia G.[1], Ricci I.[1]

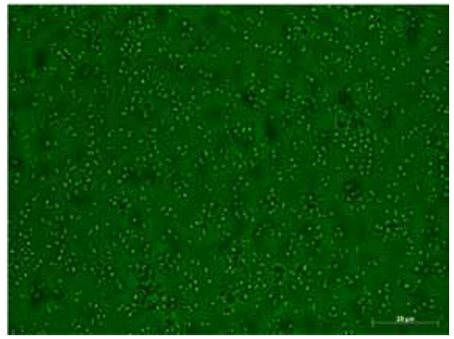
[1]Scuola di Bioscienze e Medicina Veterinaria, Università degli Studi di Camerino ~ Camerino ~ Italy, [2]Dipartimento di Patologia Animale, Igiene e Santità Pubblica Veterinaria, Università degli Studi di Milano ~ Milano ~ Italy

INTRODUCTION:
The paratransgenic approach to fight vector-borne disease provides, after the isolation of microorganisms stably associate to the insect, the engineering of symbionts to express foreign molecules against pathogen in the vector itself. For its features, the yeast Wickerhamomyces anomalus, isolated from gonads and midguts of Anopheles mosquitoes (Ricci et al., 2011, Environ Microbiol, 13:911-921), is the eukaryotic candidate that we propose for this approach in the control of malaria. Here we propose the first genetic manipulation of W. anomalus to obtain a Green Fluorescent Protein (GFP)-tagged recombinant strain very useful both in basic and applied studies.
MATERIALS AND METHODS: The genetic manipulation of the yeast has been consisted in the use of the Px4-Pex14-eGFP plasmid for the phenotypic expression of the green fluorescent protein (GFP). W. anomalus F17.12 strain isolated from An. stephensi was cultured in YPD medium, the plasmid was inserted into the yeast by electroporation (Fig.1). Stable yeast transformants were obtained by selective medium containing geneticin G418. The GFP was detected in the yeast cells by fluorescence microscopy.



Technique of electroporation

RESULTS: Experiments performed by engineered yeast expressing GFP (Fig. 2), has provided the feasibility of using W. anomalus as a tool to study important aspects of this symbiotic relationship.
The recombinant W. anomalus GFP-tagged strain allowed us to follow the localization of the yeast in the mosquito organs, and to study the dynamic of the symbiont spreading within and across vector populations (i.e. semi-field and in-field studies).



W. anomalus GFP-tagged strain

CONCLUSIONS:
Engineering of W. anomalus allowed the obtaining of a fluorescent phenotype useful to the study of symbiotic relationships as already done for the symbiotic bacterium Asaia (Favia et al., 2007, Proc Natl Acad Sci USA, 104:9047–9051). Furthermore, this manipulation toll represents the starting point to express, in future, anti-parasite effectors to control malaria and others mosquito-borne diseases.
The demonstrated transformability of the yeast indicates the possibility (i) to introduce in the plasmid molecules already known to be effective against Plasmodium or (ii) to strengthen the expression of natural antimicrobial activity of W. anomalus (Cappelli et al., accepted).

Acknowledgements: This research project has received funding from the European Union Seventh Framework Programme under grant agreement n.281222.

Keywords:
Paratransgenesis, Malaria, Mosquito

NOTES

SESSIONE PARALLELA O-08

INFEZIONI DA PROTOZOI

O08.1
EPIDEMIOLOGICAL STUDY OF BESNOITIA BESNOITI INFECTION AMONG CATTLE IN INSULAR AND NORTH-WESTERN ITALY.

Gazzonis A.L.*[1], Alvarez Garcia G.[2], Zanzani S.A.[1], Maggioni A.[1], Garippa G.[3], Rossi L.[4], Maggiora M.[4], Inverizzi A.[7], Luini M.V.[8], Dini V.[5], Tranquillo V.[6], Ortega Mora L.[2], Manfredi M.T.[1]

[1]Department of Veterinary Science and Public Health, University of Milan ~ Milano ~ Italy, [2]SALUVET, Animal Health Department, Faculty of Veterinary Sciences, Complutense University of Madrid ~ Madrid ~ Spain, [3]Department of Veterinary Medicine, University of Sassari ~ Sassari ~ Italy, [4]Department of Veterinary Sciences, University of Turin ~ Torino ~ Italy, [5]Unità Sanitaria Locale Savonese 2 ~ Savona ~ Italy, [6]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna ~ Bergamo ~ Italy, [7]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna ~ Milano ~ Italy, [8]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna ~ Lodi ~ Italy

INTRODUCTION:
Bovine Besnoitiosis is a disease in expansion in Europe both in prevalence and in distribution. Up to now, in Italy only few autochthonous outbreaks were recorded in the Northern Apennine Mountains (Manuali et al., 2011, Transbound Emerg Dis, 58:464-467; Mutinelli et al., 2011, Vet Parasitol, 178:198-198; Gentile et al., 2012, Vet Parasitol, 184:108-115). Further, a serological survey was recently carried out in Southern Italy (Rinaldi et al., 2013, Parasitol Res, 112:1805-1807) revealing a high seroprevalence (44%). According to recent indications of ESFA (2010), epidemiological surveys are necessary to monitor the presence of Besnotia besnoiti and to increase the knowledge on its biology and associated risk factors. The aims of the study were: i) to determine the frequency of Besnotia infection in Northern Italy and Sardinia; ii) to investigate a besnoitiosis outbreak in a beef farm.
MATERIALS AND METHODS:
The serosurvey was carried out in the areas in Northern Italy more suitable for cattle breeding (Lombardy, Piedmont and Emilia Romagna) and in Liguria and Sardinia regions. The investigated farms were representative of a high variety of management systems and of differences in landscape and climate. On the whole, 3141 bovine blood samples from 149 dairy and beef farms were collected between October 2012 and August 2013. Individual and farm data were registered. Additional 404 sera were collected in a positive farm in Lombardy (approximately 700 Limousine cattle bred), where suspected clinical cases were detected and investigated.
Sera were analyzed using a standardized in house ELISA; for confirmation, 200 ELISA-positive samples were randomly selected and re-tested by a tachyzoite-based Western Blot performed under non-reducing conditions (Garcia-Lunar et al., 2013, Transbound Emerg Dis, 60:59-68).
RESULTS:
ELISA seroreactors were 709 (22.5%), but only 10 samples (0.3%) from 5 farms were also positive at the confirmation test. All positive animals were found in Lombardy and Piedmont, in both dairy and beef farms (two and three, respectively). In the selected positive farm, a high prevalence (22.2%) was confirmed by Western Blot analysis. Cutaneous scarification and biopsies were taken from four seroreactors with suspected cutaneous clinical signs; cytology was not conclusive but the typical cysts were visualized in all bioptic samples. When two of these animals were slaughtered, histology and immunohistochemistry highlighted several tissue cysts of B. besnoiti in all tested organs. Testicular athrophy was also remarkably severe.
CONCLUSIONS:
Concluding, in the study area bovine besnoitiosis seems so far limited to spot areas and isolated outbreaks. In seropositive farms, however, the disease may involve several animals potentially causing economic losses. In the case of breeding bulls, besnoitiosis can lead to transient or definitive infertility.
Besides obvious epidemiological interest, serological screening with appropriate methods can be useful to detect the disease in the early phases, and eventually implement control programs.

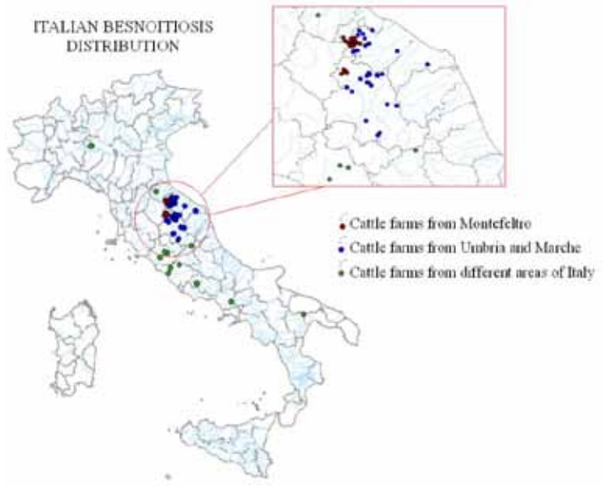
Keywords:
Besnoitia besnoiti, cattle, serosurvey

O08.2
SPREADING SURVEY OF BESNOITIA BESNOITI IN ITALIAN CATTLE POPULATION

D'Avino N.[1], Maresca C.[1], Costarelli S.[1], Filippini G.[1], Gentile A.[2], Felici A.[1], Dettori A.[1], Sebastianelli M.[1], Broccatelli S.[1], Cordovani E.[1], **Righi C.*[1]**

[1]Istituto Zooprofilattico Sperimentale Umbria e Marche ~ Perugia ~ Italy, [2]Dipartimento Clinico Veterinario Università di Bologna ~ Bologna ~ Italy

INTRODUCTION:
Bovine Besnoitiosis is a serious protozoal disease caused by Besnoitia besnoiti (Apicomplexa, Sarcocystidae).The parasite cycle is characterized by having a carnivorous animal as a definitive host while the cattle population represents the intermediate host (Diesing et al, 1988, Parasitol. Res., 75:114-117).
Despite the disease can be characterized by different symptoms, including the characteristic mucus-conjunctival cysts of the chronic phase, the asymptomatic form is the most common. Mainly the latter has been shown to be difficult to diagnose and responsible of the parasites spreading (Lepri et al, 2011, Large Animal Review, 17:145-150). The international relevance acquired by this disease in conjunction with the lack of data of this spread in the national territory led us to make this study with the aim to contribute monitoring the presence of B.besnoiti in Italian cattle population.
MATERIALS AND METHODS: Over a period of two years 2011-2012, a cross-sectional study on the prevalence of besnoitiosis was carried out. In particular 966 subjects from 37 cattle farms sharing a common restricted area for the summer pasture (Montefeltro), located between Marche, Umbria and Toscana regions, 1,233 cattle from 30 farms located Umbria and Marche (not sharing pasture) and 330 animals from 12 cattle farms from different areas of the Italy were examined. All asymptomatic subjects, older than 24 months, born and living in Italy, were serologically tested to evaluate the presence of antibodies against B.besnoiti using an indirect ELISA (PrioCHECK®Besnoitia), with sensitivity and specificity values respectively of 97.8% and 98.1%. Farms with at least one positive animal were considered as positive.



RESULTS:
503 animals out of 966 tested from the Montefeltro areas were positive for B.besnoiti with an animal prevalence rate of 52% while the farm prevalence was 94,6%. Only two farms resulted serologically negative; the in-herd prevalence ranged from 2,13% to 91,67%.
Concerning the 1,233 animals tested in Umbria and Marche, 488 were positive with an animal prevalence rate of 39,6% and the farm prevalence rate of 100%.
The subjects from different areas of the country, 97 of 330 were positive with a prevalence rate of 29,4% and 100% of farm prevalence; the in-herd prevalence ranged from 9% to 82%.
CONCLUSIONS:
Farm and animals seroprevalence are similar although in-herd seroprevalence range is very wide.
High seroprevalence in farm showed a wide spread of the disease and the pasture appears to be a risk factor. The seroprevalence evaluation, therefore, must be considered of fundamental interest for the control of the disease. In fact, the only observation of clinical cases do not permit to evaluate the real spread of the disease not allowing the identification of the large number of sub clinical or asymptomatic animals that act as reservoirs of infection. The epidemiological condition observed, indicates an urgent need to update pathogenesis and epidemiology knowledge of the besnoitiosis, in order to take timely measures to prevent further spread.

Keywords:
Besnoitia besnoiti, Seroprevalence, Italy

O08.3
PREVALENCE OF BABESIA SPP. POTENTIALLY PATHOGENIC FOR HUMANS IN IXODES RICINUS TICKS OF NORTH-EASTERN ITALY

Falcaro C.[1], Granato A.[1], Babiker A.M.A.[1], Cassini R.[2], Capelli G.[1], Montarsi F.[1], Pietrobelli M.[2], **Ravagnan S.*[1]**

[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [2]Department of Animal Medicine, Production and Health ~ University of Padua ~ Italy

INTRODUCTION:
Babesiosis is an intraerythrocytic infection caused by protozoan of the genus Babesia. Although well known as an animal disease, human babesiosis is attracting increasing attention as a worldwide emerging zoonosis (Hildebrandt et al., 2013, Infection, 41(6):1057-72). The vectors of Babesia are Ixodid ticks, with the majority of zoonotic species being transmitted by species of the genus Ixodes. Vector-based surveillance can provide an early warning system for the emergence of human babesiosis. The aims of this study were: 1) to develop a rapid biomolecular method with high sensitivity and specificity able to detect the various species of Babesia in ticks; 2) to assess the prevalence of Babesia spp. in Ixodes ricinus ticks of North-Eastern Italy.
MATERIALS AND METHODS:
To detect Babesia spp., a number of primers, targeting the 18S rRNA gene obtained from the literature, were tested. The primers BJ1 and BN2 were chosen (Casati et al., 2006, Ann Agric Environ Med, 13:65–70) able to detect the species of Babesia of interest and not amplifying fungal contaminants. A novel SYBR Green based real-time PCR assay was developed and its sensitivity and specificity evaluated. Finally, Babesia species were identified by direct sequencing of PCR amplicons. A total of 5,021 I. ricinus collected in Veneto and Friuli Venezia Giulia (FVG) in 2007 and 2008 as well as in Veneto in 2011 and 2012 were examined.
RESULTS:
The SYBR Green based real-time PCR showed both a high sensitivity (10 DNA copies in 150 µL of homogenate sample) and a good specificity (non-target organisms were not amplified). In the study area, the prevalence of Babesia spp. was found to be 0.42% (n=21). Three species were detected: B. venatorum (previously Babesia EU1), B. microti and B. capreoli. The most common species was B. venatorum (n=15; 71.4%) that was identified in Udine, Pordenone, Treviso and Belluno provinces. B. microti (n=5; 23.8%) was found in both Udine and Pordenone provinces, while B. capreoli (n=1; 4.8%) in the province of Udine.
CONCLUSIONS:
The SYBR Green based real-time PCR assay has proved to be a valuable tool to test a large number of samples in a short time, and to identify Babesia species by amplicon direct sequencing. The prevalence of Babesia spp. (0.42%) is comparable to that found in several European countries. Two out of the three Babesia spp. identified, e.g. B. microti and B. venatorum, are emergent agents of zoonotic disease. In the study area, the risk for humans of acquiring infection due to zoonotic Babesia spp. occurs in two FVG provinces (Udine and Pordenone) and in two Veneto provinces (Belluno and Treviso). The finding of B. microti has particular relevance, since it has never been reported in human cases in Europe, but it's the main causative agent of the emerging human babesiosis in the United States and around the world (Herwaldt et al., 2011, Ann Intern Med, 155:509-19). Conversely, cases of human babesiosis caused by B. venatorum have already occurred in Italy, Austria and Germany (Herwaldt et al., 2003, Emerg Infect Dis, 9:942-8; Haselbarth et al., 2007, Int J Med Microbiol, 297:197-204).
In the study area, other pathogens, especially Rickettsia spp. and Borrelia burgdorferi s.l., are highly represented (Capelli et al., 2012, Parasit Vectors, 27; 5:61), thus increasing the risk of simultaneous transmission to humans and eventually complicating their clinical pictures.

Keywords:
Babesia spp., Ixodes ricinus tick, SYBR Green based real-time PCR

O08.4
OVERVIEW ON DOURINE OUTBREAK IN ITALY; SURVEILLANCE, RESULTS OF CLINICAL AND LABORATORY MONITORING OF INFECTED HORSES, LESIONS AND DEVELOPMENT OF LABORATORY DIAGNOSIS

Pascucci I.*[1], Cammà C.[1], Di Provvido A.[1], Di Domenico M.[1], Luciani M.[1], Narcisi V.[1], Giansante D.[1], Di Francesco G.[1], Podaliri Vulpiani M.[1], Scacchia M.[1]

[1]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" Campo Boario 64100 ~ Teramo ~ Italy

INTRODUCTION:
Dourine, sexually transmitted equine trypanosomosis, was reported after 15 years in Italy Catania Province (Sicily) in May 2011. In the past the disease was first eradicated in Italy in the 1940s, but there was then a serious epidemic in the mid-70s. It was then eradicated once more, and the last case before the 2011, was notified to OIE in 1996. The present study will give an overview of the outcomes of the outbreak investigation carried out by the national reference center (CESME) at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM).
MATERIALS AND METHODS:
After the first notification in 2011 a surveillance plan was put in place in whole Italy to obtain a reliable picture of dourine prevalence and to identified as soon as possible any further sources of infection. The Italian veterinary authority decided to serologically test all adult (>2 years) male and female horses present in the whole Italian territory. In 2012 CFT was performed in those regions considered at risk on the basis of the 2011 results, in case of horses moving for sale, at the slaughterhouse in case of symptoms related to dourine and whenever an irregularity is detected in animal ID system. In 2012, 34541 samples were tested with 47 positives but none of them was confirmed. In January 2013 the MoH declared ended the surveillance activities. During the 2011 outbreaks 20 positive animals from 7 holdings were identified by trace back activities and surveillance, out of them 6 were hosted at the IZSAM experimental farm for further diagnostic investigation. Clinical monitoring was carried out and whole blood, serum, genital swabs were collected for checking the presence of trypanosomal DNA by RealTime PCR, antibodies by CFT and IFAT and anaemia. Sera from 8 infected horses have also been tested by cIB, in order to distinguish different antigen patterns. Chemiluminescent immunoblotting (cIB) and RealTime PCR methods for Trypanozoon have been set up by IZSAM.
RESULTS:
In checked horses clinical symptoms and lesions were referable to the third clinical phase of dourine, and parasitemia was sporadic, generally low and only detectable by RealTime PCR. cIB assay confirmed infected animals as positive with specific antigenic profile. Trypanosomes were isolated by inoculation of horse infected samples in rabbit scrotum
CONCLUSIONS:
Dourine diagnosis was based on epidemiological, clinical, and pathological findings, confirmed by laboratory testing. In checked horses anemia was one of the clinical symptoms most frequently observed, but parasitemia was not a constant finding except for severe clinical cases confirming that blood is not an adequate matrix for the reliable diagnosis of dourine. Anyway, the use of RealTime PCR enabled to detect even low concentrations of DNA. CFT titer appears to be related to severe clinical signs in sick animals. The results of cIB analysis suggested that this technique can be applied as a confirmatory serological test for dourine infection, but further data are required for validation. Isolation of trypanosomes on rabbit scrotum, although not providing purified material, will allow genetic analysis. This represents one of the few strains of T. equiperdum available at worldwide level.

Keywords:
Dourine, Trypanozoon, Outbreak

O08.5
CYCLOSPORA CAYETANENSIS IN ENVIRONMENTAL SAMPLES IN ITALY

Giangaspero A.*[1], Marangi M.[1], Lacasella V.[1], Lonigro A.[2]

[1]University of Foggia ~ Foggia ~ Italy, [2]University of Bari ~ Bari ~ Italy

INTRODUCTION:
Cyclospora cayetanensis (Apicomplexa, Eimeriidae) is an emerging intestinal protozoan responsible for diarrhoea in children and adults worldwide, and is transmitted by contaminated water, food or soil. In industrialized countries, cyclosporiasis has been observed in travelers and in people with no history of foreign travel, and outbreaks have also been described (Ortega et al., 2010, Clin. Microbiol. Rev. 23:218-234). Cases of cyclosporiasis have been recorded in Italy (Maggi et al., 1995, New Microbiol. 18:73–76; Masucci et al., 2008, New Microbiol., 31:281–284; Drenaggi et al., 1998, J. Travel Med. 5:153-155), and an outbreak associated with lettuce imported from Italy's Apulia region has been described in Germany (Doller et al., 2002, Emerg. Infect. Dis., 8:992–994). There is a lack of data on C. cayetanensis in environmental samples in Italy, and so this study aimed to molecularly investigate its presence in water used for irrigation and in soil and vegetables irrigated with the same water.
MATERIALS AND METHODS:
From May to December 2012, 16 treated wastewater and 16 well-water samples (100 liters each) were collected from 2 municipal wastewater treatment plants (Fasano and Noci towns in Apulia region), through a yarn-wound cartridge filter. Then, at harvest time, 2 soil pooled samples (1 kg each) were collected from each site using a soil steel cylindrical sampler to a depth of 10 cm, and 2 vegetable pooled samples (2 kg each) were collected from each site (cucumber and lettuce in Noci, lettuce and chicory in Fasano). Soil and vegetable samples were processed following the methods described by Kuczynska and Shelton (1999, Appl. Environ. Microbiol, 65:2820-2826) and Robertson and Gjerde, (2001, J. Food Protect, 64:1793-1798), modified. After DNA extraction, all samples were subjected to EvaGreen Real Time PCR and HRM analysis (Marangi and Giangaspero, 2014, Atti Soipa). A sequence of about 116 bp from the C. cayetanensis ITS-2 gene (Olivier et al., 2001, Int. J. Parasitol. 31:1475-1487) was cloned into a pEX-A vector to design a plasmid control with a range concentration from 16.5×108 to 0.165 copies/µL to construct a standard curve for quantitative analysis.
RESULTS:
Eight out of 16 (50%) treated wastewater samples from both treatment plants tested positive to C. cayetanensis throughout the period of sampling, with oocyst numbers ranging from 2 to 82/100 liters. Well water, soil and vegetable samples tested negative.
CONCLUSIONS:
This is the first finding of Cyclospora cayetanensis in environmental samples in Italy. Although this study did not detect Cyclospora in vegetables, the finding of up to 82 oocysts/100 liters in treated wastewater is a cause for concern, since this water is commonly used for agriculture. Broader-ranging studies are needed to check vegetables for Cyclospora when irrigated with treated wastewater. In the meantime, it is important to perform frequent routine monitoring of the performance of wastewater treatment plants, and to promote the use of advanced technology for water treatment in order to prevent this pathogen from putting human health at risk.

The study was supported by MIUR (Project In. T.e.R.R.A. contract No 01_01480) co-funded within the Italian Program "PON/Ricerca e Competitività 2007-2013" and PO Puglia FESR 2007-2013, Asse I, Linea 1.2. Progetto L.A.I.F.F. (codice n. 47)", Italy.

Keywords:
Cyclospora cayetanensis, treated wastewater, Italy

O08.6
EVAGREEN® REALTIME PCR (RT-PCR), PLASMID-BASED CONTROLS AND HIGH-RESOLUTION MELTING (HRM) ASSAY FOR ANALYSIS OF TOXOPLASMA GONDII AND CYCLOSPORA CAYETANENSIS IN ENVIRONMENTAL AND FOOD SAMPLES

Marangi M.*[1], Giangaspero A.[1]

[1]University of Foggia ~ Foggia ~ Italy

INTRODUCTION:
In the last few years SyberGreen® RT-PCR assays have enabled the detection and identification of protozoans of animal, human and zoonotic interest. However, this asymmetrical dye has several well-known disadvantages. The development of next-generation DNA-binding dyes such as EvaGreen, with its "just-right" affinity for ds-DNA and low or no affinity for ss-DNA and short DNA fragments (Mao et al., 2007, BMC. Biotechnol, 7:76), has made it possible to increase RT-PCR specificity, overcoming SyberGreen limitations, and at a lower cost. HRM analysis can be used to distinguish DNA sequences of the same length but different GC/AT ratio in the melting curve profile (Erali et al., 2008, Exp. Mol. Path, 85:50-58). The use of EvaGreen coupled with HRM enables detection and characterization of pathogens (Zhang et al., 2012, Parasitol. Res, 111:2157-2163).
In this study, EvaGreen and HRM were used with plasmid-based controls in order to set up a rapid and affordable assay for simultaneous detection, characterization and quantification of Toxoplasma gondii and Cyclospora cayetanensis isolates.
MATERIALS AND METHODS:
As plasmid-based controls, a fragment of T. gondii multi-copy B1 (Burg et al., 1989, J. Clin. Microbiol, 27:1787-1792) and C. cayetanensis ITS-2 genes (Olivier et al., 2001, Int. J. Parasitol, 31:1475-1487) were cloned into two different pEX-A vectors. To generate the standard curves for quantitative analysis and to assess the amplification efficiency (E), 10-fold serial dilutions of the T. gondii B1 plasmid with a range from 21.9x108 to 0.219 copies/µL and of the C. cayetanensis ITS-2 plasmid with a range from 16.5x 108 to 0.165 copies/µL were amplified and the threshold cycle value was plotted against the logarithm of their copies/µL. Three standard points of plasmid controls were tested three times in the same experiment and once a day for three other days to estimate repeatability and reproducibility. Melting analysis was performed after amplification, and the difference in melting curve shapes and melting temperature (Tm) points were used to characterize the isolates. Once the protocol had been validated, its performance was assessed by testing shellfish, water, soil and vegetable samples.
RESULTS:
A good range of linearity was observed for both plasmids, and for seven of ten tested serial dilutions; E was 123.9% with a slope of −2,857 (R2=0.987) for T. gondii B1 plasmid and 128.2% with a slope of −2,791 (R2=0.985) for C. cayetanensis ITS-2 plasmid. The intra- and inter-assay CV values for the standard points were 1.8%, 1.5%, 1.2% and 0.80%, 0.60%, and 0.93%, respectively. Tm values were 83.50°C for T. gondii and 84.50°C for C. cayetanensis. Positivity to T. gondii Type I and/or C. cayetanensis was 39.6% in shellfish and 3-12.8% in water samples (oocyst numbers: 2-82/µl of genetic eluate) (Aksoy et al., 2014, Food. Microbiol; Giangaspero et al., 2014a, E.I.D, submitted).
CONCLUSIONS:
The assay allowed rapid (<1.30 h) detection, genotyping and quantification (<5 copies/µL of DNA pathogen) of T. gondii and C. cayetanensis in water, soil and vegetable samples. The assay can be widely used in different matrixes for molecular deep analysis because of its reasonable work load, the low risk of carry-over PCR contamination and highly specific.

This study was supported by PO Puglia FESR 2007-2013, Asse I, Linea 1.2. Accordo di Programma Quadro in materia di Ricerca Scientifica. Intervento "Reti di Laboratori Pubblici di Ricerca". Progetto L.A.I.F.F. - RETE DI LABORATORI PER L'INNOVAZIONE NEL CAMPO DEGLI ALIMENTI FUNZIONALI (codice n. 47)", Italy

Keywords:
RealTime PCR-HRM, Plasmids, Protozoans

O08.7
SEROPREVALENCE AND RISK FACTORS FOR TOXOPLASMA GONDII IN SMALL RUMINANTS IN NORTHERN ITALY

Gazzonis A.L.*[1], Veronesi F.[2], Zanzani S.A.[1], Molineri G.[1], Moretta I.[2], Moretti A.[2], Piergili Fioretti D.[2], Invernizzi A.[3], Manfredi M.T.[1]

[1]Department of Veterinary Science and Public Health, University of Milan ~ Milan ~ Italy, [2]Department of Bio-Pathological Sciences and Hygiene of Animal and Food Production, University of Perugia ~ Perugia ~ Italy, [3]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna ~ Milano ~ Italy

INTRODUCTION:
Small ruminants are considered an important source of Toxoplasma gondii human infection (Kijlstra and Jongert, 2008, Int J Parasitol, 38:1359-1370); moreover, in these species T. gondii represents a major cause of infectious reproductive failure (Dubey 2009, Vet Parasitol, 163:1-14). Despite in Europe a wide range of seroprevalence was reported, only few data are available for Northern Italy. Therefore, to fill up this gap a seroepidemiological survey was conducted in goats and sheep reared in Lombardy.
MATERIALS AND METHODS:
From May 2011 to May 2013, sera from 474 goats and 502 sheep from 42 farms were collected. An audit form on farm management was filled. Sera were tested for IgG antibodies to T. gondii by indirect Immunofluorescence Antibody Assay (IFAT) using commercial antigens (MEGACOR Laboratories, Horbranz, Austria) and fluorescein isothiocyanate-labelled rabbit anti-sheep IgG and anti-goats IgG (Sigma-Aldrich, USA) as conjugate diluted 1:100 and 1:200, respectively. Sera were screened at 1:64 dilution (cut-off) and those testing positive were serially diluted. To identify risk factors, a univariate binary logistic regression analysis of the variables was performed with SPSS 19.0.
RESULTS:
Antibodies to T. gondii were found in 96.5% (28/29) goat farms and in 87.5% (21/24) sheep farms. At individual level, 198 out of 474 goats (41.7%) and 298 out of 502 sheep (59.3%) resulted positive. Seroprevalence was significantly higher in sheep than in goats (Pearson's chi-square, P=0.000). The highest percentage of infected goats was found in 4-year-old animals, whereas in animals over six years of age seroprevalence decreased. Goats sampled in eastern and western areas showed similar seroprevalence values, whereas goats from southern area had a lower probability to be infected (OR=0.41). Considering breed, Saanen goats presented the lowest seroprevalence (30.7%), whereas cross-breed exhibited the highest rate (48.7%). The risk factor "number of animals on farm" resulted highly significant (P=0.000). Animals from farms where both sheep and goats were reared had a risk to be infected 1.396 times higher than goats from farms without sheep. Goats bred in intensive farms showed lower prevalence (22.1%, OR=0.18) in comparison to those bred in extensive (45.6%, OR=0.55) or semi-intensive ones (60%, OR=1). Sampling area was by far one of the stronger predictor of T. gondii infection in sheep flock: the risk of a sheep from south-eastern area being diagnosed as seropositive to T. gondii was 3.25 times higher than a sheep from the western area. Finally, transhumant herds in comparison to semi-intensive ones appeared more at risk of T. gondii infection (66.8% vs 38.4%).
CONCLUSIONS:
The survey recorded a high T. gondii seroprevalence values. The highest prevalence was registered in sheep transhumant herds and in small family-run goat farms, speculating that these rearing systems might support the spreading and maintenance of the infection. Representing these traditional activities important resources for the conservation of the territory and its economy, it could be useful to improve management practices for a better control of the disease.

Keywords:
Toxoplasma gondii, small ruminants, serosurvey

O08.8
TOXOPLASMOSIS IN HAEMATOPOIETIC STEM CELLS TRANSPLANTED PATIENTS. IMPROVING STRATEGIES FOR DIAGNOSIS AND FOLLOW-UP

Meroni V.*[1], Genco F.[2], Colombo A.A.[3], Ripamonti F.[3], Alessandrino P.E.[3], Di Matteo A.M.[4]

[1]Dipartimento Terapia Medica e Medicina Interna università degli studi di Pavia ~ pavia ~ Italy, [2]SC Microbiologia e Virologia Fondazione IRCCS Policlinico San Matteo PAVIA ~ pavia ~ Italy, [3]Unità Trapianto, Clinica Ematologica, Fondazione IRCCS Policlinico San Matteo Pavia ~ pavia ~ Italy, [4]Dipartimento Malattie Infettive Fondazione IRCCS Policlinico San Matteo Pavia ~ pavia ~ Italy

INTRODUCTION:
Disseminated toxoplasmosis is a rare, largely underdiagnosed, life-threatening opportunistic infection. Most cases in haematopoietic stem cell transplant (HSCT) recipients develop because of reactivation of a latent pretransplant infection in seropositive recipients. Past experience at our institution includes several serious cases of cerebral toxoplasmosis. These patients, Toxo-IgG-positive before transplant, received HSCT from a seronegative donor, and lost their Toxo-specific cellular and humoral immunity. At a variable time post-transplant (range 6-8 months) they developed cerebral and/or disseminated toxoplasmosis, diagnosed by imaging and real-time PCR on cerebrospinal fluid. A program of screening and follow-up was set up in January 2013. Aim of this study is to identify any potentially predictive factor of cerebral-disseminate toxoplasmosis.
MATERIALS AND METHODS:
Since January 2013, to December 2013, we have been following up 292 adult patients that received a HSCT at Unità Trapianto, Clinica Ematologica, Fondazione IRCCS Policlinico San Matteo Pavia.
Serologic assays for specific antitoxoplasma IgG, IgM, and IgA antibodies were performed before transplant, the very day of transplant and every 15 days during the pre take period and afterwards every 3 months. Interferon gamma release assay (IGRA), real-time PCR on peripheral blood and cerebrospinal fluid (CSF) samples were performed when clinically indicated.
RESULTS:
Before transplantation 181 patients were seronegative and 111 positve for toxoplasmosis. During the follow-up 4 out of 181 negative patients turned IgG- positive. By using IgG /IgM immunoblot, we ruled out a true seroconversion and showed passive transfer of Toxoplasmosis-specific IgGs. One patient presented with an an acute infection (IgM positive,low-avidity IgG) taking place shortly before transplant. The transplant was postponed. Two out of the 111 positive patients had an asymptomatic reactivation. One of the positive patient lost all the Toxoplasma-specific antibodies present before transplant during the first month. He is being closely monitored for symptoms of disseminated toxoplasmosis and has been started on pyrimetamin-sulfadoxin.
CONCLUSIONS:
We diagnosed on time an acute infection and deferred a potentially catostrophic transplant. False seroconvertions were demonstrated, avoiding unnecessary treatment.
A subset of high risk patients was identified. These are the seropositive patients who lose their Toxo-IgG and experience a reactivation at the worst time post-transplant.

Keywords:
Toxoplasmosisis, Haematopoietic stem cells transplant, Diagnosis of toxoplasmosis

O08.9
EVALUATION OF A NOVEL RAPID ANTIGEN DETECTION TEST COMPARED TO IN HOUSE REAL-TIME PCR SYSTEM FOR DIAGNOSIS OF AMEBIASIS IN CLINICAL SPECIMENS

Formenti F.*[1], Perandin F.[1], Bonafini S.[1], Degani M.[1], Bisoffi Z.[1]

[1]Ospedale Sacro Cuore Don Calabria ~ Negrar - Verona ~ Italy

INTRODUCTION:
Amoebiasis is an important cause of death from parasitic disease worldwide. For many years, microscopic examination of stool samples has been considered to be the “gold standard” for diagnosis of many infectious parasites. Recently, Polymerase Chain Reaction (PCR) has been approved by the World Health Organization (Fotedar et al., 2007, Clin Microbiol Rev., 20:511-32) as the current method of choice for the diagnosis of Entamoeba histolytica infections. A novel, immunochromatographic antigen detection rapid test, Stick simple Crypto-Giardia-Entamoeba” (Operon), has been proposed for the diagnosis of infections caused by Cryptosporidium parvum-Giardia intestinalis-Entamoeba histolytica. We aimed to compare the preliminary results of the novel test with those obtained using in-house real-time PCR for the detection of Entamoeba histolytica / Entamoeba dispar.
MATERIALS AND METHODS:
Eighty-four samples from patients selected with Techlab or Alexon or Ab-amoeba positive results were considered for this study. Twelve samples were fluid from liver abscess and 72 were stool samples. All samples were examined with the following four methods: i) the commercial enzyme-linked immunosorbent assay, TechLab E. histolytica II; ii) the commercial enzyme-linked immunosorbent assay, Alexon; iii) in house real-time PCR (the gold standard in this study) (Verweij et al 2003), which differentiate Entamoeba histolytica from Entamoeba dispar targeting the 172 base pair region located on the small-subunit ribosomal RNA (SSU rRNA); iv) the novel one-step immunochromatographic test for detection of E. histolytica.
RESULTS:
The sensitivity and specificity showed by the novel one-step immunochromatographic test for detection of E.histolytica were 79% and 83% respectively. The novel test identified only one out of nine positive RT- PCR samples from liver abscess.

	E.histolytica (31)	E.dispar (41)	Negative (12)
Novel test	22/31	35/41	2/12
Techlab	13/31	5/41	0/12
Alexon	28/31	41/41	9/12

Results_table_1

CONCLUSIONS:
The novel one-step immunochromatographic test for detection of E. histolytica is rapid and simple to perform, and does not need any particular instrument. It showed a reasonably good sensitivity and specificity for detection of E. histolytica on fecal samples, but it seems not to work on liver abscess samples. Data on specificity should be taken with caution as the number of negatives was small. To evaluate the sensitivity and specificity we considered only the negative results (12) in real time PCR, without including E.dispar positives. A high proportion of the latter samples gave a positive result with the novel test.

Keywords:
Entamoeba, Real Time PCR, Rapid Diagnostic Test

O08.10
MOLECULAR DIAGNOSTICS OF INTESTINAL PROTOZOA IN PATIENTS WITH IRRITABLE BOWEL SYNDROME

Tosini F.[2], Biasutto D.[1], Badiali D.[1], Pozio E.[2], **Cacciò S.M.*[2]**

[1]Policlinico Umberto I ~ Roma ~ Italy, [2]Istituto Superiore di Sanita ~ Roma ~ Italy

INTRODUCTION:
Worldwide, 10-20% of the human population is affected by the Irritable Bowel Syndrome (IBS), a gastrointestinal functional disorder broadly characterized by abdominal pain or discomfort associated with altered bowel movement form and/or bowel movement frequency. Various factors are thought to be involved in the genesis of IBS, including host genetics, alteration of the gut microbiota, low-grade gut inflammation, psycho-social factors and, in about 10-15% of cases, an episode of acute gastroenteritis (post-infectious IBS). However, it has been noticed that infection with certain gastrointestinal parasites elicits symptoms that are similar to those reported by IBS patients. Thus, routine application of appropriate diagnostic tests to rule out parasitic infection is important. Here, we sought to investigate the presence and genetic identity of intestinal protozoa in a group of IBS patients and in a control group of healthy individuals.
MATERIALS AND METHODS:
A total of 50 patients, diagnosed as diarrhea-prevalent IBS (IBS-D) according to the Rome III criteria, were enrolled in this study. Each patient provided 3 fecal samples collected at alternate days. Fecal samples were also collected from a control group consisting of 22 healthy individuals matching IBS-D patients in terms of age and gender. All samples were tested by microscopy after Lugol staining, and by PCR for the following protozoa: Blastocystis spp., Dientamoeba fragilis, Giardia duodenalis, Cryptosporidium spp., Entamoeba histolytica and E. dispar. Positive PCR amplicons were sequenced to identify species/genotypes. In patients with positive test, fecal examination was repeated on new stool samples collected at least 4 weeks later. Patients with confirmed infection were evaluated by the IBS Severity Scoring System (IBS-SSS) and were treated with Metronidazole (500mg TID for 10 days). Faecal tests and IBS-SSS were repeated ≥12 weeks after treatment.
RESULTS:
All fecal samples were microscopically negative, with the exception of one patient sample where Blastocystis cysts were detected. On the contrary, PCR detected a higher prevalence of intestinal parasites in IBS-D patients compared to asymptomatic controls (18/50, 36% vs 2/22, 9%; p=0.011). Blastocystis spp. was the most frequently detected parasite and the only one associated with chronic infection. Cryptosporidium was not identified. Episodes of diarrhea with ≥4 bowel movements and/or having travelled to (sub)tropical countries were significantly associated with the presence of chronic infections. Treatment with metronidazole in patients with chronic symptomatic infection seems to be associated with an improvement of symptoms, even in the absence of parasite clearance.
CONCLUSIONS:
Molecular diagnostics of intestinal protozoa, particularly when performed on multiple stool samples, is much more sensitive than microscopy. A higher prevalence of Blastocystis spp. was observed in IBS-D patients compared to asymptomatic controls. Albeit chronic infection with Blastocystis spp. seems common in IBS-D patients, the role played by the parasite in the genesis of symptoms remains to be defined.

Keywords:
Intestinal parasites, Molecular diagnostics, Irritable Bowel Syndrome

O08.11
INTESTINAL PROTOZOA AND HUMAN GUT MICROBIOTA IN CÔTE D'IVOIRE

Berrilli F.[1], Schippa S.[2], Iebba V.[2], Santangelo F.[2], Totino V.[2], Di Cave D.[1], Di Cristanziano V.[3], **D'Alfonso R.*[4]**

[1]Department of Experimental Medicine and Surgery, Tor Vergata University ~ Rome ~ Italy, [2]Department of Public Health and Infectious Diseases, Sapienza University ~ Rome ~ Italy, [3]Institute of Virology, University of Cologne ~ Cologne ~ Germany, [4] Department of Systems Medicine, Tor Vergata University ~ Rome ~ Italy

INTRODUCTION:
To improve the understanding of the complex host-parasite interactions in recent years the study of the host-microbiota has taken on a growing role. The main purpose of this in-progress study is to investigate the gut microbiota composition in subjects living in Côte d'Ivoire. In particular, the deviations in the microbiota patterns were evaluated to assess the possible dysbiosis associated with protozoa infections.
MATERIALS AND METHODS:
Fecal samples randomly collected from subjects in three different localities in the south of Côte d'Ivoire were analyzed for the presence of intestinal protozoa by microscopic techniques and conventional PCR. Gut bacterial composition was obtained by TTGE analysis and dysbiosis was assessed by the ratio Escherichia coli/Faecalibacterium prausnitzii identified by qPCR.
RESULTS:
Stool samples were obtained from 6 males and from 15 females, aged between 1 and 74 years. Sixteen out of 21 were positive to intestinal protozoa. In particular 10 individuals were positive to Giardia duodenalis, 10 to Blastocystis hominis and 6 to Entamoeba spp. Eight subjects presented mixed infections. The molecular characterization allowed to assign 6 isolates of G. duodenalis assemblage A and 4 to assemblage B; Entamoeba isolates were attributed to the species coli (2) and dispar (1) hartmanni (3). The microbiota analysis allowed to associate the whole samples to the enterotype 2 dominated by Prevotella group. A trend in the decrease of the ratio between the potentially beneficial F. prausnitzii and the potentially pathogenic Escherichia coli was noted. Interestingly, in the subjects Giardia-positive vs Giardia-negative an increasing trend (p=0.048) of Escherichia coli was observed; conversely, in the Entamoeba spp.-positive vs Entamoeba spp.-negative patients a decrease (p=0.005) in the abundance of Escherichia coli was assessed.
CONCLUSIONS:
Few data are available on the gut microbiota composition in subjects from Sub- Saharan Africa (De Filippo et al, 2010, Proc. Natl. Acad. Sci. 107(33):14691-14696). This preliminary study represents the first report on variations in the gut microbiota related to the presence of parasites in a developing country. In the Ivorian subjects analyzed, the assignment to the enterotype 2 could be explained by a predominant carbohydrate-rich diet. In the subjects positive for G. duodenalis, the decrease of the ratio F. prausnitzii/E. coli suggests an intestinal dysbiosis. The proliferation of Escherichia coli in Giardia-positive subjects compared with those infected by non-pathogenic Entamoeba species might be explained with a gut inflammation Giardia-associated while the presence of Entamoeba species does not seem to disturb the balance in the intestinal microbiota. These preliminary data highlight the need for multidisciplinary studies to further understanding of the multiplex interactions between microbiota, host and parasites.

Keywords:
Microbiota, intestinal protozoa, Cote d'Ivoire

O08.12
INTESTINAL PARASITES IN TWO RURAL COMMUNITIES LIVING IN THE CORDILLERA PROVINCE (PLURINATIONAL STATE OF BOLIVIA)

Macchioni F.*[1], Suniga Rios F.[2], Totino V.[3], Gabrielli S.[3], Rojas Gonzales P.[2], Roselli M.[4], Bartoloni A.[4], Cancrini G.[3]

[1]Dipartimento di Scienze Veterinarie ~ Pisa ~ Italy, [2]Distrito de Salud Cordillera ~ Santa Cruz ~ Bolivia, Plurinational State of, [3] Dipartimento di Sanità Pubblica e Malattie Infettive, Università "Sapienza" ~ Roma ~ Italy, [4]Dipartimento di Medicina Sperimentale e Clinica ~ Firenze ~ Italy

INTRODUCTION:
In last years sanitary conditions of people living in Bolivia improved with more extended access to safe drinking water and sanitation (Eder et al., 2012, Rev Panam Salud Publica, 32:43-8). However, the situation is still suboptimal in some communities and undetermined in others. The research here reported, which is part of a complete parasitological screening, was aimed at collecting data about intestinal parasites affecting people of two rural communities: Bartolo and Ivamirapinta (SE Bolivia).
MATERIALS AND METHODS:
In 2013, a total of 220 faecal samples were collected and submitted to microscopic examination (directly and after Ridley concentration). Parasites were identified on the basis of their morphological features. Samples positive to Entamoeba histolytica complex and to Blastocystis were further analysed to identify the species/subtype (ST) involved in each infection. DNA was extracted and amplified (Nested-PCR and PCR followed by sequencing, respectively) following the described protocols (Solaymani et al., 2006, J Clin Microbiol, 44: 2258-61; Bohm-Gloning et al., 1997, Trop Med Int Health, 2: 771-8).
RESULTS:
In Bartolo, the overall infection rate was 81.2% (91/112), and the most commonly found protozoan was Blastocystis spp. (43.7%), followed by Entamoeba coli (37.5%), Entamoeba hartmanni (17.9%), Giardia intestinalis (14.3%), Iodamoeba butschlii (10.8%), Endolimax nana (8%), Chilomastix mesnili (8%), and E. histolytica complex (7.1%). Helminth eggs identified belonged to Hymenolepis nana (8%), Ascaris lumbricoides (3.6%) and hookworms (1.8%).
In Ivamirapinta, infection figure was 79.7% (86/108), and the most commonly found protozoan was Blastocystis (60.2%), followed by E. hartmanni (36.1%), E. coli (25%), E. histolytica complex (13%), E. nana (11.1%), I. butschlii (10.2%), G. intestinalis (10.2%), C. mesnilii (2.8%). Moreover, H. nana (2.8%) and Strongyloides stercoralis (0.9%) were found.
In Bartolo and in Ivamirapinta multiple infections amounted to 51.8% and 53.7%, respectively, and the second community proved significantly more affected by Blastocystis and E. hartmanni (P=0,02 and P=0.037, respectively). Most of the protozoa detected were scarcely pathogenic, however E. histolytica was identified in 6 subjects in the first community and in 8 people in Ivamirapinta. As for Blastocystis, in Bartolo were found ST1 (n=4) and ST3 (n=1), whereas in the other community also ST2 (n=2), beside ST1 (n=4) and ST3 (n=2), was identified.
CONCLUSIONS:
Being H. nana the most prevalent helminth found, the contamination with human fresh faeces is frequent in both communities. This finding contrasts with the almost absence of human geohelminths, probably consequence of the implemented program of preventive chemotherapy. Zoonotic opportunistic species like Balantidium coli and Cryptosporidium are not present, but most protozoa found in the presented study, G. intestinalis included, are anthropo-zooparasites that have pigs or dogs as possible additional sources of infection, therefore health educational programs should be improved.

Keywords:
Intestinal parasites, Plurinational State of Bolivia, molecular diagnostics

O08.13

DETECTION AND GENOTYPING OF FREE-LIVING AMOEBAE OF THE GENUS ACANTHAMOEBA IN WATER SAMPLES

Di Cave D.*[1], D'Alfonso R.[2], Santoro M.[1], Montalbano Di Filippo M.[1], Capolongo C.[3], Cinquepalmi V.[3], Monno R.[3], Berrilli F.[1]

[1]Department of Experimental Medicine and Surgery, Tor Vergata University ~ Rome ~ Italy, [2]Department of Systems Medicine, Tor Vergata University ~ Rome ~ Italy, [3]Department of Basic Medical Science, Neuroscience and Sense Organ, University of Bari ~ Bari ~ Italy

INTRODUCTION:
Acanthamoeba spp. are free-living amoebae widely distributed in the environment, known as opportunistic protozoan able to cause serious human diseases, including a painful and blinding infection of the cornea, the amoebic keratitis (AK), usually associated with contact lens wear. To date, the genus includes pathogenic and non-pathogenic genotypes (T1-T17) based on sequence analysis of the 18S rRNA gene. The aim of the present study was to investigate the presence of Acanthamoeba spp. from environmental sources, including well water, thermal baths and domestic tap water, in Italy and to characterize the isolates at molecular level to better understand the environmental distribution of genotypes associated with human disease.
MATERIALS AND METHODS:
In this study a total of 19 water samples (8 from thermal baths, 10 tap waters and 1 pool water) collected in the Lazio region were examined for the presence of Acanthamoeba spp. Moreover 8 Acanthamoeba isolates obtained from water samples collected in the Puglia region (2 from public ornamental fountains, 5 from artesian wells, and 1 tap waters) were analyzed. The detection protocols included in vitro cultivation on NN-agar, and molecular investigations. DNA extraction was carried out using QIAamp DNA Micro Kit (Qiagen). PCR was carried out to amplify a 405bp region from the 18S rRNA gene. To determine the Acanthamoeba sequence genotype a phenetic analysis using MEGA 5 was performed.
RESULTS:
Three out of 19 (16%) samples from Lazio (two thermal and one tap waters) resulted positive for Acanthamoeba by molecular detection; phenetic analysis allowed to assign the isolates to the genotype T4. Concerning Puglia, the phenetic reconstruction showed three monophyletic clusters corresponding to the genotypes T4, T3, and T15. In particular, the two Acanthamoeba isolates from public ornamental fountains were assigned to the genotype T4, the five isolates from artesian wells were identified as genotype T15 and that from tap water was characterized as T3.
CONCLUSIONS:
In Italy the occurrence of Acanthamoeba spp. in the environment is still poorly understood. The first report was by Scaglia et al (1987, Microbiologica, 10:171-182) from a thermal area. The only genetic data refer to Acanthamoeba strains from a freshwater pond in Southern Italy identified as genotype T16 (Corsaro and Venditti, 2010, Parasitol. Res., 107:233-238). The present ongoing study provides an initial overview of the spread of free-living Acanthamoeba genotypes in different environments in Italy. Interestingly, the presence of the T3, T4 and T15 genotypes have been already described in isolates from Italian AK patients in the same geographical area (Di Cave et al, 2009, Eur. J. Clin. Microbiol. Infect. Dis., 28:607-612; Di Cave et al., in press). These studies can help to clarify the potential pathogenic role of Acanthamoeba strains associated to water environments, essential for the development of appropriate control measures.

Keywords:
Acanthamoeba, water, genotype

NOTES

NOTES

SESSIONE PARALLELA O-09

CONTROLLO E MONITORAGGIO DI VETTORI

O09.1
MONITORING PHLEBOTOMUS SERGENTI IN URBAN, PERIURBAN AND RURAL SITES OF CATANIA PROVINCE, SICILY, AND SAND FLY POPULATION DYNAMICS

Bongiorno G.*[1], Vaccalluzzo V.[2], Severini F.[1], Lisi O.[2], Khoury C.[1], Di Muccio T.[1], D'Urso V.[2], Maroli M.[1], Gradoni L.[1], Gramiccia M.[1]

[1]Unit of Vector-borne Diseases and International Health, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy, [2]Department of Biological, Geological and Environmental Sciences, Section of Animal Biology "M. La Greca", University of Catania ~ Catania ~ Italy

INTRODUCTION:
Human cutaneous leishmaniasis (CL) by Leishmania infantum is frequently reported in Catania province with atypical clinical aspects. In order to evaluate any role of imported L. tropica as an agent of autochthonous CL, a sand fly biodynamic study was addressed in endemic CL foci located in Catania province. This area represents the unique territory in Italy where the presence of Phlebotomus sergenti, proven L. tropica vector, was reported (D'Urso et al., 2004, Parasite 11:279-283).
MATERIALS AND METHODS:
Seasonal dynamic of the phlebotomine fauna was studied in 3 urban (Catania) and 5 periurban/rural sites at the base of Etna mount (Acireale commune) where the presence of P. sergenti has been recorded. Two collecting methods were employed, sticky traps from April through December, and CDC light traps from June through October 2013. Climatic data were recorded in representative sites. All females captured by CDC traps were dissected and gut microscopically examined for the presence of Leishmania promastigotes infection to attempt parasite in vitro isolation. Sand fly females captured by sticky traps were identified and then stored frozen for subsequent nested-PCR Leishmania DNA analysis.
RESULTS: Five phlebotomine species were captured: P. perniciosus, P. sergenti, P. mascittii, P. neglectus and Sergentomyia minuta. A total of 2371 sand flies specimens were identified, of which 1127 were Phlebotomus species (47.5%), mainly represented by P. perniciosus (33.4%) and P. sergenti (13.6%). In Fig. 1 the comparative population dynamics of P. perniciosus and P. sergenti are shown for the most productive site of the latter species. The graphs show the early peak of P. sergenti (July) as compared to the late peak of P. perniciosus (August). The co-presence of the two species at low densities was recorded through October. Among 143 and 34 female specimens of P. sergenti and P. perniciosus dissected, respectively, none was found infected by Leishmania promastigotes.

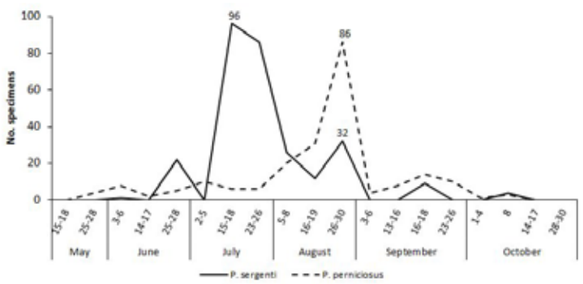


Figure 1. 2013 biodynamics of Phlebotomus perniciosus and P. sergenti in the site 1 (Pennisi, Acireale) (37° 38' 46" N, 15° 08' 20" E, altitude 306m).

CONCLUSIONS:
Sand fly fauna was studied early in 20th century in urban and rural areas of Catania province. Despite drastic environmental changes occurred in cities of the Western world during the past century, phlebotomine sand flies have shown a good adaptation to such changes. P. sergenti appears a species steadily present in Eastern Sicily but currently it does not seem to play a role of active CL vector.
Acknowledgments. This study was carried out in the frame of EDENext, a collaborative project of the 7th FP (2011-2014) funded by the European Commission under the DG Health; Contract Number: 261504.

Keywords:
Phlebotomus sergenti, Catania, Sicily

O09.2
EVALUATION OF COLLECTION METHODS FOR PHLEBOTOMUS-BORNE VIRUSES DETECTION: ISOLATION AND VIRAL RNA INTEGRITY PERFORMANCE

Remoli M.E.*[1], Bongiorno G.[2], Fortuna C.[1], Marchi A.[1], Bianchi R.[2], Khoury C.[2], Ciufolini M.G.[1], Gramiccia M.[2]

[1]Unit of Viral diseases and attenuated vaccine, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy, [2]Unit of Vector-borne Diseases and International Health, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy

INTRODUCTION:
Phlebotomus-borne (PhB) viruses are enveloped, containing a segmented RNA genome of mostly negative polarity. These viruses, mainly Phleboviruses, Vesiculovirus and Orbivirus, are involved in human disease outbreaks (Depaquit et al., 2010, Euro Surveill., 15(10):pii=19507). Because several emerging viruses have been isolated from sand flies in recent years, an important task is to monitor the spreading of PhB-viruses in Mediterranean areas through isolation and identification of these agents (Maroli et al., 2013, Med. Vet. Entomol., 27:123-147). In order to investigate on collection and storage methods of phlebotomine vectors necessary for reliable RNA detection and virus isolation, experimental viral infections of colonized sand flies were carried out and different conditions were tested.
MATERIALS AND METHODS:
Laboratory-reared Phlebotomus perniciosus was experimentally infected with Toscana Phlebovirus (TOSV, ISS-Phl3 strain) using a membrane feeding technique (Ciufolini et al., 1989, Am. J. Trop. Med. Hyg., 40:669-675). Immediately after the infected blood meal, engorged females were stored by two methods: 1) alive sand flies (AS) were immediately frozen at –80°C, mimicking the golden standard procedure usually performed by CDC light trap collection; 2) insects were put onto sticky traps left at room temperature, collected daily up 144 h and stored dry at -80°C. Batches were separated into dead sand flies contaminated by alcohol necessary for removal from papers (DS-A) and those in which contamination was avoided (DS). The specimens, collected individually or in pools, were then homogenized, centrifuged and the supernatants used both for virus isolation in VERO cells and genome identification by Reverse Transcription-PCR on N gene region (Charrel et al., 2007, Emerg. Infect. Dis., 13:465-468), and Real Time-PCR on TOSV L gene region (Pérez-Ruiz et al., 2007, J. Clin. Virol., 39:276-281).
RESULTS:
The percentage of TOSV isolation on VERO cells and RNA detection was 100% for the AS specimens, as expected. In DS specimens the performance of TOSV isolation on VERO cells was elevated at 24 h but less satisfactory thereafter, suggesting that the virus could be fairly isolated from infected wild specimens only if they were frozen 24 h from death. No TOSV isolation was obtained from DS-A specimens at any collection time. As regards RNA detection, for all conditions examined Real Time-PCR gave positive results to detect TOSV RNA until to 6 days after the infection.
CONCLUSIONS:
Recent investigations have indicated that virus diversity in the Mediterranean basin is higher than initially suspected, therefore a standardization of reliable sand fly collection and storage methods is important to harmonize entomological surveys. We confirmed that live specimens are the best target for both viral isolation and RNA identification, however methods for collection (e.g. CDC traps) are less practical. We found that sticky traps, useful to collect specimens in high numbers from different environments, are suitable for partial genome viral identification but less so for viral isolation.

Acknowledgments. This study was carried out in the frame of EDENext, a collaborative project of the 7th FP (2011-2014) funded by the European Commission under the DG Health; Contract Number: 261504.

Keywords:
Phlebotomus-borne viruses, Phlebotomus perniciosus, experimental viral infection

O09.3

COST EVALUATION AND BENEFITS OF THE ENTOMOLOGICAL MONITORING: THE EXPERIENCE OF REGIONAL SURVEILLANCE PROGRAMS FOR WEST NILE DISEASE IN NORTH-EASTERN ITALY

Montarsi F.*[1], Ravagnan S.[1], Ciocchetta S.[2], Omodeo S.G.[1], Giuliato I.[1], Mazzucato M.[1], Russo F.[3], Palei M.[4], Marangon S.[1], Capelli G.[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [2]1 Mosquito Control Laboratory, QIMR Berghofer Medical Research Institute; 2 Istituto Zooprofilattico Sperimentale delle Venezie ~ Brisbane ~ Australia, [3]Promotion and Development Hygiene and Public Health Service, Veneto Region ~ Venice ~ Italy, [4]Veterinary Public Health Service, Friuli Venezia Giulia region ~ Udine ~ Italy

INTRODUCTION:
The entomological survey is an important action of national and regional surveillance plans for vector-borne diseases and requires knowledge, specialized personnel and financial resources. In this work the costs of the entomological surveillance for West Nile Virus (WNV) (Regional Plan) in 2013 have been evaluated and benefits obtained listed.
MATERIALS AND METHODS:
The costs were considered in terms of number of people employed, work-hours (calculated as standard cost/h), material required (reagents and consumables) and travel. Costs for equipments and functioning were not considered. In 2013 a total of 66 sites were monitored through Veneto and Friuli Venezia Giulia weekly or fortnightly. Mosquitoes of 35 catches were identified every week from May to October, while the viral survey began at the end of June. Entomological surveillance was divided into four steps: mosquito collections (S1), identification and pooling (S2), detection of viruses in mosquitoes (S3) and data entry and processing (S4); for each of these steps the costs were assessed.
RESULTS:
COSTS: In total, 14 people were involved. As a seasonal average, 13,000 mosquitoes were processed per week and a person was able to identify 281.7 mosquitoes/hour. For S4 the cost for the implementation of a vector database for collecting data, processing and maps production was also calculated.
The general 2013 estimated costs for the entomological monitoring was 149.687€. Details for each steps are shown in the table.
For the year 2013 the average cost of viral research (S3) was 11.5€ per pool. However this is a variable cost depending on the total number of positivity detected.
BENEFITS: The entomological monitoring allowed to estimate the presence, density, spatial and seasonal distribution of vectors. This data are the basis for modelling (Mulatti et al., 2014, Parasites & Vectors, 7:26) and risk map building. Indeed, the areas where the vector of WNV (Culex pipiens) was more abundant well fitted the area of viral circulation. The virus detection into the mosquitoes usually anticipated the human cases and can be integrated in an early warning system (Mulatti et al., 2013, Zoonoses Public Health, 60(5):375-82). The detection of the virus into the arthropods also gives indications on their vector competence. Furthermore the viruses are genetically characterized giving insights on lineages and strains circulating in the area (Capelli et al., 2013, Vet Ital., 49(3):263-8), even in the absence of clinical cases. This latter aspect is pivotal to drive the inclusion of organs and blood donors into the viral screening system, avoiding human cases from transfusions and transplantations.

Entomological surveillance	Mosquito collection (S1)	Identification-pooling (S2)	Viruses detection (S3)	Data entry and processing (S4)
People employed, position (work/hours)	1 undergraduate (300) 3 scholarship (530) 1 entomologist (60)	1 technician (137) 5 scholarship (642) 1 entomologist (188)	1 biotechnol. (170) 2 scholarship (775) 1 technician (150)	1 technician (780); 1 office worker (100); 1 scholarship (75); 1 biotechnol. (100) 1 entomologist (170); 1 executive vet (75)
Personnel cost (EUR)	9378	14086	11134	31185
Reagents, consumables + travel (EUR)	12599+8667	500	62138	-
Total (EUR)	**30644**	**14586**	73272	**31185**

CONCLUSIONS:
Entomological monitoring is highly costly and should be justified by calculating if the benefits outweigh the costs. The economic evaluation of benefits is a systematic process which is out of the possibilities of this research team. However this work provide the background to calculate entomological costs and will be used for complete cost/benefit analyses.
This work was supported by Veneto and Friuli Venezia Giulia Regions.

Keywords:
West Nile Disease, cost-benefit, entomological surveillance

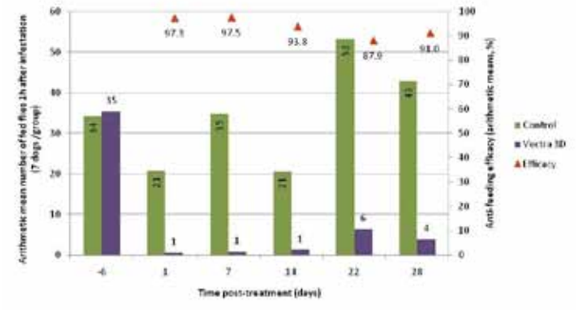
O09.4

IMMEDIATE AND RESIDUAL ANTI-FEEDING EFFICACY OF A DINOTEFURAN-PERMETHRIN-PYRIPROXYFEN TOPICAL ADMINISTRATION AGAINST SANDFLY (PHLEBOTOMUS PERNICIOSUS) INFESTED DOGS UNDER LABORATORY CONDITIONS

Varloud M.[1], Warin S.[1], **Ferrari G.*[2],** Columba M.[3], Mcgrath S.[3], Martin M.[3]

[1]Ceva ~ Libourne ~ France, [2]Ceva Salute Animale S.p.A ~ Agrate Brianza ~ Italy, [3]Charles River Laboratories Preclinical Services Ireland Ltd ~ Ballina, Co. Mayo ~ Ireland

INTRODUCTION:
Sandflies are hematophageous parasites that must perform a blood-meal for oviposition. Phlebotomus perniciosus is a European species of sandflies that can feed on dogs. It is known for transmitting Leishmania infantum. This study was conducted to assess the anti-feeding efficacy of a dinotefuran-permethrin-pyriproxyfen topical ectoparasiticide (DPP, Vectra® 3D) against non-blood fed adult female Phlebotomus perniciosus infested in dogs under laboratory conditions.
MATERIALS AND METHODS:
The protocol for this study was approved by an ethical committee. Fourteen Beagle dogs (8 male and 6 female) were allocated based on pre-treatment sandflies feeding rate (day -6) to 2 groups: an untreated control group (n=7, 10.2 ± 1.1 kg BW) and a DPP treated group (n=7, 10.5 ±1.0 kg BW). Dogs in the treated group were administered 1.6 or 3.6 mL of DPP according to label recommendations on day 0. Each dog was infested under sedation with 85 ± 5 unfed adult females and 10-20 males (Phlebotomus perniciosus) on days -6, 1, 7, 14, 22 and 28. Sandflies were removed from the exposure chamber 60-65 min after each infestation. Live female sandflies were frozen. All live and dead flies were categorised as engorged or unengorged through visual detection of blood using a stereomicroscope. Arithmetic means of engorged sandflies were calculated. Comparisons between groups were performed on each group at each time point on the mean number of engorged sandflies by ANOVA or non-parametric tests (Kruskal-Wallis) according to homogeneity and equality of variances. Veterinary examinations, bodyweight, general health observations and clinical assessments were evaluated clinically throughout the study.
RESULTS:
The DPP treatment provided ≥88% anti feeding efficacy for the duration of the four weeks assessment period. The mean number of engorged sandflies differed significantly ($p<0.05$) between control and treated groups at each time-point.
CONCLUSIONS: This study underlines the immediate and residual anti-feeding effect of DPP for one month against sandflies potentially challenging a treated dog. Topical application of DPP at the recommended dose was well tolerated.



Keywords:
sandfly (Phlebotomus perniciosus), dinotefuran-permethrin-pyriproxyfen, anti-feeding

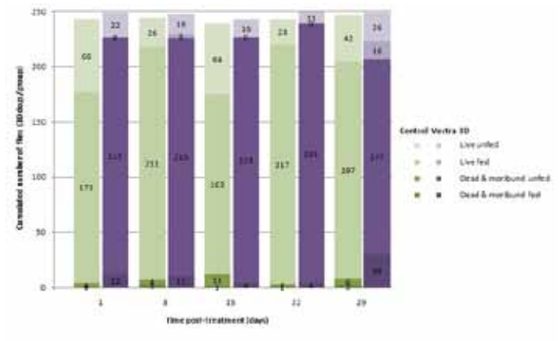
O09.5

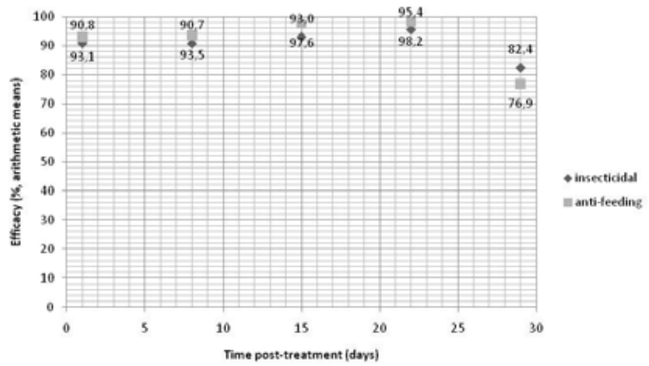
IMMEDIATE AND RESIDUAL ANTI-FEEDING AND INSECTICIDAL EFFICACY OF A DINOTEFURAN-PERMETHRIN-PYRIPROXYFEN TOPICAL ADMINISTRATION AGAINST STABLE FLY (STOMOXYS CALCITRANS) CHALLENGES ON DOGS

Varloud M.[1], Fourie J.[2], **Crippa A.*[3]**

[1]Ceva ~ Libourne ~ France, [2]ClinVet International (Pty) Ltd ~ Bloemfontein ~ South Africa, [3]Ceva Salute Animale S.p.A ~ Agrate Brianza ~ Italy

INTRODUCTION:
Stable flies are haematophageous parasites of domestic animals. Stomoxys calcitrans has a worldwide distribution with a seasonal pattern. Both males and females feed on blood during day-light with painful bites that can induce necrotizing dermatitis on dogs. This study was conducted to assess the anti-feeding and insecticidal efficacy of a dinotefuran-permethrin-pyriproxyfen topical ectoparasiticide (DPP, Vectra® 3D) administerred to dogs against unfed adult Stomoxys calcitrans challenges.
MATERIALS AND METHODS:
This protocol was approved by an independent ethics committee. Twenty mixed breed dogs were allocated based on gender and pre-treatment fly feeding success rate (day -3) to 2 groups: a control group (C, n=10, 1:1 sex ratio, 15.3 ± 3.2 kg BW) and a treated group (DPP, n=10, 1:1 sex ratio, 13.9 ±1.9 kg BW). Dogs in the treated group were administered according to label recommendations 3.6 mL of DPP on day 0. Each dog was challenged under sedation with 25 unfed adult Stomoxys calcitrans on days -4, 1, 8, 15, 22 and 29. Flies were removed from the exposure chamber 30 min after each infestation and classified as live, moribund or dead. The flies were frozen before assessment of their engorgement status through visual observation for the presence of blood in their gut through abdominal squashing on white paper. Arithmetic means of live flies and of engorged flies were calculated. Comparisons between groups were performed on each group at each time point on the geometric mean number of flies (live or fed) after log transformation (count +1) by ANOVA. The level of significance was set at p = 0.05.
RESULTS:
The DPP treatment provided insecticidal and anti-feeding efficacy against Stomoxys calcitrans flies for 4 weeks. Efficacy was >80% in 4 weeks for insecticidal efficacy and >90% in 3 weeks for anti-feeding. The mean number of flies that successfully took a blood meal differed significantly ($p<0.05$) between control and treated groups at each time-point.
CONCLUSIONS: This study underlines the immediate (24 hours) and residual (1 month) insecticidal and anti-feeding effect of DPP against stable flies on dogs.





Keywords:
stable fly (Stomoxys calcitrans), dinotefuran-permethrin-pyriproxyfen, anti-feeding

O09.6

EFFICACY AND RESIDUAL ACTIVITY OF SUNLIGHT-ACTIVATABLE PORPHYRIN FORMULATIONS, NEEM PREPARATIONS AND PORPHYRIN-NEEM COMBINATIONS AGAINST LARVAE OF ANOPHELES GAMBIAE S.L.

Ouedraogo R.[1], Yerbanga S.[1], Damiano S.[2], Sawadogo S.[1], Fabris C.[3], Lucantoni L.[2], Diabate A.[1], Coppellotti O.[3], **Lupidi G.*[3],** Jori G.[3], Ouédraogo J.B.[1], Habluetzel A.[2], Dabire R.[1]

[1]Institut de Recherche en Science de la Sante/Centre Muraz ~ Bobo-Dioulasso ~ Burkina Faso, [2]School of Pharmacy, University of Camerino ~ Camerino ~ Italy, [3]Department of Biology, University of Padova ~ Padova ~ Italy

INTRODUCTION:
Meso-tri(N-methyl-pyridyl),mono(N-dodecyl-pyrydyl)porphine is a porphyrin molecule (PO), capable to cause lethal tissue damage in insects upon ingestion and subsequent photo-activation by natural sun-light. Recent studies showed that loaded on ground animal food pellets, PO preparations are able to rapidly kill Aedes aegypti (Lucantoni et al., 2011, PLoS Negl Trop Dis. 5:e1434)and Anopheles gambiae s.l. larvae (Fabris et al., 2012, Acta Trop 123:239-43) but its residual activity did not exceed 2 days. This study aimed to investigate whether a combination-larvicide containing PO as a rapidly acting toxic agent and neem (Azadirachta indica, Meliaceae) extracts as partner components inhibiting larval development through their IGR properties, may satisfy the requirements of a fast and long acting larvicide candidate.
MATERIALS AND METHODS:
The following neem preparations were examined: powdered neem leaves (NL), bark (NB), fruit (NF) and a standardized azadirachtin-enriched commercial formulation (NeemAzal®), loaded to powdered cat food (CF). PO-neem combinations were obtained by incubating neem plant part powders and NeemAzal®-CF in 500-µM PO solutions. Batches of 60 field-caught or laboratory-reared larvae (2nd – 4th instars) were placed in plastic trays containing 500 ml of water from various types of breeding sites and 20 – 30 mg of test substances were added per tray. To expose the larvae to natural light conditions assays were conducted outdoors. Efficacy was assessed by determining larval mortality after two days of sunlight exposure and residual activity was estimated 9 days post-treatment, after having added new batches of larvae to the same trays on day 3.
RESULTS:
PO-neem combinations, particularly PO-NF and PO-NL demonstrated high efficacy in spring water yielding > 93 % larval mortality after 48 h of light exposure, comparable to the reference PO-CF. However, in breeding site waters rich in soil particles and microbiota mortality rates varied widely (10 - 88%). Yet, after 7 days of exposure, mortality rates of 77 to 100% were recorded in 3 out of the 4 tested breeding site waters. Neem preparations without PO caused moderate larval mortality after 7 days of exposure, around 50%. Inhibitory action on pupation of 4th instars larvae was revealed by neem treatment. In particular in trays treated with NeemAzal®-CF (NeemAzal® loaded at 1-10% on CF powder) and NF powder no pupae were observed up to day 11 (end of experiment). This inhibitory effect - even though not complete (2 - 13% pupation rate) - was also observed in the residual activity experiment in which new batches of larvae were added to the treatment trays on day 3.
CONCLUSIONS:
Combining porphyrin with neem, in particular with neem fruit or NeemAzal® has proven a valid approach. However, formulation studies are needed to improve palatability and to obtain larvicide candidates effective in all types of the most frequent breeding sites.

Keywords:
porphyrin, neem, Anopheles larvicide

O09.7
EFFICACY OF DISINFESTATION AGAINST ADULT OF CULEX PIPIENS MOSQUITOES IN WEST NILE VIRUS POSITIVE SITES OF NORTH-EASTERN ITALY

Carlin S.*[1], Martini S.[6], Signorini M.[2], Scremin M.[1], Nicolardi L.[3], Foroni M.[4], Russo F.[5], Capelli G.[1], Montarsi F.[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro - Padova ~ Italy, [2]Department of Animal Medicine, Production and Health, Padua University ~ Padova ~ Italy, [3]Dipartimento di Prevenzione, ULSS 10 ~ San Donà di Piave - Venezia ~ Italy, [4]Dipartimento di Prevenzione, ULSS 22, Bussolengo, Italy, [5]Promotion and Development Hygiene and Public Health Service, Veneto Region ~ Venezia ~ Italy, [6]Entostudio srl ~ Brugine - Padova ~ Italy

INTRODUCTION:
In the last years, the increase of mosquito borne diseases as Chikungunya and West Nile (WNV) has required the implementation of the entomological surveillance. In the Veneto Region an entomological monitoring is ongoing since 2009 after the emergence of WNV in 2008 (Mulatti et al., 2013, Zoonoses Public Health, 60(5):375-82; Gobbi et al., 2014, BMC Infect Dis.14:60). The USA experience suggests that a prompt disinfestation against adult mosquitoes soon after the human cases can decrease the risk of transmission (CDC guidelines, 2013). The aim of this work was to evaluate the efficacy of the disinfestation in reducing the density of adult Culex pipiens, the only recognized vector of WNV in the area.
MATERIALS AND METHODS:
In 2013 a disinfestation against adult mosquitoes was organized in sites where human cases of WNV were reported. The tests were carried out in the municipality of Jesolo (J) (Venice) and in two neighbouring municipalities, Erbè (E) and Isola della Scala (IS) (Verona). An area of 10 km^2 in J and two areas of 2 km^2 in E and in IS were treated. Two bordering areas were left untreated for control. Three sampling using CDC-CO^2 traps were performed in treated and in untreated areas, during the night (from 9 pm to 12 pm) before and after treatment. In the treated areas the following protocols were used: 1) Tetramethrin (T)10%-Piperonyl butoxide (PBO)10% (1% dilution, total volume 330l) in J, 2) Cypermethrin (C)10%-T2%-PBO15% (0.5% dilution, total volume 400l) in E, 3) C10%-T2%-PBO5% (0.5% dilution, total volume 600l) in IS. Treatments were performed through atomizer by a private company in J and by personnel of the municipality in E and IS. In the 3 sites different kind of atomizers were used to spread the insecticide.
RESULTS: The average amounts of Cx. pipiens detected in the 5 areas and the % of reduction are shown in table1. A significant reduction (ANOVA test, p=0.05) of Cx. pipiens density after disinfestation was achieved only in site IS.

Table 1 – Number and means of *Culex pipiens* before (T0) and after (T1) the disinfestation and % of reduction

Sites	Area	T0 mean *Cx. pipiens* (total no)	T1 mean *Cx. pipiens* (total no)	% of reduction
Jesolo (J)	Treated	968.0 (1936)	890.5 (1781)	-8.01%
	Untreated	1096.0 (3288)	913.0 (2741)	-16.64%
Erbè (E)	Treated	348.7 (1046)	298.0 (894)	-14.53%
Isola d. Scala (IS)	Treated	527.7 (1583)	108.7 (326)	-79.41%
Erbè/Isola d. Scala	Untreated	571.3 (1714)	510.7 (1532)	-10.62%

CONCLUSIONS:
The results show different efficacies of adult disinfestation in the sites treated, despite the use of proven efficacious formulations of insecticide. The most likely reason for the failure of efficacy lies in the different devices used to spread the insecticides. Also a rapid re-colonization of the sites from the untreated neighbouring rural areas must be considered. These preliminary results highlight the importance to follow guidelines for the correct application of disinfestation protocols and to control the efficacy of treatments in order to optimize the cost/benefit ratio of mosquito control.
This presentation was sponsored by I.N.D.I.A Industrie Chimiche spa.

Keywords:
Culex pipiens, disinfestation, West Nile Virus

O09.8
EVALUATION OF INSECTICIDE TREATMENTS AGAINST AEDES ALBOPICTUS IN THE CAMPUS OF UNIVERSITY OF ROME SAPIENZA

Caputo B.*[1], Ienco A.[1], Manica M.[2], Rosà R.[2], Bruni A.[3], Fascetti R.[3], Petrarca V.[4], Della Torre A.[1]

[1]Dipartimento di Scienze di Sanità Pubblica e Malattie Infettive, Università di Roma "Sapienza" ~ Roma ~ Italy, [2]Environment and Natural Resources Area, IASMA Research and Innovation Centre, Edmund Mach Foundation ~ S. Michele all'Adige ~ Italy, [3]Sogea srl ~ Roma ~ Italy, [4]Dipartimento di Biologia e Biotecnologie "Charles Darwin" ~ Roma ~ Italy

INTRODUCTION:
The aim of this study was to evaluate the efficacy of a common pest control strategy against Aedes albopictus within a typical urban area in Rome, where this mosquito species creates significant nuisance problems. In fact, the efficacy of these activities has been rarely evaluated in temperate areas and in particular in the Mediterranean region (Abramides et al., TRSTMH 2011, 105, 281-288). In summer 2012, in the campus of University of Roma SAPIENZA (23 Ha) we evaluated the effect on adult population dynamics of bi-monthly larvicide treatments in 166 street catch basins (Diflubenzuron, 2 g tablets with 2% of active ingredient) and of two adulticide fogging treatments (1% water-diluted TERBUTIN©: 1.5 gr pyrethrum 50% extract; 4.75 gr permethrin, 2.64 gr piperonyl butoxide for 100 gr) carried out at sunset with a 3-week interval.
MATERIALS AND METHODS:
We compared Ae. albopictus population dynamics in the treated area (sub-divided into a grid of eighteen 100x100m-cells) with that observed in an untreated 1Ha area, based on two types of weekly collections of adults specimens, i.e. 1) Sticky Traps (STs; Facchinelli et al., 2007, MVe, 2007, 21, 183–195) and 2) Catch Basin Traps (CBTs), a newly designed trap consisting of sticky panels placed vertically inside catch basins. Statistical analysis was carried out using a mixed model approach (Zuur et al 2009, Mixed Effects Models and Extensions in Ecology with R., Springer, GLMM-PQL: Generalized Linear Mixed Model-Penalized Quasi-Likelihood) to account for spatio-temporal autocorrelation of data.
RESULTS:
Results obtained by GLMM-PQL model showed that: i) counts of adult females were significantly lower in treated than in the untreated area, when considering the sampling as a whole (p-value=<0.001), while it was not so when taking into account collections carried out before the first and the second adulticide treatment; ii) female counts were significantly higher in ST than in CBT, both in untreated and in treated areas; iii) the relationship between the number of females collected in ST and CBT was not statistically different between areas, as no interactive effect was found between the two explanatory variables (i.e. sites of collections and trap methods). When the analysis by GLMM-PQL model was restricted to the treated area, female counts in two cells where a botanical garden was located were significantly higher than in all other cells.
CONCLUSIONS:
Both collection approaches revealed a strong effect on Ae. albopictus densities of the strategy applied after the second adulticide treatment. The different observed impact of the two adulticide treatments could be due to the seasonal population dynamics of the species. Interestingly, the effect of treatment was not uniformly distributed in the treated area, probably due to a different habitat suitability (e.g. mosquito breeding sites and population density, vegetation availability, etc.), and/or to a non-uniformity of fogging treatment (e.g. space barriers, wind speed and direction, etc.).

Keywords:
Vector control, mosquito, Aedes albopictus

O09.9
EVALUATION OF A NOVEL SAMPLING TOOL TO DETECT ABUNDANCE AND INFECTIVE STATUS OF MALARIA VECTOR SPECIES OF THE ANOPHELES GAMBIAE COMPLEX

Pombi M.*[1], Guelbeogo W.M.[2], Calzetta M.[1], Kreppel K.[3], Traoré A.[2], Sanou A.[2], Ranson H.[4], Ferguson H.M.[3], Mancini E.[1], Sagnon N.[2],Della Torre A.[1]

[1]Dipartimento di Sanità Pubblica e Malattie Infettive, Università di Roma "Sapienza" ~ Roma ~ Italy, [2]Centre National de Recherche et Formation sur le Paludisme ~ Ouagadougou ~ Burkina Faso, [3]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow ~ Glasgow ~ United Kingdom, [4]Vector Group, Liverpool School of Tropical Medicine ~ Liverpool ~ United Kingdom

INTRODUCTION:
Monitoring densities of adult mosquito populations is a major challenge in efforts to evaluate the epidemiology of mosquito-borne diseases, and their response to vector control interventions. In the case of malaria, collection of outdoor-resting Anophelines is rarely incorporated into surveillance and control, which is at least partially due to the lack of standardized collection tools. The need for such an approach is important also for the study of infective status of vector species in the evaluation of malaria transmission in endemic areas.
With this aim, we here present: i) the Sticky Resting Box (SRB) a sticky trap which allows passive collection of mosquitoes in both indoor and outdoor settings – and compared its performance against traditional sampling methods; ii) the estimation of infective status of mosquitoes by the optimization of a PCR protocol aimed to detect Plasmodium sp. in samples collected with the three methods.
MATERIALS AND METHODS:
and dry season of 2012 by SRBs located indoors and outdoors, and by Back-Pack aspirations inside houses (BP) and in ad hoc built outdoor pit-shelters (PIT).
The PCR protocol for Plasmodium detection is based on the one proposed for blood samples by Haanshuus et al. (2013, Malar J., 12: 26), which amplifies a 209bp sequence of an inter-genic portion between Cit1 and Citb genes and has been adapted to de-hydrated mosquito samples.
RESULTS:
Overall, almost 20,000 Culicidae specimens belonging to 16 species were collected and morphologically identified. Malaria vectors included Anopheles coluzzii (53%), An. arabiensis (12%), An. gambiae s.s. (2.0%) and An. funestus (4.5%). The diversity of species collected in the two villages was similar for SRB and PIT collections outdoors, and significantly higher for SRB than for BP indoors. The population dynamics of An. gambiae s.l. mosquitoes, as obtained by SRB-collections was significantly correlated with those obtained by the traditional methods. The predicted median estimates of An. gambiae s.l. specimens/sampling-unit/night-of-collections was 6- and 5-times lower for SRB than for BP indoors and PIT outdoors, respectively.
A subsample of 988 mosquito females (679 from Goden and 309 from Koubri) collected during 2011 rainy season were tested for their infective status, showing comparable frequencies of Plasmodium sp. infections between villages (5.6% and 5.2% in Goden and Koubri, respectively).
CONCLUSIONS:
Overall, the daily performance of SRB in terms of number of malaria vectors collected per trap was lower than that of traditionally used approaches for in- and outdoor collections. However, unlike these methods, SRB could be set up to collect mosquitoes passively over at least a week. Moreover, the successful optimization of a PCR protocol for Plasmodium spp. detection on mosquitoes collected also with SRB, make this trap a very promising tool for entomological and epidemiological studies on malaria vectors in Sub-Saharan Africa.

Keywords:
sticky trap, mosquito, monitoring

O09.10
A KILLER YEAST STRAIN IS HARBORED IN MALARIA VECTORS: NEW INSIGHTS IN THE MOSQUITO BIOLOGY AND POSSIBLE IMPLICATIONS IN THE MALARIA TRANSMISSION BLOCKING

Valzano M.*[1], Cappelli A.[1], Ulissi U.[1], Damiani C.[1], Capone A.[1], Bozic J.[1], Cecarini V.[1], Favia G.[1], Ricci I.[1]

[1]Università di Camerino ~ Camerino ~ Italy

INTRODUCTION:
The yeast W. anomalus (Wa) is considered an alluring organism for its biotechnological perspectives (Walker, 2011, Antonie Van Leeuwenhoek, 99:25-34).
Wa strains inhabit different habitats and, recently, a specific strain (WaF17.12) was isolated from malaria vector mosquito Anopheles stephensi, suggesting a possible symbiotic relationship providing host nutrition and/or protection.
Here we investigated the ability of WaF17.12 to express antimicrobial proteins called 'Killer Toxins' (WaKTs), testing the yeast killer activity against sensible microorganisms.
MATERIALS AND METHODS:
a. Yeast strains (Wa), Saccharomyces cerevisiae, Candida albicans); laboratory reared An. stephensi; WaKT extracts; the rodent malaria model (Plasmodium berghei, BALB/c mice).
b. Microbial activity tests, Western Blot, Immuno-FluorescenceAssays (IFA), ion exchange chromatography (Diethylaminoethyl (DEAE) column) and ookinete development assay.
RESULTS:
We demonstrated in vitro the antimicrobial activity of WaF17.12 against susceptible species: S. cerevisiae, C. albicans and the strain WaUM3. We tested the WaKT activity at diverse pHs and temperatures because the adaptability of the protein to different conditions is an essential requisite for potential applications of killer strains in vivo (i.e. in the mosquito host).
These antimicrobial tests have been performed using KT-WaF17.12 cultural crude extracts. Currently, we are performing the biochemical characterization and purification of this toxin to test possible inhibition on the development of Pl. berghei ookinetes in vitro and in vivo.
CONCLUSIONS:
The biological roles of Wa in mosquitoes are still unknown. Arthropod-associated strains could employ nutritional and/or protective functions mediated through antimicrobial activity against harmful organisms and pathogens (Ricci et al., 2011, Antonie Van Leeuwenhoek, 99:43-50; Wang et al., 2008, Dis Aquat Organ, 80:211-218).
The finding that WaF17.12 shows a 'killer phenotype' strengthens the hypothesis of a protective role.
Moreover, the localization of a killer strain into the female midgut of Anopheline mosquitoes is very interesting considering that this environment represents a bottleneck during the Plasmodium life-cycle making the parasite more vulnerable to the action of antagonistic microorganisms/molecules (Abraham and Jacobs-Lorena, 2004, Insect Biochem Mol Biol, 34:667-671).
Actually, we are focusing on a possible WaKT anti-Plasmodium activity inhibiting the development of ookinetes in vitro conditions.
Furthermore, our results indicate that cultivated yeasts can easily colonize mosquito tissues and continue the KT production for several weeks so, killer yeasts could be stimulated to increase the KT-secretion and reintroduced into mosquito vectors to have more efficient anti-malaria activity.
Yeasts naturally incisive against malaria should be more easily accepted by the scientific community for field applications, compared with genetically modified microorganisms predicted by paratransgenic approaches.

This research project has received funding from the European Union Seventh Framework Programme under grant agreement n. 281222 to IRENE RICCI.

Keywords:
Killer toxin, antimicrobial activity, malaria transmission blocking

O09.11
PATHOGENICITY OF A NATIVE STRAIN OF BEAUVERIA BASSIANA ON RHIPICEPHALUS SANGUINEUS GROUP TICKS UNDER LABORATORY CONDITION

Immediato D.*[1], Cafarchia C.[1], Ramos R.A.N.[1], Lia R.P.[1], Iatta R.[1], Figueredo L.A.[2], Dantas Torres F.[2], Otranto D.[1]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [2]Department of Immunology, Aggeu Magalhães Research Centre, Recife-PE, Brazil; Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy

INTRODUCTION:
Rhipicephalus sanguineus group ticks are vectors of viruses, bacteria and protozoa of human and animal concern. Acaricide drugs represent the most frequently used tools to control ticks in the environment even if they increasingly cause concerns linked to the environmental contamination and the development of resistance. Therefore, non-chemical alternative approaches for the control of ticks, such as the biocontrol by using entomopathogenic fungi are of increasing interest (Otranto and Wall, 2008, Med. Vet. Entomol., 22:291-302). Studies demonstrated the ability of Beauveria bassiana to cause mortality in other tick species such as Amblyomma cajennense and Rhipicephalus (Boophilus) microplus (Campos et al., 2010, Genet. Mol. Res., 9:1421-1430; Sun et al., 2011, Vet. Parasitol., 180:389-393; Fernandes et al., 2012, Exp. Parasitol., 130:300-305; Perinotto et al., 2012, Exp. Parasitol., 130:257-260). As far as R. sanguineus group ticks, data on the efficacy of this fungus is fragmentary (Reis et al., 2008, Ann. N. Y. Acad. Sci., 1149:239-241). Thus, this study was aimed at evaluating the in vitro effect of a native strain of B. bassiana (CD1123, University of Bari, Italy) on different life stages of R. sanguineus group ticks.
MATERIALS AND METHODS:
The bioassay was composed by 2 groups (treatment, T and control, C) of ticks for each life stage. Eggs (T n = 750; C n = 750), larvae (T n = 120; C n = 120), nymphs (T n = 60; C n = 60) and adult (T n = 30; C n = 30) of R. sanguineus sp. I (Dantas-Torres et al., 2013, Parasit. Vectors, 6:213) were submerged for 3 min in a suspension of 107 conidia/mL of B. bassiana (originally isolated from naturally infected ticks from a private dog shelter in Bari, Apulia, Italy) or in the solvent solution (i.e., sterile distilled water plus 0.1% Tween 80) for the treated and control group, respectively. All experiments were conducted in triplicate. The groups were observed for 20 days and biological parameters (i.e., mortality, hatching, moulting rate, pre-oviposition period, oviposition period and rate, egg production efficiency, and reproductive efficiency index) assessed.
RESULTS:
The effect of the B. bassiana strain herein tested showed a significantly higher mortality on eggs, larvae, nymphs and adult when compared to those of the control group ($p < 0.05$) at 5 days post-infection (PI). A 100% of mortality was registered after 15 days PI in engorged females, eggs, engorged and unengorged larvae; the highest mortality rate registered for engorged and unengorged nymphs was 88% at 10 days PI. Neither treated eggs hatched nor treated larvae moulted, but only 15% of the treated nymphs moulted. All the biological parameters of engorged females were significantly reduced ($p < 0.05$) when compared to those of the control groups.
CONCLUSIONS:
This study shows that the native strain of B. bassiana is highly effective in the control of all the developmental stages of R. sanguineus group ticks and might represent a promising biocontrol agent.

Keywords:
Rhipicephalus sanguineus, Beauveria bassiana, Biologic control

O09.12
MODELS FOR THE CONTROL AND THE THERAPY OF VECTOR-BORNE DISEASES: KILLER YEASTS AND KILLER PEPTIDES

Epis S.*[1], Damiani C.[2], Giovati L.[3], Ricci I.[2], Favia G.[2], Polonelli L.[3], Kramer L.H.[4], Bandi C.[1]

[1]Department of Veterinary Science and Public Health ~ Milan ~ Italy, [2]School of Biosciences and Biotechnology ~ Camerino ~ Italy, [3] Department of Pathology and Laboratory Medicine ~ Parma ~ Italy, [4]Department of Animal Health ~ Parma ~ Italy

INTRODUCTION:
Vector-borne diseases (VBDs) are among the most important emerging health risks for both humans and animals. In recent years arthropod vectors of diseases have spread to previously unaffected geographical areas and there has been an increasing number of cases of VBDs. Italy has also seen an expansion of VBDs and given the fact that it is one of the southernmost countries in Europe, it represents an ideal bridge for the spread from the African continent. It is widely accepted that efficient control against VBDs can only be achieved through an integrated approach that combines different strategies where traditional chemical control is integrated with biological control (Beier et al., 2008, Malar J, 7 1:S4). In addition, the onset of phenomena of resistance against several insecticide molecules and the emergence of new pathogens highlight an urgent need for alternative approaches for VBDs control.
We propose to investigate killer yeasts and their toxins, to develop novel strategies for the control and therapy of VBDs. We already know that mosquito vectors (Anopheles and Aedes) have recently been shown to harbor the yeast Wickerhamomyces anomalus (Ricci et al., 2011, Environ Microbiol, 13(4):911–921) able to produce a killer toxin active against microrganisms (KT) (Cappelli et al., In press). In addition, a killer peptide (KP), engineered from the variable region of a recombinant anti-idiotypic antibody corresponding to the 'molecular image' of yeast KT, showed a significant antibacterial and antiprotozoal activity, and immunomodulatory effects in mouse (Magliani et al., 2012, Front Microbiol, 3:190).
MATERIALS AND METHODS:
We will verify the diffusion of yeasts in field populations of different ticks and sand flies species, which represent the arthropod vectors of greatest interest in human and veterinary medicine in Italy.
Moreover, we will evaluate the in vitro inhibitory activity of KP and other peptides derived from it, against selected vector-borne pathogens (Plasmodium spp., Leishmania spp., Borrelia spp.) and the in vitro (cell mosquito lines) and in vivo (mosquitoes kept in insectary) immunomodulatory activity of KP and selected yeasts. The demonstration of the in vitro and in vivo activity of KP against the selected vector-borne pathogens would represent the starting point for the development of innovative molecules for the treatment of vector-borne infectious diseases.
RESULTS:
Our expected results are increasing the knowledge on yeasts in arthropods in order to open the way toward their development as tools for the biological/integrated control of vector-borne diseases.
As concerning the yeasts able to produce killer toxin (active against vector-borne pathogens), this system could be used, in addition to the capacity to stimulate the immune system of arthropods, to reduce the arthropod vector capacity. Moreover, the study of KP and its activity against pathogens, might also open the way toward the development of novel drugs, for the therapy of malaria and other diseases.
CONCLUSIONS:
Our work will likely open the way to studies on a variety of vector-borne diseases, of medical, veterinary and agronomic importance and will provide a general evidence of the potential utility of killer yeasts and derived peptides.

Keywords:
Symbiosis, vector-Borne Diseases, integrated Control

O09.13
DEVELOPMENT OF A PCR ASSAY TO DETECT IXODIPHAGUS HOOKERI (HYMENOPTERA, ENCYRTIDAE) IN TICKS

Campbell B.E.*[1], Giannelli A.[1], Ramos R.A.N.[1], Whittle A.[2], Dantas Torres F.[3], Wall R.[2], Otranto D.[1]

[1]Universita di Bari Aldo Moro ~ Bari ~ Italy, [2]University of Bristol ~ Bristol ~ United Kingdom, [3]Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães (Fiocruz-PE) ~ Recife ~ Brazil

INTRODUCTION:
Ticks are important arthropods affecting humans and vertebrate animals worldwide (Dantas-Torres et al., 2012, Trends Parasitology, 28:437-446). At present, the population dynamics of ticks and particularly the density-dependent biotic constraints that may be responsible for the regulation of abundance are poorly understood. However, understanding the impact of any such factors is an essential step in explaining the epidemiology of tick-borne disease. Ixodiphagus hookeri is the most well studied parasitoid of ticks and may act as an important constraint on tick population growth in some regions (Samish et al., 2004, Parasitology 129:S389-S403). Currently, the detection of larvae within ticks relies on dissection and microscopic examination, which is time consuming, dependent on the operators skills and size of the wasp. Conversely, the use of molecular methods for detecting this parasitoid provides a sensitive and rapid tool to screen tick populations, important for a better understanding of its biology and interaction with ticks. Therefore, the aim of this study is to develop a PCR assay, based on sequences obtained in Tijsse et al 2011, Parasites & Vectors 4: 228, to detect I. hookeri in ticks.
MATERIALS AND METHODS:
Genomic DNA from two adult wasps, isolated from Ixodes ricinus ticks (collected in the Parco Regionale di Gallipoli Cognato – Piccole Dolomiti Lucane, Basilicata, southern Italy) was extracted. PCR to amplify a partial 28S sequence was performed, followed by sequencing to confirm the species identity. The assay was used to screen individual I. hookeri larvae (n=20) and I. ricinus (n = 6) that were negative for I. hookeri upon microscopy. Other tick species (e.g. Dermacentor marginatus, n = 10; Rhiphicephalus turanicus, n = 10; Haemaphysalis inermis, n = 5) collected from the same area were also screened. All amplicons were sequenced.
RESULTS:
The PCR herein developed is sensitive enough to amplify 28S from individual wasp larvae and I. ricinus negative upon microscopic examination (n = 6). Amplicons were also detected in D. marginatus (n = 3) and H. inermis (n = 2). Partial 28S sequences revealed 100% nucleotide identity with I. hookeri sequences from Tijsse et al 2011, Parasites & Vectors, 4: 228.
CONCLUSIONS:
The PCR assay developed is a very sensitive, specific tool for the detection of I. hookeri in ticks. This assay can now be used to determine the prevalence and distribution of the parasitoid in different tick species in other geographical locations.

Keywords:
ticks, Ixodiphagus, parasitoid

NOTES

NOTES

SESSIONE PARALLELA O-10

PARASSITI DELLA FAUNA SELVATICA

O10.1
MOLECULAR DETECTION OF TICK-BORNE PATHOGENS IN SICILIAN WILD ANIMALS

Torina A.[1], Crucitti D.[1], Di Pasquale C.[1], Marino F.[1], De Maria C.[1], Tarantino V.[1], Randazzo K.[1], Blanda V.[1], **Caracappa S.*[1]**

[1]Istituto Zooprofilattico Sperimentale della Sicilia ~ Palermo ~ Italy

INTRODUCTION:
Arthropod-borne diseases have a great importance in human and animal health. The pathogenesis of these diseases involves several infectious agents, hosts and vectors (Harrus and Baneth, 2005, Int.J.Parasitology, 35: 1309-1318). In the last decades several arthropod-transmitted diseases are increasing due to climatic, ecological and environmental changes and the presence of wild animals in proximity to agricultural areas and human habitats has resulted in an increased association between wildlife reservoirs and arthropod vectors of human and animal pathogens (Ebani et al., 2011, J Wildl Dis, 47: 699-703).
Particularly red foxes (Vulpes vulpes) and boars are widespreading in Sicily and they are found, more often, in agricultural areas where they feed on prey such as rodents.
Babesia microti, Ehrlichia canis, Rickettsia spp. and Anaplasma spp. are arthropod-borne pathogens able to infect human and animal blood cells and they are transmitted by ticks, fleas and lice (Andrè et al., 2012, Ticks Tick Borne Dis, 3: 247-253). The aim of this study is the monitoring of Sicilian red foxes and boars for the presence of pathogen DNA belonging to Babesia microti, Ehrlichia canis, Rickettsia spp. and Anaplasma spp.
MATERIALS AND METHODS:
Post mortem spleens of 180 foxes and 6 boars coming from authorized hunting during 2013 in Sicily were analyzed. DNA was extracted from samples by a commercial kit and both conventional and nested PCR methods were performed for pathogen DNA detection. PCR amplifications were carried out using specific primers and the products were visualized after electrophoretic migration on Agarose gel.
RESULTS:
Out of 180 foxes, 107 (59%) resulted to be positive for Babesia microti, 11 (6.1%) for Ehrlichia canis and 10 (5%) for Rickettsia spp. No fox was positive for Anaplasma spp. Conversely, analyses performed on the six boar samples showed only one case positive to Anaplasma spp.
CONCLUSIONS:
In conclusion our results suggest that wildlife, in particular red foxes, are largely infected by arthropod-borne pathogens. These animals may play an important role in the diffusion of infections to the domestic species. It could speculate that the infection of these parasites in foxes might be linked to the phylogenetic proximity between foxes and dogs. Further analyses are in progress to investigate the possible role of wild animals for these and others arthropod borne pathogens maintenance and diffusion.

Keywords:
tick-borne pathogens, wild fauna, PCR

O10.2
SURVEY OF ZOONOTIC VISCERAL LEISHMANIASIS AMONG FOXES AND HUNTING DOGS LIVING IN SAME AREA OF THE CAMPANIA REGION OF ITALY

Piantedosi D.*[1], Veneziano V.[1], Di Muccio T.[2], Foglia Manzillo V.[1], Fiorentino E.[2], Scalone A.[2], Neola B.[1], D'Alessio N.[3], Gradoni L.[2], Oliva G.[1], Gramiccia M.[2]

[1]Department of Veterinary Medicine and Animal Production, University Federico II, Naples ~ Napoli ~ Italy, [2]Unit of Vector-borne Diseases and International Health, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy, [3]Istituto Zooprofilattico del Mezzogiorno, Avellino Section ~ Avellino ~ Italy

INTRODUCTION:
Zoonotic visceral leishmaniasis (VL) due to Leishmania infantum is a protozoan disease transmitted by phlebotomine sand flies widespread in southern Europe. Campania region, southern Italy, is historically considered one of the most endemic areas of Italy for both canine (CanL) and human VL. The red fox (Vulpes vulpes) is usually regarded as having a role in the occurrence of CanL in rural and peri-urban areas of the Mediterranean basin, however dedicated studies have been limited in Italy (Dipineto et al., 2007, J. Wildl. Dis., 43:518-520). Particularly, comparative studies between dog and fox populations from the same area have not been performed. The aim of the present study was to investigate on Leishmania prevalence in both dogs and foxes living in a well-known restricted territory ("Picentini area") of Salerno province, Campania region.
MATERIALS AND METHODS:
Sampling was performed in an area of about 150 km² (median altitude: 280 m/asl; human population density: 185,2/km²). Forty-six foxes were examined after being killed by hunters or, in a few cases, after car crashes from October 2012 through January 2013. Samples of spleen (SP), liver (LI) and popliteal lymph nodes (LN) were collected and examined by Leishmania nested (n) PCR (Oliva et al., 2006, J. Clin. Microbiol., 44:1318-1322). Moreover, 123 sera from autochthonous hunting dogs were collected from April through July 2012 to be examined by the indirect immunofluorescence assay (IFAT).
RESULTS:
Seroprevalence in dogs was found to be 17.9% (22/123) at the cut-off titer of 1/160. Most of the dogs (96%) did not show clinical signs suggestive for Leishmania infection. IFAT results showed also that 42/123 dogs (34.1%) were positive for anti-Leishmania antibodies at lower titers (1:40-1:80) for a total 52.0% prevalence of seroreactors. Ten foxes were found infected by Leishmania (21.7%): 4 in SP, 2 in LN and 4 on both SP and LN samples, whereas LI samples were found consistently negative. Hence, n-PCR positivity was 17.4% (8/46) for SP and 13.3% (6/45) for LN samples.
CONCLUSIONS:
Dog results confirmed the high prevalence of CanL in the studied area, similarly to other endemic area of southern Italy. The cumulative presence of specific antibodies in half of the investigated dogs suggests that the circulation of parasite is very high in this small geographic area. This result is reinforced by the high prevalence of Leishmania in foxes that could contribute as a further reservoir of infection. Due to this particular epidemiological situation, it remains unclear why in the same area only a few cases of human VL have been recorded in the past 20 years.

Keywords:
Zoonotic visceral leishmaniasis, Vulpes vulpes, hunting dogs

O10.3
COPROLOGICAL AND MOLECULAR STUDY ON THE GASTRO-INTESTINAL PARASITES OF RED FOXES IN NORTH-EASTERN ITALY

Omodeo S.G.*[1], Cazzin S.[1], Danesi P.[1], Biasion L.[1], Zannini F.[2], Simonato G.[2], Francione E.[3], Pietrobelli M.[2], Capelli G.[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [2]Department of Animal Medicine, Production and Health, Padua University ~ Legnaro (PD) ~ Italy, [3]Istituto Zooprofilattico Sperimentale delle Venezie ~ Trento ~ Italy

INTRODUCTION:
During last years red fox (Vulpes vulpes) population in Europe showed a constant spread in urban and peri-urban areas (Wandeler et al., 2003, Mol Ecol.,12(3):647-56.). The same trend to urbanization has also been observed in Italy (Casulli et al., 2005, Int J Parasitol.,35(10):1079-83). Contamination of environment with their droppings constitutes an important risk for other carnivores and for zoonotic infections (Letkova et al., 2006, Veterinarski Arhiv, 76 (Suppl.), S73-S81).
The aim of this study was to assess the prevalence of the gastrointestinal parasites of red foxes in the Triveneto regions and to compare the performance of two different coprological techniques for the detection of cestode eggs.
MATERIALS AND METHODS:
The red foxes conferred to the Institute for rabies surveillance were screened from January 2012 to December 2013. A portion of the last intestinal tract was collected from each carcass, frozen at -80°C for a minimum of 72 hours and then stored at -20°C until further analysis. A standard coprological flotation test and a filtration/sieving technique modified to enhance the likelihood to detect cestode eggs (Mathis et al, 1996, J Helminthol.;70(3):219-22) were carried out. All the cestode eggs were collected and screened by a PCR targeting Taenid species (Trachsel et al, 2007, Parasitology, 134:911-920). All the PCR positive samples were sequenced.
RESULTS:
Overall, 1508 samples were collected in the territories of Belluno (6.5%), Bolzano (31.4%), Trento (19.6%), Trieste (1.1%), Gorizia (6.6%), Udine (31.8%) and Pordenone (3.1%). Seven hundred and ninety-seven samples (53%) were positive to at least one species of parasite, i.e. Capillaria spp. (39.6%), Toxocara canis (22.4%), Coccidia (5.4%), Trichuris vulpis (2.5%) and Ancylostomatidae (1.4%). Eighty samples (5.3%) were confirmed positives for cestode eggs by PCR and the sequencing identified Taenia crassiceps (25; 31.2%), T. polyacanta (17; 21.2%), T. serialis (1; 1.2%), T. taeniaeformis (1; 1.2%), Hymenolepis spp. (3; 3.7%), H. diminuta (1; 1.2%), Mesocestoides lineatus (3; 3.7%), M. litteratus (5; 6.2%), Mesocestoides spp. (1; 1.2%), Echinococcus spp. (10; 12.5%). Thirteen sequencing results are still pending and other genetic markers will be used to deepen the Echinococcus spp. positivity. Cestodes were found in all the province but Pordenone with prevalence of 1.8% (UD), 2% (GO), 4% (BL), 7.2% (BZ), 8.8% (TN) and 11.8% (TS).
For 1311 samples both coprological techniques were performed and the filtration/sieving technique detected 57 samples positive for cestode respect 35 of the standard test (moderate agreement; k coefficient=0.55).
CONCLUSIONS:
Respect to previous studies in the same area and other part of Europe (Reperant et al., 2007, Parasitol Res., 101(3):605-11), this study show a clear predominance of monoxenous parasites respect to dixenous helminths that depend on the consumption of rodent intermediate hosts, suggesting some ecological change, such as temporal variations of the intermediate host density, and/or changes in the hunting behavior of the foxes, attracted by anthropogenic food sources. The latter hypothesis is also confirmed by the cestode negative prevalence trend going from the Alpes to the lower land (UD and PN). The filtration/sieving technique was confirmed to be a more sensitive tool for cestode collection in fecal samples.

This work was supported by the Italian Ministry of Health (project code RC-IZSVe 03/2011)

Keywords:
gastro-intestinal parasites, red fox, zoonotic risk

O10.4
A FOCUS OF ECHINOCOCCUS MULTILOCULARIS IN FOXES OF NORTH-EASTERN ITALY: AFTER TEN YEARS IS STILL THERE

Dellamaria D.[1], Trevisiol K.[1], Francione E.[1], Citterio C.[1], Simonato G.[2], Cazzin S.[1], Casulli A.[3], Deplazes P.[4], Marangon S.[1], **Capelli G.*[1]**

[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro ~ Italy, [2]Department of Animal Medicine, Production and Health, Padua University ~ Legnaro ~ Italy, [3]MIPI Department, Istituto Superiore di Sanità ~ Rome ~ Italy, [4]Vetsuisse-Faculty, University of Zurich ~ Zurich ~ Switzerland

INTRODUCTION:
Echinococcus multilocularis represents, beside rabies, the major zoonotic agent that can be transmitted from foxes to humans through contamination of the environment with the cestode eggs. The aim of this study was to detect the presence of E. multilocularis in red foxes (Vulpes vulpes) of North-Eastern Italy, where a focus of the infection was reported in 2002 in the province of Bolzano (Manfredi et al., 2002, Vet Rec., 150:757; Casulli et al., 2005, Int J Parasitol., 35:1079–83).
MATERIALS AND METHODS:
In the period 2012-2013, adult foxes conferred to IZSVe for rabies surveillance were selected from the provinces of Belluno, Bolzano, Trento, Trieste, Gorizia, Udine and Pordenone. Fecal samples were frozen at -80°C for at least 48h and then tested using a filtration/ sieving technique modified to enhance the likelihood to detect Echinococcus eggs (Mathis et al., 1996, J Helminthol.,70(3):219-22). All the cestode eggs were collected and screened by a multiplex PCR targeting Taeniid species, E. granulosus and E. multilocularis (Trachsel et al, 2007, Parasitology, 134:911-920). All the PCR positive samples were sequenced.
RESULTS: Overall, 1508 foxes were screened and 80 (5.3%) were confirmed positives for cestode eggs by PCR (see table). Among these, sequencing identified four (0.8%) samples positive for E. multilocularis from adult animals, three females and one male, collected in the province of Bolzano. The prevalence of cestodes in foxes from Bolzano and Trento provinces was significantly higher than in foxes from Friuli Venezia Giulia (FVG) region ($\chi 2$ test, $p<0.01$).

Sampling area	foxes tested	cestode eggs pos (%)	*E. multilocularis* pos (% among tested)
Bolzano	473	37 (7.8)	4 (0.8)
Trento	295	26 (8.8)	-
Trieste, Gorizia, Udine, Pordenone (FVG region)	642	13 (2.0)	-
Belluno (Veneto Region)	98	4 (4.1)	-
total	*1508*	*80 (5.3)*	*4 (0.26)*

CONCLUSIONS:
After ten years from the first report, E. multilocularis is still present close to the previous area of infection, confirming a focus of autochthonous transmission as suggested by others (Casulli et al., 2009, Int J Parasitol., 39(7):837-42). The prevalence of E. multilocularis is lower than previously detected in Bolzano province (13%) (Casulli et al., 2005, Int J Parasitol., 35(10):1079-83.). These results could be explained by ecological changes, such as temporal variations of the intermediate host density. However the different prevalence between the studies could be also due to the different biological markers used for the amplification.
For the areas apparently free from E. multilocularis, the prevalence and distribution of the other cestodes, which recognize small rodents as intermediate hosts as well, could be used for risk analysis in order to identify areas at higher risk of E. multilocularis spread. More focused surveys are ongoing in the province of Bolzano in order to spatially define the affected area and to collect data needed to evaluate the possible implementation of a continuous level of surveillance.

This work was funded by the Italian Ministry of Health (project code: RC IZSVE 03/2011)

Keywords:
Echinococcus multilocularis, red foxes, zoonosis

O10.5
PREVALENCE OF EUCOLEUS BOEHMI INFECTION IN RED FOX (VULPES VULPES) FROM ITALY

Morganti G.*[1], Veronesi F.[1], Di Cesare A.[2], Ferroglio E.[3], Piergili Fioretti D.[1]

[1]Department of Veterinary Medicine ~ Perugia ~ Italy, [2]Faculty of Veterinary Medicine ~ Teramo ~ Italy, [3]Department of Veterinary Sciences ~ Turin ~ Italy

INTRODUCTION:
In the last decade red fox abundance increased in many European countries, including Italy, not only in wild or rural areas, but also in urban settlement. This fact, as well as the increase of pets visiting wild area with their owners, greatly increases the opportunities for mutual transmission of pathogens between foxes and dogs. In fact, evidences on the role of fox as reservoir host and amplifier for a broad spectrum of dog parasites, including some emerging extra-intestinal nematodes, have been showed (Traversa et al., 2010, Parasit Vectors, 3: 62). The scant information on the prevalence of Eucoleus boehmi (syn. Capillaria boehmi) on wild life reservoirs has stimulated the present study, which aimed at determining the prevalence of nasal eucoleosis in fox populations from Central-North Italy.
MATERIALS AND METHODS:
During the hunting seasons 2012–2013, 179 red foxes, culled in the rural or sub-urban areas of central and northern regions of Italy, were examined for the presence of E. boehmi. Information on gender, age, body condition and geographical provenance of each animal was collected. The skull of each fox was inspected for the presence of adult stages of the parasite. Intact specimens were counted and subjected to the identification at the species level according to bibliographic keys (Supperer, 1953, Z F Parasitenkunde, 16: 51-55). Moreover the sediment obtained from the scrapping of the mucosa surface was microscopically and genetically examined (Di Cesare et al., 2012, J Clin Microbiol, 50: 1958-1963). Prevalence and intensity for E. boehmi infection were calculated based on necropsy results. Animal variables aforementioned were individually analyzed for association with the likelihood of E. boehmi positivity and intensity of the infection by Chi-squared test and Kruskal-Wallis test respectively.
RESULTS:
Overall 55 foxes (30.72%) were found to be infected with E. boehmi, i.e. 27 at the macroscopical inspection of the skull and 28 at the mucosal flush and scrape. Prevalence of E. boehmi infection was significantly influenced by all the independent variables tested excepted for body condition ($p>0.05$). A total of 184 specimens of E. boehmi were recovered from the examined foxes with a mean intensity of infection of 3.34 (range 1-16). A significant relationship ($p<0.05$) was found between body condition, age and increased parasitic burden. Also age significantly influenced ($p<0.05$) the intensity of infection, being higher in juveniles than in adults. The highest worm prevalence (68.47%, 126/184 specimens) was reported in the nasal passages, whereas 31.23% of the specimens (58/184) were harbored in the sinuses.
CONCLUSIONS:
The present study describes the first results on epidemiological data of E. boehmi infection in foxes from Italy. This information provides a baseline for further studies aiming at elucidating the transmission dynamics of fox-originating E. boehmi infection in pets. Specifically there is a merit in understanding which are the factors implicated in the possible dissemination of respiratory nematodes and in the risk of their expansion into sub-urban or urban environments from wildlife to dog populations living in the same geographical areas.

Keywords:
Fox, Eucoleus boehmi, Italy

O10.6
ANALYSIS OF THE HELMINTHOFAUNA OF EUROPEAN WILD CAT IN FRIULI VENEZIA GIULIA

Beraldo P.*[1], Massimo M.[1], Pascotto E.[1]

[1]Division of Veterinary Pathology, Department of Food Science, University of Udine ~ Udine ~ Italy

INTRODUCTION:
The European wild cat (Felis silvestris silvestris) has a very broad distribution, found throughout most of Africa, Europe and Asia. In Italy new data (Mattucci et al., 2013, Ecol. Evol 3, 2443–2458) show a subdivision of European wild cats in at least four genetically distinct subpopulations: in the eastern Alps, in peninsular Italy (into two distinct groups), and in Sicily are sharply differentiated. Studies on wild cat helminthofauna are rather limited (Delahay et al., 1998, J. Zool. Lond. 245, 175-183; Gherman et al 2002, Sci. Parasitol. 3, 17-21; Krone et al., 2008, Eur J Wildl Res 54, 95-100), especially in Italy (Risitano et al., 2009, Sisvet Proceeding, 174-176). This study investigated the helminthofauna of wild cat in Friuli Venezia Giulia (FVG).
MATERIALS AND METHODS:
Between 2006 and 2013, the carcasses of 17 wild cats, died as results of road accidents, were collected throughout FVG. The carcasses (frozen before examination) were submitted to biometric analysis, necropsy and parasite collection by total worm count technique. Isolated parasites were identified, sexed and counted using morphometrical keys (Durette-Desset and Pesson 1987, Ann. Parasitol. Hum. Comp. 62, 326-344; Skryabin KI et al., 1991, In: Key to parasitic Nematodes. Strongylata. E.J. Brill, Leiden, The Netherlands; Khalil LF et al., 1994, Keys to the Cestode parasites of Vertebrates. Cab International, Hertfordshire, UK). Faecal samples were collected from the recta and examined for coprological diagnosis. Descriptive statistic, including prevalence, mean intensity, mean abundance, importance value and richness were calculated for each helminth species.
RESULTS:
A total of thirteen endoparasite species were found, among which the nematodes Toxocara mystax (P=94.1%, Im=40) and Ancylostoma tubaeforme (P=76.5%, Im=48) and the cestode Taenia taeniaeformis (P=76.5%, Im=7) were the most prevalent parasites in the gut, while Eucoleus aerophilus (P=52.9%, Im=4) and Troglostrongylus brevior (P=47.1%, Im=7) in the lungs. Other helminths detected were: Molineus legerae, Aonchotheca putorii, Physaloptera rara, Trichuris sp., Macracanthorynchus catalinum, Mesocestoides litteratus, Diplopylidium sp. in the gut, and Aelurostrongylus sp. in the lung. The host population showed polyparasitism condition characterised by 5 parasite species/animal (range2-7).
CONCLUSIONS:
This study represents the first investigation on wild cat helminthofauna in FVG. The endoparasite spectrum of this wild cat population is partially comparable to that recorded in other parasitological surveys in Italy and Europe. M. legerae seems to be detected for the first time in wild cat and in FVG; while Mesocestoides litteratus has been isolated for the first time in FVG. All the wild cats were parasitized in the gastro-intestinal tract, while the 76.4% in the lung. The animal body condition score was medium/good suggesting that the wild cat parasite community in FVG is in equilibrium with host population. Nonetheless, at necropsy, the lung lesions were diffuse and marked, ascribable to verminous pneumonia. Although this study is interesting as far as a mammal species with high conservation value, the low sample size does not allow yet to achieve adequate eco-parasitological considerations.

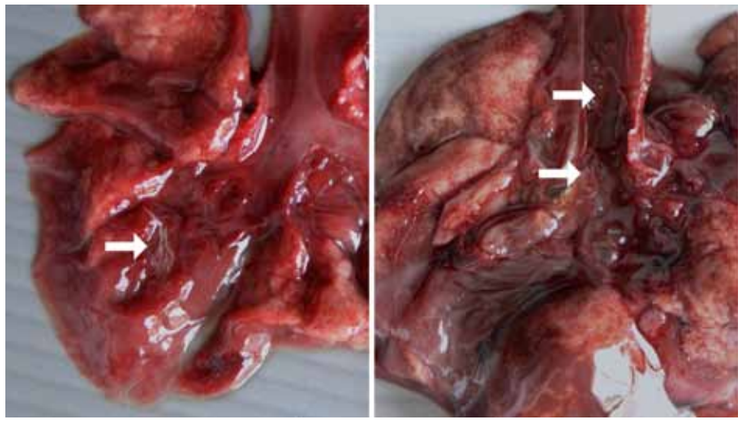
Keywords:
European wild cat, parasite community, endoparasites

O10.7
EUROPEAN WILDCATS (FELIS SILVESTRIS SILVESTRIS) AS SPREADERS OF LUNGWORMS

Falsone L.*[4], Brianti E.[4], Gaglio G.[4], Napoli E.[4], Anile S.[1], Mallia E.[3], Giannelli A.[2], Poglayen G.[5], Giannetto S.[4], Otranto D.[2]

[1]Dipartimento di Biologia Animale "Marcello La Greca", Univesità degli Studi di Catania ~ Catania ~ Italy, [2]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [3]Parco Regionale Gallipoli Cognato e Piccole Dolomiti Lucane, Basilicata ~ Matera ~ Italy, [4]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina ~ Messina ~ Italy, [5]Dipartimento di Scienze Mediche Veterinarie,Università degli Studi di Bologna ~ Bologna ~ Italy

INTRODUCTION:
In spite of the increasing number of reports of troglostrongylosis in domestic cats in Europe (Brianti et al., 2014, Vet. Parasitol., in press), no data is available on the occurrence of this parasite in wildcats. Indeed, scientific data on the lungworms of this wild felid is scarce (Krone et al., 2008, Eur. J. Wildl. Res., 54:95-100). This study aims to investigate the lungworm species infecting wildcats, in order to assess their impact on the health of this endangered carnivorous and to elucidate their role as potential bridging hosts for this parasite to domestic animals.
MATERIALS AND METHODS:
Wildcat carcasses were received from two Natural Parks situated in Basilicata and Sicily. Crops were biometrically and molecularly identified at sub-species level (Mattucci et al., 2013, Ecol. Evol., 3:2443-2458) and examined for lungworms. Morphometric data and causes of death were registered for each animal. Epidemiological indexes such as prevalence, mean abundance, mean intensity and aggregation k-index were calculated and statistically analyzed for each parasites species (Bush et al., 1997, J. Parasitol., 48:575-583).
RESULTS: A total of 21 wildcats (12 females and 9 males) were examined, being 7 yearlings and 14 adults. Based on both biometrical and molecular analysis 19 were classified as pure Felis silvestris silvestris, whereas the remaining two were hybrids. Fifteen wildcats were found positive for Troglostrongylus brevior and 7 for Eucoleus aerophilus, being 5 (23.8%) animals co-infected by both lungworm species. Epidemiological indexes calculated for T. brevior and E. aerophilus respectively are reported on the follows: prevalence: 71.4% and 33.3%; mean abundance 4.7 and 0.8; mean intensity 6.6 and 2.4; aggregation k-index 0.69 and 0.42.
The main cause of death was road casualties (66.6%) followed by illegal hunting (19.1%) and starvation (14.3%). No significant differences were recorded among animal variables neither between regions for both lungworm species. The rate of infection by T. brevior was larger in yearlings (85.7%) than in adults (64.3%), and mature worms were collected from the lungs of two 1-month-old kittens (Figure 1).



Lungs of two one month-old wildcats infected by Troglostrongylus brevior.

CONCLUSIONS:
Results of the present survey indicate that wildcats may be highly infected with T. brevior and E. aerophilus and might act as spreaders of these parasites to sympatric domestic animals. In spite of the large prevalence for lungworms herein observed, the aggregated distribution pattern recorded for both species suggests that these parasites do not represent a biological threat for the studied population (Lafferty, 2012, Philos. Trans. B., 367:2814-2827). Based on the topography, climatic features and prey spectrum of geographical regions where wildcats were collected, a similar picture might be envisaged in other wildcat populations living across the Mediterranean basin.

Keywords:
Wildcat, Troglostrongylus brevior, Eucoleus aerophilus

O10.8
OCCURRENCE OF ENDOPARASITES IN ROE DEER (CAPREOLUS CAPREOLUS) IN SPAIN: INFLUENCE OF AGE

Morrondo P.*[1], Pérez A.[1], Pajares G.[2], Prieto A.[1], Cabanelas E.[1], Panadero R.[1], López C.[1], Fernández G.[1], Díaz P.[1], Díez Baños P.[1]

[1]University of Santiago de Compostela ~ Lugo ~ Spain, [2]Asociación del Corzo Español (ACE) ~ Madrid ~ Spain

INTRODUCTION:
The main goal of the current study was to determine the possible influence of age in the prevalence and intensity of elimination of different endoparasites affecting roe deer in Spain.
MATERIALS AND METHODS:
During 2012-2013, occurrence of several parasitic infections was studied in 55 roe deer. Animals were distributed into two groups according to their age: younger than 3 years (Y=19) and adults (A=36). Flotation and sedimentation techniques were performed to determine the elimination of coccidian oocysts and trematode, cestode and gastrointestinal nematode eggs. Larval migration was used to detect bronchopulmonary nematodes. Microscopic cysts of Sarcocystis were quantified in heart muscle by using the compression method. Humoral response to Toxoplasma and Neospora was investigated by means of using direct agglutination test and competitive ELISA, respectively.
RESULTS:
Parasitic forms were found in the 60% of the investigated animals. However, no roe deer positive to Paramphistomidae or Neospora were observed.
The prevalence of Toxoplasma was similar in both age groups (Y=17.2%; A=15.8%). On the contrary, the prevalence and intensity of elimination of Eimeria oocysts in young animals 26.3%, =480±366 opg) were higher than in adults (13.8%, =300±330 opg) as well as for Sarcocystis (Y 100%; =432±796 vs A 85.7%; =78±129). Moreover, young animals shedding higher number of gastrointestinal nematode eggs (=1380±2699 epg) than the adults (=890±1807 epg), but the percentage of infection was similar in both age groups (26.3% in young and 27.8% in adults). A limited number of adult animals (2%) shed Moniezia and Fasciola eggs and Dictyocaulus larvae, making difficult the determination of the influence of age.
CONCLUSIONS:
In general, young roe deer showed higher prevalences and intensities of elimination that could suggest a certain level of age dependent protective response.

This work was supported by the Asociación del Corzo Español and by a Grant (CN2012/326) of the Xunta de Galicia for Consolidating and Structuring Competitive Research Groups.

Keywords:
roe deer, age, endoparasites

O10.9
DIRECT LIFE CYCLE ENDOPARASITES IN ROE DEER (CAPREOLUS CAPREOLUS) FROM SPAIN: INFLUENCE OF CLIMATIC FACTORS ON PREVALENCE AND INTENSITY OF ELIMINATION

Morrondo P.*[1], Pérez A.[1], Díaz P.[1], Prieto A.[1], Panadero R.[1], López C.[1], Fernández G.[1], Pajares G.[2], Díez Baños P.[1]

[1]University of Santiago de Compostela ~ Lugo ~ Spain, [2]Asociación del Corzo Español (ACE) ~ Madrid ~ Spain

INTRODUCTION:
The main objective of this study was to report the effect of edaphoclimatic conditions on the prevalence and intensity of elimination of the main direct life cycle endoparasites affecting roe deer from different areas of Spain.
MATERIALS AND METHODS:
A total of 55 roe deer were examined in this study. All of them were hunted in 2012-13 in diverse areas located on the North, Centre and South of the Iberian Peninsula and characterized by different edaphoclimatic conditions. The northern area, with an average altitude of 630 m above the sea level, has an oceanic climate; the average altitude in the Central area is about 900-1100m with a continental climate and the Southern area has a Mediterranean climate and an average altitude of 185 m.
Quantitative coprologic analysis, by using flotation and larval migration techniques, were performed to determine the number of Eimeria oocysts per gram of faeces (opg), eggs of gastrointestinal nematodes (epg) and larvae of Dictyocaulus (lpg).
RESULTS:
18.2% of the analyzed roe deer shed an average of 435±332 opg of Eimeria. The prevalence and intensity of elimination were higher in roe deer from the North (42.9%; x=566.7±448.1) than in those from the Centre (21.4%; x=200±259.8) and the South (11.8%; x=512.5±268.9), with significant differences between animals from the North and the South ($\chi2=3.96$; p=0.046). 27.3% of the roe deer exhibited high gastrointestinal nematode egg counts (1053±2059 epg). The prevalence and egg count were higher in roe deer from the North (71.4%; x=2930±2863.5) than in those from the Centre (50%; x=78.6±39.3) and the South (8.8%; x=200±218), differences in the percentages of infection between the animals from the North and the South were significant ($\chi2=3.96$; p=0.046). The percentage of roe deer excreting Dictyocaulus noerneri larvae was low (4.8%) and they were only detected (4.6±5.9) in roe deer from the North of the Peninsula.
CONCLUSIONS:
Roe deer hunted in the North of Spain presented the highest prevalence and intensity of elimination by direct lifecycle parasites, probably because edaphoclimatic conditions in this area are more favorable for the development and survival of their free-living stages.

This work was supported by the Asociación del Corzo Español and by a Grant (CN2012/326) of the Xunta de Galicia for Consolidating and Structuring Competitive Research Groups.

Keywords:
roe deer, climatic factors, endoparasites

O10.10
COMPARISON BETWEEN SEROLOGICAL AND MOLECULAR DIAGNOSTIC APPROACH TO TOXOPLASMA GONDII INFECTION IN ALPINE RED DEER (CERVUS ELAPHUS)

Formenti N.*[1], Gaffuri A.[2], Trogu T.[1], Ferrari N.[1], Pedrotti L.[3], Viganò R.[4], Lanfranchi P.[1]

[1]Dipartimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di Milano ~ Milano ~ Italy, [2]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, sezione di Bergamo ~ Bergamo ~ Italy, [3]Consorzio Parco Nazionale dello Stelvio ~ Bormio (Sondrio) ~ Italy, [4]Medico Veterinario – AlpVet ~ Verbania ~ Italy

INTRODUCTION:
The widespread Toxoplasma gondii affects several domestic and wild animal species raising public health issues related to its zoonotic value. As wild ungulates can be source of human infection, risk should be evaluated in relation to the amount of game meat available from harvest plans and besides a reliable diagnostic trial to monitoring T. gondii in free-ranging populations should be defined.
Focusing on red deer (Cervus elaphus), because of intense hunting activity in many Alpine areas and since this species is consumed even raw or undercooked, we performed a serological and molecular investigations of Toxoplasma gondii to evaluate (i) the reliability of cardiac tissue fluids as an alternative to sera in ELISA test and (ii) the applicability of three PCR protocols.
MATERIALS AND METHODS:
Overall 78 sera and cardiac tissue fluids and 159 brain tissue samples were collected for respectively serological and molecular investigations from Lepontine Alps (VB) during the hunting season 2011 and during the culling management plan scheduled by the Stelvio National Park in 2012.
A commercial ELISA kit (IDVET, Montpellier, France), validates for ruminants' sera and tissue fluids, was used and the agreement between analytical approach was assessed calculating the Kappa (K) value.
DNA was extracted with the QIAamp DNA Mini Kit (Qiagen, Italy) and the whole samples were assayed by targeting a 529 bp non-coding region (Homan et al., 2000, Int. J. Parasitol., 30:69-75) (protocol 1). Positive and doubtful results were then submitted to a single tube nested PCR (Hurtado et al., 2001, Vet. Parasitol., 102:17-27) (protocol 2) and to a PCR-RFLP using primers that identify also Neospora caninum and Sarcocystis spp. (Magnino et al., 1998, Proc. IX ICOPA:1269-1272) (protocol 3).
RESULTS:
A T. gondii prevalence of 29% and 19% was recorded respectively in sera and cardiac fluids showing a "fair agreement" (K value = 0.38) between the two matrices and a loss in sensitivity and specificity using cardiac fluids.
The whole analysed samples resulted negative for T. gondii DNA. In 15 samples doubtful PCR products with no-specific bands resulted from the protocol 1 and thus they were submitted to the second and the third PCRs. The other two protocols cleaned PCR amplified products confirming the T. gondii negativity but Sarcocystis spp. DNA was detected by protocol 3 in a 6-month-old male and in a 1-year-old female. Sequencing analysis identified Sarcocystis hjorti DNA from the calf brain tissue.
CONCLUSIONS:
The study highlights a few diagnostic difficulties in both T. gondii serological and molecular investigation in red deer. Although working with wildlife it could be easier sampling an heart than a serum sample, cardiac tissue fluids cannot be considered as an alternative of serum in this species using this ELISA test.
The doubtful results emerged using PCR protocol 1 suggest that this molecular technique can have a lower specificity than two others with red deer samples and besides protocol 3 was useful to detect the cross reaction with Sarcocystis spp.. As application of diagnostic tests developed from livestock or human to wildlife could compromise their performances, the need is to set up diagnostic methods specific for wild species mainly for pathogens of public health significance.

Keywords:
Toxoplasma gondii, red deer, diagnostic approach

O10.11
VARIABILITY AT NEUTRAL AND ADAPTIVE MARKERS IN THE ENDANGERED TATRIC CHAMOIS

Cavallero S.*[1], Vichova B.[2], Chovancová B.[3], Paule L.[4], Snabel V.[2], D'Amelio S.[1]

[1]Department of Public Health and Infectious Diseases ~ Rome ~ Italy, [2]Institute of Parasitology, Slovak Academy of Sciences ~ Kosice ~ Slovakia, [3]Tatra National Park Research Centre ~ Tatranská Lomnica ~ Slovakia, [4]Technical University ~ Zvolen ~ Slovakia

INTRODUCTION:
Rupicapra r. tatrica is a subspecies of the genus Rupicapra listed as Critically Endangered by the IUCN; it occurs as a single autochthonous population in the Tatra Mountains. Two significant bottlenecks caused population decrease (Zemanova B et al 2011, Folia Zool, 60: 70) and a continuing decline is probable because of epidemics or potential interbreeding with introduced alpine chamois in the Low Tatras. This scenario may drive to genetic variability loss, an important event for chamois inhabiting semi-isolated, fragile and fragmented habitats, with low rates of colonization and reduced gene flow between populations (Brown JH 2001, Global Ecol Biogeogr, 10: 101). Genetic variation analyses at neutral and adaptive markers may improve the understanding of natural events, geographic features, and dynamics of natural populations.
With the aim to investigate the genetic variability in Tatra chamois, three genomic regions were analysed: one adaptive marker (MHC class II DRB1 exon2) and two neutral markers (mitochondrial cytochrome B, cytb; control region, D-Loop). The Major Histocompatibility Complex is a sensitive marker for genetic variation of populations: the allelic variants influence many important biological traits. Its variability is affected by intrinsic factors, pathogens, population dynamics and it's ecologically relevant depending on host-pathogen relationships and demography. These features place MHC genes among the best candidates to study mechanisms and significance of molecular adaptation in vertebrates (Sommer, 2005, Frontiers in Zool, 2: 16; Mona et al 2008, Mol Ecol, 17: 4053; Cavallero et al, 2012, Infect Genet Evol, 12: 1020). Cytb and D-loop regions have been already investigated to infer the impact of translocations and habitat fragmentation in chamois and they are good for comparison (Crestanello et al 2009, J Hered, 100: 691).
MATERIALS AND METHODS:
Three genomic regions were analysed: MHC for 48 chamois, 12 at cytb and 26 at D-Loop using haplotype analysis, to define haplotypic composition and to estimate variability indices, in relation to population structure and possible bottlenecks. Phylogenetic analyses were carried out to infer relationships among haplotypes.
RESULTS:
Haplotype inference of exon 2 DRB1 of MHC class II in 48 Tatra samples showed 10 alleles (7 new described and 3 previously observed alleles) with a high level of homozygosity. Haplotype diversity was rather low (Hd=0.32). The most frequent allele found in Tatra Chamois (RuruDRB*28) is shared with Alpine chamois. 12 readable sequences were obtained for cytb region, showing a very low variability (2 haplotypes observed with a very low Hd=0,17) and a great similarity to previously described sequences of both alpin and tatric chamois. 23 haplotypes were observed in the D-loop region with Hd = 0.9, a value slightly higher to that previously observed both in Tatra and in Alpine chamois.
CONCLUSIONS:
Given the endangered status of the species, the trend in genetic variation in Tatra chamois population should be systematically observed, also in relation to MHC variation as an indicator of the ability to respond to eventual epidemic events. In fact, parasitic disease due to lung nematodes have contributed considerably to the morbidity of chamois in the Slovak National Park (Štefancikova et al, 2010, Galemys: 81-101). The differences in haplotype composition observed represent a strong basis to monitor the potential interbreeding with introduced alpine chamois from the Low Tatras.

Keywords:
tatric chamois, genetic variability, endangered species

O10.12
DETECTION OF CRYPTOSPORIDIUM AND GIARDIA BY ELISA ASSAY IN ALPINE CHAMOIS (RUPICAPRA R. RUPICAPRA)

Trogu T.*[1], Formenti N.[1], Ferrari N.[1], ViganÒ R.[2], Lanfranchi P.[1]

[1]Dipartimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di Milano ~ MILANO ~ Italy, [2]Medico Veterinario – AlpVet ~ VERBANIA ~ Italy

INTRODUCTION:
Giardia and Cryptosporidium are the most common enteric protozoa of humans and domestic animals and recently they are being increasingly recognised as parasites of several wild species (Appelbee et al., 2005, Trends Parasitol., 21:370-376). Therfore it emerged the importance of their monitoring in wildlife in a health ecosystem perspective. As alpine chamois (Rupicapra r. rupicapra) is the most abundant and hunted ungulate in the Italian Alps and no information are available about Giardia and Cryptosporidium in this host, we investigated the presence of these protozoa in a chamois population evaluating their spreading in relation to different age classes.
MATERIALS AND METHODS:
Faecal samples were collected from rectal ampoule of 92 hunted chamois (kids=5; yearling=33; 2-3 years=11; >3 =43), within the day of shooting during October 2013, in Lepontine Alps (VB). Faeces were frozen at -20°C and then analysed by a commercial kit ELISA searching for protozoan copro-antigens (RIDASCREEN® Giardia - R-Biopharm; RIDASCREEN® Cryptosporidium - R-Biopharm). For each subject anamnestic data reported by hunters and morpho-biometric measures were recorded during post-mortal examination.
RESULTS:
ELISA showed a prevalence of 4,3% (4/92) for Giardia, three yearlings and one 6-month-old kid tested positive, while a prevalence of 1,1% (1/92) was recorded for Cryptosporidium with a positivity in a 2 years old chamois. All the subjects were in a good body condition with normal behaviour; the post-mortem examination did not reveal any systemic macroscopic lesions. Concerning age class, data suggest a higher susceptibility of younger subjects than elder classes in agreement with domestic trend (Santin et al., 2004, Vet. parasitol., 122(2):103-17).
CONCLUSIONS:
As far as we know this is the first detection of these parasites in alpine chamois, giving evidence of the circulation of the investigated protozoa in alpine area. Despite the low prevalences recorded, the importance of protozoa monitoring should be underlined considering the increase of wild ungulate populations and their frequent interaction with livestock, mainly for mountain summer grazing, and the related economic implications. Moreover the zoonotic potential of these protozoa should be taken into consideration not only in relation to handling of carcasses by hunters and game keepers, often in field conditions without optimal cleanliness, but also regarding their transmission by water, a critical issue for all outdoor activities, increased in the latest years. Considering the well documented sanitary importance in livestock, any impact on chamois juvenile classes can not be excluded and this aspect should be deepen in relation to population dynamics. In this regard ELISA test can be considered a valuable assay for a fast screening on the presence of Giardia and Cryptosporidium, also concerning the need to develop non-invasive diagnostic techniques applicable to every wildlife reality. However, molecular studies are needed to confirm the obtained results and to provide additional information on the species/genotypes of isolates.

Keywords:
Giardia, Cryptosporidium, Rupicapra

O10.13
INTESTINAL PROTOZOANS IN RUPICAPRA SPP. POPULATIONS: BASELINE DATA IN THE FRAMEWORK OF THE RELEVANT ITALIAN PROJECT (PRIN)

De Liberato C.*[1], Trogu T.[2], Ferretti F.[3], Berrilli F.[4], Marani I.[4], Putignani L.[5], Santoro M.[5], Marangi M.[6], D'Amelio S.[7], Giangaspero A.[6]

[1]IZS Lazio e Toscana ~ Roma ~ Italy, [2]University of Milan ~ Milan ~ Italy, [3]University of Siena ~ Siena ~ Italy, [4]University of Tor Vergata ~ Rome ~ Italy, [5]OPBG ~ Rome ~ Italy, [6]University of Foggia ~ Foggia ~ Italy, [7]University Sapienza ~ Rome ~ Italy

INTRODUCTION:
Wild ungulates are affected by several protozoans which can seriously threaten their performances and well-being; it is therefore likely that selective pressure on adaptations to such parasites is high. Wildlife can also have an important role as sources, reservoirs and amplifiers of protozoans of public health significance. One aspect of the 3-year Relevant Italian Project (PRIN) 2013-2016 - 'Genomics and host-pathogen interactions in chamois' is a study of the most common intestinal protozoans affecting wild ungulates. Therefore, the aims of the present study were to investigate the presence, prevalence and parasitic burden of Eimeria, Giardia and Cryptosporidium (G/C) in Rupicapra spp. and to molecularly characterize the isolates.
MATERIALS AND METHODS:
From September 2013 to January 2014, 155 fresh faecal samples were collected from Rupicapra rupicapra rupicapra in the Western Central Alps and Pre-Alps in Lombardy (101 samples) and from Rupicapra pyrenaica ornata in the Abruzzo, Lazio and Molise National Park (54 samples). The samples were examined using standard copro-parasitological methods to detect Eimeria, and an immunofluorescence test (Kit MERIFLUOR® Meridian Diagnostic, Cincinnati, OH, USA) for microscopy detection of Cryptosporidium and G. duodenalis. Parts of ITS-1, gp60 and β-giardin genes were tested to identify Eimeria, Cryptosporidium and G. duodenalis species/genotypes, respectively.
RESULTS:
Overall, one hundred and thirty-two (85%, CI=78.6-90.3) of the animals investigated scored microscopically positive to Eimeria spp. with a burden of up to 4650 o.p.g.; seven (4.5%, CI=1.8-9.1) were found positive to G. duodenalis. In R. r. rupicapra the prevalence values were 88.1% (CI=80.1-93.7) for Eimeria and 5.9% (CI=2.2-12.5) for Giardia, while in R. p. ornata the prevalence values were 90.8% (CI=79.7-96.9) for Eimeria and 1.8% (CI=0.5-9.8) for Giardia. So far, none of the animals has resulted positive to Cryptosporidium using IFA and molecular tests.
CONCLUSIONS:
The results show that the prevalence of Eimeria and Giardia in chamois living in the study areas is non-negligible. The high prevalence of Eimeria spp. and its burden confirms previous results (Stancampiano et al., 2003, J. Mt. Ecol., 7:175–183; Martella et al., 2003, J. Mt. Ecol., 7:251-256), while the detection of G. duodenalis is noteworthy because this is the first report in Rupicapra spp.
The PRIN project is currently ongoing; broader and more in-depth data are needed regarding the Eimeria species and Cryptosporidium and Giardia species/genotypes in order to provide insights on the impact of protozoan infections on the dynamics and sustainability of chamois populations, also from a One Health perspective.

The study was supported by MIUR- Relevant Italian Project (PRIN) 2013-2016 - Genomics and host-pathogen interactions in chamois.

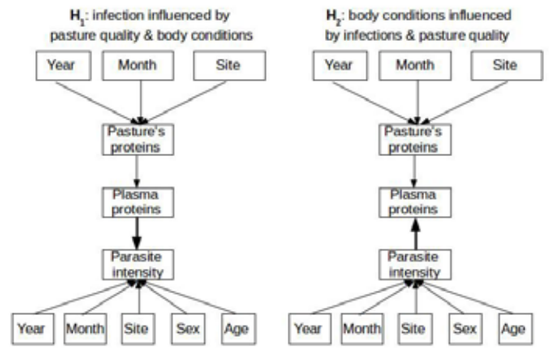
Keywords:
Rupicapra spp, chamois, intestinal protozoans

O10.14
DOES DECREASED BODY CONDITION CAUSE INCREASED SUSCEPTIBILITY TO PARASITIC INFECTION OR HIGH PARASITE INTENSITY CAUSE DECREASED BODY CONDITION? DEALING WITH THIS DILEMMA BY OBSERVATIONAL DATA

Ferrari N.*[1], Formenti N.[1], Trogu T.[1], Sartorelli P.[1], Citterio C.[2], Tarantola M.[3], Lanfranchi P.[1]

[1]DIVET, Università degli Studi di Milano ~ Milano ~ Italy, [2]Istituto Zooprofilattico Sperimentale delle Venezie ~ Belluno ~ Italy, [3] Dipartimento di Scienze Veterinarie Università di Torino ~ Grugliasco ~ Italy

INTRODUCTION: The inference of causality from observational data has several limits since directionality of the relationship between variables can not always be determined clearly. The relation between host body condition and parasite intensity represents a valid example (Belodmenico PM et al, 2009, Trends Ecol Evol, 25: 21-27) since individuals in poor conditions can be more susceptible to infections and at the same time parasitized individuals can show decreased conditions status as consequence of the detrimental effects of infection. To discriminate which of these two mechanism occurs in nature authors have approached this issue either artificially, manipulating parasite infection and body condition through additive feeding(Pedersen AB et al, 2008, J Anim Ecol, 77: 370-377) , or following individuals during time and registering the temporal changes of parasite infections and body condition in order to identify which factor change anticipates the other(Beldomenico PM et al, 2008, Proc R Soc B, 275: 1753-1759). However, for cross section observational data, the identification of the cause and consequences between these factor remains problematic.
Path analysis, through Structural Equation Modelling, deals with sequential relationships between multiple factors, allowing to estimate their effects and test theoretical models of causality (Shipley B 2000, Cause and Correlation in Biology, Cambridge Univ Press).
In this study, we test the hypothesis H1, generated by field observations where alpine chamois (Rupicapra rupicapra) from areas with declined pasture quality registered decrease in body conditions which led to increased parasite infections, against the alternative hypothesis H2 that parasites induced decline in body condition irrespective of the pasture quality (Figure).



MATERIALS AND METHODS:
In this study, we test the hypothesis H1, generated by field observations where alpine chamois (Rupicapra rupicapra) from areas with declined pasture quality registered decrease in body conditions which led to increased parasite infections, against the alternative hypothesis H2 that parasites induced decline in body condition irrespective of the pasture quality (Figure).
In 261 chamois shot during 5 hunting seasons we quantified the species composition of abomasal parasite community, blood serum metabolic profile, pasture quality through the ruminal content and individual features. We focused our attention on the intensity of infection of Haemonchus contortus, the dominant parasite species even known for its impact on protein metabolism. As a body condition proxy we focused on blood proteins and, for the pasture quality, we considered the protein content. Through SEM, we then tested the two alternative hypotheses.
RESULTS:
Results show that H1, predicting parasite infection as influenced by compromised body conditions as a result of decreased pasture quality, has a lower likelihood compared to the alternative hypothesis H2. In this respect, the parasite infection is mostly influenced by host and extrinsic factors and subsequently it compromises the host body condition, which is at the same time affected by decreasing forage quality.
CONCLUSIONS:
These results indicate that, while environmental changes may affect animal populations through reduced feeding availability, the effect of parasite infections is not influenced by these changes but sums up additionally being directly influenced by host and extrinsic factors.

Keywords:
Susceptibility, parasite impact, nutrition

O10.15

PRE-ANALYTICAL BIAS MAY SEVERELY IMPACT ON THE RELIABILITY OF COPROMICROSCOPY DATA IN CHAMOIS AND OTHER MOUNTAIN DWELLING UNGULATES

Rossi L.*[1], Victoriano Llopis I.[1], Rambozzi L.[1], Demeneghi D.[1], Diaz Alcazar E.[1]

[1]Dept. Veterinary Sciences ~ TORINO ~ Italy

INTRODUCTION:
Use of non invasive parasitological data (eg., copromicroscopic data) as hallmarks of individual and herd fitness in free-ranging wildlife is tempting due to low cost, ease of the techniques and the possibility to obtain repeated samples and/or a large sample size. With the increased emphasis on faecal egg counts analysis, there is a need to revisit the methodology and identify procedures that can be implemented as good management practices. Yet, little attention has been paid to evaluating pre-analytical factors such as collection and storage of faecal samples prior to analysis. The aim of this contribution is showing to which extent desiccation and freezing of faecal samples from Southern chamois (Rupicapra pyrenaica ornata) and Barbary sheep (Ammotragus lervia) may mirror negatively on the accuracy of parasitological results, hence on reliability of the associated conclusions.
MATERIALS AND METHODS:
Two experiments (A and B) were carried out on the effects of desiccation on fecal egg counts in two study locations: the Abruzzo, Lazio and Molise National Park, Italy (R. pyrenaica ornata) and the Sierra Espuna National Reserve, Spain (A. lervia). Fresh faeces (20 and 10 in experiments A and B, respectively) were collected from soil and refrigerated (4°C) during transport. Samples were subdivided in two aliquots: the first one (meant as the "gold standard" for eggs of gastro-intestinal nematodes and oocysts of Coccidia) was analysed in default of any additional treatment, whereas the second was incubated at 30°C 40% RU for 48 hours prior to analysis. In experiment A, "old" (partially dried) faeces collected from soil on the same date as fresh ones were used for further comparison. Counts were carried out of the eggs of gastro-intestinal nematodes (EPG), oocysts of Coccidia (OPG) and larvae of pulmonary nematodes (LPG), in Mc Master chambers (6 chambers/sample). In a third experiment (C), counts were similarly carried out of feacal samples of R. pyrenaica ornata (N=20) before and after one-month storage at -18°C. When relevant, data were analysed with non parametric statistical tests.
RESULTS:
Experiment A: incubator desiccation resulted in lower EPGs and OPGs (85 and 84% decrease, respectively), higher prevalence of larvae of pulmonary nematodes (50 vs 15%) and much higher LPGs (135 vs 4). Differences were even larger in naturally desiccated "old" samples from the same herd. Experiment B: incubator desiccation in a different model resulted in lower EPGs and OPGs (95 and 99% decrease, respectively) and lower prevalence of gastrointestinal nematodes (20 vs 80%) and Coccidia (60 vs 100%). Experiment C: one-month freezing resulted in lower prevalence of gastro-intestinal nematodes (40 vs 90%) and remarkably lower EPGs (98% decrease). Coccidia and larvae of pulmonary nematodes were not or mildly affected.
CONCLUSIONS:
Results show that pre-analytical physical factors have a significant negative effect on the detectability of parasitic eggs and oocysts and, eventually, the opposite is true for larvae of pulmonary nematodes. Noteworthy, interpretation of our parasitological data in terms of herd fitness would have been severely biased by field selection and/or storage modalities of faecal samples, eg., the chamois herd investigated in (A) would have appeared either extensively and heavily challenged by gastro-intestinal nematodes and little challenged by pulmonary nematodes based on fresh samples analysis, and just the opposite based on "old" samples.
Support was granted by the Abruzzo, Lazio and Molise National Park.

Support by the

Keywords:
wildlife, coprological data, pre-analytical bias

NOTES

NOTES

SESSIONE PARALLELA O-11A

INFEZIONI DA MICETI

O11A.1
MOLECULAR EVIDENCE OF POLYMORPHISM IN PNEUMOCYSTIS FROM WILD MICE AND SHREWS IN THE EASTERN ALPS, ITALY

Da Rold G.[1], Rizzoli A.[2], Hauffe H.C.[2], Arnoldi D.[2], Tagliapietra V.[2], Demanche C.[3], Guillot J.[4], Capelli G.[1], **Danesi P.*[1]**

[1]Istituto Zooprofilattico delle Venezie, Parasitology Laboratory ~ Legnaro (PD) ~ Italy, [2]Fondazione E. Mach, Research and Innovation Centre, Department of Biodiversity and Molecular Ecology, S. Michele all'Adige ~ Trento ~ Italy, [3]Parasitology Dept, Faculty of Biological and Pharmaceutical Sciences ~ Lille ~ France, [4]Parasitology-Mycology Dept, Dynamyc research group, National Veterinary College of Alfort ~ Maisons-Alfort ~ France

INTRODUCTION:
Pneumocystis organisms are considered as major opportunistic fungal pathogens that infect humans and a wide range of other mammalian host species. The genus comprises uncultured, host-specific parasites adapted to the alveolar epithelial cells type I where they may proliferate provoking severe pneumonitis. Thus far, five species have been described: P. carinii and P. wakefieldiae in brown rats, P. jirovecii in humans, P. murina in common mice and P. oryctolagi in rabbits.
In the present study, we detected and characterised Pneumocystis organisms in wild mice and shrews from eastern Alpes. Our objective was to assess whether Pneumocystis organisms could be used as phylogeographic markers for these mammalian species.
MATERIALS AND METHODS:
A total of 96 lung tissue samples from wild rodents and insectivores were analyzed. Specimens of Apodemus flavicollis (n=30), Apodemus sp (n=1), Myodes glareolus (n=3), Sorex antinorii (n=61), and Sorex sp (n=1) were captured as part of an large collaborative European project (EDENext) by Fondazione Edmund Mach. After DNA extraction, samples were analyzed by nested PCR, using primers for the gene encoding the mitochondrial large subunit rRNA (mtLSU rRNA) (Derouiche et al. 2009) and sequenced. The mtLSU sequences were aligned with already known Pneumocystis sequences using Clustal X (version 1.63b) and a phylogenetic analysis was performed using Neighbour Joining integrated in Mega 4 software (version 4.0).
RESULTS:
Pneumocystis DNA was detected in 58 of 96 samples (60%). Lung tissues from Apodemus flavicollis (12/30; 40%), Sorex antinorii (44/61, 72%) and Myodes glareolus (2/3; 67%) were positive. The mtLSU sequences showed marked polymorphism with seven Pneumocystis sequence types representing three distinct major clades according to the three animal species. Among Sorex genus all sequences presented a similarity of 100%, whereas six different sequence types were obtained for the genera Apodemus (n=4) and Myoedes (n=2).
CONCLUSIONS:
These preliminary observations are in agreement with previous findings, which reported similar prevalence of Pneumocystis in Apodemus and Myodes animals as well as the highest prevalence in Sorex spp., when compared with other wild mammals (Laakkonen et al., 1995, Can J Zoology, 73:961-966). The genetic polymorphism in Apodemus and Myodes has already been described and seem to be a general tendency for rodent-derived Pneumocystis. The unique sequence type detected among Sorex shrews from the eastern Alpes suggests that Pneumocystis from insectivores display a lower level of genetic polymorphism.
This study was supported by Veneto Region and partially supported by EU grant FP7-261504 EDENext (http://www.edenext.eu) and the Fondazione E Mach.
The presentation of this work is sponsored by Cutech srl BIOTECHNOLOGY

Keywords:
Pneumocystis, wild mice, Molecular polymorphism

O11A.2
SURVEY ON YEASTS IN MUCOSAE OF DOGS WITH AND WITHOUT WEAKENING PATHOLOGIES

Galuppi R.*[1], Garutti S.[1], Fracassi F.[1], Capitani O.[1], Tampieri M.P.[1]

[1]Dipartimento di Scienze Mediche Veterinarie - Università Di Bologna ~ Bologna ~ Italy

INTRODUCTION:
In human medicine many studies point out as yeasts, commensals on mucosae, can became pathogens in course of immunodeficiency, chemotherapy, cortisone therapy, endocrinopathies (Bulacio et al, 2012, J. Mycol. Med., 22: 348-353). Such risk factors were also suggested in Veterinary Medicine but, so far, only few studies about this topic were made (Tampieri et al 2008, Parassitologia, 50: 95; Bieganska et al., 2012, 18th congress ISHAM, p.177). In this paper, the yeast flora of oral, conjunctiva and rectal mucosae of dogs was examined, in relation to presence ("sick dogs") or absence ("healthy dogs") of predisposing factors such as endocrinopaty, neoplasy, cortisone therapy or other weakening pathologies.
MATERIALS AND METHODS:
One hundred eight dogs (61 "healthy" and 47 "sick") were examined; from each dog 4 swabs from oral, conjunctival and rectal mucosae were collected and inoculated in duplicate on Sabouraud Dextrose Agar (BBL) with 0.05g/ chloramphenicol (Sigma). The plates were incubated both at 25°C and 37°C ad observed daily for 7 days. The yeasts isolated were identified microscopically for Malassezia pachydermatis and with Api 20C AUX (Biomerieux Italia S.p.a, Firenze, Italy) and Dalmau plates on Yeast Morphology Agar DIFCO (Becton Dickinson Italia S.p.a, Milano, Italy) for the other yeasts.
RESULTS:
Yeasts were found in 83 dogs out of 108 examined (76.8%). M. pachydermatis was isolated in at least one sample from 78/108 dogs (73.2%), especially in rectal mucosae where also the major number of yeast species was present. Conversely, lowest yeasts isolation was from conjunctival swabs. The other yeasts isolated were: Rhodotorula spp (8.34%), Candida albicans (3.7%), C. glabrata (2.78%), Cryptococcus laurentii (1.85%), C. guilliermondii, C. parapsilosis, C. boidinii, Saccharomyces cerevisiae and Cr. albidus (0.92%). M. pachydermatis was found more frequently in healthy dogs (83.6%) compared to sick dogs (57.4%) ($X^2\gamma = 6.18$, $p>0.05$). On the contrary, other yeasts were found more frequently in sick dogs (15.22%) than in healthy dogs (6.2%) (Fisher exact $p<0.05$). In particular C. albicans, isolated only in 4 sick dogs (8.7%), was not found in healthy ones (Fisher exact $p<0.05$). In one dog with parvovirosis, C. albicans was isolated from an ulcer of oral mucosa that resolved without specific treatment after healing.
CONCLUSIONS:
M. pachydermatis was the yeast most frequently present on dogs mucosae, according to Tampieri et al. (2008 l.c.), Brito et al. (2009, Vet. J., 182: 320-326) and Santin et al. (2013, Braz. J. microbiol., 44: 139-143) and can be considered an usual component of mucosal microflora of dog. The isolation of S. cerevisiae could be explained by the administration of probiotics. C. boidinii, C. parapsilosis, Cr. laurentii, Cr albidus and Rhodotorula spp. isolated, with low number of colonies, only at 25°C, could be considered as environmental contaminants and not colonizer, contrary to C. glabrata and, especially, C. albicans that were isolated both at 25°C and 37°C, only in sick dogs and in high number of colonies. From these observations we can suppose that may be a real colonization of mucous membranes. Unlike what happens in humans, where C. albicans is a frequent commensal in the mucosa of healthy subjects, in the dog the finding of such yeast is occasional and could be a proof of organic imbalance.

Keywords:
Yeast, Mucosae, Dog

O11A.3
MALASSEZIA AND CANDIDA SPECIES INDUCING HUMAN BLOODSTREAM INFECTION

Iatta R.*[1], Otranto D.[1], Montagna M.T.[2], Cafarchia C.[1]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [2]Dipartimento di Scienze Biomediche e Oncologia Umana, Università degli Studi di Bari ~ Bari ~ Italy

INTRODUCTION:
The occurrence of bloodstream infections (BSI) by Candida spp. and Malassezia spp. increased due to the growing number of immunocompromised patients (Miceli et al., 2011, Lancet. Infect. Dis., 11:142-51). However, whilst fungemia by Candida species has been recognized as a cause of morbidity and mortality in hospitalized patients worldwide (Pfaller et al. 2012, Diagn. Microbiol. Infect. Dis., 74:323-331), the role of Malassezia spp. in determining fungemia remains largely unknown. This study reports the results of a one-year survey on Malassezia and Candida species causing BSI in patients admitted to the Neonatal Intensive Care Unit (NICU) and to the surgical pediatric ward of an Apulia hospital.
MATERIALS AND METHODS:
From July 2011 - 2012, 290 neonates or preterm infants and 17 pediatric patients with complicated post surgery features, were enrolled in the survey. After the onset of fungemia, clinical data (i.e., age, gender, underlying diseases, antifungal prophylaxis and therapy, day of catheter removal and fungemia duration) were collected in individual anamnestic files, along with skin swabs. Biological specimens and swabs were subjected to culture on Sabouraud Dextrose Agar with 0.5% chloramphenicol and Dixon Agar and incubated at 37°C for 5 days and at 32°C for 10 days, respectively. Following preliminary yeast identification by the automated system Vitek2 (Biomerieux, France), Malassezia spp. was characterized based on morphological, biochemical and molecular features (Cafarchia et al., 2011, Mol. Cell. Probes, 25:1-7).
RESULTS:
Of the 12 fungemia episodes registered, 10 came from NICU, of which 6 (2.1%) were caused by M. furfur and 4 by Candida spp. (1.4 %; i.e., 2 C. parapsilosis, 1 C. albicans, and 1 C. glabrata). The remaining 2 (11.8%) BSI cases, from the pediatric ward, were caused by M. furfur. All patients received total lipid parenteral nutrition for 38 days in average, via a central venous catheter (CVC). Seven patients were preterms (4 with M. furfur and 3 with Candida spp. fungemia) and five of them (3 with M. furfur and 2 with Candida spp. fungemia) received systemic antifungal prophylactic treatment with fluconazole (3 mg/Kg/72h). Only patients with M. furfur BSI were yeasts skin colonized. Fungemia by M. furfur and Candida spp. occurred after an average of 26 days and 42 days of hospitalization, respectively. After the onset of fungemia all patients were treated with intravenous liposomal Amphotericin B (5mg/Kg/day) and resolved Candida spp. and M. furfur BSI after 6 and 16 days, respectively.
CONCLUSIONS:
Although neither clinical features nor predisposing factors herein examined (i.e., CVC, parenteral lipid nutrition, preterm birth, extended length of hospitalization) allowed discriminating M. furfur from Candida spp. BSI, skin colonization was associated to M. furfur fungemia. Therefore lipid-supplemented culture media should be routinely used to diagnose Malassezia infection.

Keywords:
Bloodstream infections, Malassezia furfur, Candida spp.

O11A.4
ANTIFUNGAL SUSCEPTIBILITY OF MALASSEZIA FURFUR FROM BLOODSTREAM INFECTIONS

Iatta R.*[1], Figueredo L.A.[2], Otranto D.[1], Cafarchia C.[1]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [2]Department of Immunology, Aggeu Magalhães Research Centre, Recife-PE, Brazil; Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy

INTRODUCTION:
Malassezia furfur, a lipid-dependent yeast component of human skin microbiota, may occasionally cause systemic infections in immunocompromised hosts (Gaitanis et al., 2012, Clin. Microbiol. Rev., 25:106-141). Recently, the useful of a prompt and proper therapy has been assessed in clinical guidelines for the diagnosis and management of these infections (Arendrup et al., 2013, Clin. Microbiol. Infect., doi: 10.1111/1469-0691.12360). The microdilution antifungal method (CLSI BMD, M27-A3) was used for testing the antifungal susceptibility of Malassezia species (Cafarchia et al,, 2012, Vet. Microbiol., 159:536-540) although there is a need for establishing optimal broth media for M. furfur. Thus, this study aims to: i) evaluate the in vitro susceptibility of M. furfur isolates from bloodstream infections (BSI) to amphotericin B (AMB), fluconazole (FLC), itraconazole (ITC), posaconazole (POS) and voriconazole (VRC); ii) assess the optimal medium for testing the susceptibility of M. furfur.
MATERIALS AND METHODS:
A total of 36 M. furfur isolated from BSI of patients before (Group I=16) and during (Group II=16) AMB therapy or receiving FLC prophylaxis (Group III=4) were tested. Sabouraud destrose broth (SDB) with 1% of Tween 80 (SDB), RPMI 1640 buffered with MOPS and supplemented with 2% glucose, 0.4% ox bile, 1% Tween 40, 0.2% glycerol and 0.2% oleic acid (RPMI) and Christensen's urea supplemented with 0.1% Tween 80 and 0.5% Tween 40 (CUB), were used as culture media. Since tentative breakpoints have not yet been established for M. furfur, the criteria used for Candida spp. were adopted for the results interpretation (CLSI, 2008).
RESULTS:
ITC, VRC and POS displayed lower MICs values than FLC and AMB. SDB was the only medium able to detect isolates with high fluconazole MICs in patients receiving FLC prophylaxis. A high number of AMB resistant strains (from 87.5% to 100%) was registered in all groups regardless of the media used.
CONCLUSIONS:
Results suggest that SDB is a suitable medium for determining the triazole susceptibility but not for AMB. Similarly, both the drug formulation and/or the breakpoint herein applied are not useful for assessing the AMB susceptibility of M. furfur from BSI.

Keywords:
Antifungal susceptibility, Malassezia furfur, CLSI broth microdilution method

O11A.5
THERAPEUTIC ACTIVITY OF A KILLER PEPTIDE AGAINST MALASSEZIA PACHYDERMATIS OTITIS IN DOGS

Immediato D.*[1], Di Paola G.[1], Mogliani W.[2], Ciociola T.[2], Conti S.[2], Polonelli L.[2], Otranto D.[1], Cafarchia C.[1]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy, [2]Dipartimento di Scienze Biomediche, Biotecnologiche e Traslazionali, Università degli Studi di Parma ~ Parma ~ Italy

INTRODUCTION:
Malassezia pachydermatis is a commensal yeast of the skin of animals, which may become pathogenic under the influence of predisposing factors (Cafarchia et al., 2010, Med. Mycol., 45:11-15). Clinical manifestations, in domestic animals, include otitis externa/media and dermatitis, which therapeutic management is usually performed using azoles, topically or systemically in the case of severe manifestations (Negre et al., 2009, Vet. Dermatol., 20:1-12; Bond, 2010, Clin. Dermatol., 28:226-236). However, dogs may require regular maintenance of antifungal therapy to prevent relapse, therefore representing a risk for the development of resistance (Bond, 2010, Clin. Dermatol., 28:226-236; Cafarchia et al., 2012, Vet Microbiol., 155:395–402). The threat of such resistance has spurred the interest of the scientific community to discover new effective antifungal drugs. Recently a killer decapeptide (KP), engineered from the variable region of a single-chain recombinant antiidiotypic antibody, displayed in vitro and in vivo antifungal effect versus Candida yeasts (Polonelli et al., 2003. Infect. Immun., 71:6205–6212). This study aims to evaluate: i) the in vitro antifungal activity of KP against M. pachydermatis isolates and ii) the in vivo therapeutic activity versus Malassezia otitis externa in naturally infected dogs.
MATERIALS AND METHODS:
A total of 16 dogs naturally affected by M. pachydermatis otitis externa were enrolled in the study and the fungicidal activity of KP was tested in vitro on the isolated yeasts. Clinical signs (pruritus, erythema and lichenification and/or hyperpigmentation) were expressed as mean of Total Clinical Score Index (mTCIS). The therapeutic activity was evaluated on four groups of four animals each. Group A and B included animals treated topically with KP (150 µl, 2 mg/ml) three times per week or every day, respectively. Group C and D were used as controls and animals included were treated with a scramble peptide every day or left untreated, respectively. The population size of M. pachydermatis at the cytological examination (mean number of yeast cells, mYC) and cultural testing (mean number of Colony Forming Units/swab expressed as mean of Log10 CFU, mCFU) were registered daily from the first day of treatment (T0) until the achievement of two negative consecutive cultures (mCFU≤2).
RESULTS:
KP showed an in vitro fungicidal effect against M. pachydermatis isolates with an MFC90 of 1 µg/mL. The mTCIS and mYC were negative only in animals from Group B after 8 days (T+8). The mCFU decreased significantly ($p<0.05$) in Group A and B at T+4 and T+2, respectively. However, negative results (mCFU≤2) were recorded only in animals from Group B at T+8. No adverse reactions were observed in treated animals.
CONCLUSIONS:
Daily administration of KP for 8 days was safe and effective to control both clinical signs and population size of M. pachydermatis causing otitis externa, thus offering an alternative to the currently available therapeutic or prophylactic protocols for recurrent cases of Malassezia otitis in dog.

Keywords:
Malassezia pachydermatis, dog otitis, killer peptide

O11A.6
EVALUATION OF TOXICITY OF EUCALYPTUS SPP. ESSENTIAL OILS ON BEAUVERIA BASSIANA

Oliveira Arruda De Abreu G.[1], Cafarchia C.[2], Otranto D.[2], Dantas Torres F.[1], Pinto Brandão Filho S.[1], **Aguiar Figueredo L.*[1]**

[1]Centro de Pesquisas Aggeu Magalhães, Departamento de Imunologia. Fundação Oswaldo Cruz - FIOCRUZ ~ RECIFE ~ Brazil, [2] Dipartimento Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy

INTRODUCTION:
Beauveria bassiana is an entomopathogenic fungus widely studied to control arthropods of medical and veterinary concern, including phlebotomine sand flies, such as Lutzomyia longipalpis, the main vector of Leishmania infantum in the New World (Amóra et al., 2009, Bio. Control, 50:329-335). Meanwhile, Eucalyptus essential oils have also been proven effective for the control of L. longipalpis due to their insecticide properties (Maciel et al., 2010, Vet. Parasitol., 167:1-7). The use of natural compounds alternative to chemotherapeutics, for the control of phlebotomine sand flies, would benefit humans and environment by reducing chemical insecticides application. It is well known that the insecticide effect of B. bassiana may be potentiated or reduced when associated with others insecticides (Tamai et al., 2002, Arq. Inst. Biol., 69:89-96). However, no studies have demonstrated the toxicity of Eucalyptus essential oils on B. bassiana, in order to develop a biological insecticide formulation for the control of L. longipalpis. The aim of this study was to assess the toxicity of Eucalyptus essential oils of the species Eucalyptus staigeriana, Eucalyptus citriodora, and Eucalyptus globulus, on different concentrations on B. bassiana spore suspensions.
MATERIALS AND METHODS:
The B. bassiana strain URM-6702 (from Micoteca URM–UFPE, Pernanbuco, Brazil), isolated from Hypothenemus hampei (Coleoptera: Curculionidae) was cultured in Sabouraud Dextrose agar for 14 days. Spore suspensions were prepared from the plate colonies using mineral oil. The concentrations were determined using a hemocytometer and adjusted to approximately 5x104, 5x106, 5x108 spores/mL. Plates containing potato dextrose agar with essential oils at different concentrations (0.1%, 0.2%, 0.4%, 0.8% and 1.6%) of each species (E. staigeriana, E. citriodora and E. globulus) were prepared (Amóra et al., 2009, Bio. Control, 50:329-335). A total of 100 µL of each B. bassiana spore suspension were cultured on each plate in duplicate and read after 8 and 14 days of incubation. The numbers of colony-forming unit (CFU) were counted to evaluate the vegetative growth, and the conidiogenesis was evaluated by spores counting of the CFU using a hemocytometer (Tamai et al., 2002, Arq. Inst. Biol., 69:89-96).
RESULTS:
The toxicity of Eucalyptus on B. bassiana was observed according to the Eucalyptus species and to the concentration used. Eucalyptus citriodora inhibited the growth of the B. bassiana in 100% of the plates when used at the concentration of 0.4% or higher. Eucalyptus globulus inhibited the fungi growth only at a concentration of 1.6%, whereas E. staigeriana at all the concentrations tested. The vegetative growth was directly proportional to the concentration of B. bassiana spore in the suspensions. The conidiogenesis was higher on the concentrations of 0.2% for both, E. citriodora and E. globulus.
CONCLUSIONS:
Results indicate that E. staigeriana is toxic and therefore not indicated for the development of insecticide formulation in association with B. bassiana. On the other hand, E. citriodora and E. globulus were toxic dose-dependent. Therefore these essential oils at low concentrations could be useful for the development of a natural insecticide formulation in association with B. bassiana. Further studies should be encouraged towards the evaluation of the efficacy of this new insecticide formulation to control L. longipalpis.

Financial support: FIOCRUZ, CNPq and FACEPE.

Keywords:
L. longipalpis, Biological control, Entomopathogenic fungus

O11A.7

GENOTYPE AND MATING TYPE CHARACTERISATION OF CRYPTOCOCCUS NEOFORMANS ISOLATES FROM ITALIAN VETERINARY ISOLATES

Danesi P.*[1], Capelli G.[1], Cogliati M.[2], Peano A.[3], Cafarchia C.[4], Granato A.[1], Meyer W.[5]

[1]Istituto Zooprofilattico delle Venezie, Parasitology Laboratory ~ Legnaro (PD) ~ Italy, [2]Medical Mycology Laboratory, Department of Scienze Biomediche per la Salute ~ Milan ~ Italy, [3]4Departmen of Veterinary Medicine, University of Turin ~ Turin ~ Italy, [4]Department of Veterinary Medicine, University of Bari ~ Bari ~ Italy, [5]Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Sydney Medical School - Westmead Hospital,The University of Sydney, Westmead Millennium Institute ~ Sydney ~ Australia

INTRODUCTION:
Cryptococcus neoformans is a ubiquitous animal and human fungal pathogen that causes infections of the central nervous system, predominantly in immunocompromised hosts. The reproduction in C. neoformans involves cells of opposite mating types (mating type a and α), which are controlled by one locus with two alternative functional alleles (MATa and MATα). C. neoformans has been classified into three serotypes (serotype A, D and AD hybrids) and into four major molecular types named VNI, VNII, VNIII, VNIV on the basis of capsular agglutination reactions and different molecular typing techniques, respectively. As these genotypes differ in host range, epidemiology, virulence, antifungal susceptibility and geographic distribution, the aim of this study was to investigate the genetic characteristics of veterinary C. neoformans isolates in order to better understand their epidemiology and population structure.
MATERIALS AND METHODS:
Seventeen C. neoformans isolated from animals (cats n=14, falcon n=1, dogs n=2) and laboratory standard reference strains representing each of the four major molecular types of C. neoformans have been analysed (Meyer et al., 2009. Med. Mycol., 47:561–570). Isolates were cultured on Sabouraux destrose agar at 37°C for 48-72 hours. Genomic DNA was isolated and the major molecular types were determined by URA5-RFLP analysis, as previously described (Meyer et al., 2003, Emerg. Infect. Dis., 9:189–195). The digested PCR products were visualized and compared to the reference strains on 3% agarose gels, stained with ethidium bromide. Isolates with mixed patterns were considered to be potential hybrids. Mating types (MATα/MATa) were determined by specific PCR (Halliday et al., 1999, J. Clin. Microbiol., 37: 2920-2926). In addition the mating type and serotype allele pattern were determined in nine C. neoformans isolates by a multiplex PCR (Esposto et al., 2004, Clin. Microbiol. Infect., 10: 1092–1094).
RESULTS:
Molecular types VNI (n=7, 41%) and VNIV (n=7, 41%), as well as two different types of hybrids VNIII (n=2, 14%) and VNII/VNIV (n=1, 7%) where identified (Table 1). The molecular type VNI was identified from cats (n=5), a falcon (n=1) and a dog (n=1), while VNIV came from cats (n=6) and a dog (n=1). Hybrids VNIII (n=1) and VNII/VNIV (n=1) were found in asymptomatic cats, the other VNIII isolate was from the cutaneous lesion of a feline clinical case. Mating and serotype specific PCR revealed that the majority of isolates were MATα (n=15; 88%) and serotype A or D (i.e. were αA or αD). The remaining two VNIII hybrids possessed αADa (n=1) and aADα (n=1) mating and serotype.
CONCLUSIONS:
The results of this study show a similar prevalence amongst the molecular types VNI and VNIV in veterinary isolates from northern Italy in contrast to previous findings on human cryptococcosis, which reported an unbalanced molecular type distribution, with a higher prevalence of VNI in the south and VNIV in the north of the Italian peninsula (Viviani et al., 2006. FEMS Yeast Res., 6: 614–619). These new findings provided evidence that C. neoformans hybrids can be carried and spread by asymptomatic animals in the environment. Monitoring hybridization in C. neoformans is important, as it has been suggested to may represent an evolutionary driving force for increased fitness of this yeast.
This study was supported by Veneto Region

Keywords:
Genotype and mating type, Cryptococcus neoformans, veterinary isolates

NOTES

NOTES

SESSIONE PARALLELA O-11B

IMMUNITÀ E IMMUNOPATOLOGIA DELLE INFEZIONI PARASSITARIE

NOTES

O11B.1
B CELL EPITOPES OF HEMOGLOBIN IN ANISAKIS

González Fernández J.[1], **Daschner A.*[2],** Natalie N.[3], Cuéllar C.[1]

[1]Departamento de Parasitología, Facultad de Farmacia, Universidad Complutense ~ Madrid ~ Spain, [2]Servicio de Alergia, Instituto de Investigación Sanitaria, Hospital Universitario de la Princesa ~ Madrid ~ Spain, [3]Department of Immunology, Max Planck Institut für Infektionsbiologie ~ Berlin ~ Germany

INTRODUCTION:
The transport of oxygen is a recently evolved function of globins which has developed with the emergence of multicellular organisms. The hemoglobin of the midge Chironomus thummi thummi was the first characterised (inhalant) allergen. We recently showed that Anisakis hemoglobin is a potent allergen which induces specific IgE antibodies, but unexpectedly these antibodies did not recognise hemoglobin of Ascaris. Ascaris spp. are cosmopolitan nematodes which parasitise humans and pigs and have a long history of host-parasite interaction. Anisakis is not adapted to humans but to marine mammals. Our hypothesis is that different evolution in host-parasite interaction of both nematodes could have shaped hemoglobin proteins to display different IgE recognition.
MATERIALS AND METHODS:
Hemoglobin sequences of C. thummi, Ascaris suum, Homo sapiens, Sus scrofa, Anisakis pegreffi and Tursiops truncatus were obtained from the NCBI protein database. Corresponding PDB structures were obtained from rcsb.org but A. pegreffi, A. suum, T. truncatus hemoglobin were modeled using HHPred from www.proteinmodelportal.org. These models were evaluated using QMEAN server. Chi t 1 epitopes were searched on the above mentioned hemoglobin molecules. Discotope 2.0 was used for the prediction of these and other new discontinuous B cell epitopes on the hemoglobin three dimensional structures. The results were visualised on the surfaces of the molecules with PyMol and epitopes were defined. Calculations of area and electrostatic potential were obtained from Getarea 1.1 and APBS web server.
RESULTS: Five epitopes were predicted in Anisakis hemoglobin while only four were obtained in the case of Ascaris. The electrostatic potential, relevant for antigen-antibody interactions showed important differences: both Anisakis and Ascaris hemoglobin had negative potential but electrostatic potential of Anisakis hemoglobin was much more negative than Ascaris. It was also observed that most hemoglobin epitopes were located at the interfaces of electrostatic potential.
The Chi t 1 equivalent IgG binding region (Epitope 1 in Figure) was present when the other hemoglobin molecules were aligned (approximately between amino acid residues 64-82). This region showed a similar DiscoTope Score profile in the hemoglobin chain of Ascaris and Anisakis. In the case of vertebrate hemoglobin (human, pig, dolphin) this region had not enough Discotope Score to be considered B cell epitope.
Moreover, the Chi t 1 equivalent IgE binding region was also identified (Epitope 2 in Figure) but not in vertebrate hemoglobin molecules. However, the Chi t 1equivalent IgE binding regions identified on Anisakis were longer than in Ascaris hemoglobin and further their Discotope Scores were much higher. When different Discotope Score profiles were compared, no differences were observed in the first part of this region between Ascaris and human hemoglobin molecules. However, different profiles were obtained when IgE binding regions of Ascaris and Anisakis hemoglobin were compared.

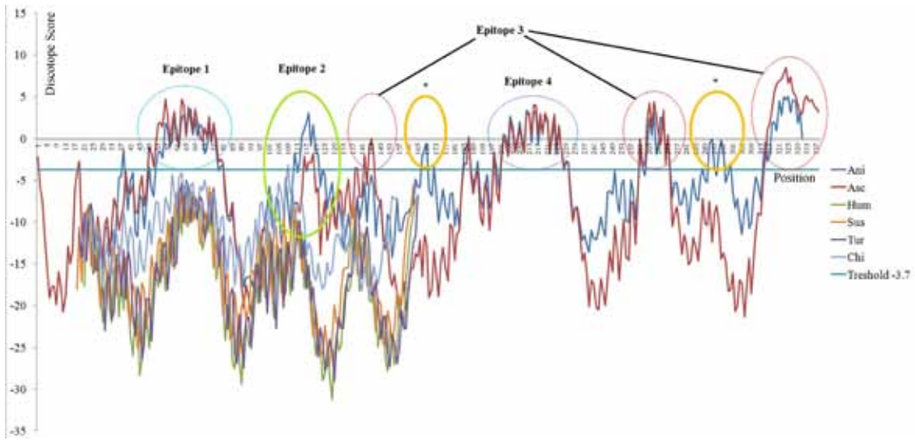


Figure 1. Discotope score of three dimensional hemoglobin from Ani (*Anisakis pegreffi*), Asc (*Ascaris suum*), Hum (*Homo sapiens*), Sus (*Sus scrofa*), Tur (*Tursiops truncatus*) and Chi (*Chironomus tummi*). Discotope treshold score for epitope identification was defined -3.7 by default. The asterisk (*) shows an epitope which is not present in *Ascaris.*

CONCLUSIONS:
A specific epitope in Anisakis, the higher negative electrostatic potential and higher Discotope Score in Anisakis haemoglobin could explain the known allergenicity of Anisakis and lack of cross-reactivity with Ascaris for the case of hemoglobin.

Keywords:
Allergy, Epitopes, IgE

O11B.2
HUMAN SENSITIZATION TO ANISAKIS PEGREFFII-ANTIGENS: COMPARISON OF TWO IMMUNOLOGICAL METHODS IN ALLERGIC ANISAKIASIS DIAGNOSIS

Mori Ubaldini F.*[1], Crisafi B.[2], Fazii P.[3], Bruschi F.[1], Mattiucci S.[2]

[1]Department of Translational Research,N.T.M.S., Università di Pisa ~ Pisa ~ Italy, [2]Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, Italy ~ Roma ~ Italy, [3]Hospital "Spirito Santo" ~ Pescara ~ Italy

INTRODUCTION:
The aim of this study was to compare two different immunological methods (namely, immunoCAP=iCAP and Western Blot=WB) to detect specific anti-Anisakis IgE recognizing the two major antigens/allergens, Ani s1 and Ani s 7 (Daschner, et al. 2014, Scand. J. Immunol., 79:120-126) to define their role in diagnosing a state of sensitization to the nematode antigens/allergens.
MATERIALS AND METHODS:
The patients for this study were recruited in the AOU Pisana, Pisa, Clinical University Hospital Umberto I, Romeand "Spirito Santo" Hospital, Pescara. The sera of 41 patients having a history of raw fish consumption and/or allergic manifestation, or resulted positive to the larval detection of Anisakis pegreffii at gastric-intestinal level, were analysed by immunoCAP=iCAP and Western Blot=WB and the sensitivity and specificity of the two methods were calculated.
RESULTS:
34 of the 41 sera tested by iCAP resulted positive (>0.1KUA/L) for the presence of Anisakis-IgE. However, among them, only 6 confirmed their positivity at the WB, with a correspondence of only 31% between the two methods. In the six cases, the iCAP and the WB were positive, with a p4>100 KUA/L and the serum reactivity to the Ani s 1-IgE and Ani s 7-IgE respectively. Among them, two patients presented the classic clinical manifestations of Gastro Allergic Anisakiasis (GAA) (Daschner et al., 2011, Trends in Parasitol, 28:9-15; Mattiucci et al., 2013, Emerg Inf Dis, 19: 496-450) with the detection of larvae penetrated in the gastric wall; the larva was identified as A. pegreffii. Whereas, 4 patients presented clinical symptoms of epigastric pain accompained by allergy, after the raw fish consumption, with or without larval detection; in these last cases the larval identification was not possible.
In addition, in three cases of gastric anisakiasis, with the larval molecular identification as A. pegreffii, the sera did not show reactivity to Ani s 1-IgE or to Ani s 7-IgE; these patients did not suffered of allergic reaction. However, a high level of iCAP reaction was observed.
CONCLUSIONS:
In 76% of cases sera with a high Anisakis-IgE concentration found at the iCAP (p4>0,35KUA/L) did not confirm positivity, using the WB. Overall, the average specificity was around the 20% for the iCAP, whilst it resulted of the 100% in the WB. The recorded sensitivity was optimal for both iCAP and WB.
Two main reasons might explain this finding: i) cross-reactive antibodies directed against non-parasite related epitopes; in this case the WB usually shows a pronounced reactivity against the 37kDa band, corresponding to a panallergenic protein ; ii) an underlying atopic state of the patient who might have IgE directed against Anisakis antigens; these cases usually present a widespread reactivity against several bands in the WB, but not against specific Ani s 1 or Ani s 7.

Keywords:
Anisakis, WB, serodiagnosis

O11B.3
PROTECTIVE ROLE OF HAEMOGLOBIN S AND C AGAINST ASYMPTOMATIC PLASMODIUM FALCIPARUM PARASITAEMIA

Mangano V.*[1], Kabore Y.[2], Bougouma E.C.[2], Verra F.[1], Sepulveda N.[3], Bisseye C.[1], Santolamazza F.[1], Avellino P.[1], Diarra A.[2], Nebié I.[2], Sirima S.[2], Modiano D.[1]

[1]Sapienza University of Rome ~ Rome ~ Italy, [2]Centre National de Recherche et Formation sur le Paludisme ~ Ouagadougou ~ Burkina Faso, [3]London School of Hygiene and Tropical Medicine ~ London ~ United Kingdom

INTRODUCTION:
HbS protects against severe and uncomplicated Plasmodium falciparum malaria and is associated with lower parasite densities in symptomatic subjects. HbC is also known to additively protect against severe malaria. However, conclusive evidence of a protective role of HbS and HbC against symptomless P. falciparum infection has not yet been obtained, as studies consisting on a single cross-sectional survey have yielded conflicting results (Taylor et al, 2012, Lancet Infect. Dis., 12(6):457–68).
MATERIALS AND METHODS:
Our study aimed at providing new insights into the protective role of HbS and HbC against asymptomatic P. falciparum infection. We conducted 5 cross-sectional surveys at regular intervals during two consecutive years, in four rural villages of Burkina Faso, West-Africa. The study involved about 2200 individuals belonging to Fulani, Mossi and Rimaibe communities (Modiano et al., 1996, Proc. Natl. Acad. Sci. U. S. A., 93(23):13206–11). P. falciparum parasitaemia (positivity and counts) has been microscopically diagnosed at each time point. Genomic DNA has been extracted from whole blood and the rs334 (A→HbA/T→HbS) and rs33930165 (G →HbA / A→HbC) Single Nucleotide Polymorphisms at the HBB locus have been genotyped using the Sequenom MassArray System. To evaluate the protective role of HbS and HbC against P. falciparum infection we compared the following phenotypes between AA (reference) and AC, AS, CC, SC subjects: i) prevalence of asexual parasitaemia at each of 5 surveys; ii) proportion of subjects infected at least once over 5 measurements; iii) number of infections over 5 measurements; iv) mean parasite density over 5 measurements; iv) proportion of subjects who remain non infected across 5 surveys.
RESULTS:
In single cross-sectional survey analysis, we observed that individuals who are heterozygotes for HbS show lower prevalences of parasitaemia compared to AA homozygotes in some but not every survey, and not always in both study populations. This observation reflects discrepancies among results of studies based on single cross-sectional surveys. However, using a longitudinal approach over the five surveys, we were able to demonstrate that AS heterozygotes show a significantly lower period prevalence of infection, lower number of infections, lower mean parasite density, and longer time to first infection than AA homozygotes both in Fulani and Non-Fulani, suggesting a protective role of HbS against the establishment of P. falciparum parasitaemia.
With respect to HbC, our data do not provide evidence for protection against any of the susceptibility phenotypes analysed at the heterozygote state, i.e. in AC subjects. Nevertheless, our data suggest a protective effect of HbC in a double mutant state, i.e. in CC homozygotes and SC double heterozygotes. The level of protection observed is similar if not higher to that observed in AS subjects. However, given the low frequencies of these genotypes, our study sample size is too small to draw solid conclusions.
CONCLUSIONS:
These observations outline a scenario made of consistent experimental and epidemiological observations. The abnormal display of parasite adhesive molecules on the surface of HbS and HbC infected erythrocytes (Fairhurst et al., 2005, Nature, 435(7045):1117–21; Cholera et al., 2008, Proc. Natl. Acad. Sci. U. S. A., 105(3):991–6), disrupting the pathogenic process of sequestration, might displace the parasite from the deep to the peripheral circulation hence promoting its elimination at the spleen level.

Keywords:
Malaria, Haemoglobinopathies, Burkina Faso

O11B.4
OUTBREAK OF TRICHINELLOSIS IN GARFAGNANA (TUSCANY): CLINICAL AND LABORATORY OBSERVATIONS

De Gennaro M.[1], **Bruschi F.*[2]**, Luciana R.[1], Mattei R.[1], Gomez Morales M.A.[3], Pozio E.[3], Luchi S.[1]

[1]Ospedale di Lucca ~ Lucca ~ Italy, [2]Università di Pisa ~ Pisa ~ Italy, [3]EURLP-Istituto Superiore di Sanità ~ Roma ~ Italy

INTRODUCTION:
In 2012, a trichinellosis outbreak involving 34 people occurred in Tuscany for the consumption of raw sausages made with pork from a hunted wild boar. The aim of this work was to describe the clinical and laboratory patterns of the infected people.
MATERIALS AND METHODS:
Between November 25 and December 31, 2012, 38 individuals (23 males and 15 females; mean age 47.8 years, range 7-80 years) living in Garfagnana, an area of the Lucca province (Tuscany), referred to the Division of Infectious Diseases and Hepatology of the Lucca hospital. Thirty-six of them reported the consumption of raw sausages prepared with either pig or wild boar meat in a 30% and 70% proportion, respectively. Only the wild boar meat resulted to be infected by Trichinella britovi larvae. Four people were not identified as cases, being completely asymptomatic, and reporting to have consumed only well-cooked sausages; whereas, 34 persons were considered true cases.
RESULTS:
Signs and symptoms appeared between 10 and 30 days after the ingestion of infected meat (mean 18 days). Fever (76.4%), facial/periorbital oedema (52.9%), myalgia (52.9%), diarrhea (45%), abdominal pains (29.4%), and diffuse erithema (24%), were the more frequently observed signs and symptoms. Eighteen patients (52.9%) showed at least 1 key sign or symptom (diarrhea, oedema, myalgia, and/or rash). Laboratory analyses showed 27 (79.4%) patients with eosinophilia (>500/µL) and 25 (73.5%) patients with increased CK and LDH levels. None of patients were hospitalized. Anti-Trichinella IgG were detected by ELISA and Western blot in serum samples collected from 8 patients 34 d.p.i., from 6 out of 8 patients 39 d.p.i., 8 out of 12 patients 43 d.p.i., from 3 patients 60 d.p.i., from 8 patients 82 d.p.i. (2 of them had been tested as negative 39 d.p.i., and 4 of them had been tested as negative 43 d.p.i.), and from 1 patient 124 d.p.i.
Since blood samples were not collected from all the persons involved in the outbreak at the same time after the ingestion of the infected sausages, the percentage of persons with eosinophilia and increased muscle enzyme values do not reflect the possible occurrence of these laboratory markers of trichinellosis in the clinical course of the infection.
All the 34 patients with a confirmed diagnosis of trichinellosis were treated with albendazole (800 mg/die for one week) in association with prednisone (25 mg/die for 10 days) then with decreasing doses of the latter. After treatment, all patients recovered with neither complications, nor hospitalization.
CONCLUSIONS:
This outbreak occurred in Tuscany, an Italian region where no human case was reported since 1993 when 4 people acquired trichinellosis for the consumption of meat from a backyard pig in the Arezzo province. This new outbreak suggests that the systematic control of all wild animals susceptible to Trichinella infection and intended for the human consumption should be implemented in this region, to prevent the introduction of the parasite in the food chain.

Keywords:
trichinellosis, outbreak, Tuscany

O11B.5
INDUCTION OF TH1 SHIFT IN IMMUNE RESPONSE IMPROVES HEALTH CONDITION OF DOGS WITH CHRONIC CANINE LEISHMANIASIS

Rossi G.*[1], Colizzi V.[2], Loria G.R.[3], Montesano C.[2], Scarpona S.[1]

[1]School of Veterinary Medical Sciences University of Camerino ~ Camerino ~ Italy, [2]Department of Biology Faculty of Science University of Tor Vergata ~ Roma ~ Italy, [3]Experimental Zooprophylactic Institute (IZS) of Sicily National Reference Center for Leishmaniasis and Anisakiasis ~ Palermo ~ Italy

INTRODUCTION:
Canine Leishmaniasis is not a closely protozoan disease, but the outcome of an aberrant immune response, since dogs showing an efficacious Th1 response are able to control the disease without need of chemotherapy. On the contrary, dogs with a prevalent Th2 immune response, with high levels of non-effective immunoglobulins, reveal the symptoms of a disease due to the damages caused by immune complexes (Barbiéri, 2006, Parasite Immunol, 28(7):329-37), difficult to control by drugs. Indeed, just the critical clinical profile of some patients, especially if in advanced kidney involvement or failure, makes the administration of antiprotozoal therapy discouraged and dangerous (Solano-Gallego et al., 2011, Parasites & Vectors, 4:86).
Starting with these assumptions, we investigated whether it was possible to clinically recover dogs with chronic disease, subclinical or overt, relapsed several times or in late-stage kidney failure, by stimulating selectively the Th1-type immune response.
MATERIALS AND METHODS:
The immunomodulatory therapy used in this study, now patented, is based on the exaltation of cell-mediated immune response, by extract of Mycobacteria (Ravn et al., 1997, J Immunol,158:1949), and the restraint of phlogosis that follows, by antiCOX-2 selective molecules (Willoughby et al., 2000, The Lancet,355(9204):646-8). This combination gives a notable expansion of cell-mediated cytotoxic effect with an increase of immune activity towards all those pathologies that require a Th1-type mechanism.
This study enrolled 116 client owned naturally infected dogs with disease manifestations, divided into two groups: group A (85 dogs) treated with the immunomodulatory therapy without any adjunctive drug with leishmanicidal activity; and group B (31 dogs) treated with a conventional therapy based on S.C. administration of meglumine antimoniate, according to all official protocols. All animals were evaluated by clinical, parasitological and immunological criteria at different times of the study, to monitor therapies efficacy and health condition of the patients. Tests performed were: clinical examination, antileishmanial antibody titration (IFAT), RT-PCR on lymph node FNA, serum proteins profile, lymphocytes CD flow cytometry, in vitro macrophages activity (NBT test and colture).
RESULTS:
Clinical and lab results demonstrated differences of both humoral and cell-mediated immune responses between the two groups. In particular, they highlighted a Th2→Th1-shift in group A, together with a very significant improvement of clinical and parasitological conditions even in critical patients, whereas group B showed fickle clinical improvement, often not long lasting, and not in critical patients, in absence of any immune shift and maintenance of a prevalent humoral response.
CONCLUSIONS:
This study demonstrated that the right approach to leishmaniasis should not be limited to fight the parasite, but aim to restore the proper mechanism of patient immune response, since the cause of the disease lies just in its aberration.

Keywords:
leishmaniasis, Th1 immune response, immunomodulatory therapy

NOTES

SESSIONE PARALLELA O-12

PARASSITI DEGLI ANIMALI DA COMPAGNIA E RISCHIO ZOONOSICO

NOTES

O12.1

ENVIRONMENTAL CONTAMINATION BY PET FAECES AND PARASITIC RISK IN THE CITY OF SASSARI (SARDINIA, ITALY)

Tamponi C.*[1], Varcasia A.[1], Del Giudice F.[1], Serra C.[1], Sanna G.[1], Dore F.[1], Pipia A.P.[1], Brianti E.[2], Scala F.[1]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari ~ Sassari ~ Italy, [2]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina ~ Messina ~ Italy

INTRODUCTION:
Pets are associated with several parasites eliminated through the feces that may have zoonotic concerns (i.e. Ancylostoma spp., Giardia duodenalis, Strongyloides stercoralis, Toxocara spp., Trichuris vulpis) or not (i.e. Isospora spp., Sarcocystis spp., Bronchopulmonary Nematodes). Environmental contamination by pet faeces is recognized as a risk for parasites transmission either to animals and humans.
The aim of this study was to evaluate the intensity of contamination by pets faeces in the city of Sassari (Sardinia, Italy), and to assess the percentage of parasitic elements in stool samples.
MATERIALS AND METHODS:
A regular grid (250m x 250m) was overlaid on the city map using the software ArcGis (Esri®). From June to November 2014, in each square of the grid were examined 200 m of roads and sidewalks for valuating the degree of fecal contamination and a systematic collection of faecal samples was performed (Risitano et al., 2007, ISTISAN Congressi 07/C5: 95). Faecal samples were examined with flotation technique using ZnSO4 (p.s. 1200). Data analysis was performed with the aid of the software Microsoft Office Excel®.
RESULTS:
One-hundred-one out of 112 (90.2%, C.I.95%: 87-93.4) monitored squares were contaminated by pets' faeces. Mean intensity of streets contamination was as high as 4.3 feces/100 mt and the 12.2% (C.I.95%: 10.1-14.3) of faeces was stepped on.
A total of 333 faecal samples were collected and 52 (15.6%; C.I.95%: 11.7-19.5) were found to be positive for parasitic elements. Forty-nine (14.7%; C.I.95%: 10.9-18.5) faecal samples were found positives for zoonotic parasites being Ancylostoma spp. (11.1%), Strongyloides spp. (4.8%) and Toxocara spp. (0.9%) the most frequent species, while only 3 samples (0.9%; C.I.95%: 0.1-1.9) tested positive for non-zoonotic parasites (Bronchopulmonary Nematodes 0.6%, Isospora spp. 0.3%).
CONCLUSIONS:
Findings of the present study evidenced as environmental contamination by pet faeces in the city of Sassari is high and wide-distributed in the all the parishes. The risk of parasitic transmission to humans is high as almost 15% of faces tested positive for zoonotic parasites. This wide contamination may represent a significant harm for most vulnerable human categories (e.g., children and immunocompromised) and should be properly addressed by local health authorities.
The prevalence here detect regarding Bronchopulmonary Nematodes may be underestimated because the method used is not the most suitable for the detection of these parasites. Moreover, tapeworms were not detected in the present study and further studies are necessary to evaluate if this could be related to the real absence of these parasites or to the possible inadequacy of the method used.

Acknowledgements: The research was funded by Regional Government of Sardinia, prot. CRP2 134 (L.R. 7, 2007); Authors thanks Mr. F. Salis, Technician, Parasitology Lab., University of Sassari for the laboratory screening of samples.

Keywords:
Environmental contamination , zoonosis, pets

O12.2
CANINE FAECAL CONTAMINATION IN UDINE AND EVALUATION OF HEALTH RISK

Beraldo P.*[2], Candusso S.[2], Mingotto F.[2], Arzese A.[1]

[1]Experimental Clinical Medicine, University of Udine ~ Udine ~ Italy, [2]Division of Veterinary Pathology, Department of Food Science, University of Udine ~ Udine ~ Italy

INTRODUCTION:
The city is an artificial ecosystem shared by humans and animals (domestic and synanthropic). The number of domestic animals in urban areas has progressively increased and the fecalization of public areas is an unpleasant inconvenience as well as significant health risk. In fact, dogs and cats are hosts to many intestinal parasites that may be transmitted to humans through direct contact with infected pets or exposure to environments contaminated with infected animal faeces. The presence of parasitic elements was investigated in canine faeces in the urban and suburban areas of Udine. Our aim was to assess the risk level for human health due to canine fecalization in Udine.
MATERIALS AND METHODS:
Four hundred-eighty faecal samples of dog were collected in six urban areas (UA) of Udine (0.25 km2) and four suburban green areas (SA). Faecal samples were tested by a floatation method, using a sucrose/sodium nitrate solution (density 1.350).
Moreover, a retrospective survey on Toxocara canis seroprevalence in humans (IgG anti-Toxocara canis - NovaLisaTM, NovaTec GmbH) was applied to patients attending Udine University Hospital.
Finally, a questionnaire survey on some aspects of dog parasitism (parasite species, awareness of zoonotic diseases and history of anthelmintic usage) was filled out by interviewing the dog owners.
RESULTS:
All the explored UA and SA of Udine contained canine faeces. In the UA 40 out of 360 faecal samples (11%) were positive for parasitic elements and in the SA 10 out of 120 (8.3%). The most frequent parasite species found in UA faecal samples were Trichuris vulpis (8.3%), followed by Ancylostoma caninum (1.9%), Cystoisospora canis (0.5%) and Toxocara canis (0.3%). In SA faecal samples A. caninum (4.2%) was the most frequently recovered species, followed by T. vulpis (1.7%) and T. canis (0.8%).
The T. canis seroprevalence in humans was derived from 196 symptomatic subjects (116 females and 80 males, aged between 1 and 92 years), in a four-year time (2009-2012) retrospective survey. Overall, Toxocara antibodies at significant titers were found in 8.7% of the selected human sample.
The 32% of interviewed dog owners annually treat the dog against intestinal helminths, while the 97% perform (annually or monthly) the prophylaxis anti-Dirofilaria, using diverse drug classes.
CONCLUSIONS:
The results showed the presence of a moderate canine faecal contamination level in the city of Udine, as well as the occurrence of canine parasitic elements, some of which are potential agents of zoonosis (T. canis and A. caninum). Similar results were obtained from other Italian urban areas although the parasite prevalence detected in canine faeces in Udine (10.4%) appear to be lower than values obtained by other authors (Poglayen and Marchesi, 2006, Parassitol., 48, 117-119; Rinaldi et al., 2006, BMC Vet. Res., 2:29; Tarsitano et al., 2010, Int. J. Environ. Res. Public Health, 7:3972-3986). With respect to T. canis, the serological survey pointed out the potential risk of acquiring toxocariasis in humans also in Udine area, thus deserving the need of further investigation. Although 75% of surveyed dog owners were aware that canine faeces are an important source of pathogens, it seems undeniable the need to implement public health education to reduce the risk of exposure for both humans and companion animals.

Keywords:
canine fecalization, zoonosis, toxocariasis

O12.3
ARE WE PERFORMING CORRECT CONTROL PROGRAMS AGAINST CANINE HELMINTHS? REMARKS ON OWNED DOG POPULATIONS FROM ROME AND PADUA PROVINCES

Frangipane Di Regalbono A.*[1], Traversa D.[2], La Torre F.[3], Cassini R.[1], Zanardello C.[4], Omodeo S.G.[4], Mutarello M.[5], Pietrobelli M.[1]

[1]Dipartimento di Medicina Animale, Produzioni e Salute, Università degli Studi di Padova ~ Padova ~ Italy, [2]Facoltà di Medicina Veterinaria, Università di Teramo ~ Teramo ~ Italy, [3]Novartis Animal Health ~ Origgio (Varese) ~ Italy, [4]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (Padova) ~ Italy, [5]Practitioner ~ Bassano del Grappa (Vicenza) ~ Italy

INTRODUCTION:
Ascarids, ancylostomatids and trichuroid are the most important parasitic nematodes affecting dogs worldwide (Traversa, 2012, Parasit. Vectors, 5:91). The aim of this study was to evaluate the presence and the prevalence of these parasites in privately owned dogs from Rome and Padua provinces, along with the evaluation of possible risk factors and Veterinarians' behavior in relation to the worm control programs suggested by the European Scientific Counsel Companion Animal Parasites (ESCCAP Guideline, 2nd Ed, 2010).
MATERIALS AND METHODS:
From February to April 2012, individual faecal samples from owned dogs were collected random by Veterinarians working in 13 Clinics, i.e. 6 in Rome and 7 in Padua provinces. Dogs that received an anthelmintic treatment within the previous three months were excluded. For each dog, a questionnaire was completed to give information on potential risk factors as follows: provenance, breed/cross-breed, gender, age, presence of enteric symptoms, home environment (apartment or gardening house), cohabitation with other dogs, walking in public green areas, and number of annual anthelmintic treatments planned by veterinarians (from 1 to 4). Samples were copromicroscopically examined with floatation using a sodium nitrate solution (density 1.3). Differences in parasite prevalence according to potential risk factors were evaluated by Pearson's chi squared test (significance level $p<0.05$). The software used was SPSS for Windows, version 16.0.
RESULTS:
A total of 493 faecal samples (257 and 236 from Rome and Padua, respectively) were collected. Overall, 46 (9.3%) dogs were positive for at least one intestinal nematode. Statistical analysis was performed for Trichuris vulpis and Toxocara canis, which showed the highest prevalence values (5.5% and 4.3%, respectively). Other recorded parasites were Ancylostoma caninum (1 dog from Rome and 1 dog from Padua), Uncinaria stenocephala (1 dog from Rome), and Eucoleus aerophilus (2 dogs from Padua). No significant differences in prevalence ($p>0.05$) were found between dogs from Rome and Padua, both for T. vulpis and T. canis (5.1% vs. 5.9% and 3.1% vs. 5.5%, respectively). The prevalence of T. canis was significantly higher in dogs ageing less than 12 months of age than in dogs 13-60 and >60 months old (8.7% vs. 3.6% and 2.7%, respectively), and cohabitating with other dogs (6.5% vs. 2.2%). With regard to worm control programs, a large ratio of questionnaires (205/493) were not filled by Veterinarians, and annual or twice yearly anthelmintic treatments were almost always (280/288 of the cases) suggested.
CONCLUSIONS:
The results of this study are in agreement with a previous survey (Capelli et al., 2006, Vet. Rec., 159:422-424), and confirm that private dogs may be infected by several nematodes. Some of them, both intestinal (e.g. T. canis, A. caninum) and respiratory (e.g. E. aerophilus) are zoonotic (Traversa, 2012, Parasit. Vectors, 5:91; Lalosević et al, 2008, Am. J. Trop. Med. Hyg., 78:14-16). Information given by Veterinarians on anthelmintic treatment planning were scant (58.4% of the questionnaires), and often in disagreement with ESCCAP guidelines. These findings endorse a negligence in worm control programs, and the risk of faecal pollution with canine parasites as demonstrated worldwide in public and urban areas (Traversa et al., 2014, Parasit. Vectors, 7:67).

Keywords:
Zoonoses, control program, helminths

O12.4
GIARDIA DUODENALIS IN SHELTER AND PRIVATELY OWNED DOGS IN NORTH-EASTERN ITALY

Simonato G.*[1], Marcer F.[1], Tessarin C.[1], Traversa D.[2], Zanardello C.[3], Ravagnan S.[3], Pietrobelli M.[1]

[1]Dipartimento di Medicina Animale, Produzioni e Salute, Università degli Studi di Padova ~ Padova ~ Italy, [2]Facoltà di Medicina Veterinaria, Università di Teramo ~ Teramo ~ Italy, [3]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (Padova) ~ Italy

INTRODUCTION:
Giardia duodenalis (syn. G. lamblia, G. intestinalis) has been described worldwide in kennelled and privately owned dogs with varying prevalence rates (Scaramozzino et al., 2009, Vet. J., 182:231-234). Molecular analyses have allowed the identification of different Giardia assemblages (Monis et al., 2003, Infect. Genet. Evol., 3:29-38). Host-specific assemblages C and D are commonly found in dogs, but the sporadic detection of the human-associated assemblages A and B suggests a potential role of dogs in the zoonotic transmission. This survey investigated molecularly the occurrence of giardiosis in shelter and private dogs.
MATERIALS AND METHODS:
From November 2008 to June 2012, individual faecal samples were collected from privately owned dogs in Padua and from dogs belonging to 8 shelters, 7 in Veneto and 1 in Friuli Venezia Giulia regions. Of 542 faecal samples (224 from private dogs and 318 from shelter dogs) copromicroscopically screened, 285 from kennelled dogs were analysed for Giardia spp. with a real-time PCR targeting the SSU-rRNA gene (Verweij et al., 2003, Mol. Cell. Probes, 17:223-225). Positive samples were subjected to a nested-PCR (Read et al., 2002, Int. J. Parasitol., 32:229-231) to confirm Giardia infection, then amplicons were sequenced and compared with sequences available in GenBank™.
RESULTS:
The overall prevalence of giardiosis was 10.1% when copromicroscopic technique was used, i.e. 3.1% (7/224) in owned dogs and 15.1% (48/318) in shelter dogs. The prevalence in shelter dogs greatly increased to 51.9% (148/285) when examined by the real-time PCR. The nested PCR confirmed 90/148 real-time PCR positive samples, 64 of which were successfully sequenced. Assemblages C (accession number DQ385548) and D (DQ385549) were found in 36 and 28 samples, respectively. Molecular analyses on privately owned dogs are still ongoing.
CONCLUSIONS:
The copromicroscopic results confirm that dogs living in confined areas have a higher prevalence of giardiosis than household dogs (Capelli et al., 2006, Vet. Rec., 159:422-424). This highlights the higher exposure of dogs to parasites due to the greater environmental contamination resulting in an heightened risk of infection (Ortuño and Castellà, 2011, Isr. J. Vet. Med., 66(3):103-107). The real time PCR found more positive samples than nested-PCR as already reported (Dado et al., 2012, Parasitol. Res., 111:2419-2422). Also, giardiosis is confirmed as a widespread infection in dogs, whose prevalence may be underestimated when evaluated only by copromicroscopy. Molecular analyses are more sensitive, also to investigate the zoonotic potential of the isolates circulating in the canine populations.

Keywords:
Giardia duodenalis, dog, real-time PCR

O12.5
ZOONOTIC PARASITES IN FECAL SAMPLES AND FUR FROM DOGS

Paoletti B.*[1], Di Cesare A.[1], Iorio R.[1], De Berardinis A.[1], Bartolini R.[1], Gatti A.[1], Traversa D.[1]

[1]Faculty of Veterinary Medicine ~ Teramo ~ Italy

INTRODUCTION:
Different zoonotic parasites may be harbored by stray and privately owned dogs. Among them, Toxocara canis, Ancylostoma caninum, Echinococcus granulosus, Assemblages of Giardia duodenalis and Cryptosporidium spp. have a fecal-oral transmission and humans can be infected either by fecal contamination of food, water or the environment or by direct contact with the infected host, according to the parasite's biology (Smith et al., 2007, Vet. Parasitol., 149:29-40). The present study evaluated the occurrence of zoonotic parasites in faeces and on fur of a number of dogs living in Italy.
MATERIALS AND METHODS:
From November 2011 to December 2013, fecal samples of 117 from kenneled dogs and 385 privately owned living in Abruzzo region (central Italy) were examined by a classical flotation method and Baermann's technique. Also, samples were examined for protozoa using a sucrose gradient centrifugation and a modified Ziehl-Neelsen stain, respectively. Twenty samples positive (11 from kenneled dogs and 9 from private animals) for G. duodenalis were genetically characterized. Additionally, 138 and 36 fur samples were collected by combing the dorsum from private and kenneled dogs living in the same area, respectively. Coat samples were examined for the presence of parasite elements and subjected to embryonation of T. canis eggs (De Savigny, 1975, J. Parasitol., 61:781-782).
RESULTS: Out of 502 fecal samples, 127 (25,29%) were found to be microscopically positive. Prevalence rates of the various parasites species are summarized in table 1. Nine private dogs were molecularly positive for Assemblage C (n=7), Assemblage D (n=1), Assemblages C+D (n=1), while 11 kenneled dogs were positive for Assemblage C (n=8), Assemblage D (n=1), Assemblages C+D (n=2). Roundworm and whipworm eggs were found on the fur of 3 and 1 private dogs respectively. Eggs of both nematodes were detected on the coat of another single private dog. These eggs were not considered viable, as no embryonation occurred.

Parasites	Positive samples	%
Giardia duodenalis	41	8,17
Cryptosporidium spp.	1	0,2
Isospora spp.	7	1,39
Sarcocystis spp.	5	1
Toxocara canis	33	6,57
Ancylostoma caninum	34	6,77
Trichuris vulpis	36	7,17
Angiostrongylus vasorum	11	2,19
Capillaria aerophila	3	0,6
Uncinaria stenocephala	1	0,2

Table 1. Positive samples and percentage for parasites found in the examined fecal samples

CONCLUSIONS:
The present findings confirm that dog faeces may contain zoonotic parasites and may be a potential risk for humans and other animals, especially when they contaminate urban environments (Traversa et al., 2014, Parasit. Vectors, 7:67). The small number of positive coat samples differs significantly from data reported in other studies conducted in Europe (Overgaauw et al., 2009, Vet. Parasitol., 163:115-122). Indeed, transmission of parasites or protozoa to humans is only possible if parasites have developed to the infective stage, if they are zoonotic and when ingestion of sufficient numbers of infective agents takes place. Thus, the role of dogs as source of human infections should be interpreted with caution.

Keywords:
Zoonotic parasites, Epidemiological survey, Dog

O12.6
FLOTAC & PETS: A SERVICE FOR PARASITOLOGICAL DIAGNOSIS IN VETERINARY PRACTICE

Maurelli M.P.*[1], Musella V.[2], Del Prete L.[3], Pepe P.[1], Noviello E.[3], Pennacchio S.[1], Rinaldi L.[4], Cringoli G.[4]

[1]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania) ~ Naples ~ Italy, [2]Department of Experimental and Clinical Medicine, University Magna Graecia of Catanzaro ~ Catanzaro ~ Italy, [3]Private Practitioner ~ Naples ~ Italy, [4]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania); Inter-University Center for Research in Parasitology (CIRPAR) ~ Naples ~ Italy

INTRODUCTION:
Infections by intestinal and pulmonary parasites in pet animals are often considered neglected with respect to their diagnosis, surveillance and control. In order to increase the awareness regarding these infections amongst vet practitioners, a diagnostic service "FLOTAC & Pets" has been activated at the Unit of Parasitology and Parasitic Diseases of the Department of Veterinary Medicine and Animal Productions, University of Naples Federico II.
MATERIALS AND METHODS:
During 2013, a total of 416 faecal samples from pet dogs were collected at 30 veterinary clinics, located in Naples (Campania region, southern Italy). Each vet was provided with Fill-FLOTAC kits for sampling. Each sample was fixed in formalin 5% and analysed using the FLOTAC dual technique (Cringoli et al., 2010, Nat. Prot., 5(3): 503–15) to evaluate the presence of intestinal and pulmonary parasites. A sodium chloride-based flotation solution (specific gravity (s.g.) = 1.20) and a zinc-suphate-based flotation solution (s.g. = 1.45) were used. Anamnestic data were also collected for each dog.
RESULTS: Out of the 416 samples examined, 150 (36.1%; 95% Confidence Interval (CI)=31.5-40.9%) showed the presence of parasitic elements (eggs, larvae, oocysts and cysts). The prevalence for each parasite is reported in Table 1. The presence of endoparasites was higher (37.5%; 95% CI=32.9-42.4%) in dogs older than 6 months. No significant differences were found with respect to sex and size of dogs.

Parasitic species/groups	Dogs (no. examined = 416)		
	No. positive	Prevalence (%)	95% CI
Isospora spp.	33	7.9	5.6-11.1
Giardia spp.	40	9.6	7.0-13.0
Ancylostomydae	10	2.4	1.2- 4.5
Toxocara canis	46	11.1	8.3–14.6
Toxascaris leonina	3	0.7	0.2–2.3
Trichuris vulpis	14	3.4	1.9 - 5.7
Crenosoma vulpis	1	0.2	0.0-1.5
Angiostrongylus vasorum	2	0.5	0.1-1.9
Dipylidium caninum	1	0.2	0.0-1.5

Table 1. Parasitic species/groups found in pet dogs in Naples.

CONCLUSIONS:
The prevalence values for endoparasites detected in the present study (36.1%) are similar with those previously conducted on dog faecal samples from the city of Naples (59.5%) (Maurelli et al., 2013, Proocedings of LXVII Convegno Nazionale S.I.S.Vet.: 38) and from the Benevento province (55.8%) (Musella et al., 2010, Parassitologia, 52 (1-2): 327).
The high prevalence found in pet dogs highlights that a faecal monitoring should be conducted regularly to identify parasitic species present and the effectiveness of control programs (www.escaap.org). It is desirable to plan such parasitological investigations also on a large scale, using advanced epidemiological and diagnostic methods in order to better target effective control strategies.

Keywords:
FLOTAC, pets, parasitological diagnosis

O12.7
CROSS-SECTIONAL SURVEY ON LEISHMANIA INFANTUM INFECTION IN DOGS IN NORTHERN SARDINIA

Pipia A.P.[1], **Varcasia A.*[1],** Tosciri G.[1], Pitzalis F.[1], Cadeddu R.[1], Sanna G.[1], Dore F.[1], Tamponi C.[1], Manunta M.L.[1], Brianti E.[2], Scala A.[1], Vitale F.[3], Piazza M.[3]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari ~ Sassari ~ Italy, [2]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina ~ Messina ~ Italy, [3]Centro di Referenza Nazionale per le Leishmaniosi (C.Re.Na.L.), Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy

INTRODUCTION:
Canine leishmaniasis (CanL) caused by Leishmania infantum is a zoonotic disease of the old and the new worlds and domestic dogs are considered the main reservoir hosts of the disease (Desjeux, 2004, Infect Dis, 27:305–318). Accurate and rapid detection of CanL is of great importance to prevent transmission (Chargui et al., 2009, Parasite, 16:65-69). In Italy CanL is considered endemic in southern and central regions, including Sardinia (Gramiccia M., 2011, Vet Parasitol, 181:23-30), even if in this island epidemiological data are scant and dated (Manunta et al., 1999, Congresso di Igiene Urbana Veterinaria, 147-153).
The aim of this study was to update the knowledge on CanL in Northern Sardinia through an cross –sectional survey in which both serological and molecular diagnoses were employed.
MATERIALS AND METHODS:
From January 2012 to December 2013, from 486 dogs peripheral blood samples and 42 conjunctival swabs (CS) were collected and tested by serology and PCR, respectively. Immunofluorescence test (IFAT) was employed in the serological diagnosis starting from a dilution of 1:40 (OIE Manual of Diagnostics Tests and Vaccines). The CS were collected from the right and left conjunctiva of each dog and DNA extracted was submitted to a nested PCR (n-PCR) as described by Di Muccio et al., 2012 (J Clin Microbiol, 50:1-9).
RESULTS:
Anti-L. infantum IgG antibodies were found in the 11.5% (56/486) of the examined dogs with titers ranging from 1:40 to 1:10,240 (male 12.9%, 33/255; female 9.9%, 23/231; $\chi 2$ =1.058 ; p =0.303). Dogs with antibodies titers ≥1:160 were 5.3% (26/486). The majority of serologically positive dogs, 78.6% (33/42), were also positive to CS (n)-PCR. The 45.2% (19/42) of dogs tested positive at both (n)-PCR and IFAT with titers ranging between 1:80 and 1:10240. Some dogs tested positive only at CS(n)-PCR (33.3%; 14/42) while others were positive only at the serology (19%; 8/42). There was no agreement between IFAT (gold standard) and (n)-PCR (K <0.001).
CONCLUSIONS:
Compared to the data reported in a previous survey in which 33% of dogs were serologically positive to L. infantum (Manunta et al., 1999, Congresso di Igiene Urbana Veterinaria, 147-153), results ot the present study suggest a decreasing trend of seroprevalence (15.2%) in Sardinia (χ^2= 94.37, p = 0.000). However, findings yield by (n)-PCR indicate that dogs are still highly exposed to this zoonotic protozoa. Our data, although not concordant with serological findings (conversely with what reported by Di Muccio et al., 2012, J Clin Microbiol 50:1-9), are in accordance with those found in cats by Veronesi et al., 2012 (Mappe Parassitologiche 18: 311).

Acknowledgements: The research was funded by Regional Government of Sardinia, prot. CRP2 134 (L.R. 7, 2007); Authors thanks Mr. F. Salis, Technician, Parasitology Lab., University of Sassari for the laboratory screening of samples.

Keywords:
Leishmania, Sardinia, dogs

O12.8
A QUANTITATIVE REAL-TIME PCR PROTOCOL FOR THE DIAGNOSIS OF CANINE LEISHMANIOSIS

Romano A.*[1], Bruno B.[2], Spina S.[1], Mignone W.[1], Ingravalle F.[1], Barzanti P.[1], Zanatta R.[2], Goria M.[1]

[1]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, ~ Torino ~ Italy, [2]Dipartimento di Scienze Veterinarie ~ Grugliasco, Torino ~ Italy

INTRODUCTION:
Canine leishmaniosis (CanL) due to Leishmania infantum is a zoonotic disease transmitted by sand flies, whereas dogs represent the main reservoir. In dogs diagnosis is usually confirmed by indirect (serological) or direct (mainly PCR) tests. However in endemic areas up to 67% of dogs can be infected by sand-flies and the diagnosis is complicated due to the high seroprevalence rate and the high percentage of PCR positive in sub-clinical and asymptomatic dogs (Solano-Gallego et al., 2001, J Clin Microbiol, 39:560-563). A gold standard direct test for CanL diagnosis is far from to be identified and several real time PCR protocols have been described (Francino et al., 2006, Vet Parasitol, 137:214-221; Mary et al., 2004, J Clin Microbiol, 42:5249-5255). The aims of this study were first to clarify if quantitative PCR (qPCR) represent a useful tool for the identification of real clinical state of infected dogs and then to evaluate if parasite load (PL) and serological titre follow clinical and pathological variations. Merging obtained data we also tried to set a cut off value to differentiate infected from sick dogs.
MATERIALS AND METHODS:
In this study 138 dogs were included, collected from 2011 to 2013 and selected from endemic areas: healthy animals and animals with symptoms compatible with CanL were visited and periodically monitored. Based on clinical-pathological data dogs are then classified as: asymptomatic (A), symptomatic or relapsing (S), partial remission (PR), total remission (TR). Dogs subjected to therapy were visited at 1, 3, 6 and 12 months and then annually. Animals with clinical relapse are being reintegrated into the monitoring protocol. For all subjects lymph node aspirate was collected and total genomic DNA was extracted using commercial kit. Three replicates of 6 different DNA concentrations (from 106/ml to 10/ml. in 10-fold serial dilutions) obtained from a culture of L. infantum (MON-1 IPT1) were used to asses the sensitivity and efficiency of the qPCR assay. TaqMan probe, PCR primers, master mix concentrations and thermal profile used were previously described (Vitale F. et al., 2004). Correlation of qPCR data, IFAT and clinical status of the dogs was assess. The median regression model was used to evaluate diagnostic tests in correlation to each clinical condition and ROC curves were built to determine the cut-off value able to define the clinical status in dogs with the lowest error.
RESULTS:
The qPCR method gave a statistically significant difference between the distributions of asymptomatic values compared to the other classes of symptomatic dogs (about 80% have values close to 0) as well as IFAT did (Fig1). Both diagnostic methods exhibited significantly different values between A and S, but seemed to be less efficient in differentiating the other 3 classes of dog (S, PR, TR). If S dogs were compared vs all classes, qPCR showed higher performance (for specificity and sensitivity) than IFAT, as stated by ROC area values (Fig2). This could indicate how IFAT gave less early informations about efficacy of treatment.

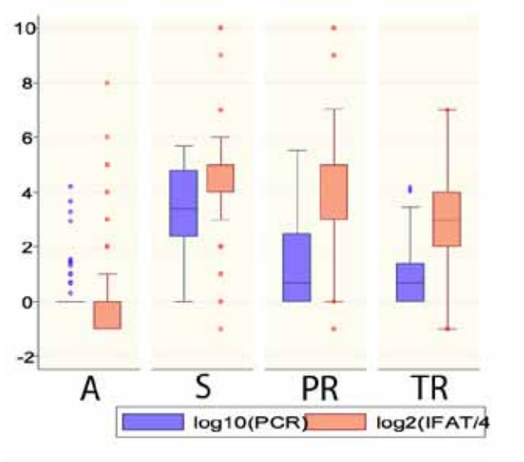


Fig1 Box-plot distribution for qPCR and IFAT

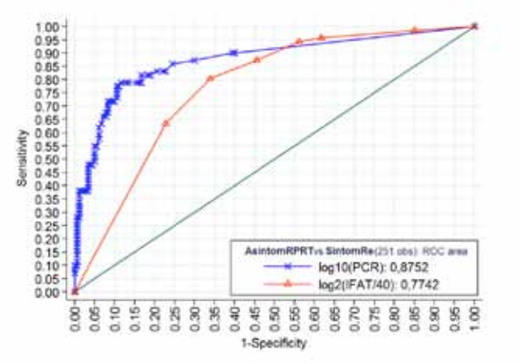


Fig2 ROC curve S vs A/PR/TR

CONCLUSIONS:
The PL, obtained by qPCR, progressively and significantly decreased in dogs with a linear trend with clinical remission, reaching values close to A. ROC curves were effective to estimate the cut-off value in order to differentiate dogs in different clinical classes and qPCR could be a useful tool to evaluate the efficacy of a therapy protocol, showing any variations in PL.

Keywords:
QUANTITATIVE REAL-TIME PCR, Canine leishmaniosis, ROC curves

O12.9
MOLECULAR IDENTIFICATION OF RICKETTSIA PATHOGENS FROM STRAY CATS AND DOGS AND THEIR FLEAS

Torina A.[1], Alaimo A.[1], **Blanda V.*[1],** Giudice E.[2], D'Agostino R.[1], D'Agostino R.[1], D'Agostino R.[1], Caracappa S.[2], Lelli R.[1]

[1]Istituto Zooprofilattico Sperimentale della Sicilia ~ Palermo ~ Italia, [2]Faculty of Veterinary Medicine, Universitá degli Studi di Messina ~ Messina ~ Italy

INTRODUCTION:
Stray cats and dogs have a crucial role in several infectious diseases human transmission, some of these, transmitted by hematophagous arthropods, like ticks or fleas, are of relevance since they could represent zoonoses (Gracia et al., 2008, Vet Parasitol, 151:312-319). Fleas, in particular, are competent vectors for numerous microbial pathogens of medical and veterinary importance. Recently, R. felis has emerged as a new human pathogen, causing flea-borne spotted fever. The bacterium was first detected in the cat flea, Ctenocephalides felis (Beugnet and Marié, 2009, Vet Parasitol, 163: 298-305) and the prevalence in fleas can be very high varying according to the ecological environment and season. R.felis has also been detected in C.canis, Pulex irritans, the human flea, and Archeopsylla erinacei.
The analysis aimed to Rickettsia spp. molecular characterization from stray cats and dogs and from fleas collected from them to investigate their potential role as reservoirs.
MATERIALS AND METHODS:
The study concerned a total of 26 cats and 13 dogs housed in Palermo Municipality shelter and their ectoparasites. For each animal, EDTA blood was uptake and fleas, if present, were harvested, kept alive for few days to allow the digestion of eventually ingested blood, rinsed with distilled water and stored in 70% ethyl alcohol. A total of 134 fleas was collected, identified (Chinery, 1998, 3th edn. Franco Muzzio Editore, Roma) and sectioned longitudinally. Halves of each flea were used for DNA extraction.
DNA was extracted from cat and dog EDTA blood and from fleas and PCRs were conducted to detect Rickettsia spp. DNA (Tzianabos et al., 1989, J Clin Microbiol, 27:2866-2868). PCR products were visualized on 1.5% agarose gel, with SYBR Safe 1X. A multi-gene assay was carried out on positive samples, amplifying regions of genes gltA (Roux et al., 1997, Int J Syst Bacteriol, 47:252-61), ompA (Oteo et al., 2006, J Clin Microbiol, 44: 2669–2671) and ompB (Choi et al., 2005, Emerg Infect Dis, 11: 237-244). PCR products were purified and sequenced. Sequences were analyzed by Bioedit software and searching for reference strains identity.
RESULTS:
A total of 134 fleas was collected from the animals, 97 of which from cats and 37 from dogs. Out of these, 132 belonged to the species C.felis (29 males and 103 females) and only two (a male and a female) to C.canis, both of them found on a dog.
DNA of Rickettsia spp. was found in 11 (28%) out of the examined stray animals, 8 cats and 3 dogs, and in 90 (67%) of the collected fleas. Rickettsia species was identified in samples resulted positive to at least two molecular markers at the multigene assay and this analysis allowed detecting R.felis and R.monacensis in two and one cats, respectively, while a very high prevalence of R.felis was observed in fleas.
CONCLUSIONS:
Sequencing analyses confirmed the frequent association between the flea C. felis and the pathogen R.felis. The finding of a cat positive to R.monacensis, a tick transmitted pathogen, suggests the possibility that stray cats could have a role in its maintenance and diffusion. Since studies performed on stray animals and their ectoparasites are not common nor easy to carry out, they can be undervalued as possible reservoirs for zoonotic agents.
Distribution patterns of flea parasitism are important tools in developing control strategies and evaluation of flea-borne disease risks in cats, dogs and humans.

Keywords:
Rickettsia, stray cat and dogs, fleas

O12.10

DOGS AS SOURCE OF TRYPANOSOMA CRUZI INFECTION IN RURAL AREAS OF THE CHACO REGION (PLURINATIONAL STATE OF BOLIVIA)

Totino V.*[1], Macchioni F.[2], Gabrielli S.[1], Fraulo M.[1], Segundo E.[3], Rojas Gonzales P.[3], Roselli M.[4], Bartoloni A.[4], Cancrini G.[1]

[1]"Sapienza" University of Rome ~ Rome ~ Italy, [2]University of Pisa ~ Pisa ~ Italy, [3]Distr. de Salud Cordillera ~ Santa Cruz ~ Bolivia, Plurinational State of, [4]Dip. di Medicina Sperimentale e Clinica ~ Firenze ~ Italy

INTRODUCTION:
Chagas disease continues to be an important sanitary problem in the Americas, even if the risk of transmission in Latin American endemic countries has been reduced by safer blood transfusions and vector-control measures applied (WHO, 2010, 75-9). Trypanosoma cruzi, the etiological agent of this anthropo-zoonosis, has been detected in more than 100 mammal species (Barretto, 1968, Rev Brasil Biologia, 28:481-94), but the role played by each species in the dispersion/maintenance of the parasite in each area of its occurrence is not completely known (Cortez et al., 2006, Expl Parasitol, 14:305-13). This is true for Bolivia, where only few studies on this topic have been undertaken (Albarracin-Velzaga, 1999, Rev Saude Publica, 3:230-6; Medrano-Mercado, 2008, Mem Inst Oswaldo Cruz, 103:423-30), and where a serosurvey carried out in Chuquisaca and Santa Cruz Dep.s detected antibodies in ≈70% of people (Gabrielli et al., 2008, Parassitologia, 50:85).
MATERIALS AND METHODS:
Aim of this paper is to evaluate the role of dogs as source of infection for kissing bugs vectors and, indirectly, for humans living in two rural Bolivian communities (Ivamirapinta and Bartolo).
By venepuncture, blood samples were drawn from 101 dogs living around people resident in study areas, and used to prepare Giemsa stained thick and thin smears, and to impregnate (300-500µL) filter papers. From these dried blood spots DNAs were extracted and a region of 330bp from kDNA was PCR-amplified (Enriquez et al., 2013, Acta Trop J, 126 (3): 211-217); amplicons were sequenced, and sequences were BLAST compared with available sequences to confirm the species.
RESULTS:
As expected, smears proved negative to the microscopic analysis whereas 24 blood spots (23.7%) were positive to T. cruzi (8/48 in Ivamirapinta and 16/53 in Bartolo). The presence of the parasite in the bloodstream of ≈24% examined dogs suggests that they can play an important role as source of infection for kissing bugs in endemic rural areas.
CONCLUSIONS:
This result disagrees with data collected in the city of Cochabamba (Medrano-Mercado et al., 2008, Mem Inst Oswaldo Cruz, 103:423-30), but it is in total agreement with studies carried out in rural areas of N-W Argentina, where dogs proved to be a source of infection for bugs more important than cats, humans and chicken (Gurtler et al., 2007, Parasitology, 134:69-82). In rural areas, the environment offers more opportunity for kissing bugs to feed on dogs, and general conditions of dogs (as malnutrition) maybe contribute to poorly control the parasite reproduction in the bloodstream. So, this animal may be the main domestic reservoir of T. cruzi and a risk factor for parasite transmission, as demonstrated by the report in this host of all the discrete typing units of T. cruzi identified in humans (Ramírez et al., 2013, Vet Parasitol, 196:216-9). Therefore, improved detection of T. cruzi infections in dogs is relevant for risk assessment. Local veterinaries should pay attention to the problem, and check dogs regularly for this parasite.

Keywords:
Trypanosoma cruzi, Bolivia, Dogs

NOTES

NOTES

POSTER 01

INFESTAZIONI DA ELMINTI

P01.1
SEROLOGICAL AND MOLECULAR DATA ON CASES OF HUMAN HYDATIDOSIS STUDIED AT THE UMBERTO I TEACHING HOSPITAL IN ROME

Totino V.*[1], Gabrielli S.[1], Mattiucci S.[1], Bartoloni A.[2], Cancrini G.[1]

[1]"Sapienza" University of Rome ~ Rome ~ Italy, [2]University of Florence ~ Florence ~ Italy

INTRODUCTION:
Echinococcus granulosus s.l. is the most common parasite (>95% of cases) causing human Cystic Hydatidosis (CH), with 2-3 million of affected people in the world (Craig et al., Lancet Infect Dis, 2007, 7:385-94). In Europe, the estimated incidence of human CH is about 0.1/100,000 inhabitants (www.efsa.europa.eu), and in Italy it reaches 1.3/100,000 population, with the highest number of reported cases from Sardinia (8-10 cases/100,000 inhabitants) ((Conchedda et al., 2010, Parasitol Int., 59:454-9). Humans are accidental host of the parasite whose metacestodes reach and develop in any anatomical site, even if their presence is mostly detected in the liver. The genetic diversity of the parasite ('lion strain' and G1-G10 genotypes, some of which actually considered as separate species) may explain difficulties sometime encountered in immunological diagnosis of CH, therefore at the Policlinic Umberto I we are studying all cases by serological and molecular point of view. This work aims to report data collected in 2006-2013
MATERIALS AND METHODS:
A total of 731 patients, coming from different foreign countries and presenting space-occupying cysts detected by imaging, were analysed by serology in order to define the nature of the cysts. Serological tests applied were: ELISA test (Cypress Diagnostics, BE) to detect specific IgG reactivity, and WB assay (LDBIO-Diagnostics, FR) to confirm and distinguish reactivity to E. granulosus or to E. multilocularis. Most of the patients reactive to E. granulosus were pharmacologically treated, whereas some of them were submitted to surgical intervention. In 22 cases we had the chance of analyse, at the molecular level, cysts removed from liver (n=18), lungs (n=2), muscle (n=1) and kidney (n=1). Cystic material was firstly examined by microscopy; then it was submitted to genomic DNA extraction and sequence analysis at two target mitochondrial genes (cytochrome c oxidase subunit 1 and the small subunit of the ribosomal RNA gene), according to protocols previously described (Varcasia et al., 2007, Parasitol Res, 101:1135-9). The obtained sequences of ≈500bp were subjected to Blast homology search (NCBI) to assess the genotype.
RESULTS:
Specific ELISA detected antibodies anti-Echinococcus in 224/731 patients (30.6%), and the WB test confirmed 192/224 (85.7%) positive results. Unconfirmed ones were mainly related to cysts located in lungs, spleen, brain, and pericardium. According to the sequence analysis, the 22 removed cysts of E. granulosus s.l. were identified as belonging to the genotype G1-G3. Therefore, in all studied patients coming from different European and African countries the most common E. granulosus "sheep strain" was found.
CONCLUSIONS:
In conclusion, CH confirms to be an important zoonotic burden for humans. Imaging plays a major role to suspect the parasitosis and prompts to further investigate by means of serology and molecular tools. However, physicians should be aware that some cases of CH may remain unconfirmed/undetected by serology, and therefore prudent therapeutic management and material collection is needed, mainly when the lesion is non-hepatic.

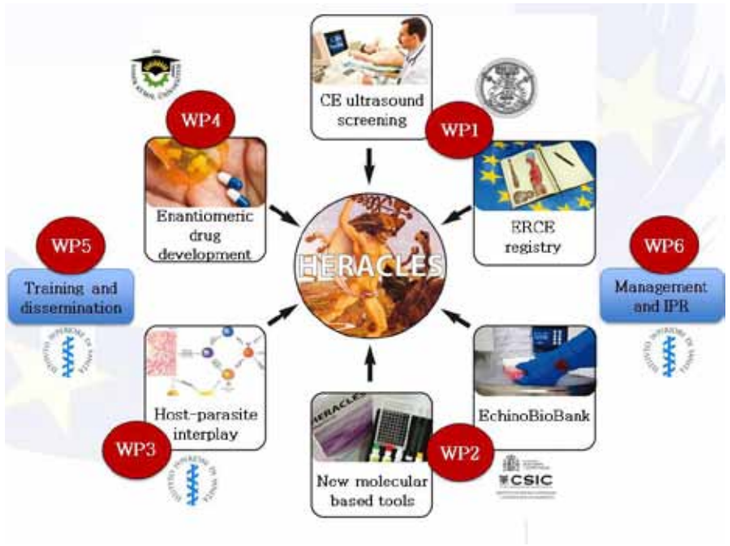
Keywords:
Echinococcus, genotyping, serodiagnosis

P01.2
HERACLES (FP7 FUNDED PROJECT ON CYSTIC ECHINOCOCCOSIS)

Casulli A.*[1]

[1]Istituto Superiore di Sanità ~ Rome ~ Italy

INTRODUCTION:
Adriano Casulli (1) Project Coordinator, on behalf of the Consortium HERACLES(2)
(1) Department of Infectious, Parasitic and Immunomediated Diseases. Istituto Superiore di Sanità, Rome Italy; (2) Project funded by the European Commission under the Grant Agreement n°602051 of the Seventh Framework Programme (FP7), HEALTH.2013.2.3.4-1. The Heracles project, focusing on Cystic Echinococcosis (CE), is designed to provide new insights into the host-parasite relationship and epidemiology, new tools for epidemiological surveillance in animals, parasite infectivity, host immunity, diagnosis, clinical manifestations, improvement of therapy, and follow up in humans. Heracles Consortium is made up of nine partners, of which seven are academic institutions, one is a small-medium enterprise (SME) and one is a service provider company. Five countries are represented: Bulgaria, Italy, Romania, Spain, and Turkey.
Lifespan of this project: 2013-2017. Total project cost: 3.879.712 €.
Consortium composition: Applicant (ISS, Italy) and Partners (UNIPV, Italy; CSIC, Spain; CCHUMFCD, Romania; HUSM, Turkey; SHATIPD, Bulgaria; NKU, Turkey; ALTA, Italy; VIRCELL, Spain).
MATERIALS AND METHODS:
- Identify the Eastern European population (EEP) affected by CE by ultrasound screening;
- Create the European Registry of Cystic Echinococcosis (ERCE);
- Establish the Echino-Bio-Bank from animal and human CE patients and related Databases;
- Set-up and validate new molecular-based tools for the detection, diagnosis and follow-up of CE;
- Identify the molecular bases associated with CE response to therapy or lack thereof, through the study of host-parasite interplay ("omic" studies);
- Increase drug bioavailability of Albendazole (ABZ);
- Train experts working in Eastern European countries (EEC), as they are crucial to fight this disease.



RESULTS:
Heracles translates the project outputs into POC-LOC (Point Of Care - Lab On a Chip) commercial tools to use in EEC. The ERCE registry for the surveillance of CE provides baseline data for future risk calculations and to establish a prospective case retrieval. A better understanding of the host-parasite interplay will ultimately be useful to improve response to treatment. The synthesis of a new enantiomeric drug based on ABZ will be followed by a phase 1 clinical trial protocol in which the pharmacokinetics, safety, and tolerability of the improved formulation will be assessed.
CONCLUSIONS:
Project stakeholders, such as the rural populations in areas where CE is endemic, will be engaged as an integral part of the project. Special events to increase awareness in the population of how CE is transmitted will be organized. The results of Heracles activities will support governments, the European Commission and related European agencies to harmonize data collection, monitoring and reporting of CE, according to the EU Legislation.

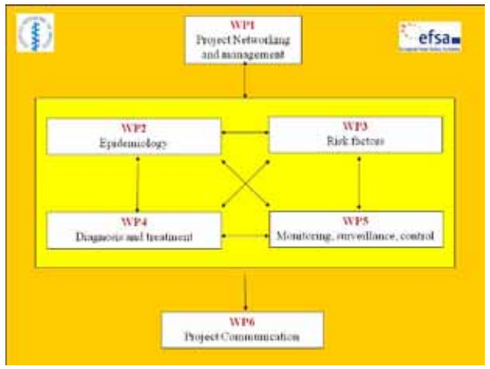
Keywords:
Cystic Echinococcosis, Seven Framework Programme of European Union, Bio-medical project

P01.3
EMIA (EFSA FUNDED PROJECT ON ECHINOCOCCUS MULTILOCULARIS)

Casulli A.*[1]

[1]Istituto Superiore di Sanità ~ Rome ~ Italy

INTRODUCTION:
Adriano Casulli (1) Project Coordinator, on behalf of the Consortium EMIA (2)
(1) Department of Infectious, Parasitic and Immunomediated Diseases. Istituto Superiore di Sanità, Rome Italy; (2) Project funded by the European Food Safety Agency (EFSA) under the Grant Agreement "GP/EFSA/AHAW/2012/01: Echinococcus multilocularis infection in animals", art 36 cooperation.
The European Commission has to review the Regulation (EU) No 1152/2011 on Echinococcus multilocularis (EM), no later than December 2016. To assist in this review, EFSA funded this project to provide a scientific opinion on EM infections in animals by the end of 2015.
Lifespan of this project: 2013-2015. Total project cost: 228.443 €.
Consortium composition: Applicant (ISS, Italy), Partners (ANSES, France; EVIRA, Finland; RIVM, Netherlands; FLI, Germany; NVRI, Poland; CSIC, Spain), the Systematic Review Advisor (Sapienza University, Rome) and International External Experts.
MATERIALS AND METHODS:
This research is conducted according to the methodological approach of the Systematic Reviews (SRs). Bibliographic searches, within "a priori protocols" were carried out using the following databases: Medline; Embase; Sci Search; Biosis; Cabi; Google Scholar. The search was restricted to eight languages from electronic databases. Databases were searched using keywords associated with the Boolean operators. Quality assessment will be performed using the following approach: 1.Randomized clinical trial: Jadad score; 2. Observational studies: Newcastle-Ottawa scale; 3. Diagnostic studies: QADAS-2.



RESULTS:
Bibliographic searches identified 10,151 scientific papers of which 5,282 were deleted because duplicates. At the end of the search 4,869 papers were identified within the eight protocols. For each protocol paper identifications are as follow:
EPIDEMIOLOGY (WP2)
- Geographic distribution and the prevalence of EM infection in animals and the importance of the different hosts species in the life-cycle of this parasite in the EU and AC. Papers identified: N=2,702; Papers selected: N=379.

RISK FACTORS (WP3)
- Risk factors for introduction and establishment of EM in free areas through movements of domestic and wildlife species involved in the EM lifecycle. Papers identified: N=458; Papers selected: N=18.
- Risk factors associated with human AE. Papers identified: N=891; Papers selected: N=63.
- Impact of EM infection in animals on public health in EU and AC. Papers identified: N=367; Papers selected: N=24.

DIAGNOSIS AND TREATMENT (WP4)
- Laboratory techniques for the detection of EM in live or dead animals. Papers identified: N=3,858; Papers selected: N=81.
- Effectiveness of available EM de-worming drugs, resulting in treatment protocols for dog, cats and ferrets. Papers identified: N=356; Papers selected: N=21.

MONITORING, SURVEILLANCE AND CONTROL (WP5)
- Monitoring and surveillance programmes of EM infection in definitive and intermediate hosts. Papers identified: N=1,230; Papers selected: N=109.
- Programmes for the eradication of EM in wildlife host species. Papers identified: N=289; Papers selected: N=33.

CONCLUSIONS:
In order to be able to provide a comprehensive and quantitative assessment of EM infections in animals, the current knowledge and data on the epidemiology and risk factors related to this disease will be collected in the European Union and adjacent countries. Information and data on the aspects listed above will be gathered by means of eight Systematic Reviews (SRs) of literature and data.

Keywords:
Echinococcus multilocularis, European Food Safety Agency (EFSA), Systematic Review

P01.4
THE ITALIAN REGISTRY OF CYSTIC ECHINOCOCCOSIS

Tamarozzi F.[1], Rossi P.[2], Galati F.[3], Mariconti M.[1], Nicoletti G.J.[1], Rinaldi F.[1], **Meroni V.*[4],** Pozio E.[2], Brunetti E.[5]

[1]Department of Clinical, Surgical, Diagnostic and Paediatric Sciences, University of Pavia, WHO Collaborating Centre for Clinical Management of Cystic Echinococcosis, Pavia, Italy ~ Pavia ~ Italy, [2]Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy ~ Roma ~ Italy, [3]SIDBAE, Information Technology, Istituto Superiore di Sanità, Rome, Italy ~ Roma ~ Italy, [4]Toxoplasmosis laboratory, IRCCS San Matteo Hospital Foundation, Pavia, Italy ~ Pavia ~ Italy, [5]Department of Clinical, Surgical, Diagnostic and Paediatric Sciences, University of Pavia, Division of Infectious and Tropical Diseases, San Matteo Hospital Foundation, WHO Collaborating Centre for Clinical Management of Cystic Echinococcosis, Pavia, Ital

INTRODUCTION:
Cystic Echinococcosis (CE) is a zoonosis with worldwide distribution, which is highly endemic in Southern and Eastern Europe, Italy included (Grosso et al., 2012, World J Gastroenterol., 18:1425-37). However, its true prevalence is largely unknown due to the lack of efficient reporting systems designed to take into account the particular features of this disease. Clinical management of CE patients is complex due to the lack of good quality evidence, the difficulty in the long-term follow-up of patients who often seek medical attention in different centres over the years and the generally poor knowledge of this infection outside referral centres, with consequent inconsistencies in clinical practices and often unnecessary procedures with associated risks and costs (Brunetti et al., 2011, PLoS Negl Trop Dis. 5:e1146). Despite being a notifiable infection in animals, being listed among the reportable professional diseases, and being subject to surveillance according to the European legislation, in Italy the notification of human cases ceased to be compulsory in 1991 and only a yearly summary of regional data is required by the health authorities. At the national level, only hospitalized cases are registered, but this system is inadequate and only detects the "tip of the iceberg", as the majority of CE cases are diagnosed and managed on an outpatient basis. No official data are transmitted to European authorities, as shown by the reports of ECDC and EFSA which continue to indicate that data from Italy are lacking; furthermore, prevalence and incidence data in livestock are incomplete (EFSA, ECDC, 2013, EFSA Journal, 11:3129). In 2012 the Italian Registry of Cystic Echinococcosis (RIEC) has been set up by Istituto Superiore di Sanità (ISS) and the WHO Collaborating Centre for Clinical Management of Cystic Echinococcosis (San Matteo Hospital Foundation, University of Pavia, Italy) and published in October 2012 on the ISS website (www.iss.it/riec). The implementation of RIEC has been the first step to address the long-standing issue of neglect and underreporting of CE in Italy, with its consequences in terms of public health, patients care and research funding. The aims of RIEC are to indicate the burden of CE in Italy; to bring to the attention of health authorities the importance of this infection; to encourage public health policies toward its control; to stimulate research on CE. Moreover, it provides an useful tool for patients follow-up and evaluation of therapeutic interventions.
MATERIALS AND METHODS:
RIEC is a prospective multicenter registry of CE patients visited from January 2012 in Italian health centres which adhered to RIEC. Representatives of hospital departments join the Registry on a voluntary basis, receiving a nominal credentials, while patients willing to participate after giving written informed consent are assigned a progressive number to avoid duplication of data entry. Records include epidemiological, clinical, and serological data
RESULTS:
As of February 2014, 346 patients were enrolled in just 11 centres, figures largely outnumbering the national reports of many endemic European countries. So far, the majority of records were entered in Pavia Hospital, WHO Collaborative Centre for the Clinical management of Cystic Echinococcosis.
CONCLUSIONS:
This work will discuss preliminary data and challenges of RIEC, that is the template for the European Registry of CE (ERCE), to be implemented within the FP7 HERACLES project.

Keywords:
Cystic Echinococcosis, Italian Registry, Reporting

P01.5
UPDATING ON ANIMAL AND HUMAN TRICHINELLOSIS IN CENTRAL APENNINES (ITALY)

Calderini P.[1], **Gabrielli S.*[2]**, Tancredi F.[1], Cancrini G.[2]

[1]Ist. Zooprofilattico Sperimentale di Lazio e Toscana ~ Rieti ~ Italy, [2]Università "Sapienza" ~ Roma ~ Italy

INTRODUCTION:
Trichinellosis is a meat-borne ubiquitarian zoonosis now considered emerging in Europe (WHO, 2004, 5:3-5). Indeed, even if meat is randomly submitted to sanitary controls, consuming infected food is an event always possible due to the poor sensitivity of the available diagnostic tests and the increased alimentary habit of consuming raw/poorly cooked meats, sylvatic ones included (Dorny et al., 2009, Vet Parasitol, 163:196-206). In addition, the global trade of foodstuff allows the presence of any trichinella species everywhere, as happens in Italy where to date have been isolated T. britovi, T. spiralis and T. pseudospiralis (Pozio et al., 1999, J. Parasitol, 85 759-61; Pozio et al., 2009, Int J Parasitol, 39:71-9). As for human infections, the most important foci detected in last years were due to meat of imported horses and of wild boars/semi-free ranged pigs; only two foci originated from fox meat.
MATERIALS AND METHODS:
This research aims to evaluate the risk of infection for people consuming raw meat that, through a chain of carnivore hosts living in Central Apennines, could arrive on our table.
Research was carried out on muscles of animal carcasses delivered, in 2008-2011, to the IZSLT (Sect. of Rieti) to be submitted to necropsy, collected from areas where trichinellosis had been detected in foxes, wolfes (Cagnolati et al., 1959, Parassitologia, 1:78; Stancampiano et al., 1994, in: Trichinellosis. Campbell, Pozio, Bruschi Eds: 585-9) and in people (Frongillo et al., 1992, Eur J Epidemiol, 8:283-8). Trichinellosis was evaluated by means of: i) in vitro digestion (IVD); ii) molecular diagnostics (MD: PCR and sequencing) as described by Pozio et al. (2003, Methods Mol Biol, 216:299-309); iii) serology (ELISA test) applied to diafragmatic juice of wild boars. Moreover, 396 sera of people living in the same area were submitted to an ELISA test followed by confirming WB in case of positive results.
RESULTS:
Results concerning animals are summarized in table1.
Only one wolf proved positive to parasitological analyses (the species involved was T. britovi), and serology evidenced antibodies anti-Trichinella in 1% of the examined wild boars. As for humans, 11/396 sera (2.8%) proved positive to ELISA test but only 3 of them were confirmed as positive by the WB then applied (overall prevalence 0.76%).
Low sensitivity of parasitological analyses to directly evidence the parasite in muscles is not surprising, and underlines the useful contribute of serology in order to evaluate the presence of trichinella in possible hosts, mainly when parasitized by poorly prolific (and pathogen) species like T. britovi.
CONCLUSIONS:
In conclusion, serology detected reactivity to Trichinella antigens in few edible animals and people resident in the same area. That means: a not high risk of human infection exists; however, it must be considered. Therefore, it is: i) useful to go on with animal controls; ii) necessary to improve both people education about alimentary habits and physician knowledge about clinical manifestation that could suggest trichinellosis.

ANIMALS	IVD Pos/Ex	MD Pos/Ex	ELISA Pos/Ex
Wild boar	0/1962	0/1962	2/200
Pig	0/3507	0/3507	-
Equids	0/242	0/242	-
Wolf	1/17	1/17	-
Fox	0/4	0/4	-
Bear	0/1	0/1	-
Porcupine	0/2	0/2	-
Badger	0/1	0/1	-

Table 1

Keywords:
Trichinella, infection risk, Central Appenine

P01.6
MIXED INFECTION BY AELUROSTRONGYLUS ABSTRUSUS AND TROGLOSTRONGYLUS BREVIOR IN A KITTEN FROM SARDINIA REGION (ITALY)

Morandi B.[1], Galuppi R.[1], Tamponi C.[2], Pipia A.P.[2], Parigi M.[1], Pietra M.[1], **Poglayen G.*[1]**

[1]Dipartimento di Scienze Mediche Veterinarie - Università Di Bologna ~ Bologna ~ Italy, [2]Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Italy ~ Sassari ~ Italy

INTRODUCTION:
Aelurostrongylus abstrusus (Strongylida, Angiostrongylidae) is regarded as important lungworm specie of domestic felids. Recently also Troglostrongylus spp. (Strongylida, Crenosomatidae), has been described in domestic cats with growing number of cases in Southern Italy (Brianti et al., 2014, Vet. Parasitol, S0304-4017: 00061-2), having an indirect life cycle, which overlaps that of the better known A. abstrusus. Adult worms of both genera have distinct morphology and localization within the lung tissue of the definitive hosts. The presence of these parasites in the airways of the definitive hosts may cause significant respiratory signs. This study describes a mixed infection by these lungworms in a kitten coming from Sardinia (Capo Comino), but resident in the province of Bologna.
MATERIALS AND METHODS:
The fecal sample of a 3 months old small cat was sent on October 8, 2013 at the Dept. of Veterinary Medical Sciences, University of Bologna, Italy, for routine examinations. Copromicroscopical examination carried out with a floating solution (PS 1300) and by Baerman apparatus, showed the presence of larvae (L1). The kitten was clinically evaluated and subjected to X-ray exams. Larvae found in each microscopic field were morphometrically and morphologically examined individually and identified based on their size and appearance of the tail and the head. DNA was extracted from L1 larvae and then processed by PCR amplyfing ITS2 ribosomal RNA as described by Tamponi et al. (2014 in press).
RESULTS:
The morphology and the size of the larvae was indicative for mixed infection of A. abstrusus and Troglostrongylus spp.. Clinical examination was normal and chest X-ray showed a diffuse alveolar-interstitial pattern with an increased soft tissue opacity in the right caudal lobe. ITS2 sequences showed a 100% homology those of T. brevior and A. abstrusus. The kitten firstly was treated with Prednisone 2.5 mg for 5 days and Imidacloprid 10%/Moxidectin 1% Spot-on (Traversa et al., 2009, Parasitol. Res., 105: s55-s62). Fecal examination repeated on October 30, 2013 tested still positive while, at x ray the pulmonary framework was improved; the kitten was treated again with the spot-on. After a further period spent in Sardinia, on January 28, 2014 fecal examination revealed again the presence of larvae and a third treatment, increasing the dosage on the spot on was performed. The last control on March 6, 2014 tested negative and the clinical pictures allowed us to be considered the cat healed.
CONCLUSIONS:
Lungworm parasitic infection of the cat are usually reported as due to A. abstrusus, while there are scarce reports of other lung nematodes; this could be due to the lack of accurate evaluation of morphometrical features of L1 during coprological diagnosis. This case in a cat coming from Sardinia confirms the spreading of these parasites, and in particular of T. brevior in this island as recently reported in other surveys (Tamponi et al. 2014 in press). This genera is usually reported as few pathogen, unlike A. abstrusus. In our case, despite the double infestation, the subject showed no obvious clinical signs, even if the framework of lung was altered and improved following therapy.

Keywords:
Aelurostrongylus abstrusus, Troglostrongylus brevior, Cat

P01.7
UPDATES ON METASTRONGYLOID LUNGWORMS INFECTING CATS OF SARDINIA (ITALY)

Tamponi C.*[1], Varcasia A.[1], Visco S.[1], Sulcis D.[1], Frau V.[1], **Sanna G.[1],** Dore F.[1], Pipia A.P.[1], Scala A.[1]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari ~ Sassari ~ Italy

INTRODUCTION:
Among the parasite species of the super-family Metastrongyloidea infecting the lungs of felids, Aelurostrongylus abstrusus is the most frequent, but recently other metastrongyloids such as Troglostrongylus brevior and T. subcrenatus (Strongylida: Crenosomatidae) have been reported in different areas of Italy (Brianti et al., 2012, Parasit. Vectors., 5:178-189; Annoscia et al., 2014, Vet. Parasitol., 199:172-178; Di Cesare et al., 2013, Parasitol. Res., 113:613-618) and also in Sardinia island (Tamponi et al., 2014, Vet. Parasitol., in press).
Aim of this study was investigate the presence of A. abstrusus and Troglostrongylus spp. in cats from Sardinia by combining copro-microscopical and biomolecular techniques.
MATERIALS AND METHODS:
An epidemiological survey was carried out between July 2011 and March 2014 in Sardinia (Italy), in order to achieve epidemiological data on parasites infesting broncho-pulmonary system of cats. One hundred and fifty-four (154) individual faecal samples were recovered and examined with qualitative Baermann technique. First stage larvae obtained with this method were subsequently identified using morphometric keys(Sloss et al., 1994, Veterinary clinical parasitology, 6th edn., 17-44; Gerichter, 1949, Parasitology, 39:251-262). An aliquot of the Baermann sediment of each positive sample was examined with biomolecular techniques amplifying cox1 and 18S genes using previously described protocols.
RESULTS:
Based on Baermann results, 29.9% (46/154) of examined cats were infected by broncho pulmonary nematodes. A. abstrusus was the most frequent specie 26% (40/154), whereas larvae of Troglostrongylus spp. were found in 5.2% (8/154) of positive samples. Frequencies of infestation were slightly higher in female cats (33.3%) than in males (25.7%) ($p>0.05$). The mean age of positive animals was of 20.1 months (SD: ±26.9), more specifically the mean age of positive for A. abstrusus was 22.1 months (SD: ±28.2), and for those positives for T. brevior was 7.5 months (SD: ±7.6); the difference is not statistically significant (t-test=2.81; p=0.008), probably due to the small number of positive samples to T. brevior. Biomolecular investigation confirmed the morphological diagnosis for all the A. abstrusus positive samples while Troglostrongylus spp. positive samples were molecularly identified as Troglostrongylus brevior.
CONCLUSIONS:
The overall prevalence of metastrongyloids infection here detected (29.9%) indicates that metastrongyloid lungworms are frequent parasitic agents in the studied cat population with a prevalence of A. abstrusus (26%) higher than those reported in other European Countries (Barutzki & Schaper, 2012, Parasitol. Res., 112:855-861).

Acknowledgements: The research was funded by Regional Government of Sardinia, prot. CRP2 134 (L.R. 7, 2007); Authors thanks Mr. F. Salis, Technician, Parasitology Lab., University of Sassari for the laboratory screening of samples.

Keywords:
cats, brochopulmonary, Sardinia

P01.8
EPIDEMIOLOGICAL UPDATES ON DICROCOELIUM DENDRITICUM IN SHEEP OF SARDINIA

Sanna G.[1], Varcasia A.[1], Pipia A.P.[1], Dore F.[1], Maccioni V.[1], Fenu G.[1], Tamponi C.[1], Muntoni S.[1], Salis F.[2], **Scala A.*[1]**

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari ~ Sassari ~ Italy, [2]Practitioner, Martini Zootecnica ~ Sassari ~ Italy

INTRODUCTION:
Dicrocoeliosis, caused by Dicrocoelium dendriticum is a hepatic parasitic disease of clinical and economical significance in ruminant breeding which causes direct losses due to confiscation of parasitized livers and indirect losses due to hepatobiliary alterations produced by the parasites and the costs associated with anthelminthic treatments. In Sardinia island, where are raised almost 3,228,878 dairy sheep, the parasitosis seems widespread according slaughterhouse records but no recent data on this parasitic disease have been published.
The aim of the present survey is to fill this gap of knowledge with an epidemiological survey in order to assess the prevalence of Dicrocoelium dendriticum in sheep of Sardinia (Italy) through a coprological study and direct examination in slaughtered animals.
MATERIALS AND METHODS:
The coprological study was carried out in Sardinia from 2011 to 2013 on 381 Sarda sheep farms with a semi-extensive breeding; 15 individual faecal samples were collected on each farm, divided into three pools and analysed with a qualitative method, through sedimentation and floatation in Zinc Sulphate solution (PS = 1.35) in centrifuge (2000 rpm x 10 minutes). At the same time, faecal samples from 121 sheep farms were analysed using a quali-quantitative method, the Flotac® technique.
At the slaughterhouse, livers from 356 sheep were examined for the presence of adult specimens of Dicrocoelium dendriticum. The parasite count was carried out in all the parasitized animals and positive sheep were divided into two ranges: ≤ 50 and >50.
RESULTS:
The coprological survey carried out on 381 sheep farms showed a prevalence of 22% (84/381). The faecal pools of the 121 sheep farms analysed with the quali-quantitative method (Flotac®) showed an average levels of 61.4 (± 105.5) Eggs Per Gram (EPG) of faeces, while 4.9 % of farms showed values >150 EPG.
The direct parasitological examination at slaughterhouse allow to highlight a prevalence of 24.7% (88/356) for Dicrocoelium dendriticum in the sheep liver. In particular 60.2% of positive livers (53/88) have a number of parasites ≤ 50 and 39.8% (35/88) > 50 specimens.
CONCLUSIONS:
Our results show a remarkable presence of D. dendriticum in Sardinia, with an overall prevalence of 22% which is consistent with data reported by Sánchez-Andrade et al., 2003 (Prev. Vet. Med. 57: 1–5) in Sardinia, which found a 24.5% prevalence ($\chi2=0,16$; $P= 0.684$). Conversely, prevalence found at the slaughterhouse (24.7%) is quite greater compared to that observed (10%) by Scala et al., 2000 (proceedings 8th FEMESPRUM., 8: 239-243) ($\chi2=26,13$; $P<0.0001$).
However individual EPG means (61.4) and 60.2 % of positive livers with a number of parasites ≤ 50, showed a moderate infection in sheep of Sardinia.
Anyway the report of 4.9 % positive animals with EPG levels >150, indicate a zootechnic risk situation according to Ambrosi, 1995 (Parassitologia Zootecnica. Edagricole, Bologna) that require a careful prophylactic and therapeutic control.

Acknowledgements: Regional Government of Sardinia, CRP-60126 (L.R. 7, 2012) and Mr. F. Salis, Technician, Parasitology Lab., University of Sassari.

Keywords:
Dicrocoelium dendriticum, sheep, Sardinia

P01.9
PARASITOLOGICAL SURVEY ON STRONGYLES AFFECTING HORSES AND CATTLE IN THE ABRUZZO REGION

Di Cesare A.*[1], Iorio R.[1], Sconza S.[1], Paoletti B.[1], Bartolini R.[1], Traversa D.[1]

[1]Faculty of Veterinary Medicine ~ Teramo ~ Italy

INTRODUCTION:
Gastro-intestinal strongylosis of horses and ruminants are of major importance in veterinary medicine in terms of sanitary importance and economic losses caused to the livestock industry (Charlier et al., 2009, Vet Parasitol, 164:70-79; Matthews, 2011, Equine Vet J, 43:126-132). Horse small strongyles (i.e. "cyathostomins") are key horse parasites, for their worldwide distribution and severe pathogenic impact, and the spread of drug-resistant populations (von Samson-Himmelstjerna G, 2012, Vet Parasitol, 185:2-8). Although cattle strongyles often cause asymptomatic infections, they may induce severe economic losses in terms of productivity (Charlier et al., 2009, Vet Parasitol. 164:70-79). This study evaluated the presence and distribution of horse and cattle strongyles in animals bred in the Abruzzo region, using classical and molecular diagnostic methods.
MATERIALS AND METHODS:
Ten cattle farms and 10 horse stables were selected on the basis of criteria of density and history of intestinal parasitoses. Individual fecal samples collected in each site were subjected to fecal examinations with flotation and McMaster techniques. Positive cattle and horse samples were subjected to a PCR amplyfing the Internal Trascribed Spacer 2 (Newton et al., 1998, Vet Parasitol, 69:1-15) of cattle nematodes and to a RLB using specific probes for cyathostomins, respectively (Traversa et al., 2007, J Microbiol, 45:2937-2942).
RESULTS:
Of the 500 cattle examined, 34 (6.8%), 31 (6.2%), 6 (1.2%), 3 (0.6%), 3 (0.6%) and 1 (0.2%) were positive for Strongylidae, Eimeria spp., Trichuris spp., Ascarididae, Strongyloides spp. and Paramphistomidae, respectively. Out of 237 horses, 109 (46.0%) were positive for Strongylidae and 10 (4.2%) for Ascarididae. The molecular analysis showed polispecific infection in both cattle and horses. Thirty and 25 samples from cattle were positive for Ostertagia ostertagi (88.2%) and Cooperia oncophora (73.5%). The most frequently identified cyathostomins were Cylicocyclus nassatus (83.5%), Cylicostephanus longibursatus (81.0%), Cyathostomum catinatum (79.8%), Cylicocyclus goldi (77.6%) and Cyathostomum pateratum (69.0%).
CONCLUSIONS:
Although the number of positive cattle in the study area is not particularly high, the pathogenic potential of these parasites should be always taken into appropriate account for the economic losses they may cause. Additionally, the herein results indicate that horse cyathostomins are present in the study area with high levels of contamination of the pastures and properties. This study confirmed that the RLB is powerful for epidemiological surveys on cyathostomins, especially for its ability to simultaneously analyse multiple samples against multiple species. Further studies are warranted to validate a similar assay for cattle strongyles as well. This study, although on a small area, provides new epidemiological data on the distribution of these parasites of large animals, towards further control programs of intestinal strongyloses in bovine and equine populations.
This work was funded by Special Project "Progetto Eccellenza Ricercatori", University of Study of Teramo – Tercas Foundation.

Keywords:
intestinal strongyloses, horse, cattle

P01.10
PRELIMINARY DATA ON GASTROINTESTINAL PARASITES OF GRAZING CATTLE OF GALLURA, SARDINIA, ITALY

Muntoni S.[1], Zidda A.[1], Varcasia A.[1], Boasso A.[1], Sanna G.[1], Tamponi C.[1], Pipia A.P.[1], Dore F.[1], **Scala A.*[1],** Sini A.[2]

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari ~ Sassari ~ Italy, [2]Associazione Regionale Allevatori della Sardegna, Sassari, Italy

INTRODUCTION:
In Sardinia, cattle breeding (cow calf line) is still carried out with extensive techniques in large grazing areas for meat production. Gastro intestinal parasites are a significant threat to the productivity of grazing livestock throughout much of the world, and unfortunately data on epidemiology and diffusion of these parasites in Sardinia are scant and dated (Scala et al., 2001, Atti società italiana di buiatria, 33: 311-317). This study aimed to estimate the prevalence of gastrointestinal nematodes and the intensity of infection in grazing dairy cattle from small and medium-sized farms in the Gallura region, Sardinia, Italy.
MATERIALS AND METHODS:
One hundred and twenty-nine faecal samples of grazing cattle (Limousine, Charolaise and crossbred with Sarda) of eight different locations of a single farm with over 600 acres of extension in the municipality of Berchidda (Gallura region, North-East of Sardinia Island). were collected and examined. Faecal samples were collected directly from rectum and once in laboratory examined with quali-quantitative technique through the use of FLOTAC®, according to the protocol described by Cringoli et al (2000). Animals were stratified according to farm location and age groups (0.5; 1; 2; 3-5; 6-10; > 10), evaluating EPG in the different groups and evaluating the zootechnic risk as described by Ambrosi (1995). Statistical elaboration was performed using software Epi-info V.2.0 and Minitab 16.
RESULTS:
An overall prevalence of 93.8% (121/129) for endoparasites has been found in examined animals. In particular a prevalence of 28% (36/129) for Eimeria spp., of 61.2% (79/129) for Calicophoron daubney, and of 22.5% (29/129) for Dicrocoelium dendriticum was highlighted. Gastro intestinal Nematodes (GIN) were found in 69% (89/129) of the examined samples, while Nematodirus and Trichuris spp. were found respectively in 1.5% and 0.8% of animals. Only in 2.5% of positive animals to C. daubney, shown an EPG value over the threshold of zootechnic risk (250 EPG). Regarding GIN infections, 6.7% of animals shown EPG values between 100 and 250 EPG. Average levels of EPG and prevalences for GIN stratified for age class differed significative between them (respectively H=16.24; p=0.006 and χ^2 trend=4.56; P=0.03), where animals < 6 months of age showed the highest values. Prevalences for D. dendriticum stratified for age class differed significative between them (χ^2 trend=6.775; P=0.009), where animals of two years of age shown the higher prevalence (38.5%). Significative differences in EPG means have been found between the eight location monitored (p>0.05), while only GIN shown statistically significative differences between prevalence (χ^2 trend=7.435; P=0.006).
CONCLUSIONS:
The survey has highlighted a poly-parasitological situation inside examined animals, even if the parasitic charges revealed no particular risk situations for livestock monitored, a clear sign that the management and density of livestock on pasture are at present optimal, making an anthelmintic intervention superfluous.

Acknowledgements:
Regione Sardegna, Misura 124 "Ichnusa Bubula" (Prot. 0004543) and Mr. F. Salis, Lab. Technician, Parasitology, University of Sassari.

Keywords:
Cattle, Sardinia, parasites

NOTES

POSTER 02

PARASSITOSI TRASMESSE DA ARTROPODI

P02.1
MOLECULAR IDENTIFICATION OF RICKETTSIA SPECIES IN IXODES RICINUS SAMPLED IN SOME NORTHEASTERN, CENTRAL AND INSULAR AREAS OF ITALY

Castro L.R.[1], **Gabrielli S.*[2],** Iori A.[2], Cancrini G.[2]

[1]Universidad del Magdalena ~ Santa Marta ~ Colombia, [2]Università "Sapienza" ~ Roma ~ Italy

INTRODUCTION:
Rickettsiae are intracellular bacteria maintained in nature by Ixodid ticks through the trans-stadial and trans-ovarial transmission; therefore, their geographical distribution coincides to the distribution of the carrier ticks (Parola, 2001, Clin Microbiol Infect, 7:80-3). Several species, like Rickettsia conorii in Europe, are long since well-known causative agents of diseases, however, molecular approach enabled recent identification in European countries of other rickettsiae of the spotted fever group of interest in animal or human health (Oteo et al., 2012, Ticks Tick Borne Dis, 3:270-7). In Italy, some rickettsial species have been identified in Ixodes ricinus (Beninati et al., 2002, Emerg Infect Dis, 8:983-6; Pajoro et al., 2010, Parassitologia 1, 2:178) and in Dermacentor marginatus specimens (Selmi et al., 2009, J Med Entomol, 46:1490-3), and Rickettsia monacensis and Rickettsia massiliae have been isolated from patients (Madeddu et al., 2012, Emerg Infect Dis, 18: 702-703; Vitale et al., 2006, Emerg Infect Dis, 12:174-5). However, available data about the species present in the country and their distribution on the territory are few and fragmentary. The aim of the present study was to increase knowledge about Rickettsia species present in I. ricinus sampled in some Northeastern, Central and Insular areas of Italy.
MATERIALS AND METHODS:
The aim of the present study was to increase knowledge about Rickettsia species present in I. ricinus sampled in some Northeastern, Central and Insular areas of Italy.
A total of 376 ticks, collected directly from the vegetation in five Italian sites (surroundings of Belluno, Perugia, and Rieti, and in Pianosa and Capri islands), were screened for DNA of the gltA gene of Rickettsia, as previously described (Regnery et al., 1991, J Bacteriol, 173:1576-89). Amplifications were followed by 2% agarose-gel electrophoresis and visualized with GelRed® under UV-light. Positive PCR products were purified and nucleotide sequences of positive samples were obtained and compared with reference sequences from GenBank.
RESULTS:
Positive templates were 75 (20%). Samples showed 98-100% blast identity to four rickettsiae of the spotted fever group of zoonotic concern: Rickettsia monacensis, Rickettsia raoultii, Rickettsia massiliae, and Rickettsia helvetica.
R. raoultii was only found in samples from Pianosa, R. monacensis and R. helvetica were only identified in ticks from Rieti, and R. massiliae was only evidenced in one sample collected in Perugia.
CONCLUSIONS:
I. ricinus, one of the most abundant tick species in Italy, confirms to be a Pandora's vase and, having a large record of attacking humans, a fearful possible source of infections. The results here reported enlarge the number of rickettsial species potentially dangerous for animals and people carried/transmitted by this widespread tick; therefore physicians should be aware of the possible transmission of these further bacteria, and should be well trained on the clinical features of corresponding infections in order to intervene, when needed, with appropriate treatment.

Keywords:
ickettsia, Ixodes ricinus, Italy

P02.2
DETECTION OF RICKETTSIAL PATHOGEN IN TICKS FROM SARDINIA

Chisu V.[1], Foxi C.[1], Piredda M.[1], Mura A.[1], **Cabras P.*[1],** Masala G.[1]

[1]Istituto Zooprofilattico Sperimentale della Sardegna ~ Sassari ~ Italy

INTRODUCTION:
Tick-borne rickettsioses are caused by obligate intracellular bacteria belonging to the spotted fever group (SFG). In Sardinia, the number of notified clinical cases of SFG Rickettsiosis, presumably MSF, has been 11.9 for every 100 000 inhabitants, in contrast to the national average of 2.1 (Beninati et al., 2002, Emerg.Infect.Dis., 8:983-986). In recent years, three other tick-borne pathogens, R. massiliae, R. aeschlimannii and R. slovaca, and a rickettsia of unknown pathogenicity, Candidatus Rickettsia barbariae, have been identified from ticks collected in Sardinia (Mura et al., 2008, Clin. Microbiol. Infect., 14: 1028-1033; Masala et al., 2012, Ticks Tick-borne Disease, 3: 393–395). In this study, we aim to update the repertoire of tick-borne rickettsiae in Sardinia by using molecular detection of rickettsiae in ticks.
MATERIALS AND METHODS:
A total of 254 adult ticks were collected in June 2010 from domestic dogs, goats, horse and wild boar in Sassari and Ogliastra province. Ticks were identified by morphological key, homogenated and DNA was extracted using Qiagen protocol. Rickettsial DNA was detected by standard PCR using oligonucleotide primers for the citrate synthase (gltA) and outer membrane protein A (ompA) genes presented in all spotted fever group Rickettsiae. PCR products were purified and sequenced. All sequences were assembled, edited and compared with those of the rickettsiae present in the GenBank database.
RESULTS:
The collected ticks were identified morphologically as Rhipicephalus turanicus (211 specimens), Rh. sanguineus (17 specimens), Dermacentor marginatus (14 specimens) and Hyalomma marginatum marginatum (12 specimens). Rickettsial DNA was found in 13 of the 254 ticks (5%) examined using the both specific-target genes by PCR.
Two Rh. turanicus ticks collected from goats (1%; 95% CI 0.16-3.75) contained rickettsial DNA with 100% similarity to the 630-bp ompA fragment of Candidatus rickettsia barbariae. Moreover, one Rh. turanicus ticks from goats (0.5%; 95% CI 0.02-3.02) contained rickettsia with an ompA gene 99.8% (620/621) similar to that of R. massiliae. Three Rh. sanguineus ticks (17.6%; 95% CI 4.67-44.20) from dogs possessed an ompA gene with 100% nucleotide similarity to that of R. conorii israelensis. In addition, in two Rh. sanguineus ticks (11.8%; 95% CI 2.06-37.75), R. massiliae was identified with 100% similarity. The sequence analyses of Rickettsia DNA detected in four D. marginatus ticks (28.6%; 95% CI 9.58-58.00) from wild boar showed 100% similarity with R. slovaca. One H. m. marginatum tick (8.3%; 95% CI 0.44-40.25) from horse contained rickettsial DNA with 99.8% (559/560) similarity to R. aeschlimannii.
CONCLUSIONS:
In this study, we identified the presence of one more pathogen, R. conorii israelensis, to add to the four human pathogenic rickettsiae previously found in Sardinia, R. massiliae, R. aeschlimannii, R. slovaca and R. monacensis (Mura et al., 2008, Clin. Microbiol. Infect., 14: 1028–1033; Masala et al., 2012, Ticks Tick-borne Disease, 3: 393–395; Madeddu et al., 2012, Emerg. Infect. Dis., 18, 702-704). Clinicians in Sardinia should be informed that several pathogenic rickettsiae are prevalent on the Island.

Keywords:
Vector tick, tick-borne disease, Rickettsia

P02.3
DOG SUBCUTANEOUS DIROFILARIOSIS BY DIROFILARIA REPENS IN CENTRAL ITALY

Di Cesare A.*[1], Iorio R.[1], Paoletti B.[1], Di Giulio E.[2], Bartolini R.[1], La Torre F.[3], Traversa D.[1]

[1]Faculty of Veterinary Medicine ~ Teramo ~ Italy, [2]Ambulatorio Veterinario James Herriot ~ Roseto degli Abruzzi, Teramo ~ Italy, [3] Novartis Animal Health S.p.A ~ Varese ~ Italy

INTRODUCTION:
The mosquito-transmitted nematode Dirofilaria repens is the causative agent of subcutaneous dirofilariosis in dogs, other animals and humans. The infection in dogs may be asymptomatic although cutaneous signs, i.e. dermatitis, subcutaneous nodules, itching and allergic reactions, could occur. In the last years D. repens has emerged and spread in various countries, including Italy, with a rise of cases in dogs and humans (Otranto et al., 2013, Parasit Vectors, 6:16). This report describes the incidence of new cases by D. repens in dogs living in an endemic area, evaluated during a field clinical study performed to investigate the efficacy of an oral formulation containing milbemycin oxime/praziquantel for the chemoprevention of the infection.
MATERIALS AND METHODS:
The study was carried out from April 2012 to July 2013 in four regions of Italy endemic for D. repens. One hundred and twenty five dogs enrolled in the study and negative for D. repens by Knott's and PCR methods were not treated with any product effective on D. repens less than 4 weeks prior to study start and for the whole study. Additionally, the dogs did not receive any repellent treatment, were treated monthly with a spot-on combination of metaflumizone and amitraz (Promeris®, Zoetis) and were vaccinated for Leishmania infantum (CaniLeish®, Virbac). By June 2012 (Day 0) blood samples were collected from the 125 dogs at days 28±2, 56±2, 84±2, 168±2, 196±2, 224±2, 252±2, 280±2, 308±2 and 336±2. The samples were subjected to a Knott's test and to a specific PCR assay as previously described (Traversa et al., 2010, Vet Parasitol, 179:107-112) and all animals which become microfilariemic received a rescue treatment, which showed a 100% efficacy in treating the infection.
RESULTS:
Out of the 125 dogs, six (4.8%) become infected by D. repens during the study, both at the microscopic and genetic assays.
CONCLUSIONS:
The incidence herein detected is similar to the infection rate (i.e. 5.6%) described in a previous epidemiological study carried out in central Italy (Traversa et al., 2010, Vet Parasitol, 169:128-32). These results confirm the presence of D. repens in the examined areas and that dogs are realistically exposed to the infection during the mosquito season. Given the impact of D. repens in canine and human health, it is advisable that veterinary practitioners take in account subcutaneous dirofilariosis in dogs and that the disease is included in the differential diagnosis of canine skin diseases. Indeed, a continuing veterinary education and awareness is advisable to avoid the spread of this nematode in both endemic and free areas and to minimize the risk of exposure for dogs and humans.

Keywords:
Dirofilaria repens, subcutaneous dirofilariosis, dog

P02.4
DETECTION OF LEISHMANIA AMASTIGOTES IN THE ASCITIC FLUID OF A HIV-POSITIVE PATIENT

Mariano A.[1], Rianda A.[1], Iacomi F.[1], Vincenzi L.[1], Abdeddaim A.[1], Baiocchini A.[1], Del Nonno F.[1], **Paglia M.G.*[1],** Zaccarelli M.[1], D'Offizi G.[1], D Offizi G.[1]

[1]Istituto NAzionale Malattie Infettive "L. Spallanzani" ~ Roma ~ Italy

INTRODUCTION:
Visceral leishmaniasis has been frequently reported in HIV-positive patients from areas in which leishmaniasis is endemic, including several cases in which Leishmania was detected in atypical anatomical sites.
MATERIALS AND METHODS:
PCR for detection of Leishmania DNA was performed on peripheral blood and ascitic fluid. Leishmania-specific primers, which amplify a nuclear repetitive genomic sequence, were used. Identification of Leishmania at the species level was obtained by restriction enzyme analysis with HaeIII.
RESULTS:
In this case, a 54-year-old HIV-positive Italian male patient was admitted to our Institute following several weeks of progressive weakness and a new onset ascitis. He was on a successful regime of antiretroviral therapy, with undetectable HIV-RNA and a CD4 count of 340/mmc. The patient was HCV and HBV negative, with no alcohol issues, haemoglobin was 8.6 g/dl, leukocytes 1600/mmc, platelets 49000/mmc; gamma-globulin 3.2 gr/dl, albumin 1.6 gr/dl; proteinuria was present (2 gr/day). A CT scan revealed enlargement of both liver and spleen and diffuse lymphoadenopathies.
Microscopic examination of ascitic fluid and of a neck lymphonode revealed macrophages containing intracellular amastigotes consistent with Leishmania species. PCR analysis performed on peripheral blood and ascitic fluid was positive for Leishmania infantum. Anti-Leishmania serology was negative. He was then successfully treated with intravenous liposomal amphotericin B.
CONCLUSIONS:
Clinicians and pathologists with HIV patients should be aware of the possibility of detecting Leishmania amastigotes in atypical anatomical sites.

Keywords:
Leishmania, ascitic fluid, HIV-positive patient

P02.5
MOLECULAR DETECTION OF HEPATOZOON CANIS IN HEALTHY HUNTING DOGS FROM MAREMMA, TUSCANY

Nardoni S.*[1], Papini R.[1], Fognani G.[1], Stefani F.[2], Mugnaini L.[1], Rocchigiani G.[1], Mancianti F.[1]

[1]Dipartimento di Scienze Veterinarie ~ Pisa ~ Italy, [2]Clinica Veterinaria Costa d'Argento ~ Orbetello (Grosseto) ~ Italy

INTRODUCTION:
Hepatozoon canis, transmitted by Rhipicephalus sanguineus, the brown dog tick, is distributed throughout the Old World. Infections are usually asymptomatic but may range from subclinical and chronic, to severe and life-threatening especially in presence of concurrent infections (Allen et al., 2011, Vet Clin North Am Small Anim Pract., 41:1221-38). Data about infection prevalence in asymptomatic dogs from Italy are scanty. H. canis infection has been reported in Central and North-Eastern Italy with low prevalence (0.4%) (Cassini et al., 2009, Vet. Parasitol., 165: 30–35). The other reports from this country have been carried out in a shelter located in southern Italy (Otranto et al., 2011, Parasit Vectors, 4: 55), or are dealing with symptomatic animals (Gavazza et al., 2003, Revue Méd. Vét., 154, 565-571; Marchetti et al., 2009, Vet Clin Pathol., 38: 121-125).
MATERIALS AND METHODS:
A PCR survey was conducted to investigate the prevalence of H. canis infecting hunting dogs from Tuscany Maremma, with no history of recent tick treatments and with no overt clinical manifestations. The animals (N=117), of both genders (69 males and 48 females), aged from 8 to 132 months (median 54 months, SD ± 31.5) were selected among dogs presented at veterinary clinic because of wild boar attacks during hunting activity.
A blood specimen was drawn from each animal and used for PCR purposes. A fragment of the 18S rRNA gene was amplified by PCR, using the primers HepF (5'-ATACATGAGCAAAATCTCAAC-3') and HepR (5'- CTTATTATTCCATGCTGCAG-3') to detect the presence of Hepatozoon sp., as described by Inokuma et al, 2002 (Vet Parasitol. 26; 106:265-71). PCR products obtained from positive samples were then sequenced and analyzed. Sequences were assembled and corrected by visual analysis of the electropherogram using Bioedit v.7.0.2 30, then compared with those available in GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST) to assign the species. Differences between gender and infection were evaluated by chi square test.
RESULTS:
Thirty-eight animals out of 117 (32.4%) scored positive for parasite DNA. Twenty-eight males (40.6%) and 10 females (20.8%) were positive to Hepatozoon. Infection rate was significantly higher in male subjects.
CONCLUSIONS:
H. canis appeared very spread in investigated dogs. This parasite was recovered in similar surveys in 16.7% of foxes from Siena province (Gabrielli et al., 2010, Vector Borne Zoonotic Dis., 10:855-859), in 57.8% from dogs living in the same shelter with high vectorial prevalence (Otranto et al. 2011). Other surveys from Mediterranean basin report prevalences ranging from 3.3% in Spain (Tabar et al., 2009) to 11.8% (Vojta, 2009 Int J Parasitol., 39:1129-36). The sampling period (November to January) coincided with the period of increased possibility in finding infected animals, presumably following the dynamic population of Rh. sanguineus (Dantas-Torres et al., 2012, Parasitol Res, 110:695-698). The prevalence significantly higher in males is in agreement with (Mundim et al., 2008, Vet Parasitol., 153 :3-8), while disagrees with Beaufils et al., 1988 (Pract Med Chir. Anim. Comp, 23: 281-293).

Keywords:
Hepatozoon canis, PCR, dog

P02.6
MONITORING PHLEBOTOMUS-TRANSMITTED VIRUSES IN MARCHE REGION, ITALY

Remoli M.E.*[1], Fortuna C.[1], Marchi A.[1], Bucci P.[1], Argentini C.[2], Bongiorno G.[3], Gradoni L.[3], Maroli M.[3], Superti F.[4], Tinari A.[4], Gramiccia M.[3], Ciufolini M.G.[1]

[1]Unit of Viral diseases and attenuated vaccine, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy, [2]Unit of Global Health, Therapeutic Research and Medicines Evaluation Department, Istituto Superiore di Sanità ~ Roma ~ Italy, [3]Unit of Vector-borne Diseases and International Health, MIPI Department, Istituto Superiore di Sanità ~ Roma ~ Italy, [4]Unit of Ultrastructural Infectious Pathology, Technology and Health Department, Istituto Superiore di Sanità ~ Roma ~ Italy

INTRODUCTION:
Phlebotomus-borne (PhB-) viruses are mainly belonging to the genus Phlebovirus, Vesiculovirus and Orbivirus. The PhB-viruses transmitted by sand flies are cause of meningitis, encephalitis and febrile illnesses. To monitor the stability of the PhB-viruses "foci" and to investigate about the possible circulation of different viral variants, surveillance activity was carried out at the end of summer 2012 in a known Italian PhB-viruses focus in Fermo commune (Marche region) monospecific for Phlebotomus perfiliewi (Ciufolini et al., 1992, Parassitologia, 34, Suppl. 1: 7-8).
MATERIALS AND METHODS:
Thirty pools, for a total of 900 Phlebotomines (540 Females), collected using CDC light traps in Fermo commune, were studied for PhB-viruses presence. For each pool, all identified as P. perfiliewi, we performed virus isolation on VERO cells and two nested-PCR using degenerated primers targeting regions of the L (Sanchez-Seco et al., 2003, J. Med. Virol., 71:140-149) and N (Charrel et al., 2007, Emerg. Infect. Dis., 13:465-468) genes. The PCR products obtained by L and N genes were sequenced and aligned with other Phlebovirus sequences by CLUSTAL within DAMBE software and analyzed in MEGA 5.2. Tissue culture samples showing a cytopathic effect (CPE) were also processed for electron microscopy (EM) examination. Plaque Reduction Neutralization Test was carried out on positive pools using mouse specific antisera against Phleboviruses belonging to the Sandfly fever Naples (SFNV) group.
RESULTS:
Phlebovirus RNA was detected in 7/30 pools (6 pools of females and 1 of males) and all showed also a CPE in VERO cells. EM micrographs showed viral characteristics compatible with virus belonging to the Bunyaviridae family. The sequences analysis showed that 1 isolate clustered in Toscana virus (TOSV) subgroup and the other six (5 females and 1 males pools) showed a high distinction from all known Phleboviruses analyzed both at nucleotide and aminoacid level. By using specific primers for Phleboviruses within the SNFV complex, we confirmed that the 6 Fermo isolates belong to Naples Serocomplex. Serological studies corroborated these results suggesting that these isolates belonged to a novel Phlebovirus, tentatively named Fermo virus.
CONCLUSIONS:
This study provided genetic evidence that TOSV continue to circulate in P. perfiliewi populations in Central Italy. Interestingly, we isolated the novel Fermo virus also from male sand flies pool suggesting that even in this virus the transovarial transmission occurs under natural conditions. Further investigations are planned to monitor the Fermo virus circulation by seroprevalence surveys and investigations on possible association with neurological or febrile summer diseases.

This work was supported by EDENext, a collaborative project of the 7th FP (2011-2014) funded by the European Commission under the DG Health; Contract Number: 261504.

Keywords:
Phlebovirus, Surveillance, Phlebotomus perfiliewi

NOTES

POSTER 03

FARMACORESISTENZA

P03.1

FREQUENCY OF CYP2C8*2 AND CYP2C8*3 ALLELES IN UGANDAN CHILDREN EXPOSED TO MALARIA

Paganotti G.M.*[1], Cosentino V.[1], Gramolelli S.[1], Tabacchi F.[1], Russo G.[1], Coluzzi M.[1], Romano R.[1]

[1]Department of Public Health and Infectious Diseases, "Sapienza" University, Roma ~ Italy

INTRODUCTION:
Plasmodium falciparum malaria is a priority in public health mainly in sub-Saharan Africa and particularly in Uganda where it represents one of the most important parasitic diseases. Vector control and drug therapy remain the primary goals to contrast the infection. Unfortunately, P. falciparum has selected resistant strains for the majority of the molecules used in anti-malarial therapy. Moreover, the cytochrome P450 2C8 (CYP2C8), a polymorphic enzyme that mainly contributes to the hepatic metabolism of amodiaquine (AQ) and chloroquine (CQ), shows genetic variants (CYP2C8*2, rs11572103, 805A/T and CYP2C8*3, defined by the presence of two SNPs: rs11572080, 416G>A and rs10509681, 1196A>G) that has been associated with higher rate of drug resistant parasites in the infected host. CYP2C8*2 is the variant most common in East and West Africans and is associated to a poor metabolizer phenotype in subjects carrying at least one copy of the defective allele. CYP2C8*3 is a very defective allele and is only known to be present in Zanzibar islands (Tanzania) as regard Africa. The aim of this study is to investigate cytochrome CYP2C8*2 and *3 distribution and allele frequency in two Ugandan populations exposed to P. falciparum malaria.
MATERIALS AND METHODS:
The samples analysed in the present study were collected during cross-sectional surveys performed in Uganda (Namalu and Rupa health centres, Kakoliye and Nadunget primary schools -Karamoja region; Makindiye children centre – Kampala region). A total of 261 children and adolescents had been enrolled and genotyped for the single nucleotide polymorphisms rs11572103,, rs11572080 and rs10509681. Fingerpick blood samples were spotted on Whatman grade 1 filter papers at the time of the field survey and then air dried before being separately stored in sealed plastic containers. The PCR-RFLP technique was used to discriminate the wild-type from the defective alleles.
RESULTS:
The CYP2C8*2 allele frequency (± SE) in rural sites of Karamoja region (North-Eastern Uganda) was 0.10 ± 0.02, while was 0.13 ± 0.03 in the Kampala suburbs (central Uganda). Both the genotype distributions are in Hardy-Weinberg equilibrium, and the allele frequencies are not statistically different (Yates corrected $\chi 2=0.89$, $P=0.346$). The overall CYP2C8*2 frequency in all sites was 0.10 ± 0.02. Genotype frequencies were in Hardy-Weinberg equilibrium ($\chi 2=1.52$ $P=0.221$). The allele CYP2C8*3 is absent for all the populations studied (frequency = 0.00).
CONCLUSIONS:
Our study demonstrated that CYP2C8*2 allele is widespread in Africa and is present at an appreciable frequency also in Uganda, an area of hyperendemic malaria transmission. Moreover, the absence of the CYP2C8*3 allele is a confirmation that it is a marker of genetic admisture of the Zanzibari population with a Caucasoid component. Antimalarial treatment in Uganda is based on artemisinin combination therapies (ACTs) with artesunate (AS) plus AQ being used as second line ACT. Consequently, the presence of the CYP2C8*2 allele may be a potential co-factor in the onset of adverse side effects associated with AQ administration. Furthermore, we emphasize the risk related to the presence of CYP2C8*2 in selecting AQ-resistant strains, since the interplay between host and parasite genetic variation could be similar to that of CQ.

Keywords:
cytochrome, anti-malarial therapy, Ugandan children

NOTES

NOTES

POSTER 04

CONTROLLO DELLE PARASSITOSI

P04.1
POUR-ON EPRINOMECTIN IS AN EFFECTIVE TREATMENT FOR NATURAL HAEMATOPINUS ASINI INFECTION IN DONKEYS (EQUUS ASINUS)

Veneziano V.*[1], Mariani U.[2], Neola B.[1], Cimmino R.[1], Di Loria A.[3], Piantedosi D.[1], Gokbulut C.[4]

[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Italy ~ Napoli ~ Italy, [2]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy ~ Portici ~ Italy, [3]Department of Health Science, University of Magna Græcia, Catanzaro, Italy ~ Catanzaro ~ Italy, [4]dDepartment of Medical Pharmacology, Balikesir University Turkey ~ Balikesir ~ Turkey

INTRODUCTION:
Donkeys and horses may be infested with two species of louse, the chewing louse, Werneckiella equi and the sucking louse Haematopinus asini. Louse infestation often leads to alopecia, anorexia, restlessness, loss of body condition and anaemia (Veneziano et al., 2012, Parasitol Res, 111:967-973). Eprinomectin (EPM), a member of the avermectins class, is marketed as a pour-on formulation for use in cattle. Therapeutics, such as antiparasitic compounds, are often administered to donkeys based on dosage and intervals recommended for horses and cattle, because very few drugs have donkey-specific label indications (Grosenbaugh et al. 2011, Equine Vet Educ, 23:523-530). The aim of the present study was to evaluate the efficacy of EPM pour-on against natural infestation of H. asini on donkeys.
MATERIALS AND METHODS:
The trial was performed in a donkey farm located in Southern Italy. In the absence of standardized guidelines for the quantification of lice on equids, the WAAVP guidelines used to evaluate the efficacy of ectoparasiticides in ruminants (Holdsworth et al., 2006, Vet Parasitol, 136:45-54) and the louse counting procedures described for donkey (Veneziano et al., 2012) were used. On the day before treatment, louse counts were performed on 15 naturally infested donkeys by recording the individual louse count at seven louse predilection sites: head, neck/mane, shoulders/withers, foreleg, back, hindleg and tailhead/rump. On day 0 the animals received EPM pour-on (Eprinex, Merial) at the manufacturer's recommended cattle dose (0.5 mg/Kg body weight). There was no untreated control group for animal welfare reasons. Louse counts were performed weekly (day –1, 7, 14, 21, 28, 35, 42, 49 and 56) by summing all predilection site counts. The efficacy (%) was determined using Abbott's formula: Efficacy=100x[(C-T)/C] (T=louse count after the treatment and C=louse count before the treatment). The Body Condition Score of each animal was determined prior to treatment (day -1) and at the end of study (day 56) using the BCS chart (The Donkey Sanctuary, 2003).
RESULTS:
In total, 589 H. asini were recorded from the inspection of the study donkeys on day -1 (mean 39.3). More than the half of the louse burden was found in the area along the neck/mane (40.6%) and shoulders/withers (25.8%). The head, back, foreleg, tailhead/rump and hindleg sites contained 17.2, 12.5, 2.3, 1.4 and 0.2% of the counted lice, respectively. For all post-treatment days, no lice were counted at the inspection sites or during whole body inspections; resulting in an efficacy of 100% for days 7-56. Considering the total efficacy of treatment, no statistical analysis was performed. The BCS values were in the normal range for donkeys. No abnormal animal health conditions related to treatment were observed during the study.
CONCLUSIONS:
This trial demonstrates that EPM at the manufacturer's recommended cattle dose was completely effective and user-friendly for the treatment of H. asini infestation on donkeys.

Keywords:
Haematopinus asini, Donkey, Eprinomectin

P04.2
FIELD EFFICACY STUDY OF MOXIDECTIN POUR-ON AGAINST NATURAL HAEMATOPINUS TUBERCULATUS INFESTATION ON BUFFALO (BUBALUS BUBALIS) AND INFLUENCE OF THE TREATMENT ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCES

Veneziano V.*[1], Cimmino R.[2], Miotto L.[3], Bassini A.[3], Claps S.[4], Rufrano D.[4], Varricchio E.[2], Neglia G.[1]

[1]Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Italy ~ Napoli ~ Italy, [2]Department of Sciences and Technologies, University of Sannio ~ Benevento ~ Italy, [3]Zoetis ~ Roma ~ Italy, [4]Consiglio per la Ricerca e la Sperimentazione in Agricoltura - Unità di Ricerca per la Zootecnia Estensiva ~ Bella Muro, Potenza ~ Italy

INTRODUCTION:
Haematopinus tuberculatus is a sucking louse of buffaloes and other ruminants. Louse infestation often leads to skin irritation, anemia and loss of body condition. On buffalo, formulations marketed for cattle have been tested against H. tuberculatus, mainly macrocyclic lactones (MLs) such as ivermectin, doramectin and eprinomectin (Veneziano et al, Parasitol Res, 2013, 112:2907-2912). Moxidectin (MOX) is a second generation MLs with potent endectocide activity. In Italy MOX is marketed as a pour-on formulation for cattle, with 6 days milk-withdrawal time. The aim of this study was to assess the efficacy of MOX pour-on against H. tuberculatus on infested buffaloes and the influence of the treatment on milk production and reproductive activity.
MATERIALS AND METHODS:
The study was performed on 64 buffaloes (46 pluriparous + 18 primiparous) in southern Italy, naturally infested by H. tuberculatus. One day before treatment (day -1) louse counts were performed on animals. Buffaloes were divided in 2 groups, according to milk yield recorded in the previous year (for pluriparous) and at random for primiparous and louse counts. On day 0 (1 week before parturition), the MOX-group (n=32: 23 pluriparous + 9 primiparous) received Cydectin 0.5% Pour On (Zoetis) at the manufacturer's recommended dose rate. The Control (C) group (n=32: 23 pluriparous + 9 primiparous) received pour-on saline solution. The parasitological investigations were performed weekly on 20 buffaloes (10 in each Group). Louse counts were performed from day -1 until day 84 at 8 predilection sites on each buffalo, according to the WAAVP guidelines (Holdsworth et al, 2006, Vet Parasitol, 136:45-54). Body condition score (BCS) was recorded at the start and at the end of the trial. Milk production was daily recorded by an automatized milking machine. Fertility rate and intercalving period were also calculated. Statistical analysis was performed by ANOVA and Chi Square test.
RESULTS:
On day-1 an average of 80 and 87 lice were counted in MOX and C groups, respectively. During the trial, MOX was well tolerated by all animals and was completely effective (100%) throughout the study. BCS was higher (P=0.08) in MOX Group compared to C Group at the end of the study (7.0±0.1vs7.3±0.1, in C and MOX Groups, respectively). Total milk production throughout the experimental period was significantly (P<0.01) different between Groups (10.35±0.1 vs 10.98±0.1 kg, in C and MOX Group, respectively) in pluriparous (10.70±0.1 vs 11.03±0.1 kg in C and MOX Group, respectively) and primiparous (9.70±0.1 vs 10.80±0.2 kg in C and MOX Group, respectively). The fertility rate recorded in pluriparous buffaloes was similar between groups (83.0 vs 88.0% in C and MOX Groups, respectively), whereas a significantly higher fertility rate was observed in primiparous animals (50.0 vs 78.0%, in C and MOX Group, respectively). The intercalving period (days) was also significantly (P<0.01) lower in treated compared to untreated buffaloes (504±13 vs 427±13, respectively), in both primiparous and pluriparous. These results may be explained by the higher dry matter ingestion and the lower negative energy balance that animals encountered during the first phase of lactation after treatment.
CONCLUSIONS:
This trial suggests that MOX is effective, safe compound for the treatment of buffaloes with louse infestation. MOX can be used as antiparasitic drug in buffalo farms providing convenience for treatment in periparturient period, probably reducing the stress of the first phase of lactation.

Keywords:
Haematopinus tuberculatus, Buffalo, Moxidectin

P04.3
A QUESTIONNAIRE SURVEY ON WORM CONTROL PRACTICES IN HORSES IN ITALY

Papini R.*[1], De Bernart F.[1], Sgorbini M.[1]

[1]Dipartimento di Scienze Veterinarie ~ Pisa ~ Italy

INTRODUCTION:
Intestinal parasitism is a widely recognized problem in horses (Uhlinger 2007, Vet Clin Equine, 23:509-517). Though anthelmintics have a pivotal role in controlling the effects of parasites, there is a paucity of data on how horse breeders and owners use anthelmintics in Italy (von Samson-Himmelstjerna et al., 2009, Parasit Vectors, 2:S3).
MATERIALS AND METHODS:
A sample of 152 horse breeders and owners were surveyed in a cross-sectional study by using an interview-based questionnaire regarding farm structure, horse management, and parasite control practices.
RESULTS:
It was found that more than half of the farms had access to grazing areas. Pasture hygiene was not practiced on many farms or was inadequately performed. Anthelmintics were used in almost all of the farms. The frequency of treatment in some farms was surprisingly high (every 4-8 weeks). Planning of the anthelmintic treatment strategy for most of the farms was responsibility of breeders, owners, or personnel. In many cases the live weight to estimate the anthelmintic dose was determined by visual appraisal or was not determined at all. Macrociclic lactones were the most commonly used drugs and the majority of the interviewed had been using the same class of anthelmintics for the last two years. Treatment in most farms did not depend on the epidemiology of parasites. Coprological examinations after treatment were rarely performed while faecal egg count reduction test was never performed.
Some farms had experienced problems caused by endoparasites. Most of the breeders and owners ranked the importance of worm infections on their own farms as high. In many cases anthelmintic resistance was not perceived to be a problem. Most breeders and owners ranked the information about endoparasite control from veterinary practitioners as high.
CONCLUSIONS:
Based on these results, departures from best practices in anthelmintic administration were observed. These could enhance the development of anthelmintic resistance (Coles, 2002, Vet Rec, 151-165-169). Therefore, horse breeders and owners need to be encouraged to know the problem of anthelmintic resistance, to monitor the resistance status of nematode populations on their farms, and to develop strategies aimed at maintaining the effectiveness of the existing anthelmintic drugs. For this purpose, the capacity of veterinary practitioners should be targeted to communicate information relating the best practices in gastro-intestinal helminth control. This study provides a picture of worm control practices employed by horse breeders and owners in Italy and allowed us to identify risk factors for future studies on development of anthelmintic resistance in this country.

Keywords:
Equine anthelmintics, Questionnaire, Italy

P04.4
DIAGNOSTIC PATHWAY FOR PATIENTS WITH HEPATIC CYSTIC ECHINOCOCCOSIS: A RETROSPECTIVE ANALYSIS OF A SAMPLE OF SARDINIAN CASES

Conchedda M.*[1], Caddori A.[2], Bortoletti G.[1], Cagetti M.[1], Caredda A.[1], Seu V.[1]

[1]Università di Cagliari ~ Cagliari ~ Italy, [2]P.O. SS. Trinità ~ Cagliari ~ Italy

INTRODUCTION:
Cystic echinococcosis (CE) is endemic in Sardinia and despite being on the decline, incidence in humans (Conchedda et al, 2010, Parasitol International 59: 454-9) and prevalence in sheep (Conchedda et al, 2012, Acta Tropica 122:52-8) remain high. The economic and social costs of CE are significant, both at the individual and community level. The most tangible costs are expensive clinical treatments for humans comprising diagnostic procedures, surgery and/or chemotherapy, hospitalization, and economic loss during convalescence. Diagnostic pathways and related costs are difficult to evaluate, considering the long latency and pauci-symptomaticity of the disease, especially hepatic CE.
The aim of this study was to investigate the diagnosis pathway of clinical cases of hepatic CE, which account for > 70% of total locations, in order to estimate the direct costs of human CE.
MATERIALS AND METHODS:
A retrospective analysis was conducted on the medical records of a sample of 81 Sardinian patients with a mean age of 49 years (10-78), who had undergone surgical treatment for hepatic CE. A single cyst was detected in 75% of patients, most frequently in the right lobe, while 25% had several cysts also in other organs. Diagnostic pathways were recorded and related costs estimated.
RESULTS:
About 40% of patients in the sample were asymptomatic and cysts were incidentally discovered during imaging carried out for other reasons. Around 45% complained of a dull ache in the right hypochondrium and about 15% showed signs of complications. 20% of patients had relapsed 4-30 years post-surgery, mostly on the lungs.
Regarding medical imaging, the diagnosis pathway involved ultrasound scanning for virtually all patients, which is useful for the functional classification of cysts (CL, CE1, CE2, CE3, CE4, CE5) and for determining intrahepatic topographic position. CT scans were also frequently required to detect small cysts and show site and morphology of mainly CE 4 and CE5 cysts in greater detail. Vice versa magnetic resonance imaging was rarely performed as it does not provide much additional information though for simple cysts the pathognomonic circular rim of the pericyst can be observed.
Endoscopic retrograde cholangiography was performed on patients with suspected intra-biliary rupture to investigate the condition of bile ducts. X-ray imaging, performed on 20% of the sample continues to be valuable for showing upward displacement of the diaphragm, possibly indicative of a hydatid cyst in the liver, and for revealing cyst calcification. Serology tests were also performed on 25% of patients to confirm the diagnosis.
CONCLUSIONS:
The direct costs of human CE in Sardinia in terms of hospital stays alone have been estimated at about 4 million euros over 5 years (Conchedda et al., 2010, Parasitol. International,59: 454–9), but chemotherapy at home, rehabilitation and diagnostics also need to be considered. This study suggests additional specific costs of around €150-250 per surgical patient for specific diagnosis costs, possibly increased by repeated tests due to the long latency of the disease. Indirect tangible as well as intangible costs should also be considered.

Keywords:
cystic echinococcosis, costs, humans

P04.5
COMPARISON OF TWO POUR-ON FORMULATIONS OF IVERMECTIN AGAINST GASTROINTESTINAL WORMS, FLEAS AND LICE IN NATURALLY INFECTED STRAY DOGS

Ibarra Velarde F.*[1], Vera Montenegro Y.[1], Ambía Medina J.[2], Sánchez Morales K.[1], Ochoa Galvan P.[1]

[1]FMVZ-UNAM ~ Mexico city ~ Mexico, [2]Lab. Salud Animal ~ Mexico city ~ Mexico

INTRODUCTION:
Infections with intestinal parasites (IP), fleas and lice in dogs and cats are very common all over the World. Macrocyclic lactones, such as avermectin and milbemycins, show a high anti-parasitic activity against nematodes, fleas and lice in dogs. In Mexico, where disease-carrying IP, fleas and lice in dogs are abundant, no controlled test on the impact of pour- on ivermectin has been carried out. The aim of the present study is to compare the efficacy of two pour-on ivermectin formulations against IP, fleas and lice in naturally infested stray dogs.
MATERIALS AND METHODS:
Eighteen stray dogs were selected of intestinal parasites or adult fleas and lice. On day 0, the dogs were divided into 3 groups (G) of 6 animals each. Group 1 (G1) received Vermisan Pour-on applied percutaneously at 500 mg/kg-1. G2 received Dermodex pour-on applied at 200 mg-1/Kg, according to label instructions. G3 served as a control. Simple fecal McMaster method was carried out on days 0, 7, 14, 21 and 28 to determine the percentage of Egg Per Gram of Feces (EPGF). On day 30 after treatment, the necropsy of animals was performed to obtain the intestine and to collect and identify the adult worms. In addition, the number of fleas and lice after treatment was also individually recorded. Efficacy was measured as the percentage reduction in the EPGF, adult worms, as well as in the number of fleas and lice from the treated groups relative to the untreated control. The information obtained was submitted to the Kruskall-Wallis test and the Wilcoxon sign test, using the Scientific Analysis System (S.A.S) packet, 2004.
RESULTS:
The efficacy of Vermisan Pour-on through the EPG reduction against ascarids was 100, 75.5, 98.7 and 100%, and for Ancylostoma 100, 87.5, 83.3 and 79%, for days 7, 14, 21 and 28, respectively. The data obtained for Dermodex showed a lower efficacy. The ascarid reduction of EPG were 46.6, 100, 91.6 and 90.2% and for Ancylostoma 50.7, 47.1, 42.3 and 50.4%, respectively. Specimens such as Dypilidium caninum, Taenia taeniaeiformis, Toxocara canis, Toxascaris leonina and Ancylostoma caninum were collected at necropsy. The percentage of efficacy for Vermisan Pour-on was 100% for T. canis and A. caninum. For Dermodex the efficacy obtained against adult worms was 62.4% for T. canis and 76.4%, for A. caninum. Efficacy against fleas and lice.- The percentage reduction for Vermisan Pour-on on fleas was 67.9, 78.8, 91.5 and 96% and for Dermodex 57.7, 66.7, 59.2 and 71.1%, respectively. The statistical analysis showed significant differences betwen groups (p<107), (p<.0076), (p<0024) and (p<.001) for days 7, 14, 21 and 28, respectively, indicating that the efficacy produced by Vermisan Pour-on on fleas was higher. In the case of lice, both compounds showed 100% efficacy
CONCLUSIONS:
Vermisan Pour-on showed better percentages of efficacy than Dermodex either in egg reduction or in the elimination and intestinal parasites as well as in the case of fleas. Both compounds showed 100% efficacy against lice. Further studies should be conducted to determine if there is any toxicity in the use of pour-on presentations of these ivermectins on dogs since the available data up to now is limited.

Keywords:
Pour-on Ivermectins, Endo and Ectoparasites, Stray dogs

P04.6
IN VITRO ANTHELMINTIC EFFECT OF TROPICAL PLANT EXTRACTS ON EXCYSTED FLUKES OF FASCIOLA HEPATICA

Alvarez Mercado J.M.[1], Ibarra Velarde F.[1], **Vera Montenegro Y.*[1],** Alonso Díaz M.A.[1], Avila Acevedo G.[2]

[1]FMVZ-UNAM ~ Mexico city ~ Mexico, [2]FES-Iztacala- UNAM ~ Mexico city ~ Mexico

INTRODUCTION:
Fasciolosis, caused by Fasciola hepatica, has a worldwide distribution affecting cattle, and other animals. Recent studies have shown that plants and their components such as Artemisia mexicana, Mentha piperita, Achillea millefolium, Allium sativum, Piper nigrum, and Carica papaya have parasiticidal effects against F. hepatica. The objective of the present study was to evaluate the anti-Fasciola hepatica effect of fifteen plants extracts from Veracruz, Mexico.
MATERIALS AND METHODS:
Fresh leaves of Acacia cornígera, Acacia farnesiana, Artemisia absinthium, Artemisia mexicana, Bocconia frutescens, Cajanus cajan, Cordia spp, Hibiscus rosa - sinensis, Lantana camara, Leucaena diversifolia, Melia azedarach, Mentha sp, Ocimum basilicum, Piper auritum and Teloxys ambrosioides were collected from villages in Veracruz, Mexico. To determine the antihelmintic effect of the 15 plant extracts on mortality of excysted flukes a series of in vitro experiments were undertaken. Newly excysted flukes were obtained by the artificial excysment of F. hepatica metacercarie according to Ibarra and Jenkins (1984). Those plant extracts with in vitro fasciolicide efficacy higher than 80 % were re-evaluated twice in order to confirm the results, and a concentration of 375 mg/L was added to calculate the lethal concentration to kill 50%, 90% and 99% of the flukes (LC50, LC90 and LC99). All procedures were performed under aseptic conditions using a laminar flow hood. The flukes under study were examined at 24, 48 and 72 hours post-exposure. Activity was measured by comparing the survival of the treated flukes relative to those of the control group. The effectiveness of the plant extracts was assessed with the formula of Abbott, (2004). When an extract showed an in vitro efficacy greater than 80%, it was considered to possess fasciolicide activity. A Kruskal-Wallis test, P <0.05 was used to determine significant differences (STATGRAPHICS v.16.1.17) and a PROBIT test was performed with POLO PLUS (LeOra Software, 2003) to determine the LC50, LC90 and LC99 of the extracts that showed in vitro fasciolicide efficacy.
RESULTS:
Flukes placed in the control wells remained alive and healthy throughout all the tests. Five plant extracts at different dose levels effectively killed the trematodes (P<0.05, Table 1). At a dose of 500 mg/L, Cajanus cajan, Lantana camara and Piper auritum had an efficacy of 100%. While Bocconia frutescens and Artemisia mexicana had a 100% efficacy since a dose of 125 mg/L. Melia azedarach, showed an efficacy of 13% at a concentration of 500 m/L. The five extracts that showed in vitro flukicide activity greater than 80% are indicated in table 2. These were evaluated for a second time including a concentration of 375 mg/L to determine the LC50, LC90 and LC99. Lethal concentration estimates at 50%, 90% and 99% for exposed flukes to plant extracts. The slope, LC 50, 90 and 99% in Artemisia mexicana, Cajanus cajan and Bocconia frutescens tested plant extracts are shown in Table 3. Artemisa mexicana showed significantly lower LC50, LC90 and LC99 estimates than C. cajan and B. frutescens.
CONCLUSIONS:
Of the fifteen extracts tested, five showed promising in vitro fasciolicide efficacy, thus indicating that they could possibly be strong candidates for further testing and analyses aimed at demonstrating their real potential for liver fluke control in ruminants.

Table 1. Anti-fluke *in vitro* effectiveness of plant extracts.

Extract	Efficacy (%)[c]			
	0 mg/l	125mg/l	250 mg/l	500 mg/l
Cajanus cajan	0[a]	0[a]	0[a]	100[b]
Lantana cámara	0[a]	0[a]	0[a]	100[b]
Bocconia frutescens	0[a]	10±0.1[a]	100[b]	100b
Melia azedarach	0[a]	7±0.11[a]	7±0.11[a]	13±0.11[a]
Pipor auritum	0[a]	0[a]	0[a]	100[b]
Artemisia mexicana	0[a]	100[b]	100[b]	100[b]

Different letter between columns indicates statistically significant differences. Significant at p < 0.05 level. Control—nil mortality.
[c] average of three replicates ± standard deviation.

Table 2. Second Assessment of Anti-fluke *in vitro* effectiveness of plant extracts.

Extract		Efficacy (%)[c]				
		0 mg / L	125 mg / L	250 mg / L	375 mg / L	500 mg / L
Artemisia mexicana	n=10	0[a]	93±0.06[b]	100[a]	100[a]	100[a]
Bocconia frutescens		0[a]	0[a]	100[b]	100[b]	100[b]
Lantana camara		0[a]	0[a]	0[a]	93±0.06[b]	100[b]
Piper auritum		0[a]	0[a]	0[a]	83±0.06[b]	100[b]
Cajanus cajan		0[a]	0[a]	0[a]	93±0.06[b]	93±0.06[b]

Different letter between columns indicates statistically significant differences. Significant at p < 0.05 level. Control—nil mortality.
[c] average of three replicates ± standard deviation.

Table 1, 2

Table 3. Lethal Concentrations from plant extracts with fasciolicide efficacy *in vitro*.

Extract	LC_{50} (mg/L)	UCL-LCL	LC_{90} (mg/L)	UCL-LCL	LC_{99} (mg/L)	UCL-LCL	S.E	x^2(df=10)
Artemisia mexicana	92.85	42.16-124.50	210.44	166.78-306.78	410.04	288.46-1135.26	±2.197	5.893
Cajanus cajan	382.73	327.13-444.12	570.09	479.89-908.48	788.9	603.92-1768.30	±3.653	15.258
Bocconia frutescens	369.96	318.77-419.83	529.94	457.78-748.36	710.34	567.74-1298.47	±3.813	14.702

LC_{50}—lethal concentration that kills 50% of the exposed flukes, LC_{90}—lethal concentration that kills 90% of the exposed flukes, LC_{99}—lethal concentration that kills 99% of the exposed flukes, UCL: upper confidence limit; LCL: lower confidence limit; x^2—Chi-square; df: degree of freedom. Significant at $p < 0.05$ level.

Table 3

Keywords:
Plant Extracts, Fasciola hepatica, Anthelmintic in vitro activity

P04.7

THE ADULTICIDE EFFECT OF A COMBINATION OF DOXYCYCLINE AND IVERMECTIN IN DIROFILARIA IMMITIS-EXPERIMENTALLY INFECTED DOGS WHICH IS ASSOCIATED WITH REDUCTION IN LOCAL T REGULATORY CELL POPULATIONS

Passeri B.[1], Vismarra A.[1], **Kramer L.H.*[1],** Bazzocchi C.[2], Bacci C.[1]

[1]Dipt. Scienze Medico Veterinarie ~ Parma ~ Italy, [2]Dipt. Patologia Animale ~ Milano ~ Italy

INTRODUCTION:
worms in otherwise immune-competent hosts. Several recent studies have indicated an important role for regulatory T cells (Treg) in inducing hypo-responsiveness to filarial infection (McSorley et al., 2008, J. Immunol, 181:6456-6466; Korten et al., 2011, Parasitology, 138:35–45). Furthermore, it has been reported that successful adulticide treatment may also depend on changes in the immune response to infection and a reduction of immune-tolerance (Mai et al., 2006, Clin Exp Immunol, 147: 504–512) The present study was aimed at evaluating Treg cell populations in Dirofilaria immitis-experimentally infected dogs treated with different treatment protocols and compared to untreated controls.
MATERIALS AND METHODS:
Briefly, lung tissue conserved in formalin from a previous study of D. immitis-experimentally infected dogs (Bazzocchi et al., 2008, Int. J. Parasitol, 38:1401–1410) was used. Treatment protocols are reported in Table 1. Each group consisted of four dogs. The right caudal lobe of each lung was sampled and processed for immuno-histochemistry using an anti-Foxp3 primary antibody (1.200; eBioscience, Italy). Positive cells were examined and enumerated. The total number of cells for 5 fields at 40X magnification was determined for each dog and the mean for each group was calculated.
RESULTS:
Figure 2 reports the results of Treg cell populations in the different treatment groups. Dogs treated wuith a combination of doxycycline and ivermectin showed a significant ($p = < 0.05$) reduction in Treg cell populations compared to the other treatment groups. Interestingly, while treatment protocols for groups 1 and 2 showed an adulticide effect of 20% and 9% respectively, a combination of the two resulted in the death of 80% of adult worms at necropsy. Dogs treated with melarsomine showed a 100% adulticide effect, but without significant reduction in Treg cell populations.
CONCLUSIONS: It has been hypothesized that the intracellular endosymbiont Wolbachia is responsible for inducing tolerance to filarial infections and the removal of the bacteria through specific antibiotic treatment may cause a shift in the immune response. Here, the authors show that antibiotic treatment in D. immitis infected dogs, when combined with ivermectin, results in a significant reduction of regulatory immune cells.

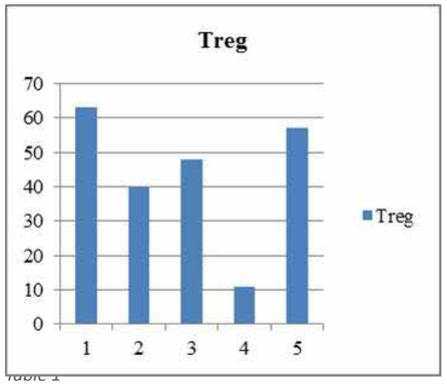


Table 1

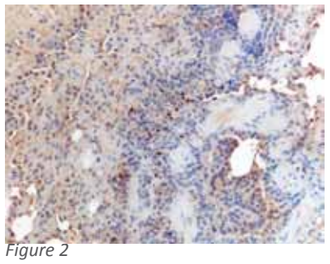


Figure 2

Keywords:
Dirofilaria immitis, Ivermectin, Doxycyline

NOTES

POSTER 05

ENTOMOLOGIA MEDICA E VETERINARIA

P05.1
IN-EMERGENCY SURVEILLANCE FOR FLAVIVIRUS PERFORMED AFTER A HUMAN CASE OF WEST NILE VIRUS IN CENTRAL ITALY

Antognini E.*[1], Canonico C.[1], Barchiesi F.[1], Ruschioni S.[2], Moascatelli F.[1], Moresi M.[3], Gavaudan S.[1]

[1]Istituto Zooprofilatico Sperimentale Umbria Marche ~ Perugia ~ Italy, [2]Università Politecnica delle Marche ~ Ancona ~ Italy, [3]Azienda Sanitaria Unica Regione Marche ~ Ancona ~ Italy

INTRODUCTION:
In summer 2011, in the city of Ancona (Marche, Italy) a case of human WNDv fever was hospitalized. Full genomic sequencing identified the virus as belonging to the high virulent lineage 2 (Bagnarelli et al., 2011, Euro Surveill., 16:43).
On the basis of the epidemiological survey, the virus was probably introduced by mosquitoes through shipping trades in the harbor. In fact, WNDv Lineage 2 was widely spreading at that time in Greece but not yet in Italy. Entomological surveillance was carried out in the harbor were the man lives and works, with the aim to detect the WNDv in mosquitoes as soon as index human possible case was reported.
MATERIALS AND METHODS:
The risk Area was recognized; every mosquitoes breeding site was removed and the inextinguishable sites were disinfested and then monitored. Seven CDC's CO2 traps and four BG sentinel traps® with BG Lure attractant were used during 22 night and day captures. Mosquitoes were submitted to the laboratory twice a day.
Captures of each trap was examined in order to identify the mosquitoes species by morphological keys and their relative abundance; then the screening by RT-PCR was performed on pools composed at least by ten insects. The presence of flavivirus RNA was confirmed by whole genomic sequencing.
Each pool was analyzed for flavivirus by RT-PCR and positive samples have been confirmed by whole genome sequencing.
RESULTS: An overall of about one hundred and forty Culex pipiens and one hundred and thirty Aedes albopictus, not engorged female mosquitoes were analyzed.
Mosquito-flavivirus was found in five pools of Culex pipiens and in four pools of Aedes albopictus.
Usutu virus was found in three Culex pipiens pools.
West Nile Virus was never been detected by RT-PCR.
Usutu virus was belonging the same strain that had caused a large scale outbreak, previous year, in blackbirds (Turdus merula) in the studied Area.



Traps installed

DATE	ZONE	N° TRAP	TRAP	MOSQUITO	PCR FLAVIVIRUS
30/9/11	HOUSE	S3	Night CDC	C.pipiens	not detected
30/9/11	HOUSE	S3	Night CDC	Ae.albopictus	not detected
30/9/11	Harbor	P3	Night CDC	C.pipiens	Usutu - Mosquito flavirus
1/10/11	HOUSE	S3	Night CDC	C.pipiens	Mosquito flavi virus
1/10/11	HOUSE	BG F1	Day BG sentinel	Ae.albopictus	Negativo
1/10/11	Harbor	P3	Night CDC	C.pipiens	Usutu - Mosquito flavirus
1/10/11	Harbor	P3	Night CDC	Ae.albopictus	Mosquito flavirus
1/10/11	Harbor	BG P	Day BG sentinel	Ae.albopictus	not detected
1/10/11	HOUSE	S2	Night CDC	C.pipiens	DETECTED (unidentified.)
1/10/11	HOUSE	S2	Night CDC	Ae.albopictus	DETECTED (unidentified.)
1/10/11	HOUSE	BG F1	Day BG sentinel	Ae.albopictus	not detected
2/10/11	Harbor	P1	Night CDC	C.pipiens	DETECTED (unidentified.)
3/10/11	HOUSE	BG F1	Day BG sentinel	Ae.albopictus	not detected
3/10/11	Harbor	BG P	Day BG sentinel	Ae.albopictus	DETECTED (unidentified.)
3/10/11	HOUSE	BG F1	Day BG sentinel	Ae.albopictus	not detected
5/10/11	HOUSE	S4	Night CDC	C.pipiens	Usutu
5/10/11	Harbor	S3	Night CDC	C.pipiens	not detected
6/10/11	HOUSE	S3	Night CDC	C.pipiens	not detected
6/10/11	HOUSE	BG F1	Day BG sentinel	Ae.albopictus	not detected
6/10/11	Harbor	P2	Night CDC	C.pipiens	not detected
6/10/11	HOUSE	BG F1	Day BG sentinel	not detected	
17/10/11	Harbor	P3	Night CDC	C.pipiens	not detected
17/10/11	Harbor	P3	Night CDC	Ae.albopictus	Mosquito flavivirus
17/10/11	HOUSE	S1	Night CDC	C.pipiens	not detected
18/10/11	Harbor	P1	Night CDC	C.pipiens	not detected
18/10/11	Harbor	P1	Night CDC	Ae.albopictus	not detected
21/10/11	Harbor	P1	Night CDC	Ae.albopictus	not detected
21/10/11	Harbor	S4	Night CDC	C.pipiens	not detected
TOTAL FLAVIVIRUS					10

Results

CONCLUSIONS:
In-emergency entomological surveillance plan is a fundamental tool to measure the width of outbreak's area and a "just in time" pest control is addressed to reduce the spreading of the virus.

Keywords:
West Nile disease, Vector borne disease, surveillance

P05.2
FIRST REPORT OF BITE BY YELLOW SAC SPIDER (CHEIRACANTHIUM PUNCTORIUM, VILLERS, 1769) IN MAN, IN SOUTHERN ITALY (BASILICATA REGION)

Cafiero M.A.*[1], Raele D.A.[1], Mancini G.[1], Galante D.[1], Palazzo L.[2], Quaranta V.[2]

[1]Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Entomologia Sanitaria Italy ~ Foggia, ~ Italy, [2]Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Sez. Diagnostica ~ Potenza ~ Italy

INTRODUCTION:
Among the arthropods identified at the Istituto Zooprofilattico di Puglia e Basilicata (IZSPB), spiders from synanthropic environments are scarce, with only 2 Lycosa sp. and 1 Argiope sp. specimens, in a period of 15 years. In Italy, where some species of potentially dangerous spiders are present, documented cases of spider bite inflictions on humans are also rare. It was deemed interesting to summarize a case of aggression from spider in man.
MATERIALS AND METHODS:
In the early days of August 2011, the IZSPB (Section of Potenza) was contacted by the local hospital emergency department to identify a spider suspected of having bitten a man. The patient, male, 48 years-old, lived in a cottage located in the periurban area of the Potenza city. The victim reported that while he was performing outdoor activities, he was intrigued by unusual yellow colour of a spider motionless on the surrounding wall of the garden of his house. During capture activities, he was bitten on the tip of index finger of the right hand. Immediately, the man experienced a local pain comparable to "pinprick or wasp" sting. During the next ten minutes, the pain and the numbness spread to the hand and then up the arm. Worried, the victim went to the hospital for medical assistance. Diagnosis was "spider bite" and corticosteroids and anti-inflammatory drugs (NSAIDs) were administered. After about 4 hours, symptomatology appeared clearly improved. The pain and the skin lesion completely disappeared within 24 hours and 3 days, respectively.
RESULTS: First time, the arthropod was identified at the IZSPB as a male specimen of Cheiracanthium sp (Araneae: Miturgidae) using morphological keys (Trotta A., 2005, Memorie Soc. Entomol. Ital., 83: 3-178). Subsequently, the spider was identified as C. punctorium (Villers, 1769) by an experienced aracnologist (Fulvio Gasparo, Trieste).



Cheiracanthium punctorium, male, dorsal view showing habitus (Photo: Fulvio Gasparo, Trieste)

CONCLUSIONS:
In Italy, seventeen species of spiders, belonging to 13 families, are medically important; they represent about 1% of the national aracnofauna (Trotta, 2005, Memorie Soc. Entomol. Ital., 83: 3-178). However only 7 species, including the yellow sac spiders C. punctorium and C. mildei can be not lethal but dangerous for the human health, both widespread in peninsular Italy. Documented events of spider bites attributable to both these species are rare, with six reports in Italy in 1990-2012 years (Papini, 2012, J.Venom Anim Toxins incl Trop Dis, 18: 350-354), of which only one case in Southern Italy (Apulia region), involving C. mildei (Pepe et al., 2002, Thalassia Salent, 26: 45-51). Attacks are an accidental event and they occur in May-September when male specimens move more in search of females or females tend to be aggressive defending their egg sacs. Diagnosis of spider envenomation is usually based on patient's history, symptomatology and presence of bite. The spider is usually not collected by the victim or it results damaged due to crushing or, in the case, it is not identified by an expert in spider recognition. The presence of both these spiders in human habitats is due to their wandering behavior. The dangerousness of the bite coupled with scarcity of data, draw more attention on this topic. It also suggests to report detailed information on verified spider bites to improve the knowledge of the danger to man of the species of zoonotic interest.

Keywords:
Cheiracanthium punctorium, Spider bite, Human

P05.3
REARING OF AEDES (FINLAYA) KOREICUS [HULECOETEOMYIA KOREICA] (DIPTERA: CULICIDAE) UNDER LABORATORY CONDITION

Camuffo S.[1], **Ciocchetta S.*[2]**, Carlin S.[1], Scremin M.[3], Pietrobelli M.[4], Mutinelli F.[1], Montarsi F.[1], Capelli G.[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [2]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy; Mosquito Control Laboratory, QIMR Berghofer Medical Research Institute ~ Brisbane ~ Australia, [3]Entostudio snc ~ Brugine (PD) ~ Italy, [4]Department of Animal Medicine, Production and Health, Padua University ~ Legnaro (PD) ~ Italy

INTRODUCTION:
The aim of this study was to establish an in-laboratory system to breed Aedes (Finlaya) koreicus, an exotic invasive mosquito detected for the first time in Italy in 2011 (Capelli et al. 2011, Parasites & Vectors, 4:188). An established laboratory colony can provide useful information on the biology of the species, as well as a high number of pathogen-free insects required both for the validation of biomolecular methods and to study pathogens transmitted by mosquitoes.
MATERIALS AND METHODS:
The breeding was started with the collection of larvae in the field. The Ae. koreicus colony was maintained into insectarium and was reared under laboratory standard condition (temperature: 25 ± 1°C; relative humidity: 60 ± 10%; light-dark: 16-8 h). The insectarium consists of two rooms with double doors and mosquito nets, equipped with two shelves and a table. The larvae are kept in trays containing rainwater and are fed daily with dry food for cats; when they reach the fourth stage are transferred into smaller containers and then placed in cages for adults emerging. For adults rearing cages of two sizes are used: 50x50x50 cm and 30x30x30 cm. The adults are fed daily with cotton soaked in sugar water (sucrose 10%) and apple slices and about twice per month with blood provided by the artificial system Hemotek Feeding System™ and by human volunteers.
After blood meal a black jar of 500 ml with rainwater and a masonite stick are placed in the cages for oviposition.
RESULTS:
The average number of eggs laid by each female is 60-70; the eggs placed in the water for hatching after 8-105 days of storage from oviposition were able to develop. The hatching of eggs lasted from 1 to 4 days with an average hatching percentage of 88.7% (range 77-100%).
The larval cycle lasted on average 9.6 days (range 6-19 days) and the pupal stage 2.7 days (range 2-4 days); the average sex ratio males/females was 40:60 M:F.
The average life span of adults with supply of sugar and blood was 73.6 days (range 21-94 days) and the average mortality in the first 10 days is 0.04% mosquitoes/day (range 0-0.5% mosquitoes/day). The egg laying can occur from 3 to 44 days after the blood meal.
CONCLUSIONS:
Starting from larvae collected in the field, mosquitoes were reared until the F2 generation. The main limiting factor was relative humidity; the highest mortality occurred when relative humidity dropped below 30%. In conclusion under the reported conditions the rearing of Ae. koreicus can be successfully conducted, the life cycle can be completed and the colony can be maintained over time.
Funding: this work was supported by the Veneto Region and IZSVe
The presentation of this work is sponsored by I.N.D.I.A Industrie Chimiche spa

Keywords:
Aedes koreicus, mosquitoes rearing, invasive mosquitoes

P05.4
FIRST REPORT OF TROPICAL RAT MITE INFESTATION BY ORNITHONYSSUS BACOTI (ACARI: MESOSTIGMATA) IN HUMANS IN URBAN AREA, IN ITALY (APULIA REGION)

Mancini G.[1], Raele D.A.[1], Galante D.[1], **Cafiero M.A.*[1]**

[1]Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata ~ Foggia ~ Italy

INTRODUCTION:
The tropical rat mite (TRM), Ornithonyssus bacoti, Hirst 1913, is an obligate temporary blood-sucker, common on rodents. It can occasionally bite mammals including humans and cause a non-specific dermatitis. We report a case of urban TRM dermatitis diagnosed by identifying the parasite.
MATERIALS AND METHODS:
In November 2013, the Istituto Zooprofilattico di Puglia e Basilicata (IZSPB) was contacted to identify arthropods collected in a cottage in a city in Southern Italy and suspected to be the origin of pruritus and bites in a 2-year-old child and her 71-year-old grandmother. Since March 2013 the baby began complaining of diffuse pruritic dermatitis. In June, the family left home for holidays and symptoms disappeared. In October, after returning from vacation, more severe symptoms occurred. For the first time, the grandmother also suffered from the same symptoms after one night in the same habitation and when she came back home 10 days after. Four physicians were consulted who diagnosed dermatitis by mites (3/4) and by Streptococcus spp. (1/4) respectively. Antibiotics and cortisone were prescribed unsuccessfully. Worried by the failure of the medical treatments, the mother refused any therapy. By an internet search with keywords “dermatitis, man, mites” she came across scientific articles on skin lesions by the red mite, Dermanyssus gallinae and she inspected her daughter and habitation for mites. On November 17, she brought arthropods-like parasites to the IZSPB.



Two-year-old child with bites from Ornithonyssus bacoti on the armpit.

RESULTS:
Parasites were identified such insects without medical importance. However, because the history was suggestive of infestation from zoonotic ectoparasites, an inspection of the flat was performed by IZSPB staff. On November 25, dust samples collected from fireplace and under the cot were found positive for mites identified as O. bacoti (O.b.), according to Baker morphological keys (Baker, 1999, Mites and ticks of domestic animals, The Natural and History Museum, London, The Stationery Office). The source of the mites was found in an abandoned mice’s nest close to fireplace. Removal of nest and disinfestation were followed by complete regression of the symptoms.



Female of Ornithonyssus bacoti collected from the fireplace.

CONCLUSIONS:
Mesostigmatid mites are a cause of human infestation and O.b. can be one of the possible species. Episodes of TRM dermatitis in city-dwellers are rarely documented in Europe (Beck, Pantchev, 2009, Parasitäre Zoonosen, Hannover, Schlütersche Verlagsgesellschaft mbH & Co. KG); in these cases wild and pet rodents were the source of the mites. In Italy, TRM has been registered in relation to an episode of occupational dermatitis in 4 medical researchers handling laboratory rats infested from feral rats (Cafiero et al, 2011, 21stECCMID/27thICC, 17:218) and to an infestation in a mouse collected in suburban area (Raele et al, 2012, Atti XIV SIDiLV: 461-464); both the cases occurred in Apulia region. However, because physicians/dermatologists have difficulties identifying this epizoonosis, more cases could be. It suggests to draw more attention to this subject. The TRM should be regarded as an occupational hazard and as an underrated public health issue, in man in metropolitan areas. In our opinion, Mesostigmatid mites of medical interest, including O. bacoti, should be also listed as zoonotic agents in all regulations regarding the human health.

Keywords:
Ornithonyssus bacoti, Human infestation, Italy

P05.5

ASSESSING THE VECTOR COMPETENCE OF CULEX PIPIENS POPULATIONS FOR WNV: SET UP OF AN ARTIFICIAL INFECTION SYSTEM AND GENETIC CHARACTERIZATION OF INFECTED MOSQUITOES

Di Luca M.*[1], Severini F.[1], Toma L.[1], Boccolini D.[1], Fortuna C.[1], Remoli M.E.[1], Fedorova M.V.[2], Bucci P.[1], Benedetti E.[1], Montarsi F.[3], Platonov A.E.[2], Ciufolini M.G.[1], Romi R.[1]

[1]Dep. Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità ~ Rome ~ Italy, [2]Central Institute of Epidemiology ~ Moscow ~ Russian Federation, [3]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy

INTRODUCTION:
In Europe Culex pipiens seems to play the primary role in maintenance, amplification, and transmission of West Nile virus (WNV), both in rural and urban ecosystems. However, it is still a matter of debate whether Cx. pipiens is a complex of two sibling species or different bioforms, characterized by different behavior. When in sympatry, the two forms may interbreed producing hybrid offspring, which could exhibit intermediate ecological features. Consequently, the discrimination of two forms and hybrids and the assessment on their involvement to WNV circulation become a focal point not only for ecological and epidemiological studies, but also for risk assessment and public health strategies.
MATERIALS AND METHODS:
Within the EuroWestNile Project, we planned to assess the vector competence of Cx. pipiens for WNV, under BSL3 conditions, by using a membrane feeding apparatus. To set up and optimize the method, preliminary experiments without virus were carried out with natural and synthetic membranes, using different blood sources at different conditions.
RESULTS:
Variances in the capability to feed on this apparatus were observed, nevertheless several wild and lab-established populations were successfully fed with infected blood meals: from Italy, Frascati and Caffarella (Rome, Latium), Zafferana (Catania, Sicily) and Legnaro (Padua, Veneto), and from Russia, Sochi and Volgograd. Legnaro and Volgograd populations came from two WNV endemic areas. Under controlled conditions, the infected colonies were maintained throughout the experiment to evaluate the WNV extrinsic incubation period. At selected days post infection, 5-10 fed females were dissected: bodies and legs + wings were analyzed to evaluate the Infection Rate and the Dissemination Rate, respectively. Transmission Rate was estimated by detecting WNV in the saliva of the potential infected specimens (Dubrulle et al. 2009, PLoS ONE 4(6): e5895. doi:10.1371/journal.pone.0005895).
In addition, to define the genetic composition of the tested populations, a molecular investigation was implemented using CQ11 as marker, able to distinguish pipiens form, molestus form and hybrids (Bahnck & Fonseca 2006, Am J Trop Med Hyg 75(2):251-5).
Our findings indicated that all Cx. pipiens populations tested for WNV became infected with different efficiency; in particular Legnaro, Sochi and Volgograd populations showed a higher competence.
Each population assessed for WNV susceptibility was molecularly characterized and the two bioforms and hybrids occurred alone or together at different frequencies. In addition, our preliminary results showed that all three genotypes were found infected with WNV, with no significant difference in viremia.
CONCLUSIONS:
However, the genetic composition of some Cx. pipiens colonies seems to change over time, due to a long-term establishment at Insectary conditions that selects the molestus component of each population. So we believe that it would be appropriate to directly infect wild populations, collected during the potential WNV transmission season.

This research was founded by EU Grant HEALTH-F3-2011-261391 EuroWestNile.

Keywords:
Culex pipiens complex, West Nile Virus (WNV), CQ11

XXVIII Congresso Nazionale SoIPa, Roma 24-27 giugno 2014

NOTES

NOTES

POSTER 06

PARASSITI DELLA FAUNA ACQUATICA

P06.1
MOLECULAR IDENTIFICATION OF METACERCARIAE IN FRESHWATER FISH

Cazzin S.*[1], Da Rold G.[1], Pretto T.[2], Arcangeli G.[2], Marcer F.[3], Danesi P.[1], Capelli G.[1], Manfrin A.[2]

[1]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (PD) ~ Italy, [2]Istituto Zooprofilattico Sperimentale delle Venezie ~ Adria (RO) ~ Italy, [3]Department of Animal Medicine, Production and Health, University of Padua ~ Legnaro (PD) ~ Italy

INTRODUCTION:
Fish-borne trematode diseases are widespread all over the world, especially in the Asian countries (Sohn, 2009, Korean J Parasitol, 47:S103-S113). These parasites are characterized by a complex life cycle in which several animal species are involved: fish and snails are generally the intermediate hosts in which the larval stages development occurs while piscivorous birds, humans and several carnivorous hold the adult worm. In freshwater fish the parasites evolve in metacercariae that infest muscles or other organs. Some species are zoonotic and it is important to discriminate the human pathogen parasites from others. However, the morphological identification of metacercariae is very difficult due to the similarity of shapes, structures and dimensions of many species. The aim of this study was to develop a molecular tool for the rapid diagnosis of these fish parasites.
MATERIALS AND METHODS:
During routine diagnostic activity fish were tested for metacercariae by enzymatic digestion of 100 g of muscles. Visible parasites from muscles or organs were isolated using a stereoscopic microscope.
For the molecular identification three primer sets were designed, by using published sequences of the 18S region of fish trematodes belonging to different orders: Echinostomida, Opisthorchiida, Plagiorchiida and Strigeidida (Dzikowski et al., 2004, Dis Aquat Org, 59:35-41). One pair amplify a segment of approximately 1156 bp while the other two amplify shorter regions included in the longest one. All the couple of primers were tested with conventional PCR using DNA from metacercariae and the best results were obtained with the pair amplifying a segment of 546 bp. Each metacercaria was singularly analysed by conventional PCR with AmpliTaq Gold® DNA Polymerase Kit (Quiagen) and sequencing. In case the sequencing results of the first PCR screening indicated trematodes of the family Opisthorchiidae, the samples were also tested with specific primer for this family (Muller et al, 2007, Parasitol Res, 100:905-909) in order to detect the genus/species of zoonotic interest such as Opistorchis felineus.

RESULTS: The molecular analysis applied to parasites isolated from four species of freshwater fish provided the identification of six trematode species belonging to three different orders (table 1).

Table 1

Trematodes species	Family	Order	N° and species of positive fish
Pseudamphistomum truncatum	Opisthorchiidae	Opisthorchiida	6 *Tinca tinca*
Centrocestus formosanus	Heterophyidae	Opisthorchiida	1 *Morone saxatilis* and *Morone chrysops* hybrid
Petasiger phalacrocoracis	Echinostomatidae	Echinostomida	2 *Tinca tinca*
Holostephanus dubinini	Cyathocotylidae	Strigeidida	29 *Tinca tinca*
Clinostomum complanatum	Clinostomidae	Strigeidida	1 *Squalius cephalus*
Ichthyocotilurus spp.	Strigeidae	Strigeidida	7 *Perca fluviatilis*

CONCLUSIONS:
The molecular method proved to be a good candidate for the fast identification of fish metacercariae. The specificity of this primer set needs to be optimized using other different species of trematodes. The data presented cannot provide prevalence of the trematode species in the fish tested due to the nature of sampling.

Keywords:
Metacercariae, Freshwater fish, Molecular identification

P06.2
FIRST REPORT OF THE MICROSPORIDIAN ENTEROSPORORA NUCLEOPHILA IN ITALIAN FARMED GILTHEAD SEABREAM (SPARUS AURATA)

Caffara M.*[1], Sirri R.[1], Gustinelli A.[1], Mandrioli L.[1], Fioravanti M.L.[1]

[1]Department of Veterinary Medical Sciences Alma Mater Studiorum University of Bologna ~ Bologna ~ Italy

INTRODUCTION:
In order to study the first case of emaciative syndrome due to an enteric microsporidian infection in gilthead sea bream farmed in sea cages in Italy, a parasitological and histopathological study have been carried out.
Enterosporora nucleophila is a microsporidian parasite of the family Enterocytozoonidae responsible for an emaciative syndrome in farmed gilthead sea bream (Sparus aurata), recently described in Spain (Palenzuela et al., 2014, Int. J.Par., 44: 189-203).
MATERIALS AND METHODS:
In December 2013 a disease outbreak occurred in sea caged gilthead seabream in Italy, affecting 0+ year fish with very low mortality rate and showing clinical signs such as lethargy, anorexia and pronounced weight loss up to cachexia.
Twenty gilthead sea bream (mean weight 32 g) were sent by the farmer to the DIMEVET for diagnostic purposes. All the fish were necropsied, and subjected to parasitological examination. Samples of intestine were fixed at the sampling site in 10% buffered formalin and processed for routine histology. Four µm sections were stained with H&E and Luna, according to AFIP manual, and evaluated for histopathology. Intestinal tissue was also subjected to molecular analysis; a fragment of the 18S rDNA was amplified by PCR and then sequenced.
RESULTS:
At necropsy the fish showed emaciation, skin and fin erosive lesions, gill necrosis and keratitis. Hindgut showed a moderate dilatation of the lumen in association with the presence of whitish casts. Results of parasitological examinations allowed to detect, besides gill monogeneans and gall bladder myxozoans at low intensities, the presence of many small clusters of minute spores referable to microsporidians in intestinal scrapings and wall fresh mounts. The sequences obtained from infected intestines showed 99.9% identity with E. nucleophila. In all cases a severe mucosal atrophy with total folds flattening was detected; in all histological sections a limited number of mast cells and lymphocytes was found within lamina propria and submucosa (mild enteritis) accompanied by a mild mucous cells hyperplasia. Multifocally within enterocytes and rodlet cells, the nucleus and/or cytoplasm contained central drop-like and clusters of microsporidian spores tightly packed, more evident with Luna stain.
CONCLUSIONS:
In recent years fish microsporidioses have become increasingly important among the factors threatening aquaculture, both for their economic impact and for lack of effective/authorized treatments. The newly described emaciative syndrome caused by E. nucleophila, microsporidian unique in infecting intestinal epithelium of gilthead seabream and showing, in addition to intracytoplasmic, an intranuclear development in this host, may represent an emerging pathology for the Mediterranean mariculture and should be urgently investigated and understood in terms of biology, epidemiology and pathogenesis. In the cases studied the severe mucosal flattening could be interpreted as an effect of a chronic insult, not only related to the microsporidia infection; other concurrent predisposing factors could be then considered into the development of this disease.

Keywords:
Enterospora nucleophila, Gilthead sea bream Sparus aurata, Italy

P06.3
EPIDEMIOLOGY AND LIFE CYCLE OF CYSTIDICOLA FARIONIS (NEMATODA: CYSTIDICOLIDAE), PARASITE OF SALMONIDS SPREADING IN ITALY

Cavazza G.[1], **Gustinelli A.*[1]**, Menconi V.[1], Caffara M.[1], Fioravanti M.L.[1]

[1]Department of Veterinary Medical Sciences Alma Mater Studiorum University of Bologna ~ Bologna ~ Italy

INTRODUCTION:
A parasitological survey has been carried out in wild and farmed salmonids from northern Italy in order to clarify the current diffusion of the swimbladder nematode Cystidicola farionis (Nematoda: Cystidicolidae), reported since 2006 in wild salmonids from Adige river in the Bolzano province.
MATERIALS AND METHODS:
From July 2012 to September 2013 a total of 103 trouts have been examined, in particular 99 rainbow trout (Oncorhynchus mykiss), 6 wild from Adige river in Bolzano province, 63 and 30 rainbow trout from a farm in Vicenza province (F1) and a farm (F2) located in Trento province, respectively; and 4 wild brown trout (Salmo trutta fario) from Adige river in Bolzano province. All the fish were weighted, measured and then subjected to parasitological examination, with more attention to swim bladder. Parasites were isolated, rinsed in deionized water, fixed in 70% ethanol and clarified by Amman's lactophenol for morphological studies. Prevalence values (%), Mean Intensity (MI) and Abundance (A) have been calculated according to Bush et al., 1997, J.Parasitol., 83: 575-583.
RESULTS:
Sixty-four (64.6%) out of 99 rainbow trout were positive for nematodes (MI=13.4,A=8.64); 35 (55.5%) out of 63 fish (MI=12.6, A=7) from F1, 26 (86.6%) out of 30 (MI=14.7, A=12.7) from F2 and 3 (50%) out of 6 (MI=53, A=26,5) from the wild. Only 1 (25%) out of the 4 wild brown trout were parasitized by 31 nematodes. All the parasites were identified as Cystidicola farionis. In F1, 4 samples of parasite amphipods described as intermediate host of C. farionis, have been collected upstream and within the tanks by a plankton net. Amphipods were transported alive to the laboratory and then examined for the presence of larval nematodes at the dissection microscope. The larvae were isolated, fixed in 5% buffered formalin and submitted to morphological study. A total of 4505 amphipods, all identified as Echinogammarus spp., have been examined. One hundred-four larvae referable mainly to third stage, L3 of C. farionis were found (MI=1.22, ranging from 1 to 5 larvae/amphipod) in 85 (1.9%) amphipods. A slightly higher positivity (2.1%) for C. farionis larvae has been observed inside the farm compared to upstream (1.7%), and the highest prevalence value (8.4%) has been observed in the month of October.
CONCLUSIONS:
In Europe the occurrence of C. farionis is documented in several northern and central European countries, including the European part of Russia, but its report in Italy is quite recent (Gustinelli et al., 2008, Ittiopatologia, 5:87-97). On the basis of this study C. farionis spread from Adige river to other water systems involving also trout farms with a potential impact on their commercial value. Infected farms show common features such as a not-intensive management, with particular reference to low biomass density and integrative feeding, leading the fish to feed on natural preys as amphipods and allowing the development of C. farionis life cycle.
The increasing presence of C. farionis in Italian salmonid species confirms its stable introduction in both freshwater wild and farming environments and give a strong warning on the hazard of the introduction of not-monitored fish and their exotic parasites that could become a risk factor on national scale.

Keywords:
Cystidicola farionis, Oncorhynchus mykiss, Salmo trutta fario, Italy

P06.4
PARASITOLOGICAL FINDINGS IN CETACEANS STRANDED IN SICILY

Currò V.*[1], Costa A.[1], Disclafani R.[1], Torina A.[1], La Cavera E.[1], Vicari D.[1], D'Amelio S.[2], Caracappa S.[1]

[1]Istituto Zooprofilattico Sperimentale della Sicilia ~ Palermo ~ Italy, [2]Sapienza University ~ Roma ~ Italy

INTRODUCTION:
In recent decades, various episodes of mortality of cetaceans in different parts of the world occurred. During 2013 a further phenomenon of abnormal mortality of marine mammals in the Tyrrhenian Sea caused a massive stranding of cetacean especially dolphins and 37 animals were found along the Sicilian coasts. The animals were brought to the Sicilian Istituto Zooprofilattico Sperimentale to investigate about the probable cause of death. During the last few cases of massive stranding the mortality have been linked to viruses like morbillivirus concerning especially young subjects with an immune system not fully developed, or animals debilitated by non-viral agents including parasites that are considered among the primary causes of individual or massive stranding and mortality. This report describes the parasitological finding in cetaceans involved in the episode which has affected the Italian regions bordering the Tyrrhenian Sea. It could provide a contribute to the knowledge of parasitofauna of marine mammals in the Italian seas.
MATERIALS AND METHODS:
Between february and october 2013 along the coast of Sicily a total of 37 marine mammals were collected. They were represented by: S. coeruleoalba (32subject) species, T. truncatus (2),D. delphis(1), P. macrocephalus (1), and a dolphin not identified (as the head was absent it has not been possible to determine the species).During the necropsy the parasitic forms were collected and were identified through microscopic and biomolecular techniques.
RESULTS:
Among the 37 animals examined, 18 were positive for parasites. According to the available literature (Klimpel et al, 2011, Polar. Biol., 34:899-906), the parasitic species most represented were larval stages of cestodes and adults and larvae of anisakid nematodes, free in the lumen of the stomach especially in the first compartment. Concerning the cestodes, in the 100% of animals in subcutaneous adipose tissue of the perigenital region and along the sides, there was a considerable presence of larvae of Monorygma grimaldii and Phyllobothrium delphini (Aznar et al, 2007, Int. J. Paras., 37:243-255; Agusti et al, 2005, J. Paras, 91: 83-92), some sample of Tetrabothrius forsteri and a specimen of Synthesium tursionis in the intestine. In four subjects some larvae of Skrjabinalius guevarai were found in the bronchial and bronchiolar lumen (Measures, 2001, II Ed. Iowa State Univ Press: 279-300). Anisakid nematodes were collected by stomach lumen from 4 individuals of S. coeruleoalba and one of T. truncatus. All the parasites, morphologically identified as Anisakis genus, were identified by PCR-RFLP as belonging to A. pegreffii and A. physeteris (D'Amelio et al, 2000, Int J Parasitol, 30:223).

N. PROG.	SITE OF FINDING	PARASITE	ANATOMIC TRACT	HOST SPECIES
1	Milazzo (ME)	Phyllobothrium delphini	Subcutaneous adipose tissue in the perigenital region	S. coeruleoalba
		Anisakis physeteris	Stomach lumen	
2	Milazzo (ME)	Phyllobothrium delphini	Subcutaneous adipose tissue in the perigenital region	S. coeruleoalba
		Tetrabothrius forsteri	Small intestine	
		Skrjabinalius guevarai	Bronchial and bronchiolar lumen	
3	Scicli (RG)	Tetrabothrius forsteri	Small intestine	S. coeruleoalba
4	Patti (ME)	Skrjabinalius guevarai	Bronchial and bronchiolar lumen	N.I.
5	Tremestieri (ME)	Tetrabothrius forsteri	Small intestine	T. truncatus
		Anisakis pegreffii	Stomach lumen	
6	Patti (ME)	Monorygma grimaldii	Subcutaneous adipose tissue in the perigenital region	S. coeruleoalba
		Anisakis pegreffii	Stomach lumen	
		Synthesium tursionis	Intestine	
7	Enna (EN)	Anisakis pegreffii	Stomach lumen	S. coeruleoalba
		Skrjabinalius guevarai	Bronchial and bronchiolar lumen	
8	Oliveri (ME)	Phyllobothrium delphini	Subcutaneous adipose tissue in the region perigenital	S coeruleoalba
9	Castelbonnano dei Sardi (TP)	Anisakis pegreffii	Stomach lumen	S. coeruleoalba
		Synthesium tursionis	Intestine	
		Phyllobothrium delphini	Subcutaneous adipose tissue in the perigenital region	
10	Mazzara del Vallo (TP)	Skrjabinalius guevarai	Bronchial and bronchiolar lumen	T. truncatus
11	Campobello di Mazara (TP)	Phyllobothrium delphini	Subcutaneous adipose tissue in the perigenital region	S. coeruleoalba
12	Milazzo (ME)	Anisakis pegreffii	Stomach lumen	S. coeruleoalba
		Synthesium tursionis	Intestine	
		Tetrabothrius forsteri	Small intestine	
13	Torre Faro (ME)	Tetrabothrius forsteri	Small intestine	S. coeruleoalba
		Phyllobothrium delphini	Subcutaneous adipose tissue in the perigenital region	
14	Fontane Bianche (SR)	Phyllobothrium delphini	Subcutaneous adipose tissue in the perigenital region	S. coeruleoalba
15	Isili Marina (ME)	Phyllobothrium delphini	Subcutaneous adipose tissue in the perigenital region	S. coeruleoalba
16	Gioiosi (ME)	Skrjabinalius guevarai	Bronchial and bronchiolar lumen	S. coeruleoalba
17	Fontane Bianche (SR)	Phyllobothrium delphini	Subcutaneous adipose tissue in the perigenital region	S. coeruleoalba
18	Messina	Anisakis pegreffii	Stomach lumen	S. coeruleoalba

Tab 1 Host and parasitic species,origin and anatomic tract of finding

CONCLUSIONS:
Some parasitic species in fact may cause serious injuries especially in the case of massive or multiple infestation performing negatively on the ability to echolocation, and when multiple organs are involved, also may cause disorders in the homeostatic balance leading to the death of the subject (Balbuena et al., Elsevier: 133-140; Evans et al, 2001, Kluwer Academic, Plenum Publishers, N.Y.; Raga et al, 2008, II ed, Academic Press, San Diego: 821-830).

Keywords:
Parasite, cetacean, Tyrrhenian Sea

P06.5
A SURVEY ON CESTODES IN ITALIAN LACUSTRINE FISH

Gustinelli A.*[1], Prearo M.[2], Bernardoni E.[1], Menconi V.[1], Caffara M.[1], Cavazza G.[1], Righetti M.[4], Quaglio F.[3], Fioravanti M.L.[1]

[1]Department of Veterinary Medical Sciences Alma Mater Studiorum University of Bologna ~ Bologna ~ Italy, [2]State Veterinary Institute of Piedmont, Liguria and Valle d'Aosta ~ Torino ~ Italy, [3]Department of Comparative Biomedicine and Nutrition, University of Padova, Italy ~ Padova ~ Italy, [4]State Veterinary Institute of Piedmont, Liguria and Valle di Aosta ~ Torino ~ Italy

INTRODUCTION:
AIM: Data concerning the presence of cestodes in wild fish populations in Italian Lakes are scarce and fragmentary. In the framework of a Research Project funded by the Ministry of Health on zoonotic helminths in freshwater fish, a parasitological survey aimed to update the existing data on the presence of Diphyllobothrium spp. (Cestoda: Diphyllobothriidea) in Italy has been carried out in fish populations from Italian lakes, widening data collection also to the presence of not zoonotic tapeworms.
MATERIALS AND METHODS:
During the period January-November 2013, from 5 Italian lakes (Maggiore, Como, Iseo, Garda, Piediluco), 775 fish belonging to 6 species have been sampled by professional fishermen: 553 perch (Perca fluviatilis), 91 shad (Alosa fallax lacustris), 66 whitefish (Coregonus lavaretus), 46 burbot (Lota lota), 10 pike (Esox Lucius) and 9 tench (Tinca tinca). Fish were weighted, measured and submitted to parasitological examination. Parasites were isolated, washed in saline solution and fixed in 70% ethanol for morphology. Plerocercoid larvae were also submitted to molecular identification. Prevalence (P%) values, Mean Intensity (MI) and Abundance (A) have been calculated according to Bush et al., 1997, J. Parasitol., 83: 575-583.
RESULTS:
Concerning Diphyllobothriid plerocercoid larvae positivities were found in perch from Maggiore Lake (P%=10.53, MI=1.00, A=0.11), Como Lake (P%=23.97, MI=1.34, A=0.32) and Iseo Lake (P%=12.79, MI=1.68, A=0.17); pike from Como (P%=40.00, MI=6.00, A=2.40) and Iseo Lakes (P%=100, MI=3.50, A=2.33); burbot from Como (P%=17.39, MI=5.75, A=1.00) and Iseo Lake (P%=19.05, MI=6.75, A=1.29). All the fish from Garda and Piediluco lakes and all the shad and whitefish were negative for diphyllobothriid plerocercoid larvae. All the larvae found in perch, pike and burbot are identified as Diphyllobothrium latum by morphology and confirmed by PCR and sequencing.Concerning adult cestodes found in fish intestine, Proteocephalus percae has been isolated from perch, pike and burbot, confirming perch as definitive host and pike and burbot as post-cyclic hosts. Proteocephalus longicollis has been isolated from whitefish, representing the most suitable host besides salmonids and from shad in which the major part of parasites were subadult. In tench from Piediluco lake the adult cestodes has been identified as the caryophyllidean Monobotrhium wageneri, commonly observed in tight clusters of parasites and causing a huge inflammatory reaction in massive infections.

Lake	Species	+/Exam.	P%	MI	A
Maggiore	Perch	26/38	68,42	3,81	2,61
	Shad	19/28	67,86	29,63	20,11
	Whitefish	21/26	80,77	32,24	26,04
Como	Perch	8/121	6,61	2,63	0,17
	Burbot	1/23	4,35	3,00	0,13
	Pike	0/5	0	-	-
Iseo	Perch	29/219	17,26	4,14	0,72
	Shad	27/53	62,79	29,30	16,72
	Whitefish	5/15	33,33	32,40	10,80
	Burbot	1/21	4,76	1,00	0,05
	Pike	2/4	50,00	3,00	1,50
	Tench	0/1	0	-	-
Garda	Perch	0/91	0	-	-
	Shad	7/10	70,00	48,57	34
	Whitefish	2/25	8,00	1	0,10
	Pike	0/1	0	-	-
Piediluco	Perch	0/33	0	-	-
	Tench	7/8	87,50	28,57	25,00

Table 1: quantitative data on adult cestodes found in fish from different Italian lakes

CONCLUSIONS:
Data collected represent a contribution to the knowledge on the diffusion of cestodes in wild freshwater fish in Italy. Concerning D. latum plerocercoid larvae, perch, pike and burbot are the only hosts reported in Italy so far and lakes Maggiore, Como and Iseo confirm to be endemic environments, instead of Garda Lake which seems to be D. latum free, but more fish from additional sampling areas have to be investigated to confirm these data. .

Keywords:
Cestodes, Diphyllobothrium latum, Italy

P06.6
SEASONAL DIFFERENCES BETWEEN PLATYHELMINTH PARASITE ASSEMBLAGES OF SCOMBER COLIAS (OSTEICHTHYES: SCOMBRIDAE) FROM THE WESTERN MEDITERRANEAN SEA

Mele S.*[1], Villora Montero M.[2], Vivas M.[3], Morán Gálvez J.T.[2], Perez Del Olmo A.[2], García E.[3], Montero F.E.[2], Merella P.[1], Garippa G.[1]

[1]Parassitologia e Malattie Parassitarie, Dipartimento di Medicina Veterinaria, Università di Sassari ~ Sassari ~ Italy, [2]Unidad de Zoología Marina, Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València ~ Valencia ~ Spain, [3]Centro Oceanográfico de Murcia, Instituto Español de Oceanografía ~ San Pedro del Pinatar ~ Spain

INTRODUCTION:
The Atlantic chub mackerel Scomber colias is a pelagic fish inhabiting the Atlantic Ocean and the Mediterranean Sea. Few studies deal with the temporal distribution of the parasites of this fish (Akmirza 2003, Turkish J. Marine Sciences, 9:187-195; Costa et al., 2007, J. Helminthol., 81:33-38), and the seasonal variability of its parasite fauna from the western Mediterranean Sea is unknown. The current study describes the seasonal distribution of the platyhelminth parasites of S. colias from the western Mediterranean Sea.
MATERIALS AND METHODS:
Parasites were collected from 57 Atlantic chub mackerel (total length 226-366 mm) from the Gulf of Alicante (western Mediterranean Sea) in spring (n = 30 specimens), summer (n = 15), and winter (n = 12) of 2011. Prevalence and mean intensity of infection of each parasite species were calculated, and the seasonal differences evaluated (Reiczigel and Rózsa, 2005, Quantitative Parasitology Web1.0, Budapest). The following descriptors were calculated at infracommunity level: mean species richness (mean S), mean Brillouin's diversity index (mean HB), mean evenness (mean E), dominance index of Berger-Parker (d) and mean number of parasite per host (mean N).
RESULTS: Fourteen parasite species/taxa were found: Grubea cochlear, Kuhnia scombercolias, Kuhnia scombri, Pseudokuhnia minor, Halvorsenius exilis, Lecithocladium sp., Nematobothriinae gen. sp. 1, Nematobothriinae gen. sp. 2, Nematobothriinae gen. sp. 3, Nematobothriinae gen. sp. 4, Nematobothrium faciale, Nematobothrium filiforme, Nematobothrium scombri, Opechona sp.
The infracommunity richness increased from winter (mean S = 2.5, mean N = 10.4) to summer (4.3, 51.7). Pseudokuhnia minor was the dominant species (d = 0.32-0.80).
The analysis of infracommunity diversity showed that the summer samples had higher diversity (mean HB = 075, mean E = 0.45) than the winter (mean HB = 0.42, mean E = 0.22) and spring ones (mean HB = 0.69, mean E = 0.42).
The prevalence of K. scombercolias, N. filiforme and Opechona sp. had significant differences ($p \leq 0.01$) between seasons (Fig. 1). The mean intensity of P. minor was higher in spring and summer than in winter.

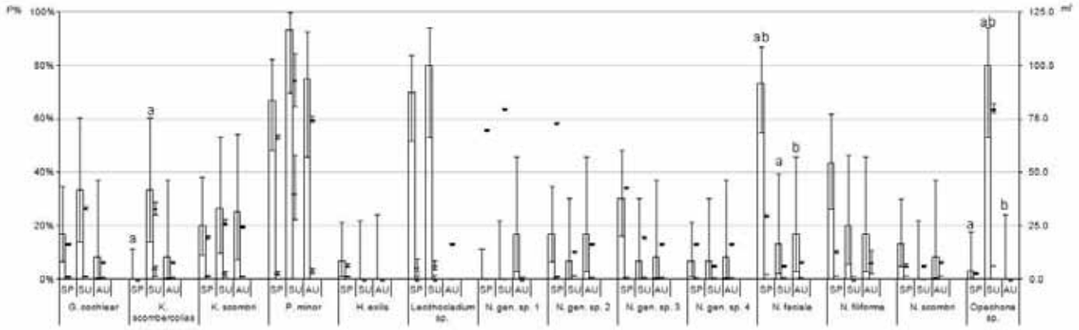


Fig. 1. Prevalence (P%) and mean intensity (mI) of platyhelminth parasites of S. colias from the western Mediterranean Sea. Abbreviation: SP, spring; SU, summer; AU, autumn; N. gen. sp., Nematobothriinae gen. sp.

CONCLUSIONS:
The results showed that didymozoids are the dominant group of the platyhelminth assemblage of S. colias from the Gulf of Alicante. In fact, several didymozoid taxa were recorded for the first time in the Mediterranean Sea (H. exilis, Nematobothriinae gen. sp. 1, Nematobothriinae gen. sp. 2, Nematobothriinae gen. sp. 3, and Nematobothriinae gen. sp. 4) and some of them could be new species. The seasonal variation of the richness and the mean number of parasites per host seems to be related with the higher values of infection of monogeneans and the abundance of the dominant species. In fact, the mean intensity of P. minor was higher in summer than in the other periods, confirming that the environmental conditions can influence its seasonal distribution (Costa et al. 2007, J. Helminthol., 81:33-38). Therefore, to understand the seasonal changes of the parasite fauna of S. colias, the parasite assemblages of more areas of the Mediterranean Sea should be studied.

Research supported by the Spanish Government project PARATUN AGL2010-20892 and the Master and Back 2012-2013 project of the Regione Autonoma della Sardegna.

Keywords:
Atlantic chub mackerel, Western Mediterranean Sea, Parasite assemblage

P06.7
MOLECULAR IDENTIFICATION OF ANISAKIS SPP. (NEMATODA: ANISAKIDAE) IN STRANDED CETACEANS FROM THE MEDITERRANEAN SEA, NE ATLANTIC OCEAN AND SE-SW PACIFIC WATERS, WITH INSIGHTS INTO THE HOST-PARASITE CO-PHYLOGENETIC ASPECTS

Mattiucci S.*[1], Cipriani P.[2], Paoletti M.[2], Marcer F.[3], Frantzis A.[4], Brownlow A.[5], Davison N.[5], Mcgovern B.[5], Webb S.[6], Dougnac C.[7], Nascetti G.[2]

[1]Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, Italy ~ Roma ~ Italy, [2] Department of Ecology and Biology (DEB), Tuscia University, Viterbo, Italy ~ Viterbo ~ Italy, [3]Dept. Experimental Veterinary Science, Padova University ~ Padova ~ Italy, [4]Pelagos Cetacean Research Institute ~ Vouliagmeni ~ Greece, [5]Scottish Marine Animal Scheme, SRUC Veterinary Services Drummondhill Inverness Scotland ~ Inverness ~ United Kingdom, [6]Cawthron Institute ~ Nelson ~ New Zealand, [7]Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile ~ Santiago ~ Chile

INTRODUCTION:
The application of genetic/molecular markers to nematodes of the genus Anisakis, parasites of cetaceans in all world's seas and oceans, has advanced the understanding of their systematics, taxonomy, ecology and phylogeny. These methodologies have allowed the detection of new species and outline the co-evolutionary processes between Anisakis spp. and their definitive hosts (Mattiucci & Nascetti, 2008, Adv Parasitol, 66: 47-148; Mattiucci et al., 2014, J Parasitol, 100:199-214).
Aim of the present work was to identify and characterize the Anisakis species from their cetacean host, and enhance the study of the relationships among and between ecologically related anisakid nematodes with respect to their hosts, resulting in a co-phylogeny mapping.
MATERIALS AND METHODS:
Several adult specimens of Anisakis spp. collected from different cetacean species stranded along the coasts of Mediterranean Sea (Stenella coeruleoalba, Tursiops truncatus and Ziphius cavirostris from off Italian coast, Physeter macrocephalus and Z. cavirostris from off Greece coast), NE Atlantic Ocean (P. macrocephalus and Lagenorhynchus albirostris from Scotland waters; Globicephala melas from off Faroe Islands), SE Pacific Ocean (G. melas and Z. cavirostris from off coast of Chile), and SW Pacific Ocean (Grampus griseus and G. melas from New Zealand waters) were identified by multilocus allozyme electrophoresis and sequenced at two mitochondrial genes (mtDNA cox2, rrnS) for their identification to the species level. The genetic structure of Anisakis spp. specimens was compared with those of the nine Anisakis spp. already genetically characterized (Mattiucci et al., 2014).
RESULTS:
The adult specimens of Anisakis analysed from P. macrocephalus from Greece belonged to the species A. physeteris; a mixed infection by A. physeteris and A. simplex (s. s.) was found in P. macrocephalus from Scotland, while specimens from the L. albirostris stranded in the same area belonged to A. simplex (s. s.). The specimens collected from Z. cavirostris from Greece showed a mixed infection by A. physeteris and A. pegreffii. The dolphins S. coeruleoalba and T. truncatus stranded along Italian coast were infected by A. pegreffii. G. melas from the SE and SW Pacific waters showed a mixed infection with A. berlandi and A. pegreffii, while Z. cavirostris was infected by A. ziphidarum. G. melas from off coast of Chile was found infected by A. pegreffii and A berlandi, while Z. cavirostris by A. ziphidarum. The G. griseus stranded along New Zealand coasts were infected by A. pegreffii and A. berlandi. Co-plylogenetic elaboration of the host-parasite association between cetaceans species and their Anisakis spp. as endoparasites, have been performed and discussed.
CONCLUSIONS:
The results achieved enlarge the knowledge on the geographical distribution and hosts of the Anisakis spp., improving information on their systematics and ecology. The host-parasite association observed further support the possible co-evolutionary scenarios that have been proposed for these endoparasites with respect to the phylogeny of their hosts.

Keywords:
anisakis, cetacean, co-evolution

P06.8
A CASE OF SPARGANUM INFECTION IN AN ITALIAN DIVER

Bracaglia G.*[1], Ranno S.[1], Santoro M.[1], Del Chierico F.[1], Boldrini R.[1], Cerroni L.[2], Sangueza O.[3], Paco B.[4], Diociaiuti A.[1], El Hachem M.[1], Callea F.[1], Putignani L.[1]

[1]Ospedale Pediatrico Bambino Gesu' ~ Roma ~ Italy, [2]Facolta' di Medicina, Universita' di Graz ~ Graz ~ Austria, [3]Wake Forest Baptist Health ~ Greensboro ~ United States, [4]Unit of Dermathology ~ Peru' ~ Peru

INTRODUCTION:
Sparganosis is a parasitic zoonosis, rarely detected in Europe, caused by the plerocercoid infective larva (Sparganum) of pseudophyllidean tapeworms belonging to the genus Spirometra. Only six cases have been discussed in the literature, four from Italy and two from France. We report a case of sparganosis for a diver in Italy.
MATERIALS AND METHODS:
A skin biopsy from a diver was characterized at the Unit of Pathological Anatomy Bambino Gesù. We observed the slides for parasitological advice on the tip of Dr Sangueza and Dr Bravo. We have designed primers for molecular PCR analysis. A concerted diagnosis was provided by International collegues and by our Parasitology laboratory, thanks to the coordination of the Unit of Pathological Anatomy.
RESULTS:
Microscopic finding was consistent with the description of a larvae section of Sparganum spp. In addition we have designed some primers for the PCR detection but the amplification failed probably because of not enough amount of extracted-DNA.
CONCLUSIONS:
The patient began therapy showing improvements.

Keywords:
Sparganum spp., rare case, diver

P06.9
A PARASITOLOGICAL SURVEY IN ELASMOBRANCHS FROM ADRIATIC SEA

Menconi V.[1], **Gustinelli A.*[1],** Caffara M.[1], Fioravanti M.L.[1]

[1]*Department of Veterinary Medical Sciences Alma Mater Studiorum University of Bologna ~ Bologna ~ Italy*

INTRODUCTION:
Elasmobranches play a key-role as definitive hosts in the complex life cycle of several parasitic species being at the top of the food chain. Despite their biological importance, studies on the parasitofauna of sharks and rays are rather difficult to be carried out, as most of them are endangered species and specimens collection and examination are sporadic and mainly limited to biodiversity and environmental monitoring research projects.
MATERIALS AND METHODS:
During 2013, in collaboration with the Lab of Marine Biology and Fishery, University of Bologna, located in Fano (Ancona province), Italy, a parasitological survey has been carried out on elasmobranchs caught in the Adriatic sea for stocks evaluation purposes. A total of 184 fish from georeferenced fishing stations and belonging to 10 species have been examined: 50 Galeus melastomus, 43 Scyliorhinus canicula, 34 Raja clavata, 27 Squalus acanthias, 11 Mustelus punctulatus, 8 Mustelus mustelus, 5 Raja miraletus, 3 Myliobatis Aquila, 1 Torpedo maculata, 1 Torpedo marmorata. All fish were weighed, measured and submitted to parasitological examination focused on the gastrointestinal tract. Parasites were cleaned in water and fixed in alcohol 70%, then mounted and clarified in Amman's lactophenol for morphological identification. Prevalence (P) values, Mean Intensity (MI) and Abundance (A) have been calculated according to Bush et al,. 1997, J. Parasitol., 83: 575-583 and are showed in the table 1.
RESULTS:
All the individuals of the remaining species, Galeus melastomus, Myliobatis aquila, Torpedo maculata and Torpedo marmorata were negative for the presence of parasites.
All the digenean trematodes have been referred to one species, Ptychogonimus megastomus (Rudolphi, 1819), already reported in M. mustelus and R. clavata but never described in Mustelus punctulatus. This represents to our knowledge a new host-record.
Concerning cestodes, the specimens isolated belongs to two trypanorhinch species, in particolar Nybelinia lingualis in S. canicula and Eutetrarhynchus ruficollis in M. punctulatus, while the tapeworm isolated from S. canicula was not well preserved and impossible to be identified.
Nematodes are the most represented endoparasites in our survey as they have been isolated from the intestine of all the six positive fish species. In S. canicula nematodes were identified as Proleptus obtusus, already reported in this fish species, while in R. clavata, S. acanthias, M. punctulatus, M. mustelus and R. miraletus, the parasites isolated seem referable to Pseudanisakis spp., but further morphological analyses need to be carried out to confirm this preliminary study.
In addition in S. acanthias and M. mustelus, the presence of Hysterothylacium sp. and Acanthocheilus sp. has been respectively observed.

Fish species	Digeneans	Cestodes	Nematodes
Scyliorhinus canicula	-	P=2.3%, MI=2, A=0.04	P=20.9%P, MI=6.2, A=1.30
Raja clavata	P=55%, MI=14.5, A=0.8	-	P=16.6%, Mi=6.1, A=1.02
Squalus acanthias	-	-	P=7.4%, MI=2, A=0.14
Mustelus punctulatus	P=27.2%, MI=2.3, A=0.63	P=9%, MI=1, A=0.09	P=63.6% MI=20.1, A=12.8
Mustelus mustelus	P=12.5%, MI=3, A=2.5	-	P=75%, MI=43, A=32,25
Raja miraletus	-	-	P=20%, MI=2, A=0.4

Table 1 – quantitative data of parasites found in 6 out of 10 species of elasmobranchs

CONCLUSIONS:
The data collected during this survey give a preliminary sight on the parasitofauna present in elasmobranchs species not easy to be investigated and add new information on the host specificity of some parasitic species,such as Ptychogonimus megastomus, to our knowledge for the first time reported in M. punctulatus. Furthermore identification at species level and morphological data on nematodes such as Pseudanisakis spp., should contribute to improve the knowledge on this poorly known genus.

Keywords:
Elasmobranchs, cestodes digeneans nematodes, Adriatic Sea

P06.10
IDENTIFICATION OF ANISAKID LARVAE IN FISHERY PRODUCTS FROM NORTH ATLANTIC SEA USING MORPHOLOGICAL AND MOLECULAR MARKERS

Costa A.*[1], Cammilleri G.[1], Buscemi M.D.[1], Graci S.[1], Ferrantelli V.[1]

[1]*National Reference Centre for Anisakiasis ,Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy*

INTRODUCTION:
Anisakid nematodes, in particular those belonging to the genera Anisakis and Pseudoterranova, are of medical and economic importance, due to public health implications and for the subsequent effects on the marketability of fishery products. Larval forms constitute in fact a risk for human health when ingested through raw or undercooked fish.
The life cycle of anisakid nematodes involve an important and diverse array of marine hosts with a certain degree of specificity in their geographic distribution and their host preference in major areas of the marine ecosystem (Mattiucci et al 2008 Advances in Parasitology, 66: 47-148).
The accurate identification of anisakid nematodes is important both to deepen the knowledge on their taxonomy, and ecology and for the assessment of their relative epidemiological role.
MATERIALS AND METHODS:
Authors report preliminary data on the occurrence of Pseudoterranova sp and Anisakis sp larvae in fishery products collected from local markets and belonging to the species Gadus morhua, Lophius piscatorius, Brosme brosme and another fish species not indicated: the species and the provenance (North Atlantic areas) were indicated on the label. Larval forms, isolated with visual inspection and digestion method, were morphologically identified at genus level by light microscopy and at species level by PCR-DNA markers based on ITS rDNA (D'Amelio et al., 2000, Int J Parasitol, 30:223) and mtDNA cox2 sequence analysis (Valentini et al, 2005, J Parasitol, 92:156).
RESULTS:
Out of 11 fish fillets examined, 5 (45.5%) were positive and a total of 80 anisakid larvae were collected. The larvae isolated from Brosme brosme (n.42) were morphologically identified as L3 stages of Anisakis Type I (sensu Berland 1961): in the molecular method, digestion profiles with the PCR-RFLP of ITS using endonucleases HinfI and HhaI, allowed the identification as Anisakis simplex s.s.
The larvae isolated from other fish species were classified by light microscopy as Pseudoterranova decipiens s.l. The molecular approach based on mtDNA cox2 sequence analysis identified the larvae as P. decipiens s.s. and P. krabbei (Table 1).
The obtained DNA sequences were aligned, through the BLAST algorithm, with the reference sequences present in literature (Mattiucci et al, 2012, Molecular Detection of Human Parasitic Pathogens: 645). Phylogenetic analyses were carried out on mitochondrial (cox2) and sequences representing the combine dataset.
There is a correlation between the distribution of the parasite and the origin of the fish product, also between the fish's lifestyle and its parasite too. In fact, Pseudoterranova spp. has adapted to the climatic conditions of the North Sea, where it can find a low temperature range of the water column. Pseudoterranova is unable to float and adhere to the substrate through their caudal termination, preferring fishes related to the life of the fund as intermediate host species, such also L. piscatorius. Unlike Anisakis due to their buoyancy capacity, are able to infest demersal intermediate hosts such as B. brosme.
CONCLUSIONS:
The consumption of raw, undercooked or marinated fish represent a health risk for the infection by zoonotic anisakid when the fish are imported from endemic areas.
Moreover the identification of parasitological fauna in fish products could be helpful for the traceability of products.
Table 1 Host species and anisakid specimens genetically identified

Host species	Samples tested		Parasites tested	Id species
Merluccius hubbsi	5	-		
Merluccius capensis	1	-		
Gadus morhua	1	1	30	*P. krabbei*
*	1	1	2	*P. decipiens s.s.*
Brosme brosme	1	1	42	*Anisakis simplex s.s.*
*	1	1	4	*P. krabbei*
Lophius piscatorius	1	1	2	*P. krabbei*
	11	5	80	

*fish species not indicated

Table 1

P06.11
MOLECULAR CHARACTERIZATION OF ANISAKIS SPP FROM FISHES COLLECTED IN THE SICILIAN SEA

Costa A.*[1], Graci S.[1], Cammilleri G.[1], Sciortino S.[1], Gaglio G.[2], Ferrantelli V.[1]

[1]National Reference Centre for Anisakiasis, Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy,, [2]Department of Veterinary Sciences, University of Messina, Italy

INTRODUCTION:
Anisakis spp larvae are parasitic nematodes present in marine fish and cephalopods: the accidental consumption of infected raw or undercooked seafood may cause a clinical disease in humans (anisakiasis). The accurate identification of anisakid nematodes is important for study their ecology and epidemiology, for diagnosis and control and to screen the safety of fish products. During the last decades the use of nuclear ribosomal ITS allowed the identification and description of anisakid nematodes (D'Amelio et al, 2000, Int J Parasitol, 30:223) and to recognize the heterozygote genotype between A. simplex sensu stricto and Anisakis pegreffii (Abollo et al, 2003, Infect Genet Evol, 3:175). The aim of the present work was to investigate, using RFLP genetic analysis, the prevalence of Anisakis spp in fish species collected at different sites off the Sicilian coasts. The fish species sampled were Lepidopus caudatus, Trachurus trachurus, Scomber scombrus, Merluccius merluccius, Engraulis encrasicolus, Sardina pilchardus, Lophius piscatorius, Phycis phycis and the blunthead puffer Sphoeroides pachygaster. The last host species has not been so far checked for anisakids.
MATERIALS AND METHODS:
A total of 680 larval forms were collected from the body cavities of the specimens: the larvae were morphologically identified at genus level by light microscopy as L3 stages of Anisakis belonging to Type I and to Type II (sensu Berland, 1961 Sarsia, 2:1-50) and fixed in 70% ethanol for molecular characterization. After DNA extraction, the genomic region of the ribosomal DNA, spanning the ITS-1, the 5.8S and the ITS-2, was amplified by PCR and subjected to RFLP analysis using the two restriction enzymes (HhaI, Hinf I) for species identification (D'Amelio S et al, 2000, Int J Parasitol, 30:223-226).
RESULTS:
The Type I larvae were identified as belonging to A. pegreffii (92.0%), A. simplex s.s. (3.8%), A. typica (0.4%), A. ziphidarum (0.1%) whereas the Type II larvae were all found to belong to A. physeteris (1.2%) (Table 1). Specimens with a heterozygote restriction pattern between A. pegreffii and A. simplex s.s. with HinfI digestion (2.5%), were also observed and analyzed by sequencing of the entire ITS region: the resulting electropherograms were analyzed using Chromas 2.13 software and their DNA sequences were put BLAST with reference sequences to confirm the presence of both nucleotides (C/T) in the correct position (Abollo et al 2003 Infect Genet Evol 3: 175).Larvae of A. pegreffii were collected in all fish species examined. Larvae of A. simplex s.s., were particularly isolated from specimens of S. scombrus captured in the west-south coasts of Sicily. All the larval forms of Anisakis identified as A. physeteris were collected from teleost fish captured in the Ionian Sea. A. typica were identified in two samples of S. scombrus captured in the north-west coasts (coinfestation with A. pegreffii) and in one of T. trachurus sampled in the east coast of Sicily whereas A. ziphidarum were isolated from P. phycis caught in the south coast. Two larvae isolated by one specimen of Sphoeroides pachygaster were identified as A. pegreffii.
CONCLUSIONS:
The result obtained improve the picture of the occurrence of larval forms of Anisakis species in the marine ecosystem of Sicily, an island in the center of Mediterranean, and adds new data of the presence of the recombinant genotype or putative hybrids in this area.
Table 1 Prevalence (%) of Anisakis species larvae identified by PCR-RFLP

Host species	N of parasites tested	*A. pegreffii*	*A. simplex s.s.*	*Hybrid genotype*	*A. typica*	*A. ziphidarum*	*A. physeteris*
Lepidopus caudatus	195	95.9% (187)		4.1%(8)			
Trachurus trachurus	232	97.0% (225)		0.9%(2)	0.4% (1)		1.7% (4)
Scomber scombrus	105	69.5% (73)	24.8(26)	2.9% (3)	1.9% (2)		0.9% (1)
Merluccius merluccius	62	95.2% (59)					4.8% (3)
Engraulis encrasicolus	70	94.3%(66)		5.7 % (4)			
Sardina pilchardus	6	100% (6)					
Lophius piscatorius	5	100% (5)					
Phycis phycis	3	75% (2)				25% (1)	
Sphoeroides pachygaster	2	100% (2)					
Totale	680	92.0% (625)	3.8% (26)	2.5% (17)	0.4%(3)	0.1%(1)	1.2%(8)

Table 1

NOTES

NOTES

POSTER 07

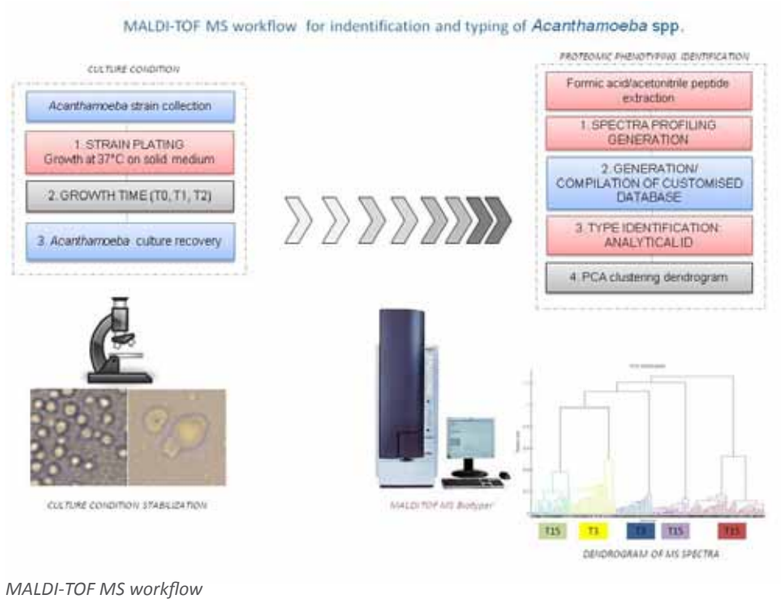
BIOLOGIA E FILOGENESI MOLECOLARE APPLICATE A PARASSITI E VETTORI

P07.1
A MALDI-TOF MS-BASED ASSAY FOR THE IDENTIFICATION AND TYPING OF ACANTHAMOEBA SPP.: A NEW TOOL IN DIAGNOSTIC PARASITOLOGY

Accardi C.[1], Del Chierico F.[1], Santoro M.[1], D'alfonso R.[2], Berrilli F.[2], Muraca M.[1], Di Cave D.[2], **Putignani L.*[1]**

[1]Bambino Gesù Children's Hospital ~ Rome ~ Italy, [2]Università Tor Vergata ~ Rome ~ Italy

INTRODUCTION:
Acanthamoeba is a genus of amoebae frequently found in fresh water and other habitats. Most species are free-living bacterivores, but some are opportunistic pathogens that can cause infections in humans and other animals.
Particulary, Acanthamoeba keratitis is a rare disease in which amoebae invade the eye cornea. It may result in permanent visual impairment or blindness. It is nearly always associated with contact lens use, as Acanthamoeba can survive in the space between the lens and the eye. Here we report on the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) profiling for the identification and typing of Acanthamoeba strains compared to PCR-based genotyping assay.
MATERIALS AND METHODS:
Ten Acanthamoeba strains were collected at the Tor Vergata University of Rome from biological samples, and grown under low concentrations of nutrient agar media containing living Escherichia coli for 7 days at RT. Each strain was genotyped by conventional PCR assay for the amplification of the 18S rRNA gene using the genus specific primers JDP1 and JDP2. At time of collection, 3 and 7 days after (T0, T1, T2), cell growth was evaluated by microscopy, in Bambino Gesù Hospital. For the MALDI-ToF-based assay, colony cells were mixed thoroughly in 300 ml of distilled water and added by absolute ethanol (900 ml). Then, the recovered pellets, were air dried after centrifugation. The dry pellets were mixed thoroughly with 20 mL of 70% formic acid (Sigma-Aldrich, Milan , Italy) and with the same volume of acetonitrile (Sigma-Aldrich , Milan, Italy). One mL of supernatant was placed on a polished steel target MSP 96 (Bruker Daltonik GmbH, Bremen, Germany) and left to dry at RT. Subsequently, each sample was overlaid with 1 mL of the matrix. The matrix-sample was co-crystallized by air drying at RT. Twenty-four spectral measurements (500 laser shot) for each sample were performed with a Microflex LT mass spectrometer (Bruker Daltonics), using FlexControl software (version 3.0, Bruker Daltonics). The entire set of the 240 spectra replicae, from each time point, was analyzed by MALDI biotyper 3.0 for clustering and PCA analyses.
RESULTS:
For the 2 genotypes, T3 and T15, all the spectra at T0 were employed for the creation of a mean spectrum (MSP), by standard MSP creation method (FlexAnalysis software). Final MSP spectra were assigned to the correct taxon node of the taxonomy tree into the MALDI-TOF MS library and used to perform analytical ID of Acanthamoeba isolates (MALDI Biotyper 2.0, Bruker Daltonics). All the strains were correctly identified with a score comprised from 1.7 to 2.0. The Clustering analysis of each replica at different time points showed an independent grouping for each strain, based on genotype and time course point.
CONCLUSIONS:
The MALDI-TOF MS-based analysis for the identification and typing of Acanthamoeba represents a substantial improvement in diagnostic parasitology. The strength of this method is, indeed, to overcome PCR-based genotyping, allowing peptide fingerprinting-based typing.



MALDI-TOF MS workflow

Keywords:
MALDI-TOF MS-based assay for the identification and typing, Acanthamoeba spp., Diagnostic parasitology

P07.2
COMPARATIVE STUDY OF GIARDIA LAMBLIA, CRYPSTOSPORIDIUM PARVUM, ENTAMOEBA HISTOLYTICA AND DIANTAMOEBA FRAGILIS IN FAECAL SAMPLES BY REAL-TIME PCR APPROACH

Bracaglia G.*[1], Ranno S.[1], Santoro M.[1], Losito L.[2], De Maio L.[2], Muraca M.[1], Putignani L.[1]

[1]Ospedale Pediatrico Bambino gesù ~ Roma ~ Italy, [2]R-Biopharm ~ Milano ~ Italy

INTRODUCTION:
Enteric parasites are responsible for several intestinal parasite infections (IPIs), especially in children. Common IPIs, frequently detected in stools are due to protozoa (e.g., Giardia lamblia, Entamoeba histolytica, Cryptosporidium spp.). The current diagnostic mainly relies on microscopy. However, molecular procedures can be introduced in routine protocols to increase sensitivity.
MATERIALS AND METHODS:
A total of 400 faecal samples from daily routine will be included in this study. Each stool samples, collected and fixed in 10% formalin, will be observed under a 40x-100x light microscope after concentration, in accordance with Ritchie and stained with Lugol. Micrometric eyepiece will be used for exact determination of cystic diameter and parasite dimensions. Prior microscopy, an aliquot of each faecal sample will be immediately processed through rapid immunocromatographic test (ICT), Rida quick immunochromatographic dipstick tests Cryptosporidium/Giardia/Entamoeba. All samples will be processed according to routine barcode-based automatic DNA extraction procedures without dedicated additional treatment. At the same time, these samples will be tested with a multiplex q-real time-PCR RIDAGENE parasitic stool panel for the detection of common parasites: Cryptsoporidium parvum, Entamoeba histolytica, Giardia lamblia, Dientamoeba fragilis.
RESULTS:
The aim of this study will be to evaluate the q-real time-PCR RIDAGENE parasitic stool panel (Cryptsoporidium parvum, Entamoeba histolytica, Giardia lamblia, Dientamoeba fragilis), compared to reference methods, in order to assess its efficiency and sensitivity as a molecular methodology in diagnostic routine.
CONCLUSIONS:
The experiments are currently in progress.

Keywords:
Real time RIDAGENE multiplex kit, intestinal parasites, molecular diagnostic

P07.3
A THREE-YEARS SURVEY ON THE OCCURRENCE OF TICK-BORNE PATHOGENS TRANSMITTED BY IXODES RICINUS IN NORTH-EASTERN ITALY

Da Rold G.*[1], Ravagnan S.[1], Babiker A.M.A.[1], Piccolo S.[1], Porcellato E.[1], Tessarin C.[2], Dal Pont M.[3], Delai N.[4], Berto M.[5], Danesi P.[1], Montarsi F.[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro ~ Padova ~ Italy, [2]Department of Animal Medicine, Production and Health, University of Padua, Legnaro ~ Padova ~ Italy, [3]ULSS 1, Public Health Department of Belluno ~ Belluno ~ Italy, [4]ULSS 2, Public Health Department of Belluno ~ Feltre (BL) ~ Italy, [5]C.T.A Feltre, Corpo Forestale dello Stato ~ Feltre (BL) ~ Italy

INTRODUCTION:
During the last decades the proliferation and spread of the tick Ixodes ricinus has been associated with the alteration of the alpine ecosystem (Sanogo et al., 2003, Ann NY Acad Sci; 990:182-190). In this study the occurrence of pathogens transmitted by ticks (TBP) in the Belluno Province (North-Eastern Italy) has been investigated from 2011 to 2013.
MATERIALS AND METHODS:
The survey was conducted in 30 sampling sites located in 17 municipalities. Tick sampling was performed by standard dragging in both permanent sites, monitored monthly from April to November, and in itinerant sites, visited only once or twice. Each tick was morphologically identified. DNA/RNA were extracted from single adults and from pools of nymphs or larvae and screened for the main TBPs. Three TaqMan RT-PCRs were used to detect tick-borne encephalitis (TBE) (Schwaige, 2003, J.Clin. Virolo, 27:136-145), Candidatus Neoehrlichia mikurensis (Jahfar et al., 2012, Parasit Vectors, 5: 74) and Rickettsia spp. (Labruna et al., 2004, JMed Entomol., 41:1073-1081). Positive samples were confirmed with traditional PCRs (Diniz et al., 2011, JClin Microbiol, 49:2059; Choi et al., 2005, Emerg Infect Dis, 11-2: 237-244). A RT-PCR Sybr Green (Casati et al., 2006, Ann Agric Environ MeD, 13: 65–70) was used to detect Babesia/Theileria spp. and conventional PCRs were used for Anaplasma phagocytophilum (Massung et al., 2003, Clin Microbiol, 41(2):717-22) and Borrelia burgdorferi sl. (Skotarczak et al, 2002, Ann Agric Environ Med, 9(1):25-8). All the positive results were confirmed by sequencing. Homology searches were done with the BLAST algorithm (NCBI).
RESULTS:
A total of 1719 ticks were collected: 1210 nymphs, 319 larvae and 190 adults (sex ratio 1:1). The TBPs identified and their prevalence are shown in Table 1. The most prevalent pathogens in the area were A. phagocytophilum, Borreliae and Rickettsiae. TBE and Babesia/Theileria spp. were not found. Only one pool of larvae was positive for R. helvetica in 2013. In two permanent sites a double co-infection of A. phagocytophilum-R. helvetica, B. burgdorferi-R. helvetica, B. afzelii-R. helvetica occurred in two females and one male ticks. A significant variation of prevalence among years was found for A. phagocytophilum and B. burgdorferi ss.

Table 1 - Overall adult prevalence (P) and nymphs estimated rate of infection (ERI) of TBPs in *Ixodes ricinus*

	2011		2012		2013	
	nimph pos pools n=53 (ERI)	pos adult n=70 (P)	nimph pos pools n=56 (ERI)	pos adult n=80 (P)	nimph pos pools n=44 (ERI)	pos adult n=40 (P)
A. phagocytophylum	22 (5.8%)	7 (10%)	5 (1.16%)	2 (2.5%)	4 (1.35%)	5 (12.5%)
B.burgdorferi s.s.	9 (2.05%)	18 (25.7%)	1 (0.22%)	0	0	0
B. afzelii	2 (0.43%)	1 (1.4%)	4 (0.92%)	3 (3.8%)	3 (1.0%)	1 (2.5%)
B. garinii	1 (0.21%)	0	0	0	1 (0.33%)	0
R.helvetica	21 (5.45%)	2 (2.9%)	17 (4.42%)	8 (10.0%)	10 (3.62%)	2 (5.0%)
R.monacensis	1 (0.21%)	0	2 (0.45%)	2 (2.5%)	0	0
Cand. N. mikurensis	3 (0.65%)	3 (4.29%)	7 (1.66%)	0	5 (1.71%)	0

CONCLUSIONS:
The results highlight that humans residing or attending the area monitored are at greater risk of exposure to A. phagocythophilum, Borrelia and R. helvetica. The risk of exposure is also variable through the years, likely due to the variations of tick densities and host populations acting as reservoirs (birds and small mammals) or amplifying hosts of the tick population (wild ruminants). The presence of co-infected ticks can explain eventual human cases presenting not classical or atypical clinical manifestations.
Funding: Veneto region
The presentation of this work is sponsored by Bayer Animal Health

Keywords:
Pathogens, North Est Italy, Molecular screening

P07.4
STAGE-SPECIFIC PROTEIN EXPRESSION IN THE OOCYST/SPOROZOITE OF TOXOPLASMA GONDII

Esposito Abate R.[1], Possenti A.[1], Cherchi S.[1], Bizzarro T.[1], Messina V.[1], Tinti M.[1], Di Matteo S.[1], Pozio E.[1], Dubey J.[1], **Spano F.*[1]**

[1]Istituto Superiore di Sanità ~ Roma ~ Italy

INTRODUCTION:
Despite the central role played in disease transmission, the sporozoite of Toxoplasma gondii is, among the parasite's infective stages, the less characterized at the molecular level. The existence of oocyst/sporozoite-specific proteins was first documented in 1985 by serological and biochemical methods (Kasper et al., 1985, J. Clin. Invest. 75:1570-1577), yet no stage-specific protein was identified until 2004, with the characterization of the sporozoite surface antigen SRS28 (Radke et al., 2004, Mol. Microbiol., 52:93-105), followed by the cloning of three oocyst wall proteins (TgOWP1, TgOWP2 and TgOWP3) (Possenti et al., 2010, Int. J. Parasitol., 40:1639-1649] and the immunogenic micronemal protein TgERP [Hill et al., 2011, J. Parasitol., 97:328-337). Recently, two large-scale mass spectrometry studies (Fritz et al., 2012, PLoS One 7:e29955; Possenti et el., 2013, BMC Genomics, 14:183) shed light on the proteome of the T. gondii oocyst/sporozoite and on the protein repertoire expressed by this stage in a developmentally-regulated manner. In one of these two studies, we identified a subset of 154 putative oocyst/sporozoite-specific proteins, including nearly forty enzymes involved in metabolism and energy production, as well as several secretory proteins presumably implicated in the interaction with the host cell and/or the external environment. Various members of this latter protein class are currently being characterized in our laboratory.
MATERIALS AND METHODS:
The characterization of the above mentioned subset of novel oocyst/sporozoite proteins employed a wide array of molecular and biochemical techniques, e.g., cDNA cloning, RT-PCR, immunoprecipitation and western blot. For western blot and immunolocalization studies, T. gondii oocysts (60% fully sporulated, 40% non sporulated) of strains Me49 (genotype II) or VEG (genotype III) were purified from cat feces by sucrose gradients, mechanically ruptured and excysted with 1.5% sodium taurocholate. When required, sporozoites were used to infect human foreskin fibroblast monolayers and cultured for 4-6 weeks to obtain spontaneous differentiation into tachyzoites and bradyzoites. Alternatively, in vitro tachyzoite-to-bradyzoite differentiation was induced by ariginine starvation. Specific murine antibodies were raised against histidine-tagged recombinant forms of individual proteins expressed in bacteria.
RESULTS:
We provide an overview of the studies currently in progress in our laboratory aimed at the characterization of novel oocyst wall components and of sporozoite-specific proteins potentially involved in the interaction with the host cell and its invasion. The proteins under study include: i) the tyrosine-rich oocyst wall protein (TROWP), a novel constituent of the T. gondii oocyst wall which adds to the cysteine-rich proteins of the TgOWP family (Possenti et al., 2010, Int. J. Parasitol., 40:1639-1649); ii) five members of the LCCL protein family, a group of multimodular secretory molecules with sugar-, lipid- and protein-binding domains expressed in T. gondii sporozoites; iii) the mucin-like protein MUC1, encoded by a multi-gene locus possibly subject to stage-specific antisense regulation and iv) paralogs of the moving junction constituents AMA1 and RON2.
CONCLUSIONS:
The studies herein described have the potential to shed new light on T. gondii oocyst wall structure and physicochemical properties, as well as on fundamental aspects of sporozoite biology.

Keywords:
Toxoplasma gondii, oocyst, sporozoite

P07.5
HIGH RESOLUTION MELTING ANALYSIS COUPLED TO REAL TIME PCR FOR DETECTION AND QUANTIFICATION OF NOSEMA CERANAE AND NOSEMA APIS IN HONEY BEES

Loiacono M.[1], Albonico F.[1], Guidi A.[1], Cersini A.[2], Formato G.[2], Corsi N.[3], Colombo M.[3], **Mortarino M.*[1]**

[1]Dipartimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di Milano ~ Milano ~ Italy, [2]Unità Operativa di Apicoltura, Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana ~ Roma ~ Italy, [3]Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Università degli Studi di Milano ~ Milano ~ Italy

INTRODUCTION:
Nosemosis is a gut disease of honey bee, Apis mellifera, caused by the fungal microsporidia Nosema apis and N. ceranae. These two species are morphologically similar but differ in epidemiological pattern and virulence. Proper control strategies of the infection by beekeepers rely on correct species differentiation. N. apis and N. ceranae spores are not easily distinguishable under microscopic examination, requiring molecular analysis for species identification. High Resolution Melting Analysis (HRMA) is a molecular technique allowing single-nucleotide discrimination that is increasingly used in diagnostic microbiology and parasitology for species identification and genotyping (Reed et al., 2007, Pharmacogenomics 8:597–608).
This work aimed to design a qualitative and quantitative assay for the simultaneous detection and discrimination of N. ceranae and N. apis infection in honey bees, based on HRMA coupled to quantitative real time PCR (qPCR).
MATERIALS AND METHODS:
Samples of 25-30 adult honeybees as a balanced pool of individuals from each of 5-10 colonies were collected from apiaries during field investigation. Honey bee crushings underwent microscopic examination to assess the presence of Nosema spp. spores. For microscopically-positive samples, DNA extraction and Nosema DNA amplification through PCR for species identification were performed using standard protocols (Martin-Hernandez et al., 2007, Appl Environ Microbiol 73:6331-6338). The primer pair for qPCR-HRMA amplification was designed in a region of Nosema 16S rRNA gene, where primer annealing sequences are conserved whereas the interposed sequences shows no intraspecific variability but interspecies variability at different positions. The assay protocol included a real time PCR reaction, a subsequent DNA high resolution melting process, and a normalization step for data analysis.
The analysis was performed on the EcoTM Real-Time PCR System (Illumina, Inc., San Diego, CA, USA). The obtained amplicons were sequenced and similarity analysis was performed by using BLAST database search and GenBank. N.ceranae and N. apis amplicons were cloned into plasmids, and proper plasmid diluitions were prepared to serve as external reference curves for qPCR quantitation.
RESULTS:
Each honey bee sample positive to microscopical evaluation was also positive to real time PCR-HRMA even after proper dilution with high amplification efficiency and sensitivity. The analysis of high resolution melting curves allowed clear discrimination of the two Nosema species, and mixed infection, according to the different melting temperature of the corresponding amplicons.
CONCLUSIONS:
This work validated a new qPCR-HRMA protocol assay for infection assessment of the two Nosema species affecting honey bees. This method could be particularly suitable for routine diagnostics applied to field investigation, as a quick and sensitive single step protocol using an unique primer pair and saturating dye without sequence specific probes. This allows a shorter analysis time, a reduced cost, and a comparable amplification efficiency for both targets, especially useful in case of simultaneous infection with the two Nosema species.

This study was supported by Regione Lombardia, "STRANOVA" project.

Keywords:
honey bee, Nosema ceranae and Nosema apis, qPCR-HRMA

P07.6
IDENTIFICATION OF ANTIMICROBIAL ACTIVITIES IN THE SALIVA OF THE MALARIA MOSQUITO ANOPHELES GAMBIAE

Lombardo F.*[1], Gargiullo C.[1], Arcà B.[1]

[1]Sapienza University of Rome Department of Public Health and Infectious Diseases ~ Rome ~ Italy

INTRODUCTION:
Female mosquitoes feed on blood to acquire nutrients to develop eggs. Both male and female mosquitoes need plant sugar to survive, to fly and to enhance reproduction. To obtain blood, hematophagous insects necessarily have to break their host's skin, which is a barrier to pathogen infection: skin surface microbes might contaminate host circulation, and therefore the ingested blood meal. On the other hand, sugar sources are similarly often contaminated by several microbial pathogens naturally living on nectar and honeydew. Perhaps for this reason, a common finding in sialotranscriptomes of blood feeding arthropods is an overabundance of proteins known to have antimicrobial activity and a large number of still unknown antimicrobial families.
The aim of the work is the identification and characterization of novel antimicrobial activities in the salivary glands of the malaria vector Anopheles gambiae.
MATERIALS AND METHODS:
To express recombinant proteins in a eukaryotic system, we have employed an in vitro wheat germ transcription/translation procedure. cDNAs encoding for 16 putative antimicrobial peptides were cloned in an expression vector designed to add an His-tag at the C-terminus of the recombinant proteins. To verify the correct expression and abundance of recombinant proteins (range of protein concentrations between 5 to 20 micromolar), Western blot and Coomassie Staining (or Silver Staining) were performed.
Bacterial growth inhibition assays were performed using a 96-well plate platform and a microplate reader to monitor bacterial growth in the presence of recombinant peptides.
Quantitative Real time RT-PCR was also performed to investigate the response of candidate genes to immune challenge triggered by sugar meals infected with Gram positive, Gram negative bacteria and yeast.
RESULTS:
From the An. gambiae sialome, we have compiled a short sub-catalogue of putative antimicrobials salivary candidates (16 genes). Selection criteria were the following: i) sequence features (length, biochemical properties, similarities with members of antimicrobials families) and ii) expression profiles. Three categories of genes were indeed considered: i) genes expressed specifically or principally in the females glands (possibly involved in the defence against pathogens on human skin); ii) genes expressed in both sexes (encoding for factors possibly playing a protective role during sugar feeding) and iii) genes expressed in the salivary glands and in other organs.
We have successfully synthesized fourteen recombinant proteins using the in vitro wheat germ transcription/translation system. Preliminary results indicate that some of the recombinant proteins exert antimicrobial activities as observed by bacterial growth inhibition assays. Moreover, transcriptional modulation was evidenced for some of the candidates upon bacterial challenge.
CONCLUSIONS:
Preliminary results of this work show that antimicrobials activities are encoded by short peptides expressed in the mosquito salivary glands. Even though most of these peptides do not show any sequence similarity with proteins in databases, novel antimicrobial features were revealed and further studies are required to confirm our data and to understand likely ways of action.

Keywords:
Anopheles gambiae, saliva, antimicrobial peptides

P07.7
GENOTYPING OF TOXOPLASMA GONDII ISOLATED FROM WILD AND DOMESTIC ANIMALS IN ITALY

Mancianti F.[1], Nardoni S.*[1], Mugnaini L.[1], Rocchigiani G.[1], Papini R.[1]

[1]Dipartimento di Scienze Veterinarie ~ Pisa ~ Italy

INTRODUCTION:
Toxoplasmosis is a zoonotic infection caused by Toxoplasma gondii, an opportunistic protozoan belonging to the phylum Apicomplexa. Up to one-third of the human population in the world is chronically infected, and toxoplasmosis has been targeted one among the five parasitic diseases as priorities for public health action. Human infections are primarily asymptomatic, but lymphadenopathy or ocular toxoplasmosis may occur in some patients. T. gondii infection in pregnant women may lead to abortion, stillbirth, or other serious consequences in newborns . In immune-compromised patients, toxoplasmosis can be fatal if not treated, and the reactivation of a latent infection can cause life-threatening encephalitis. The population structure of T. gondii is complex and shows distinct geographic patterns. Approximately 90% of the T. gondii isolates from Europe and North America can be classified into three distinct clonal lineages (types I, II, and III strains) that differ remarkably in pathogenicity. In addition, a fourth clonal lineage has been revealed in Nord America and a higher genetic variability has been detected based on newer markers for genetic characterization. The acute pathogenicity of T. gondii strains has been characterized in murine infections. The high pathogenicity of type I lineage is well represented by the commonly used laboratory strain RH, since the primary inoculation of a single infectious organism into a mouse is the cause of death in 8–12 days. Instead, lineages II and III are considered low pathogenic in the mouse model. To the best of our knowledge scanty data are present about genotypes circulating in animals in Italy. In this survey data about types isolated from seropositive animals are reported.
MATERIALS AND METHODS:
Different tissue samples (heart, brain, faeces, blood) from n. 46 seropositive animals (3 waterfowls, 8 foxes, 15 cats, 10 goats, 4 sheep and 6 jennies) were processed for DNA extraction and subsequent amplification by n-PCR to screen T. gondii DNA. Direct genotypic characterization of T. gondii strains was performed by PCR amplification of 11 genetic markers (SAG1, 3-SAG2, alt. SAG2, SAG3, BTUB, GRA6, C22-8, C29-2, L358, PK1, and Apico) and thereafter was analyzed by restriction fragment-length polymorphism. The classification in not clonal or atypical types was performed considering the homogeneity of SAG markers, when differences among these markers were recorded, parasites were considered as atypical.
RESULTS:
Clonal genotype I was recovered from 1 fox, 1 goat and 7 cats, genotype II was identified in 1 jenny and 1 cat respectively, and genotype III occurred in 4 goats, 5 jennies and 7 cats. Not clonal genotype I was present in 2 sheep and 1 goat, not clonal genotype III was observed in 2 waterfowl and foxes, respectively, as well as 1 sheep. Atypical genotypes were observed in 1 waterfowl and 1 sheep, respectively, in 5 foxes and 4 goats.
CONCLUSIONS:
The present results would confirm the hypothesis that clonal genotypes occur more frequently in domestic animals, as well as the presence of pure clonal types in cats would be in agreement with the possible a female-biased sex ratio in T. gondii.

Keywords:
Toxoplasma gondii, PCR-RFLP, Genotypes

P07.8
LOCALIZATION AND QUANTIFICATION OF INTRACELLULAR SYMBIONT MIDICHLORIA MITOCHONDRII IN ORGANS OF IXODES RICINUS TICKS

Mereghetti V.[1], Martin E.[1], Sassera D.[2], Mariconti M.[3], Bazzocchi C.[1], Bandi C.[1], Bacchetta R.[4], **Epis S.*[1]**

[1]Department of Veterinary Science and Public Health ~ Milan ~ Italy, [2]Department of Biology and Biotechnology "Lazzaro Spallanzani" ~ Pavia ~ Italy, [3]IRCCS San Matteo ~ Pavia ~ Italy, [4]Departement of Biosciences ~ Milan ~ Italy

INTRODUCTION:
Midichloria mitochondrii is an intracellular bacterium member of the recently described family of the Rickettsiales, Candidatus Midichloriaceae, that encompasses a wide group of intracellular bacteria associated with ticks (Montagna et al., 2013, Appl Environ Microbiol., 79:3241-8).
This bacterium has been found in different species of ticks; in particular, in Ixodes ricinus tick, it has been detected in 100% of the examined I. ricinus females across its geographical distribution and in about 50% of males. M. mitochondrii has been observed not only in the cell cytoplasm, but also inside the mitochondria, within the intermembrane space (Sacchi et al, 2004, Tissue Cell., 36:43-53). Up to now, we know that this symbiont is present in the ovary and salivary glands of the I. ricinus tick (Mariconti et al, 2012, Microbiology, 158:1677-83; Epis et al., 2013, Ticks Tick Borne Dis., 4:39-45) but no studies have so far investigated whether M. mitochondrii is spread and quantified in other organs of the tick. In particular, taking in consideration indirect evidences that suggest that the bacterium could be transmitted to vertebrates during the blood meal of the tick, the analysis of salivary glands should be of extreme interest. Moreover the localization of M. mitochondrii in the body of the arthropod and its ability to diffusion are unknown.
MATERIALS AND METHODS:
In this work we investigated the localization/quantification of M. mitochondrii in the body of the tick using immunofluorescence assay with specific anti-FLID antibody for M. mitochiondrii detection and quantitative Real-Time PCR in different organs of I. ricinus. Two genes were amplified: the gene of M. mitochondrii gyrB coding for gyrase B and the nuclear gene of I. ricinus cal coding for the protein Calreticulin. Adult female ticks both "semi-engorged" (i.e. a tick at half of its blood meal) and "non-engorged" (i.e. a tick that didn't start its blood meal yet) were analyzed.
RESULTS:
M. mitochondrii was detected in different body organs of the ticks. Furthermore differences in bacterial burden between the two analyzed life cycle periods of the tick were found. In fact an increase of M. mitochondrii burden, such as one of classical pathogens transmitted by the tick, can be noticed during the meal in salivary glands and rostrum.
CONCLUSIONS:
This study thus offered further evidences that M. mitochondrii is transmitted by ticks to vertebrates through saliva. Interestingly each trachea of the analyzed I. ricinus was positive for M. mitochondrii: this suggests that the respiratory tract can be used by M. mitochondrii as a dissemination way in the tick. This datum was described in previous works for organisms belonging to Rickettsia genus; Baldridge and colleagues (2007, J Invertebr Pathol., 94:163-74) experimentally infected tick of the specie Ixodes scapularis (a tick with American diffusion) with a cultivable Rickettsia monacensis able to produce the green fluorescence protein (GFP). After experimental infection, fluorescent R. monacensis were found distributed in the respiratory system of ticks; this result allowed the researchers to consider the tracheal system as a way of dissemination of bacteria and a possible "reservoir" during moulting. Similarly, Midichloria may also use the respiratory system as a way to spread within the I. ricinus tick.

Keywords:
Ticks, Rickettsiales, Real time PCR

P07.9

ESTABLISHING THE GOLGI COMPLEX OF CRYPTOSPORIDIUM PARVUM BY USING THREE PROTEINS AS SPECIFIC MARKERS

Nocita E.[1], Pozio E.[1], **Tosini F.*[1]**

[1]Department of Infectious, Parasitic and Immunomediated Diseases Istituto Superiore di Sanità ~ Rome ~ Italy

INTRODUCTION:
Apicomplexans possess unique vesicular organelles, micronemes, dense granules and ropthries, dedicated to the invasion of the host cell through the transportation of secreted proteins. Otherwise the Golgi complex, which is a membranous structure highly conserved in most eukaryotes, is the central connection of vesicular transport both towards the cytoplasmic organelles and the secretive vesicles. Recently, it has been demonstrated that a Golgi-like apparatus plays a crucial role in the protein trafficking of Plasmodium falciparum and in the biogenesis of the apical secretory organelles of Toxoplasma gondii. However, many aspects of the vesicular network of the Apicomplexa remain unclear and further investigations would be indispensable to reveal all the connections between the Golgi apparatus and the apicomplexan secretory vesicles. The presence of Golgi apparatus in Cryptosporidium species is still a controversial issue and, in some reports, Cryptosporidium is described as a Golgi-lacking organism. It is conceivable that a better understanding of the C. parvum Golgi apparatus would provide useful insights into the vesicular system of this parasite. Here, we identified three C. parvum “Golgi proteins” by similarity with proteins of related Apicomplexa and localized them in the sporozoite cell.
MATERIALS AND METHODS:
Gene homologs for various Golgi proteins were identified in CryptoDB by keywords and Blast search. Three of the identified ORFs were cloned in expression vectors to obtain 6his-tagged proteins in Escherichia coli and purified by affinity chromatography. The recombinant proteins were used to produce specific antisera in mice. Native proteins were identified in the sporozoite lysate by Western blot assays and their localization determined by immunofluorescence.
RESULTS:
Twenty-one proteins highly homologous to various proteins associated with the Golgi functions were detected.
Three putative genes of C. parvum, namely: Sec23, GRASP and SORTLR, were selected for their similarity with the homologs in P. falciparum and T. gondii, and for the absence of introns in their coding sequence. The expression of these three genes (CpSec23, CpGRASP, and CpSORTLR) was confirmed by RT-PCR on total RNA from sporozoites. Antisera for the putative Golgi proteins were tested for their specificity on recombinant proteins and sporozoite lysate.
The native proteins expressed in C. parvum were identified by immunoblot assays with the total lysate from sporozoites probed with the antisera for these recombinant proteins. Anti-Sec23 antibodies recognized a band of 70 kDa in sporozoites; whereas, anti-Sortilin antibodies bound a unique band of 80 kDa. Remarkably, antibodies for Grasp recognized a triplet of bands of approximately 90 kDa, suggesting the presence of different variants of this protein similarly to the GRASP homolog of P. falciparum.
By immunofluorescence, the CpSec23 protein appeared to be broadly diffused in the cytoplasm; whereas, CpGRASP and CpSORTLR were accumulated in a vesicle-like structure close and anterior to the nucleus.
CONCLUSIONS:
We identified three proteins, namely CpGRASP, CpSec23 and CpSORTLR, associated to the Golgi apparatus and we demonstrated their expression in C. parvum sporozoites. Two of these Golgi markers, CpGRASP and CpSORTLR, localize in spherical vesicle-like structure close to the nucleus, suggesting that at the sporozoite stage, the C. parvum Golgi-like complex is constituted by a unique spherical vesicle.

Keywords:
Cryptosporidium parvum, Golgi Complex, GRASP protein

P07.10

FIRST EVIDENCE OF RECOMBINATION IN THE X-CHROMOSOME CENTROMERIC REGION OF THE MALARIA MOSQUITOES ANOPHELES GAMBIAE AND AN. COLUZZII (DIPTERA: CULICIDAE).

Pichler V.*[1], Weetman D.[2], Mancini E.[1], Pombi M.[1], Vicente J.[4], Rodrigues A.[3], Dinis J.[3], Petrarca V.[1], Pinto J.[4], Della Torre A.[1], Caputo B.[1]

[1]Istituto Pasteur-Fondazione Cenci-Bolognetti, Dipartimento di Sanità Pubblica e Malattie Infettive, Università di Roma “La Sapienza”, Italy. ~ Roma ~ Italy, [2]Vector Group, Liverpool School of Tropical Medicine, United Kingdom ~ Liverpool ~ United Kingdom, [3]Instituto Nacional de Saúde Pública, Ministério da Saúde Pública ~ Guinea Bissau ~ Guinea-Bissau, [4]UEI Parasitologia Médica /CMDT&UPMM, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa ~ Lisbona ~ Portugal

INTRODUCTION:
Anopheles gambiae and An. coluzzii -the most important mosquito species transmitting malaria in sub-Saharan Africa- are defined based on specific SNPs in the multi-copy IGS chromosome-X linked rDNA region (della Torre et al., 2001, 10:9-18.). In fact, the species are mostly differentiated in the centromeric regions of chromosome-X and of the two autosomes, which have been hypothesized to represent “islands of speciation” containing the genes responsible for reproductive isolation (Turner et al., 2005, PLoS Biol. 3:e285). In their range of sympatry in West and Central Africa, hybrids between the two species are found at frequencies <1%, but in the westernmost part of the range, such as The Gambia and Guinea Bissau, hybrids have been observed with frequencies >20% (Oliveira et al., 2008, J Med Entomol, 45:1057-63; Caputo et al., 2011, PLoS One, 15 6:e16415). The aim of our work was to evaluate inter-specific genomic differentiation and frequency of recombination events along the X-centromere in the core of the putative secondary contact region, focusing the analysis on males which being hemizygous for the X-chromosome, allow to distinguish heterozygosity from recombination.
MATERIALS AND METHODS:
We genotyped 263 males collected in 2010 in three sites in Guinea Bissau for: i) the IGS diagnostic locus; ii) the insertion of a SINE retrotransposable element specific for An. coluzzii (Santolamazza et al. 2008 Malar J. 25:163); iii) a 57 bp-insertion in intron 4 of cytochrome CYP4G16 gene (Turner et al., 2005, PLoS Biol. 3:e285) specific for An. gambiae. Moreover, more than 800 SNPs (34 SNPs of which species-specific and located in the X centromeric region) were genotyped in a subset of 59 males.
RESULTS:
We observed: i) inter-specific differentiation only on chromosome-X centromeric region, the rest of the genome being undifferentiated; ii) intra-individual mixed IGS arrays in nearly 12% of the whole male sample, probably indicating the occurrence of introgression events; iii) unexpected recombination among IGS, SINE and CYP4G16 in 24% of males, including 13% of individuals showing recombination between SINE and CYP4G16, despite the close proximity of these two loci (7 Kb). Moreover, results from SNP-array suggest that recombination in the X-centromeric region is mostly restricted in the area nearby the SINE insertion, which could represent a hot-spot of recombination.
CONCLUSIONS:
The presented first evidence of recombination restricted to peculiar genomic regions within the most divergent genome region of A. gambiae and A. coluzzi (i.e. X-centromere) will be discussed in the frame of the current hypothesis on their recent speciation.

Keywords:
malaria vectors, speciation, recombination

P07.11
PRELIMINARY DATA ON TOXOPLASMOSIS IN PIGS OF SARDINIA.

Pipia A.P.[1], Varcasia A.[1], Pau M.[1], Seu S.[1], Sanna G.[1], Dore F.[1], Tamponi C.[1], Salis F.[2], Grimaldi C.[3], Salvatore S.[4], **Scala A.*[1]**

[1]Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari ~ Sassari ~ Italy, [2]Practitioner, Martini Zootecnica ~ Sassari ~ Italy, [3]ASL n.8, Sardegna ~ Cagliari ~ Italy, [4]ASL n.1, Sardegna ~ Sassari ~ Italy

INTRODUCTION:
Although pigs usually did not show any clinical signs, they are quite susceptible to Toxoplama gondii infection, and the pork meat is considered among the principal source for human infection (Dubey et al., 1991, J Parasitol, 77: 517-521; Cook et al., 2000, Br Med J, 321: 142-147). In Sardinia island are raised approximately 170.000 pigs of which 6.000 reared in family farms with traditional extensive methods.
The aim of the present study was to investigate the presence T. gondii in brains of pigs raised and slaughtered in family farms of Sardinia island with molecular techniques.
MATERIALS AND METHODS:
The survey was carried out in Sardinia during the pig home slaughtering campaign in 2013.
Eighty-four pig brains (n sows=77; = n gilts=7) were collected after slaughtering during the Azienda Sanitaria Locale (ASL) Official Vets inspection from 56 breedings. All brain samples were homogenized and an aliquot identified and properly stored at -20°C until DNA extraction with a commercial kit (High Pure PCR Template Preparation Kit, Roche Diagnostics, Mannheim, Germany). All samples were tested with a nested PCR targeting the multicopy 18S 5.8S rRNA internal transcribed spacer (ITS1) region as described by Hálova et al. 2011(Zoonoses Public Health, 60: 168-173).
RESULTS:
The 42.8% (36/84) of the examined brain samples were positives to the nested PCR for T. gondii. To confirm the nature of the amplified fragments, PCR products were sequenced, showing total homology with the T. gondii ITS-1 region. Positives samples were found in 53.6% (30/56) of monitored breedings. Positives samples were detected mainly in gilts (71.4%; 5/7) than sows (40.2%; 31/77) ($\chi 2$ Yates corrected=1.431; P=0.231). The presence of cats was reported in 46.6% (14/30) and in the 69.2%(18/26) of positive and negative breedings respectively ($\chi 2$=2.895; P=0.088 - Pearson Correlation=-0.227; P=0.092)
CONCLUSIONS:
Brain can be considered a good matrix for the detection of DNA of T. gondii in this animal species. Juránková et al. (2014, Food Microbiol, 38: 167-170) demonstrate in an experimental study that brain is the predilection site of T. gondii in pigs followed by lungs, heart and dorsal muscles. The high prevalence of T. gondii DNA (42.8%) herein found represent an alarming results and confirmed that pigs can be considered, also in the island, an important source of infection for humans as recently reported in pigs raised in Portugal (Esteves et al., 2014, Vet Parasitol, 200: 8-12). In our study the presence of cats in pigs family farms not seems to represent a possible risk factor for Toxoplasmosis; this could depend by the fact that pig can become infected also after the consumption of rodents and birds, milk of infected sows, congenital transmission or cannibalism (Turcekova et al., 2013, Acta Parasitol, 58: 361-366).

Acknowledgements: CIRPAR; Mr. F. Salis, Technician, Parasitology Lab., University of Sassari.

Keywords:
Toxoplasma, pigs, PCR

P07.12
GLOBAL PROTEOMICS OF THE OOCYST/SPOROZOITE OF TOXOPLASMA GONDII REVEALS SIGNIFICANT STAGE-SPECIFIC TRAITS

Possenti A.[1], Fratini F.[1], Fantozzi L.[1], Pozio E.[1], Dubey J.[2], Ponzi M.[1], Pizzi E.[1], **Spano F.*[1]**

[1]Istituto Superiore di Sanità ~ Roma ~ Italy, [2]United States Department of Agriculture ~ Beltsville ~ United States

INTRODUCTION:
Human toxoplasmosis can be acquired either congenitally or via the oral route. In the latter case, transmission is mediated by two distinct invasive stages, i.e., encysted bradyzoites present in raw or undercooked meat or sporozoites enveloped in environmentally resistant oocysts shed with cat feces. Despite its crucial epidemiological role, the sporozoite is the less characterized at the molecular level among the infectious stages of Toxoplasma gondii, mostly due to the difficulty in producing and handling oocysts. The existence of oocyst/sporozoite-specific proteins was first documented nearly thirty years ago by serological and biochemical methods, yet no stage-specific protein was identified until 2004. In recent years, several groups applied different high-throughput proteomic techniques to the study of the tachyzoite of T. gondii, with >5000 distinct proteins identified, accounting for ~65% of the global predicted proteome. On the contrary, the proteome expressed by the sporozoite remained largely unexplored.
MATERIALS AND METHODS:
Toxoplasma gondii oocysts (60% fully sporulated, 40% non sporulated) of strain VEG (genotype III) were purified by sucrose gradient from cat feces, mechanically ruptured, excysted with 1.5% sodium taurocholate and subjected to various treatments to obtain total protein lysates. These were analysed in three independent experiments by one-dimensional gel electrophoresis coupled to liquid chromatography-linked tandem mass spectrometry.
RESULTS:
We produced a dataset of 1685 non reduntant proteins accounting for ~21% of the total predicted proteome. Proteins with nuclear (25%), cytoplasmic (22%) and mitochondrial (13%) localizations were highly represented, as well as those associated with parasite's secretory routes (27%). Of 1685 proteins identified, 914 (52%) were classified according to the MIPS functional catalogue database, with the highest number of proteins being involved in protein fate (192), metabolism (177), cellular transport (119) and cell rescue, defence and virulence (111). Approximately 94% of the 1685 oocyst proteins were supported by mRNA expression microarray data, which allowed to identify proteins encoded by genes upregulated at various stages of sporulation. Most importantly, the comparison with the extensively covered proteome of the tachyzoite revealed the presence in our dataset of 211 putative oocyst/sporozoite-specific proteins, some of which were validated by Western blot. The analysis of this protein subset highlighted significant differences between the oocyst/sporozoite and the tachyzoite of T. gondii, particularly in the metabolism of lipids and amino acids and in the expressed complement of moving junction proteins.
CONCLUSIONS:
Although much remains to be done to map the complete proteome expressed by the oocyst/sporozoite of T. gondii, the present study shed light for the first time on the metabolic organization of this environmentally resistant form, unveiling stage-specific differences with respect to the extensively studied tachyzoite. Several enzymes involved in amino acid biosynthesis, fatty acid beta-oxidation, branched amino acid and amylopectin degradation were found to be specifically expressed in the oocyst, consistent with a strong commitment of this parasite stage to a host-independent life style. In addition, the stage-specific expression of AMA1 and RON2 isoforms points to the existence in the sporozoite of alternative moving junction complexes.

Keywords:
Toxoplasma gondii, sporozoite, proteomics

P07.13
GENOTYPING OF BLASTOCYSTIS HOMINIS FROM FAECAL SAMPLES IN ROME

Ranno S.*[1], Bracaglia G.[1], Santoro M.[1], Del Chierico F.[1], Accardi C.[1], Muraca M.[1], Putignani L.[1]

[1]Ospedale Pediatrico Bambino Gesù ~ Roma ~ Italy

INTRODUCTION:
Blastocystis hominis is a common anaerobic intestinal protozoan found in both human and animals. It is one of the most prevalent parasites isolated from patients suffering from diarrhea, flatulence, constipation and vomiting. Moreover, its pathogenicity and pathophysiology remain controversial to date. B. hominis is characterized by a high genetic diversity with at least 17 subtypes (STs). Several studies are evaluating the pathogenic role of these subtypes. In this regard, we are in the process to analyze faecal samples positive for Blastocystis hominis in order to identify strains corresponding to particular symptoms and to suggest a probable pathogenic role, by co-correlation analyses.
MATERIALS AND METHODS:
From January to December 2013, we collected 100 faecal samples positive for B. hominis by microscopy. To identify the various subtypes of B. hominis we are analyzing all faecal samples by PCR to confirm the presence of parasite. The PCR positive samples will be processed by sequencing with STS primer sets.
RESULTS:
The aims of this study is to identify the distribution of the parasite and to characterize its subtypes to understand its role in pathogenesis or gut disbyosis.
CONCLUSIONS:
The experiments are currently in progress.

Keywords:
Blastocystis hominis, Subtyping, gene Sequencing

P07.14
A LYOPHILISED REAL-TIME PCR SYSTEM FOR LEISHMANIA SPP SCREENING

Ranno S.*[1], Bracaglia G.[1], Gramegna M.[2], Turner L.[2], Santoro M.[1], Muraca M.[1], Putignani L.[1]

[1]Ospedale Pediatrico Bambino Gesù ~ Roma ~ Italy, [2]SENTINEL CH ~ Milano ~ Italy

INTRODUCTION:
PCR is a sensitive and rapid method for the diagnosis of Leishmania spp. infections and can be performed on a variety of biological samples, including peripheral blood, lymphnode, bone marrow and skin. Standard PCR requires amplicon electrophoretic analysis and is usually not suitable for quantification of the template DNA. For these reasons new real-time methods have been developed. Our group is testing a lyophilized system of Real time PCR for Leishmania spp screening.
MATERIALS AND METHODS:
In 2013, a total of 415 samples were analyzed for Leishmania screening with both STAT-NAT Lyophilised DNA-real time PCR amplification mix (SENTINEL) and a commercial endpoint nested-PCR kit. Before amplification all samples were processed according to routine barcode-based automatic DNA extraction procedures, without dedicated additional treatment for molecular analysis.
RESULTS:
Ten samples positive in nested-PCR resulted also positive with STAT-NAT Lyophilesed DNA-real time PCR amplification. We have introduced the Real-time PCR lyophilised amplification screening in our diagnostic routine, because of its rapidity (about 2 hours versus 5 hours) and theoretical absence of contaminations.
CONCLUSIONS:
We are setting a protocol for quantitative STAT-NAT Lyophilezed DNA-real time PCR amplification mix (SENTINEL) in order to introduce an efficient, rapid and quantitative molecular test for patient diagnosis and follow-up.

Keywords:
STAT-NAT Lesihmania spp. Lyophilesed DNA-real time PCR amplification, rapid molecular diagnosis, follow-up

P07.15

DESIGN AND APPLICATION OF SUBGENOTYPING FOR THE EPIDEMIOLOGIC STUDY OF CRYPTOSPORIDIUM SPP. AND MICROSPORIDIA INFECTION IN PEDIATRIC PATIENTS: STUDY OF ISOLATES FROM BAMBINO GESÙ CHILDREN'S HOSPITAL MISSION IN DODOMA (TANZANIA)

Reddel S.[1], Del Chierico F.[1], Concato C.[1], Muraca M.[1], Callea F.[1], **Putignani L.*[1]**

[1]Bambino Gesù Children's Hospital ~ Rome ~ Italy

INTRODUCTION:
Cryptosporidium spp. and Microsporidia are opportunistic coccidian parasites, which complete their life cycle both in humans and animals, through zoonotic and anthroponotic transmission, causing sickness and severe diarrhea. While in the immunocompetent host the infection is self-limiting, in immunocompromised (HIV-infected individuals) and malnourished people the infection can be severe, long-lasting and even fatal. Our aim is to identify Cryptosporidium and Microsporidia in stool samples from HIV-positive children and calves who is living in the community "Villaggio della Speranza", Tanzania, hence detecting possible zoonotic transmissions.
MATERIALS AND METHODS:
Stool samples were collected from 10 calves and 120 children in loco and freshly shipped to Bambino Gesù Children's Hospital. Each stool sample was analyzed for Cryptosporidium positivity by Ziehl-Neelsen (ZN) microscopy. For molecular PCR-based analysis, the samples were submitted to 5 freeze-thaw cycles and then the DNAs were automatically extracted by biorobot EZ1 (Qiagen). For Cryptosporidium detection samples were assayed by GP60-based PCR, sequencing and probing against NCBI non-redundant databases. For Microsporidia detection, samples were analyzed by modified Trichromic stain and by PCR-based 18S rDNA locus assay.
RESULTS:
For children, 25 out of 25 analyzed samples, resulted negative and 4 claves out of 10 samples resulted positive by ZN microscopy. For GP60 amplification analysis, the yielded products of expected size were obtained for 6 claves out of 10 samples and for 4 children out of 16 samples analyzed. The analysis of the 104 remaining samples is still in progress.
CONCLUSIONS:
The results obtained by microscopy were confirmed by PCR-based assay for Cryptosporidium isolates from calves sample while for human isolates. The GP60-based methods resulted more sensitive than microscopy. Moreover, this approach represents a useful survey tool to subgenotype lineages and to elucidate genetic richness of Cryptosporidium pediatric isolates, especially in these depressed areas. Also, to improve Microsporidia detection, Real time-based assays are in progress to identify, by melting curves analysis, the following species: Encephalitozoon cuniculi, Encephalitozoon helem, Encephalitozoon intestinalis and Enterocytozoon bieneusi.

Keywords:
Cryptosporidium spp., Microsporidia, subgenotyping

P07.16

NEW MOLECULAR METHODS IN DIAGNOSTIC PARASITOLOGY: PCR PROCEDURES USING LYOPHILIZED SYSTEMS FOR ORO-FAECAL PARASITES

Santoro M.*[1], Bracaglia G.[1], Ranno S.[1], Gramegna M.[2], Ventura L.[2], Muraca M.[1], Putignani L.[1]

[1]Ospedale Pediatrico Bambino Gesù ~ Roma ~ Italy, [2]SENTINEL CH ~ Milano ~ Italy

INTRODUCTION:
Traditional parasitological diagnostics is based on microscopic analysis. Moreover, molecular methods have been recently introduced in order to increase sensitivity and to evaluate molecular epidemiology of the parasites. In this study we report on protocols for rapid molecular detection of oro-faecal parasites.
MATERIALS AND METHODS:
Samples from parasitological routine of Bambino Gesu' Children Hospital, Rome were analyzed by both microscopy and molecular procedures. For PCR analysis we evaluated a lyophilized systems-STAT NAT DNA-mix (SENTINEL), home-made and adopted to provide a molecular affordable protocol.
RESULTS:
We have found a good performance of the lyophilized systems and we are setting the kits for rapid screening of several parasites.
CONCLUSIONS:
These procedures have been already introduced in our routine workflow.

Keywords:
Lyophilized systems, Rapid molecular analysis, Parasitological diagnostics

P07.17

PARASITOLOGICAL DIAGNOSIS OF HALICEPHALOBUS GINGIVALIS INFECTION IN A FATAL CASE OF MENINGOENCEPHALITIS OCCURRED IN A ITALIAN SADDLE HORSE

Veronesi F.*[1], Lepri E.[1], Passamonti F.[1], Morganti G.[1], Mandara M.T.[1]

[1]Department of Veterinary Medicine, University of Perugia ~ Perugia ~ Italy

INTRODUCTION:
In the present work parasitological findings and results of a phylogenetic analysis of the DNA sequence data obtained from Halicephalobus gingivalis specimens detected in an equine fatal case of halicephalobiasis occurred in Italy, were described.
MATERIALS AND METHODS:
On the mid-August 2013, a 7-year-old Italian Saddle gelding horse, housed at an equestrian club of the Marche region, was referred at the Perugia Veterinary Teaching Hospital after a 10 days period of illness characterized by cranial nerve deficits, progressive and severe dynamic ataxia and a reduced mentation status. Due to the rapidly worsening of the clinical conditions the animal was euthanized. At the post-mortem examination several large and grayish-white granulomatous lesions consisting of multinucleated giant cells and epithelioid macrophages, were observed in inguinal lymph node, kidneys and myocardium; moreover a non suppurative meningovascular lesions affecting prosencephalus, cerebellum and pons were detected. A large number of eggs, larval, and adult nematodes were observed within granulomatous lesions and in the perivascular spaces of the central nervous system; similar parasitic specimens were occasionally seen also at the cytological examination of the cerebrospinal fluid. Small pieces of unfixed pathological material were placed in PBS (pH 7.4) at room temperature to allow the migration of nematodes out of the tissue; the surrounding liquid was collected, centrifuged and the sediment was examined at the microscope, after Lugol staining. Moreover to clarify the genetic identity of the nematodes, DNA was extracted from a pool of 15 adult specimens using a commercial kit (QIAamp® Tissue Kit) and a fragment of 670 bp of the nuclear large subunit ribosomal RNA gene (LSU rDNA) was amplified by PCR, according to Nadler et al. (2003, Int J Parasitol, 33: 1115-1125). The PCR product was sequenced in both the directions and the sequence was aligned using ClustalW (http://www.ebi.ac.uk/Tools/clustalw2/index.html) with sequences of representative free-living and parasitic isolates. The aligned sequence data were analyzed by the Neighbor-joining method using the software package MEGA 5 (http://www.megasoftware.net/).
RESULTS:
The parasites were identified as Halicephalobus sp. based on morphologic features (Anderson et al, 1998, Parasite, 5:255–261) and confirmed by specific PCR. Phylogenetic analysis revealed that the isolate formed a clade with H. gingivalis isolates from the USA, Canada and Japan and showed the highest identity (99.8%) with a Japanese strain (accession number AB288935.1).
CONCLUSIONS:
Up to date only few reports of equine Halicephalobus infection are referred in Italy (Di Francesco et al, 2012, Veterinaria Italiana, 48: 437-442). The case here presented represents the first Italian report in which H. gingivalis infection was confirmed both by morphological and DNA-based approaches and a phylogenetic relationship among national and international strain was assessed. Such genetic approaches are of utmost importance because they have the potential to aid in identifying basic factors involved in the circuit of transmission of a parasite with veterinary and zoonotic fatal implications.

Keywords:
Halicephalobus gingivalis, Horse, Italy

XXVIII Congresso Nazionale SoIPa, Roma 24-27 giugno 2014

NOTES

NOTES

POSTER 08

INFEZIONI DA PROTOZOI

P08.1
GLOBALIZATION EFFECTS ON PARASITES DISTRIBUTION

Bracaglia G.*[1], Ranno S.[1], Santoro M.[1], Del Chierico F.[1], Tchidjou H.[1], Callea F.[1], Muraca M.[1], Putignani L.[1]

[1]Ospedale Pediatrico Bambino Gesù ~ Roma ~ Italy

INTRODUCTION:
Protozoa and helminths are responsible for several gastrointestinal infections (GI) especially in children. Common GI parasites, frequently detected in stools are either protozoa (e.g., Giardia lamblia, Entamoeba histolytica, Cryptosporidium spp.) and soil-transmitted helminths (STH) (e.g., Ascaris, whipworms, and hookworms). Recently, even in industrialized countries, risk of helminth infections has been associated to wastewater and excreta contamination, in agricultural settings, suggesting water as a key factor for worm infection. The diffusion effect of globalization contributes to the spread of some parasite also in Italy.
MATERIALS AND METHODS:
For parasitological analysis three stool samples from each patient, collected at alternate days and fixed in 10% formalin, were observed under a 40x-100x light microscope after concentration, in accordance with Ritchie and stained with Lugol. Micrometric eyepiece was used for exact determination of cystic diameter and parasite dimensions. Prior microscopy, an aliquot of each faecal sample was immediately processed through rapid immunocromatographic test. All samples were processed according to routine barcode-based automatic DNA extraction procedures without dedicated additional treatment for molecular analysis.
RESULTS:
We report two cases of these infections in adopted children: 1) an asymptomatic child with a double infection of Giardia lamblia and Trichuris trichiura at the onset and with a chronic Trichuris trichiura infection detected at later visits; 2) and a child treated for recurrent atopic dermatitis for several months but actually affected by a chronic Strongyloides stercoralis infection.
CONCLUSIONS:
These cases highlight necessity to introduce targeted parasitological investigations for adopted children during pediatric controls.

Keywords:
Gastrointestinal infections, adopted children, protozoa and helminths

P08.2
LUMINEX GASTROINTESTINAL PATHOGEN PANEL (XTAG GPP) FOR THE SIMULTANEOUS DETECTION OF PARASITES, VIRUSES AND BACTERIA IN HUMAN STOOL SAMPLES FROM COTE D´IVOIRE

Di Cristanziano V.[1], Timmen Wego M.[1], Kaiser R.[1], Pfister H.[1], Di Cave D.[2], Berrilli F.[2], **D´alfonso R.*[3]**

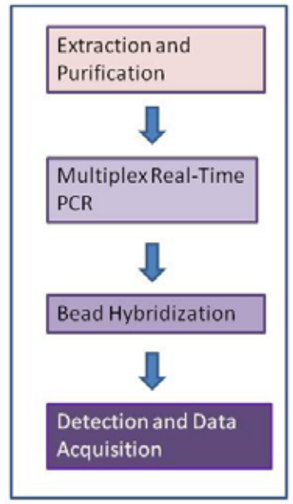
[1]Institute of Virology, University of Cologne. ~ Cologne ~ Germany, [2]Department of Experimental Medicine and Surgery, University of Rome "Tor Vergata" ~ Rome ~ Italy, [3]Department of Systems Medicine, University of Rome "Tor Vergata" ~ Rome ~ Italy

INTRODUCTION:
In the contest of a multidisciplinary study proposed to investigate the gut microbiota in Sub-Saharan Africa, we analyzed 15 human fecal samples collected in the Clinical Laboratory of the Don Orione Center in Bonoua (Côte d´Ivoire). To complete the analysis of microbiological gut composition, 15 of the most common human enteric pathogens were concurrently analyzed using the xTAG GPP from Luminex, a qualitative bead-based multiplexed molecular diagnostic test (Figure 1a) [Claas et al., 2013, J. Microbiol. Biotechnol., 23:1041-1045; Gray et al., 2014, Epidemiol. Infect., 142:1-11]. To our knowledge this research represents one of the first attempts to apply this technology to patients coming from developing countries.

Figure 1a Panel of bacteria, viruses and parasites of xTAG GPP

Bacteria	Parasites
S. boydii, S.sonnei, S. flexneri, S. dysenteriae	*G. duodenalis*
V. cholerae	*E. hystolitica*
C. jejuni, C. lari and C.coli	*C. parvum* and *C. hominis*
Salmonella spp.	**Viruses**
C. difficile toxin A/B	*Rotavirus* A
Y. Enterocolitica	*Norovirus* GI/GII
E. coli O157	*Adenovirus* 40/41
Enterotoxigenic *E.coli* (ETEC) LT/ST	
Shiga-like Toxin producing *E.coli* (STEC) stx1/stx2	

Figure 1b Workflow of xTAG GPP



MATERIALS AND METHODS:
After collection, the samples were transported to Germany for the subsequent extraction of the nucleic acids using VERSANT® kPCR. The xTAG GPP workflow is described in Figure 1b. In order to confirm the reliability of the xTAG GPP and the preservation of the viral nucleic acids during the transport to Europe the samples were parallel analyzed by Rota-/Adeno-/Astrovirus antigen tests as well as by Real-Time PCR for the detection of Norovirus, Adenovirus, Enterovirus, Parechovirus and Hepatitis E Virus. We performed also the molecular characterization of G. duodenalis and Entamoeba spp. isolates by conventional PCR.
RESULTS:
Overall, it was detected at least one pathogen in 9 out of 15 samples. All positive samples from patients under 12 years revealed a mixed infection (Table 1). The xTAG GPP identified 8 positive samples. The viral isolates detected by xTAG GPP were confirmed by the other methods, with the exception of the sample M18, resulting weakly positive for Adenovirus in Real-Time PCR. Among the parasitic isolates 2 isolates of Giardia were not detected by xTAG GPP. The molecular characterization assigned the 6 G. duodenalis isolates to Assemblage A or B. Two Entamoeba isolates were assigned to the non-pathogen coli or dispar species, which are not included in the xTAG GPP.

ID	Age (years)	xTAG GPP	Real-Time PCR	*Giardia* PCR and Genotyping	*Entamoeba* PCR and Genotyping	*Rota-/Adeno-/Astrovirus* Antigen test
M1	8	-	-	-	-	-
M5	8	*E.coli* 0157 *G. duodenalis*	-	*G.duodenalis* Assemblage A	-	-
M7	10	*E.coli* 0157	-	*G.duodenalis** Assemblage B	*E. coli*	-
M8	5	*Campylobacter* ETEC *G.duodenalis*	*Coxsackievirus B***	*G.duodenalis* Assemblage A	*E. dispar*	-
M9	3	*G.duodenalis*	*Coxsackievirus A13*** *Parechovirus*	*G.duodenalis* Assemblage B	-	-
M11	9	-	-	-	-	-
M12	40	-	-	-	-	-
M13	30	-	-	-	-	-
M14	12	*Norovirus* GI/GII	*Norovirus* GI *Enterovirus 114***	*G.duodenalis** Assemblage A	-	-
M15	12	-	-	-	-	-
M16	39	*Norovirus* GI/GII *Rotavirus* A	*Norovirus* GII	-	-	*Rotavirus*
M17	50	*G.duodenalis*	-	*G.duodenalis* Assemblage B	-	-
M18	41	-	*Adenovirus**	-	-	-
M19	67	-	-	-	-	-
M21	24	*Shigella*	-	-	-	-

Table 1
Summary of results obtained by xTAG GPP, Real-Time PCR (*Adeno-/Noro-/Entero-/Parecho-/Hepatitis E Virus*), *Giardia* and *Entamoeba* PCR and genotyping, *Rota-/Adeno-/Astrovirus* Antigen tests.

*no detected by xTAG GPP
**Enterovirus isolates were sequenced at Robert Koch Institute

CONCLUSIONS:
The present data highlights the potential of xTAG GPP to detect simultaneously in a single test a panel of gastrointestinal pathogens, including parasites, bacteria and viruses. Considering the burden of the environmental enteropathies in developing countries, with a very high rate of co-infections in particular in the infant population, this study let´s hope a wider application of xTAG GPP in endemic areas. The use of this technology allowed us to deepen knowledge of gut microbiota composition in people living in the south of the Cote d'Ivoire, to better understand the multiplex interactions between microbiota, host and pathogens.

Keywords:
xTAG GPP, Cote d´Ivoire, gastrointestinal pathogens

P08.3
PRELIMINARY ASPECTS OF THE MOLECULAR EPIDEMIOLOGY OF CRYPTOSPORIDIUM INFECTIONS IN CHILE

Mercado R.*[1], Peña S.[1]

[1]*Parasitologia, Facultad de Medicina, Universidad de Chile ~ Santiago ~ Chile*

INTRODUCTION:
Cryptosporidium hominis and C. parvum intestinal protozoan infections are the most prevalent found in Chile, both are anthroponotic and zoonotic agents causing diarrhea and affecting public health in humans and economic loses in cattle industry.
MATERIALS AND METHODS:
We studied human (12) and bovine (30) Cryptosporidium positive fecal samples by using PCR followed by sequencing of the SSU rRNA 18S gen fragment and the 60 kDa glycoprotein (gp60) gen fragment.
RESULTS:
Nine of the 12 humans samples were positive to 18S gen and 33% (3/9) of the samples analyzed were C. parvum, 66% (6/9) were C. hominis. Neonatal calves showed a 100% 18S C. parvum gen fragment positivity and this finding is expected in 0-3 week old neonatal calves. Only 5 of the 18S human positive samples were amplified by gp60 PCR and sequenced. These samples had a wide variety of C. hominis gp60 subtypes: IdA16 (2), IdA15 (1), IaA12R1 (1). Gp60 IIc subtype appears in one sample (IIcA5G3b), indicating anthroponotic cycle of C. parvum in immunocompromised people in Chile. Ten samples were positive to gp60 PCR and sequenced. Subtype family IIa of C. parvum appears to be the only gp60 subtypes affecting neonatal calves. Subtype IIaA15G2R1 were the most representative gp60 subtype in the 60% of the sequences analyzed. Furthermore, there are differences in gp60 subtype spatial distribution in neonatal calves. In central Chile C. parvum IIaA17G4R1 appears in almost all cases analyzed. Instead, in south Chile, gp60 subtype IIaA15G2R1 is the gp60 subtype affecting calves.
CONCLUSIONS:
These findings suggest that a few Cryptosporidium subtypes are infecting susceptible hosts in Chile, especially in neonatal calves and open an opportunity to define the epidemiology and better control of the disease.

Keywords:
Cryptosporidium hominis, Cryptosporidium parvum, gp60

P08.4
DETECTION AND GENETIC CHARACTERIZATION OF GIARDIA DUODENALIS IN RABBITS

Paoletti B.*[1], Di Cesare A.[1], Frangipane Di Regalbono A.[2], Iorio R.[1], Bartolini R.[1], Averaimo D.[1], Traversa D.[1]

[1]Faculty of Veterinary Medicine ~ Teramo ~ Italy, [2]Department of Animal Medicine,Production and Health ~ Padova ~ Italy

INTRODUCTION:
Giardia duodenalis is distinct in eight genetic groups, i.e. Assemblages A to H. They differ in their host distribution (Monis et al., 2009, Trends Parasitol, 25:93-100) and, importantly, Assemblages A and B are often associated with human infections. While the genetic identity of G. duodenalis Assemblages has been investigated in several animals, few studies have addressed their occurrence in rabbits. A survey conducted in Europe revealed a prevalence of 5.9% of giardiosis in these animals (Pantchev et al., 2005, Tierarztl Praxis, 4:296-306) and some evidences have shown that rabbits harbor mainly the zoonotic Assemblage B (Levecke et al., 2011, Vet Parasitol, 177:166-170). The present study investigated the occurrence of G. duodenalis in rabbits from Abruzzo region of central Italy, towards an evaluation of these animals as potential zoonotic reservoirs.
MATERIALS AND METHODS:
Individual fecal samples were collected from 167 rabbits (23 privately-owned, 4 from pet shops, 128 from rural farms, 12 from intensive farms). Individual stool samples were examined for the presence of G. duodenalis using a sucrose gradient centrifugation (Roberts-Thomson et al., 1976, Gastroenterology, 71:57-61). Genomic DNA was extracted from concentrated suspension of G. duodenalis cysts from selected microscopically positive samples. The DNA was submitted to three nested PCRs, i.e. specific for a 175 bp region of the small subunit (SSU)-rDNA, a 511 bp fragment of the gene encoding for the β-giardin protein (bg) and a 530 bp of the gene encoding for the triose phosphate isomerase (tpi) (Read et al., 2002, Int J Parasitol, 32:229-331; Lalle et al., 2005, J Parasitol, 91:203-205; Sulaiman et al., 2003. Emerg Infect Dis, 9:1444-1452). The PCR products were sequenced and all the sequences were compared with those available in the GenBankTM database.
RESULTS:
Out of 167 fecal samples, 4 (2.5%), collected each in different rural farms keeping animals in cages with no outdoor access, were microscopically positive for G. duodenalis cysts. The infected animals showed no clinical signs. The SSUrDNA sequences corresponded to Assemblage B (i.e. 100% homology with GenBank HG425134.1). Two samples scored positive for the bg and tpi genes and the sequencing confirmed their identity as Assemblage B, i.e. 100% with the sub-Assemblage BI (GenBankTM Acc. No HQ397719.1; Acc. No KC960631.1).
CONCLUSIONS:
The infection rate herein observed is lower than that found from other areas in Europe (Pantchev et al., 2005, Tierarztl Praxis, 4:296-306). Despite no sound evidence has supported the zoonotic transmission of Assemblage B between humans and animals, case control studies have showed that a contact with farm animals may be related to increasing infection rates of human giardiosis (Hoque et al., 2002, Int J Infect Dis, 6:191-197; Hoque et al., 2003, Epidemiol Infect, 131:655–662). Considering that Rabbits are both kept as pets and bred for human consumption, more extensive studies from different areas are necessary to better characterize the risk of transmission for giardiosis between human and rabbits and to assess its potential public health significance.

Keywords:
Giardia duodenalis, rabbit, epidemiology

P08.5
ANIMAL TOXOPLASMOSIS: A SEROEPIDEMIOLOGICAL SURVEY ON A GEOGRAPHIC AREA OF TUSCANY REGION

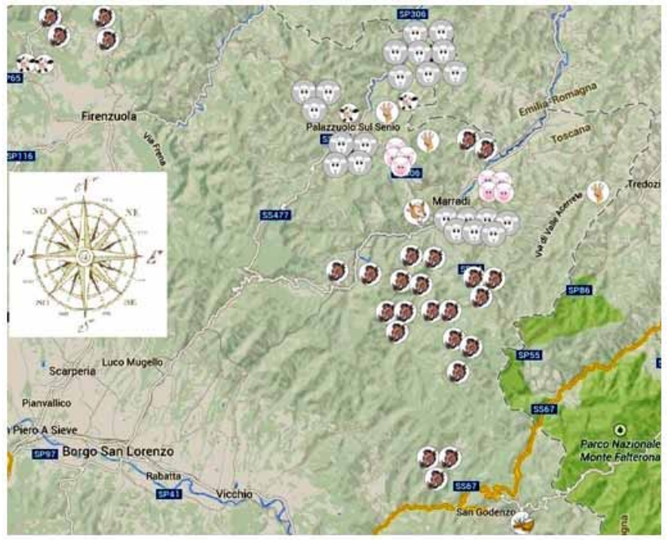
Moretta I.[2], Poglayen G.[1], Parigi M.[1], Catani M.[3], Morandi B.[1], **Piergili Fioretti D.*[2]**

[1]Dipartimento di Scienze Mediche Veterinarie ~ Bologna ~ Italy, [2]Dipartimento di Medicina Veterinaria ~ Perugia ~ Italy, [3]AUSL 10 D ~ Firenze ~ Italy

INTRODUCTION:
Toxoplasmosis represents the most common parasitic zoonosis worldwide and, despite the European Union has issued a Directive (Dir. 2003/99/EC, implemented in Italy by the DL n. 191, April 4, 2006) including the Toxoplasmosis in the list of zoonosis to be monitored according to the epidemiological situation, the national epidemiological data are so scarce that reliable information to EFSA (European Food Safety Agency) is almost impossible to provide. Most of the currently Italian data are from the Ce.Tox. (National Reference Center for Animal Toxoplasmosis) and, therefore, related to the seroprevalence of toxoplasmosis in animal species reared in Sicily.
In order to improve the knowledge on the distribution of Toxoplasmosis, a seroepidemiological survey was conducted from wild-life and domestic species living in a small mountain geographic area of Tuscany region.
MATERIALS AND METHODS:
Serum samples from 97 cattle, 59 sheep, 24 goats, 56 pigs, 87 wild-boars, and 12 wild ruminants (10 roe deer, 3 red deer, 1 fallow deer), living in the area near to the National Park of “Foreste Casentinesi, Monte Falterona e Campigna”, were obtained through the collaboration with the local Veterinary Public Services and the hunters’ association. Anamnestic informations about the origin of the animals were recorded.
All samples collected from domestic and wild-life animals were tested for the detection of anti-Toxoplasma gondii (IgG) antibodies respectively by IFAT (Immunofluorescence Antibody Test) (MegaScreen FLUO Toxoplasma g., Megacor) and by MAT (Microscopic Agglutination Test) (Toxo-Screen DA, Biomérieux).
RESULTS: The results of the serological survey according to the animal species are summarized in Table 1; the number of positives are reported on the Map 1.

Animal species	N° animals Examined	Serological test performed	Cut-off	Percentage of positivity (%)
Cattle	97	IFAT	1/64	4.1 %
Sheep	59	IFAT	1/64	37.3 %
Goat	24	IFAT	1/64	4.2 %
Swine	56	IFAT	1/16	14.3 %
Wild boar	87	MAT	1/25	29.9 %
Deer	12	MAT	1/25	33.3 %

Table 1: Seroprevalence of *T. gondii* according to the animal species investigated and the serological approach used.



Map 1

CONCLUSIONS:
The map of the positivity shows an important cluster of T. gondii infection extended to the northwest borders of the National Park of “Foreste Casentinesi, Monte Falterona e Campigna”, and including mainly wild boars but also some deer and domestic animals i.e. sheep and pigs. In this area of the Tuscany lives a small population of wild cat (Felix silvestris) and a specimen of Lynx lynx, probably abandoned (ISPRA, 2008, Biologia e conservazione dei felidi in Italia; www.parcoforestecasentinesi.it), that may represent a source of dissemination of the protozoan oocysts across the territory.
Although the number of analyzed domestic animals does not represent an appropriate sample size, the prevalence recovered in sheep (37.3%) is consistent and higher than that found in the pig population (14.3%). Concerning pigs, we tested all the adults animals bred in this area: these animals come from factory farms and are raised in this area only 3-4 months for finishing. This could justify the lower seroprevalence than in sheep, which are native animals and graze throughout their life in this territory.
Moreover, it is interesting to highlight the high positivity found in deer (33.3%), a selective grazer species, compared to that found in wild boar (29.9%), an omnivorous scavenger species.
Our effort to show the distribution of T. gondii in term of geographic area seem to be interesting and suggestive of deeping that we are already planning.

Keywords:
animal toxoplasmosis, Tuscany, epidemiological survey

P08.6
BOVINE BESNOITIOSIS: REVIEWS OF SYMPTOMATIC CASES

Righi C.*[1], Maresca C.[1], Scoccia E.[1], Sylla L.[2], Filippini G.[1], Gentile A.[3], Manuali E.[1], Pavone S.[1], Agnetti F.[1], Gobbi M.[1], Mangili P.[4]

[1]Istituto Zooprofilattico Sperimentale Umbria e Marche ~ Perugia ~ Italy, [2]Dipartimento di Medicina Veterinaria Università di Perugia ~ Perugia ~ Italy, [3]Dipartimento Clinico Veterinario Università di Bologna ~ Bologna ~ Italy, [4]Istituto Zooprofilattico Sperimentale Umbria e Marche ~ Pesaro ~ Italy

INTRODUCTION:
Bovine besnoitiosis, caused by the cyst-forming apicomplexan parasite Besnoitia besnoiti, is a chronic and debilitating disease with consequent severe economic losses. According to the symptoms, infected animals are classified into three different classes: 1) animals with typical signs of the disease 2) animals with sclero-conjunctival cystic lesions 3) animals which do not show any sign (Jacquiet et al.,2009, Journées Nationales, G.T.V, 1213-1217). Asymptomatic animals can help to spread the pathogen. Nowadays, the disease is considered to be re-emerging by the European Food Safety Authority. This paper presents an update of symptomatic animals recorded in the last six years in Italy.
MATERIALS AND METHODS:
Over the years 2009-2014 native symptomatic animals were enroled in the study. A network was made between practitioner colleague, Veterinary Universities and other Italian public institutes of animal disease survey with the aim to report all suspected cases of besnoitiosis.
Diagnosis were made base on following procedures:
- Detailed anamnesis
- Clinical examination
- Serological test (ELISA)
- Histopathological examination
- Andrological examination.

RESULTS: 15 bovines, 7 bulls (5 Limousine , 1 Chianina, 1 Romagnola) and 8 cows (6 Limousine, 1 Holstein, 1 Chianina) with an average age of 22,2 months (DS ± 8,3) and clinical signs of besnoitiosis were recorded.
14 subjects were beef cattle while 1 was a dairy cattle, most of the animals (10/15) sharing a common pasture with other cattle farms.
All cases with a seropositive diagnosis, showed clinical signs characterized by weight loss (15/15), weakness (11/15), sclera-conjunctival cysts (13/15) and skin thickening especially in the head, periocular, neck and genital regions (15/15).
The andrological examinations highlighted chronic orchitis and atrophy of the seminal vesicles (2/3), azoospermia (2/3) and teratozoospermia (1/3).
Histologically samples were characterized by B.besnoiti tissue cysts as circular bodies containing bradyzoites in dermis, panniculus and underlying muscle layer.



A: Periocular cutaneous thickening



B: Sclera-conjunctival cysts (arrows)



C: Cysts spread in the subcutaneous tissue

Clinical signs

CONCLUSIONS:
The number of symptomatic animals found is relatively low if compared with the study of seroprevalence done in the same period in our country (0,6%) (Gollnick et al, 2010, Letter. Vet. Rec. 166, 599). The question is: must the besnoitiosis in Italy be considered endemic? Under this light, in agreement with the literature, the proportion of clinical manifestation recorded in our study is comparable to the endemic countries, in contrast to the areas where the disease whose incidence of symptomatic cases varies from 15-20% is emerging (Jacquiet et al., 2009, Journées Nationales G.T.V., Nantes, 1213-1217).
The nature of the clinical signs, however, is non specific and diagnosis can be difficult and confused with other diseases (Jacquiet P.,2010,Veterinary Parasitology, 174:30).
From the above, we can say how, in addition to the signalment of new symptomatic cases, the assessment of seroprevalence plays a crucial role to resolve the epidemiological gap while, in order to prevent the spreading of the disease, a global farm strategy as testing new animals and controlling the movimentation are needed.

Keywords:
Besnoitiosis, symptomatic animals, economic losses

P08.7
DROPLET DIGITAL PCR FOR QUANTITATIVE DETECTION OF LEISHMANIA INFANTUM: PRELIMINARY DATA

Romano A.*[1], Ingravalle F.[1], Barzanti P.[1], Zanatta R.[2], Goria M.[1]

[1]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta ~ Torino ~ Italy, [2]Dipartimento di Scienze Veterinarie ~ Grugliasco, Torino ~ Italy

INTRODUCTION:
The digital PCR is a new technology that enables precise quantification of target nucleic acids in biological samples, allowing to measure absolute quantities by counting nucleic acid molecules encapsulated in discrete, volumetrically defined, water-in-oil droplet partitions. This technique is a direct detection method and relies in limiting partition of the PCR volume, such that a positive result in a large number of microreactions, indicates the presence of a single target molecule in the starting sample. Canine leishmaniosis (CanL) is a zoonotic disease due to Leishmania infantum, transmitted by sand flies. A gold standard direct test for CanL diagnosis is far from to be identified and several real time PCR protocols have been described (Francino et al., 2006; Mary et al., 2004). Aim of this work was to test the digital PCR approach to detect and quantify L. infantum load in canine samples.
MATERIALS AND METHODS:
Four ten-fold standard serial dilutions of DNA obtained from a culture of L. infantum (MON-1 IPT1, from 104 to 10 leish/mL) were previously submitted to real time PCR quantification and then assayed by a digital PCR protocol. The same was done with 30 lymph node aspirate samples collected from dogs with a diagnosis of CanL characterized by different parasite loads (pL): 8 samples with a high pL (~106 leish/mL at the qPCR) were assayed in digital PCR in 1 replicate; 6 samples with a medium/high pL (from 104 to 105 leish/mL) in 2 replicates, 6 samples with medium/low pL (from 10 to 1.5•103 leish/mL) in 3 replicates; 8 samples with low pL (from 5 to 102 leish/mL) in 4 replicates; 3 samples from dogs that, after specific therapy, were in clinical remission, without clinical-pathological abnormalities and with absence of parasites on qPCR, were also included and tested in 5 replicates. Digital PCR was performed in a Droplet Digital PCR (ddPCR) QX100 platform (Bio-Rad, CA) and it was conducted using "QLEISH primers and probe" (Vitale F. et al., 2004) in a total reaction volume of 20 µl of "Droplet PCR Supermix" (Bio-Rad). Analysis of the ddPCR data was performed with QuantaSoft software (Bio-Rad).
RESULTS:
The ddPCR response was linear over the dynamic range of the DNA standards (y=0.954x-1.802), covering all the range in the 4 orders of magnitude with an excellent efficiency (R2=0.9995). The 104 leish/mL DNA standard was directly quantified at 1100 copies of DNA target/µL. Considering that ddPCR target is a sequence of kinetoplast DNA (kDNA) replicated in 103 copies per parasite genome, the resulting quantification is 1000 leish/mL. This discrepancy was recorded constantly in all standards and clinical samples. It could be related to the fact that the target copy is not a standalone sequence in kDNA: so each droplet could encapsulate several target copies detected as single positive signal underrating the final pL.
CONCLUSIONS:
This underestimation gap could be overcome with an appropriate step of enzymatic DNA digestion prior to amplification. The ddPCR confirmed real time data and detected positive pL in a qPCR negative animal. The ddPCR technology provided good reproducibility with conventional qPCR in L. infantum quantification and it promises high performances in direct quantification without requiring a calibration curve. It would be used in follow-up to reliably monitor the pL, allowing to correlate variations with therapy efficacy or potential relapses in simpthomatic CanL.

Keywords:
Droplet Digital PCR, real time PCR, L. infantum

P08.8
USEFULNESS OF PCR-RFLP IN DETECTION AND IDENTIFICATION OF LEISHMANIA SPECIES

Paglia M.G.*[1], Vulcano A.[1], Bellagamba R.[1], Ghirga P.[1], Toffoletti A.[1], Di Caro A.[1]

[1]INMI Lazzaro Spallanzani ~ Roma ~ Italy

INTRODUCTION:
Leishmaniasis, a vector-borne zoonotic disease transmitted by sand fly bites, is a public health concern in many countries of the world. In the countries of the Mediterranean basin, especially Spain, France and Italy, both visceral and cutaneous forms of leishmaniasis are observed in humans. The most common methods of diagnosing visceral (VL) and Old World cutaneous leishmaniases (CL) are classic parasitologic methods such as microscopic direct examination and in vitro culture of bone marrow aspirates. Since these techniques are time-consuming and cannot be used to differentiate the various Leishmania species, we used a rapid method, PCR-RFLP, for detection and identification of the species of parasites from samples of peripheral blood, bone marrow and skin biopsies.
MATERIALS AND METHODS:
From January 2004 to December 2013, sixty-two samples belonging to 56 patients with the clinical suspicion of VL, 6 samples belonging to 6 patients with the clinical suspicion of CL, and four samples belonging to two patients with the clinical suspicion of both VL and CL, for a total of 72 samples from 64 patients, all reporting signs and symptoms and/or risk factors related to leishmaniasis, were analyzed in our laboratory with PCR-RFLP, using peripheral blood samples or bone marrow aspirates and skin biopsies. DNA extraction was performed using QIAmp DNA Blood Mini kit (Qiagen) in accordance with the manufacturer's instructions. For formalin-fixed and paraffin-embedded (FFPE) tissues, 5-10 mmc slices were deparaffinated by treatment once with xylol and twice with ethanol. After drying of the tissue, manufacturer's protocol was followed. Leishmania-specific primers, which amplify a nuclear repetitive genomic sequence, were used (Minodier et al, 1997 J. Clin. Microbiol., 35:2551-5). Identification of Leishmania at the species level was obtained by restriction enzyme analysis with HaeIII.
RESULTS:
Fifty-six patients were diagnosed as having VL. The primary diagnosis of VL was done on peripheral blood for 49 of 56 patients (87.5%) and on bone marrow aspirates for the remaining patients (12.5%). Two patients had confirmed both VL and CL. Six patients had CL. The diagnosis of CL was confirmed on skin biopsies. By means of PCR-RFLP analysis, a single 250-bp band suggestive of L. infantum infection was observed for all subjects.
CONCLUSIONS:
PCR-RFLP assay is a rapid, reliable, sensitive and routine-adapted method that allows a secure diagnosis of VL with a sample (peripheral blood) that can be obtained by non-invasive means. The assay is also applicable to other types of samples: bone marrow aspirates and skin biopsy specimens. In conclusion, this method could be used as an alternative system of screening individuals with suspected VL and CL, or as a tool for monitoring the efficacy of treatment and the appearance of relapse of subclinical disease.

References: (1) Minodier P. et al, 1997 J. Clin. Microbiol., 35:2551-5.

Keywords:
Leishmania, PCR-RFPL, blood sample

P08.9
NOVEL ARTEMISININ DERIVATIVES AS POTENTIAL ANTILEISHMANIAL AGENTS

Basilico N.*[1], Parapini S.[2], Corbett Y.[2], D'alessandro S.[2], Grande R.[3], Parvez M.A.K.[4], Haynes R.K.[5], Taramelli D.[2]

[1]Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche, Università di Milano, Via Pascal 36, 20133 Milano, Italia, [2] Dipartimento di Scienze Farmacologiche e Biomolecolari, Università di Milano, Via Balzaretti 9, Milano, Italia, [3]Azienda Ospedaliera L. Sacco, Polo Universitario UOC, Microbiologia Clinica, Virologia, Diagnostica delle Bioemergenze, Milano, Italia, [4]Department of Microbiology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh., [5]Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom 2520, South Africa

INTRODUCTION:
Leishmaniasis is one of the major tropical parasitic diseases, transmitted to humans by sandflies. The World Health Organisation (WHO) estimate that 350 million people are at risk of contracting leishmaniasis. In the absence of an effective vaccine, chemotherapy remains the only weapon to fight the infection. However, costs, toxicity and resistance to conventional drugs, make very urgent the need for new drugs with selective toxicity against the parasite.
Artemisinin derivatives are the mainstay of antimalarial therapy. It has been reported that artemisinins have anti-leishmanial activity, as well, both in vitro and in vivo at micromolar concentrations.
To contribute to the search of new drugs for leishmaniasis, here we describe the antileishmanial activity of two novel artemisinin derivatives: artemisone, and GC12 against promastigotes and intramacrophages amastigotes of L. infantum. Artemisinin and dihydroartemisinin were also used as controls.
MATERIALS AND METHODS:
Promastigote stage of L. infantum strain MHOM/TN/80/IPT1 (kindly provided by Dr. M. Gramiccia and Dr. T. Di Muccio, ISS, Roma) and L. tropica (MHOM/IT/2012/ISS3130) were cultured in RPMI 1640 medium supplemented with 15% heat-inactivated fetal calf serum and 2 mM L-glutamine at 22°C. The antileishmanial activity was evaluated using the MTT assay after 72 hours of drug treatment and the IC50 was calculated for each compound. Intracellular amastigotes were obtained after infection of PMA differentiated THP-1 cells with promastigotes. After 72 hours of incubation in the presence of drugs, the cultures were fixed, stained with Giemsa and the percentage of infected macrophages in control and in drug treated cells was determined by light microscopy. Drug sensitivity assay on P.falciparum and cytotoxicity on mammalian cell were tested using the pLDH and MTT assay, respectively.
RESULTS:
Artemisinin derivatives were cytotoxic against promastigotes of both L.infantum and L. tropica with IC50 values in the micromolar range, in line with previous studies. The most active compound was the new derivative GC12. Artemisone was less active; however, it was also the less cytotoxic on mammalian cells. Mild activity was displayed also against intracellular amastigotes. All the artemisinins displayed low toxicity on PMA-differentiated THP-1 and, as expected, their antiplasmodial activity was very good, with IC50 values in the low nanomolar range.
CONCLUSIONS:
Taken together, these data indicate that artemisinin derivatives have promising anti-leishmanial activity. Further work is required in order to select drug candidates against both malaria and leishmania.

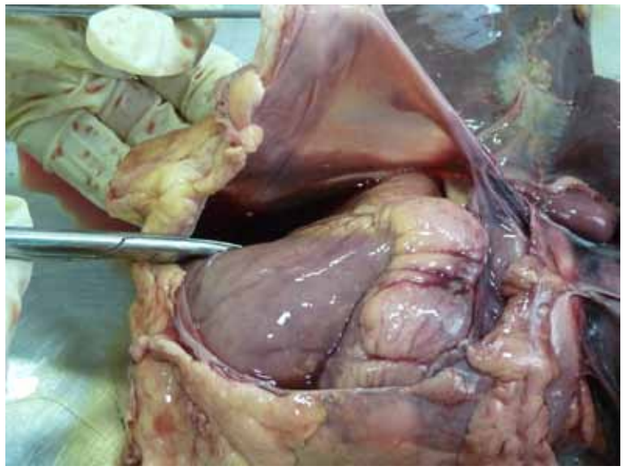
Keywords:
leishmaniosis, artemisinin derivatives, antileishmanial drugs

P08.10
STUDY OF AN EXOTIC TICK BORNE DISEASE BY INFECTION OF SHEEP WITH BALL 3 E. RUMINANTIUM "VACCINE" PERFORMED IN NAMIBIA: CLINICAL SYMPTOMS, GROSS LESIONS AND MOLECULAR DIAGNOSIS

Pascucci I.*[1], Cammà C.[1], Di Domenico M.[1], Di Mattia T.[2], Molini U.[3], Scacchia M.[1], Pini A.[1]

[1](1) Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" Campo Boario 64100 ~ Teramo ~ Italy, [2]Private Veterinarian ~ Teramo ~ Italy, [3]Central Veterinary Laboratory- Ministry of Agriculture Water and Forestry- ~ Windhoek ~ Namibia

INTRODUCTION:
Heartwater (HW) is a tick-borne rickettsiosis, caused by Ehrlichia ruminantium (in the past known as Cowdria ruminantium), and transmitted by hard ticks of the Amblyomma genus. The disease is widespread in sub Saharan Africa and its economic impact on livestock is high, similar to that of East Coast fever and African Trypanosomosis and it represents a major obstacle to the improvement of ruminants production. Even if the introduction and the establishment of HW and its vectors in Mediterranean area is unlikely, the risk of introduction cannot be excluded also because one of the most important vector, A. variegatum, has been reported in the Mediterranean area probably introduced by migratory birds. In addition it's to consider that A. variegatum and E. ruminantium have just spread outside southern Africa into the Caribbean where A. variegatum colonization has been boosted by favorable ecological conditions, leading to the establishment of the tick and of the infection in the area.
The study described was conducted in the framework of the activities of the mandate of the National Reference Centre for Exotic Diseases at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZSAM) in cooperation with Namibian Veterinary Services, in order to acquire knowledge on clinical signs, lesions and diagnostic skills for HW.
MATERIALS AND METHODS:
Ten Dorper Sheep, five years old, were selected for the trial, they were infected by intravenous inoculation of infective blood containing the Ball 3 stock of E. ruminantium (OBP) commonly used for the immunization procedure called "infection-treatment". Sheep were kept under observation till 36 days p.i., and temperature registered in order to observe clinical signs. Blood samples were collected and tested by specific E. ruminantium PCR by amplifying a fragment of the pCS20 gene. Necropsy was also carried out and organs taken for PCR test.
RESULTS:
The incubation period ranged from day 11 to day 15 p.i., when pyrexia was first recorded. Temperature peaks varied between 39.9°C and 41.3°C. Mortality occurred between day 17 and day 36 pi. Clinical signs observed were attributable to each of the four clinical forms described in literature
Respiratory symptoms and typical lesions were observed (idrotorax and idropercardium). PCR performed on blood samples were positive for all 10 animals when temperature was ≥ 39.1°C. Among the tissue samples tested, brain was the more frequent positive for E. ruminantium DNA.



(idropericardium)

CONCLUSIONS:
Results from PCR on blood confirm the short and transient bloodstream phase of E.ruminantium infection, while brain has been confirmed as organ of choice for laboratory diagnosis on dead animal. The study allowed to describe clinical symptoms and lesions of HW and to improve diagnostic skills for HW. It has been confirmed that Ball3 stock, improperly called vaccine, is actually pathogenic, able to produce clinical disease and livestock losses if infected animals are not properly and timely treated with tetracycline.

Keywords:
Exotic tick borne disease, diagnostic skills, Ehrlichia

P08.11
NOSEMA CERANAE : A TWO-YEARS MONITORING PROJECT IN AOSTA VALLEY

Ferroglio E.*[1], Ugonino P.[1], Carlin L.[2], Gerbelle C.[2], Zanet S.[1], Trisciuoglio A.[1], Ragionieri M.[2]

[1]University of Turin, Dept. Veterinary Sciences ~ Grugliasco ~ Italy, [2]Regione Valle d'Aosta ~ Aosta ~ Italy

INTRODUCTION:
Nosema ceranae is a microsporidian parasite that affects adult honeybees Apis mellifera causing a reduction of colony's productions, depopulation of affected families. N. ceranae has spread rapidly across the world and it is considered to be one of the risk factors related to "Colony Collapse Disorder". Depite being native of Asia, it has been present in Europe since at least 1993. The goal of the present study is to describe the trend of infection with N. ceranae over a period of two years in a previously exempt region in Northern Italy (Aosta Valley).
MATERIALS AND METHODS:
The research project involved 101 bee-hives from 9 apiaries. During the two-year monitoring project the hives were sampled twice at the end of summer (August-September), in November, and in April. From each hive a pool of 20 adult bees was analyzed for the presence of spores (manual counting in Burker's chamber) and was tested by PCR for N. ceranae/N. apis.
RESULTS:
N. apis was never detected in any of the tested samples. The prevalence of infection with N. ceranae increased from a minimum of 20% to 45.5% in the first and second summer samplings. GIS-based analysis evidenced that hives located in areas exposed to solar radiation high and with an area of 3 km2 from agricultural activities were statistically less infected with N. ceranae. Bees of the local Carniolan subspecies (Apis mellifera carnica) were statistically less infected than Italian bee Apis mellifera ligustica.
CONCLUSIONS:
In the Aosta Valley Region N. ceranae has widely replaced N. apis as parasite of adult honeybees. The parasite, widely present throughout the region, seems to be affected by environmental/managing factors as well as by genetic traits of bees.

Keywords:
Nosema ceranae, Nosema apis, PCR

NOTES

POSTER 09

CONTROLLO E MONITORAGGIO DI VETTORI

P09.1
ANTIBIOTIC TREATMENT OF THE TICK VECTOR IXODES RICINUS: EFFECTS ON MIDICHLORIA MITOCHONDRII

Cafiso A.[1], Serra V.[1], **Bazzocchi C.*[1],** Epis S.[1], Comandatore F.[1], Gaiarsa S.[1], Bandi C.[1], Mariconti M.[2], Plantard O.[3], Sassera D.[4], Pajoro M.[1]

[1]DIVET, University of Milan ~ Milan ~ Italy, [2]IRCCS; Policlinico S. Matteo ~ Pavia ~ Italy, [3]Epidemiology and Risk Analysis in Animal Health, LUNAM Université ~ Nante ~ France, [4]DBB, University of Pavia ~ Pavia ~ Italy

INTRODUCTION:
The tick Ixodes ricinus is widely recognized as the most important ectoparasite in Europe. Widespread across the entire continent, I. ricinus is a vector of many pathogens, including bacteria, viruses and protozoa. I. ricinus hosts a symbiotic bacterium, Midichloria mitochondrii ascribed to the Midichloriaceae family (Rickettsiales) (Montagna et al., 2013, Appl. Environ. Microbiol. 79:3241-48).This bacterium is present in all stages of I. ricinus development, with a prevalence of 100% and it is transmitted to the tick host through the blood meal. Indeed, humans and animals parasitized by I. ricinus develop antibodies against this bacterium (Mariconti et al., 2012, Pathog. Glob. Health. 106:391-96; Bazzocchi et al., 2013, Parasit Vectors. 6:3503) and the DNA of M. mitochondrii has been amplified in the blood of parasitized vertebrates. No studies have been conducted on the fate of M. mitochondrii in the host and no information are available on the symbiosis between the bacterium and the tick.
The aim of the project is to investigate the effect of the antibiotic tetracycline on the amount of M. mitochondrii after microinjection of adult females of I. ricinus and after a blood meal of larvae on treated and control gerbils.
MATERIALS AND METHODS:
Samples have been collected by colleagues in Nantes (France) and sent us for subsequent analysis. Two fully engorged females collected on roe-dear in France have been microinjected with a single dose of tetracycline (15ng/mg). Three fully engorged females have been used as controls. After oviposition, larvae have been fed on control gerbils (one for each tick) or on gerbils treated with tetracycline (one for each tick). The gerbil treatment consisted of 10mg/kg of antibiotic injected two times for forced feeding; subsequently tetracycline 1.2% (w/v) has been administered to gerbils in drinking water for 6 days. Larvae before and after the blood meal have been stored at -80°C until use.
DNA from larvae after oviposition (ten sample for each tick for a total of 50 larvae) and larvae after the blood meal on each gerbil (for a total of 100 larvae) was extracted by using a commercial kit. Burdens of M. mitochondrii in each samples were estimated by quantitative PCR. Target genes for the absolute quantification were: gyrB gene for M. mitochondrii and calreticulin for I. ricinus. Statistical analyses have been performed.
RESULTS:
Burden of M. mitochondrii is expressed as the ratio gyrB/cal x1000. The obtained results show a large variability in the amount of bacteria presents in unengorged larvae derived from different I. ricinus tick probably due to the variability of the number of M. mitochondrii in each adult female. Furthermore, microinjection of tetracycline doesn't seem to reduce the amount of bacteria in larvae; despite this, the number of produced eggs is decreased. Encouraging results have been obtained testing the presence of M. mitochondrii after the blood meal on control gerbils and gerbils treated with tetracycline. The ratio gyrB/cal x1000 indeed shows a decreasing of bacteria in engorged larvae derived from treated animals.
CONCLUSIONS:
The role of M. mitochondrii in the tick I. ricinus is still unknown. The reduction of bacteria in larvae after the blood meal on treated gerbils open the way to the study of the possible role of M. mitochondrii in the tick development stages

Keywords:
Midichloria mitochondrii, antibiotic treatment, real time PCR

P09.2
MONITORING HIPPOBOSCA EQUINA (DIPTERA: HIPPOBOSCIDAE) IN THE REGIONAL PARK BOSCHI DI CARREGA: ECOLOGICAL EVIDENCES

Defilippo F.[1], Maioli G.[1], Tomeo A.[2], Gilioli S.[2], Calzolari M.[1], Bonilauri P.[1], **Dottori M.*[1],** Corradi M.[2]

[1]Istituto Zooprofilattico Sperimentale Lombardia e Emilia Romagna ~ Reggio Emilia ~ Italy, [2]Ente di Gestione per i Parchi e la Biodiversità Emilia Occidentale ~ Parma ~ Italy

INTRODUCTION:
Hippobosca equina is a well known haematophagous fly that infests ruminants in different parts of the world, but is generally considered to be nonpathogenic. However, H. equina, can cause blood loss and serve as vector for pathogenic organisms, phoretically transport lice (Ischnocera) and mites (Acari). High infestation could have negative effects on domestic and wild animals. This study try to clarify some aspects of the ecology of these ectoparasites in the Regional Park Boschi Di Carrega, wich reported previously high prevalence of H. equina in roe deer (Young et al., 1993, J. Wildl. Dis., 29:278-283).
MATERIALS AND METHODS:
In this study we evaluated the abundance of H. equina in the period ranging from spring-autumn of 2008. We chose 11 different sites based on particular ecological characteristics (i.e. farms, presence of cattle, horses or wild mammals) and on vegetation and hydrological characteristics (presence/absence of creeks). We used blue panels (one trap per site) for the capture of insects (BIOPLANET ColorTRAP B).
The data were correlated with thermo-pluviometric reports and relative humidity obtained from the meteorological station of the Park.
RESULTS:
Of 11 sites all were positive for H. equina presence, with an average number of specimens for trap of 2,11. The highest catches were obtained in sites where the wooded vegetation was most intense and there was a strong presence of wildlife (70% of positive panels and average of specimens for each sampling 11,8) with the greatest number of insect on the trap (n=68) at the end of September. In the period tested an increase in the density occured with temperatures lower than 25°C and low precipitation.
CONCLUSIONS:
This study suggests that the density of H. equina would be vulnerable not only to the host but also to the influences of climate, This study suggests that the density of H. equina would be vulnerable not only to the host but also to the influences of climate, because we observed a density increase in case of low temperature (T<25°C) and precipitation. Moreover, it was demonstrated that the puparia of this species were more resistant to cool rather than warm (T>24°C) temperature extremes (Bennet, 1961, Can J Zoology 39: 379-406). Our results show that understanding the H. equina ecology is useful for the management and control of this ectoparasite.

Keywords:
H. equina, ecological evidences, wildlife

P09.3
EFFECT OF NIGHT TIME-INTERVALS, HEIGHT OF TRAPS AND LUNAR PHASES ON SAND FLY COLLECTION

Gaglio G.*[1], Brianti E.[1], Napoli E.[1], Falsone L.[1], Dantas Torres F.[2], Tarallo V.D.[3], Otranto D.[3], Giannetto S.[1]

[1]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina ~ Messina ~ Italy, [2]Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães (Fiocruz-PE) ~ Recife ~ Brazil, [3]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari ~ Bari ~ Italy

INTRODUCTION:
Trapping methods play an important role while studying phlebotomine sand fly populations. Climate and other abiotical variables may affect the seasonality and the activity of phlebotomine sand flies. In addition, scant information is available on the effect of lunar phases on the biology of these insects. In this study we monitored the activity of phlebotomine sand flies in a highly endemic area for canine leishmaniasis in order to gain data on their seasonality and to assess the effect of the night time-intervals, height of traps from ground and lunar phases on their abundance.
MATERIALS AND METHODS:
Phlebotomine sand fly collections were conducted in the teaching farm of the University of Messina by using commercial light traps. To assess their diversity and seasonal activity light traps were placed biweekly from May to November 2011. The nocturnal activity of phlebotomine sand flies was investigated by using one light trap at 100 cm above the ground and by changing the net bag every 2 hours from 20:00 to 06:00 for three consecutive days. The effect of the trap height from ground on the number of sand flies captured was assessed using three light traps, which were placed at different heights (i.e., 50 cm, 100 cm, 150 cm above the ground) for 30 consecutive days. In this last experiment, data on lunar phases were also recorded throughout the 30 days.
RESULTS:
Overall 1,072 phlebotomine sand flies belonging to five species (i.e., Phlebotomus perniciosus, Phlebotomus neglectus, Phlebotomus sergenti, Phlebotomus perfiliewi, and Sergentomyia minuta), four of which are proven vectors of Leishmania spp., were captured. The most abundant species was P. perniciosus (73.3%) followed by S. minuta (23.3%). The highest number of phlebotomine sand flies was collected in August and September with a peak in the collection of males recorded during the evening (i.e., from 20:01 to 22.00). No correlation was found between the abundance of capture and the daily mean temperature ($R2 = 0.42$; $P = 0.110$), whilst a significant negative correlation was observed with the mean relative humidity ($R2 = 0.78$; $P = 0.008$). The number of phlebotomine sand flies collected at 50 cm above the ground was significantly higher ($P = 0.041$) than that captured at 150 cm. This finding was significant for specimens of P. perniciosus ($P < 0.001$), while no significant difference was recorded for the number of S. minuta captured at the three trapping heights. The frequency of P. perniciosus and S. minuta collected during full moon phase was significantly higher than those collected in the other moon phases ($P < 0.0001$).
CONCLUSIONS:
Results of this study confirm the seasonal activity of P. perniciosus in Sicily from May to October. Males of P. perniciosus were more active from 20.01 to 22.00, whereas females were evenly active at different time-intervals, suggesting that the likelihood of capturing as well as the risk of being bitten is more or less constant throughout the night. Light traps placed at 50 cm above the ground seem to be more suitable for collecting P. perniciosus, and a higher activity of P. perniciosus and S. minuta was recorded during full moon phase. All these variables may significantly affect the capture of phlebotomine sand flies by light traps and should be considered in future studies on the ecology of these species in the Mediterranean area.

Keywords:
sand fly, biology, light trap

P09.4
MONITORING OF CIMEX LECTULARIUS IN PISA AREA DURING THE PERIOD 2006-2013

Macchioni F.*[1], Manca M.[2], Biasci A.[2], Gianchecchi M.[2], Magi M.[1]

[1]Dipartimento di Scienze Veterinarie ~ Pisa ~ Italy, [2]Entomox, Company of disinfestation and rat extermination ~ Pisa ~ Italy

INTRODUCTION:
Bed bugs (Cimex lectularius and Cimex hemipterus) are blood-sucking insects that readily bite humans. Infestations of this insect are rapidly increasing worldwide. Health consequences include nuisance biting and cutaneous and systemic reactions (Giorda et al., 2013, Veterinaria Italiana, 49(4): 335-40; Dogget et al, 2012, Clin Microbiol Rev, 25(1): 164-92).
MATERIALS AND METHODS:
From 2006 the presence of bed bugs in the area of Pisa was monitored by Entomox (Company of disinfestation and rat extermination). The specific identification of arthropods was conducted in the Department of Veterinary Science of Pisa following the identification keys of Chinery (Field guide insects of Britain and Northern Europe. 1993, 3rd ed. London: Harper Collins Publisher).
RESULTS:
From 2006 up to the present 64 calls for bed bugs have been received with a total of 173 interventions with disinfestation's treatments: 4 infestations in 2006, 5 in 2007, 2 in 2008, 5 in 2009, 7 in 2010, 14 in 2011, 13 infestations in 2012, 14 in 2013. All collected specimens were identified as C. lectularius. The samples were collected mainly during spring and summer and for each infestation occurred 2 or more interventions for eradicate the insect. The principal calls were by private citizens that reported bites and dermatological reactions, hotels, motels and bed and breakfast. Bed bugs were found in most of the cases in the bedroom.
CONCLUSIONS:
As clearly evidenced in this study in the last three years it has been an increase of calls and of disinfestation's treatments. This trend could be due primarily to the increase of international travels since bed bugs can be passively transported over long distances by clothing and luggage (Delaunay et al., 2011, Clin Infect Dis, 52:200-10). Furthermore second-hand furniture can be an easy way to transfer bed bugs (Romero et al., 2007, J Med Entomol, 44:175-8). The widespread use of air conditioning, which uniforms the temperature and avoids extreme variations (Doggett et al., 2004, Environ Health, 4: 30-8), creates ideal conditions for the bug development. Bed bugs resurgence should be considered an emerging public health problem. Adequate surveillance of bed bug populations is important for future monitoring and control.

Keywords:
Cimex lectularius, Bed bugs, infestation

P09.5
ENTOMOLOGICAL SURVEILLANCE ON MOSQUITOES IN PIEDMONT REGION (NORTH-WESTERN ITALY), 2011-2013

Pautasso A.[1], Francese D.R.[1], Radaelli M.C.[1], Prearo M.[1], Bertolini S.[1], Vitale N.[1], Desiato R.[1], Verna F.[1], **Pintore M.D.*[1],** Mosca A.[2], Maurella C.[1], Chiavacci L.[1], Casalone C.[1]

[1]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d' Aosta ~ Torino ~ Italy, [2]IPLA Istituto per le piante da legno e l'ambiente ~ Torino ~ Italy

INTRODUCTION:
Mosquito borne diseases (MBDs) are a group of infections representing an emerging threat worldwide for both humans and animals. Originally endemic in tropical regions, in recent years they expanded their geographical range, mainly due to globalization, climatic and environmental changes.
These factors facilitate the introduction and establishment of exotic mosquitoes species and pathogens in new areas (Scholte et al., 2008, Med Vet Entomol, 22:352–358; Medlock et al., 2011, Vector Borne Zoonotic Dis., 2:435-47).
Piedmont region is located in North-western Italy. It is a MBDs free area bordering infected regions (South-eastern of France and the Italian Emilia Romagna region), and shows habitat and climatic features suitable to the introduction of exotic mosquitoes and MBDs that they can transmit, as already evidenced in several European countries (Schaffner et al., 2009, Med Vet Entomol., 23:448-51; Seixas et al., 2013, Mem Inst Oswaldo Cruz., 108 Suppl 1:3-10).
In this work we describe the entomological surveillance carried out from 2011 to 2013 in Piedmont in order to gain a better knowledge on mosquitoes distribution and to early detect the introduction of exotic invasive species.
MATERIALS AND METHODS:
The whole Piedmont territory was divided into 73 equal squares 20x20km. For each square sited under 600 meters a.s.l. at least one trap was located in fixed sampling sites. They were selected according to risk factors that can affect MBDs epidemiological cycle: proximity to wetland areas, presence of hosts and habitat features (according to the Corine Land Cover classification).
Entomological surveillance was performed during the mosquito season of each year with weekly or fortnightly samplings. Mosquitoes were trapped using CO2 CDC dry ice-baited traps. Furthermore in 2013, 8 BG sentinel traps with the BG-Lure were placed into national and international trade areas, in order to monitor the introduction of exotic invasive mosquitoes. Mosquitoes were identified to species level by morphological standard classification keys (Stojanovic and Scott, 1997, USA: Ed.Stojanovich CJ & Scott HG). Mosquito species were grouped according to their vector competence (EFSA, 2009).
RESULTS:
During the surveillance period, a total of 99271 mosquitoes were collected with CO2 CDC traps. They belong to 14 different species. According to their vector competence, four species were classified with high level of competence, three species with moderate and seven species with low (Table 1).
A total of 3084 mosquitoes were collected in 2013 with BG sentinel traps. They belong to 6 different species. According to their vector competence, 3 species were classified with high level of competence and 3 species with moderate (Table 2). No exotic mosquito species were found.

Mosquito species	N	%	Vector competence
Culex pipiens	47996	48,35%	High
Ochlerotatus caspius	33958	34,21%	Moderate
Culex modestus	7262	7,32%	High
Anopheles maculipennis s.l.	5528	5,57%	Moderate
Aedes vexans	1950	1,96%	Moderate
Aedes albopictus	1191	1,20%	High
Culex theileri	20	0,02%	High
Other	1366	1,39%	
Total	99271		

Tab 1: Mosquito species collected in Piedmont (from 2011 to 2013) with CO_2 CDC traps and their vector competence. Other species include: *Ochlerotatus geniculatus, Culex* sp., *Aedes cinereus, Ochlerotatus cantans, Anopheles plumbeus, Culiseta subochrea, Aedes* sp., *Culiseta longiareolata, Culiseta* sp., *Culiseta annulata*

Mosquito species	N	%	Vector competence
Cx. pipiens	1192	38,65	High
Ae. albopictus	680	22,05	High
Oc. caspius	894	28,99	Moderate
An. maculipennis s.l.	246	7,98	Moderate
Ae. vexans	69	2,24	Moderate
Cx. modestus	3	0,1	High
Total	3084		

Tab 2: Mosquito species collected in Piedmont with BG sentinel traps in 2013 and their vector competence

CONCLUSIONS:
The entomological surveillance enforced in Piedmont, was designed in order to know the real vector distribution even in absence of clinical cases of MBDs. The results showed that most mosquitoes trapped had moderate-high vector competence and might be able to acquire and transmit the most common MBDs. The availability of continuous data on mosquito population, its spatial distribution and abundance in the study area, where no autochthonous cases of MBDs in humans or animals have so far been reported, provides important information to be used in case of an epidemic emergency, to set up focused anti-vectorial plan and to draw risk maps useful for public authorities.

Keywords:
mosquito borne diseases, invasive species, surveillance

P09.6
ASSESSMENT AND DETERMINATION OF LC50 OF CARVACROL AND SALICYLIC ACID ANALOGUES WITH ACARICIDE ACTIVITY IN LARVAE AND ADULT TICKS OF RHIPICEPHALUS (BOOPHILUS) MICROPLUS

Ramírez Lubianos C.[1], **Ibarra Velarde F.*[2],** Pérez Morales H.[1], Manjarrez A. N.[1], Salgado Zamora H.[3], Ortega C. L.[1]

[1]UAM-Xochimilco, México city, Mexico, [2]FMVZ-UNAM ~ Mexico city ~ Mexico, [3]IPN. México city, México

INTRODUCTION:
Boophilus microplus is a tick that causes huge economic losses in cattle. Carvacrol and salicylic acid have been evaluated in different strains of Boophilus. In the present research carvacrol analogues were selected (carvacrol methyl ether (1) and carvacrol acetate (2)), as well as salicylic acid analogues ((2,5-dihydroxybenzoic acid (3), ethyl 2,5-dihydroxybenzoate (4), methyl 2,5-dihydroxybenzoate (5), 2-(α, α'-dimethoxymethyl) phenol (6), ethyl 2-methoxybenzoate (7) and alcohol 2-hydroxybenzyl (8)), isomers of salicylic acid were also considered, such as propyl p-hydroxybenzoate (9) methyl p-hydroxybenzoate (10), were also considered to assess their acaricide activity in vitro.
MATERIALS AND METHODS:
Carvacrol, salicylic acid and 2, 5-dihydroxybenzoic acid (3) were purchased from manufacturers. Carvacrol and salicylic acid analogues above mentioned were prepared as described in the literature. Adult ticks were obtained from previously infected cattle. Larval immersion test (LIT) and Adult immersion test (AIT) were carried out according to Bravo et al. (2008). Lethal concentration 50 (LC50) was calculated using a Probit regression. Regarding AIT, all tests were performed in triplicate, and reporting only the media and standard deviation. A one-way ANOVA was performed and in case of detecting significant differences a Dunnett test was performed.
RESULTS:
The acaricide activity of salicylic acid analogues and isomers in B. microplus larvae was evaluated, employing the LIT technique (Table 1). Where the ANOVA gave a F value of 0.91, no significant differences between the salicylic acid analogues were indicated. Ethyl 2-methoxybenzoate (7) was the most potent compound followed by ethyl 2,5-dihydroxybenzoate (4), while 2 - (α, α'-dimethoxymethyl) phenol was the least potent. The first 4 compounds were submitted to evaluation with the AIT. With the data of the selected compounds, parameters such as ovoposition reduction (OR, Table 3), hatching inhibition rate (HR, Table 4) and efficacy (E, Table 5) was determined. Regarding OR, the ANOVA gave an F value of 9.26, indicating a statistical difference between the parent compounds and the analogues. However, no significant difference between the analogues was found. In the case of HR, the ANOVA gave an F value of 3.78, thus indicating with the Tukey test a significant difference between the compounds and salicylic acid, demonstrating that salicylic acid is not an effective compound on adult ticks nor is E with an F value of 8.23.
CONCLUSIONS: All evaluated compounds presented better acaracide activity at the larval stage as compared with the adult stage of B. microplus, ethyl 2-methoxybenzoate (7) and ethyl 2,5-dihydroxybenzoate (4), being the best candidates to continue their study.

Table 1 Effect of salicylic acid and its analogues on *R. (B.) microplus* larvae in vitro (% mortality) by LIT

Conc. (%)	Salicylic acid	2,5-dihydroxybenzoic acid (3)	alcohol 2-hydroxybenzyl (8)	propyl *p*-hydroxybenzoate (9)	methyl *p*-hydroxybenzoate (10)
1	92.62±6.17	27.62±5.42	15.96±2.98	34.14±4.33	79.31±7.52
0.5	12.88±1.32	13.68±3.06	1.89±0.78	10.71±1.28	49.47±7.34
0.25	0	2.18±0.84	0	0	15.50±2.97
0.125	0	0	0	0	4.21±1.22
0.0625	0	0	0	0	1.19±0.49

Table 2. CL_{50} of each compound evaluated in larvae *R. (B.) microplus in vitro*.

Compuesto	CL_{50} (µmol/mL)
Carvacrol	59.72
carvacrol methyl ether (**1**)	6.00
carvacrol acetate (**2**)	5.01
Salicylic acid	**
2,5-dihydroxybenzoic acid (**3**)	35.69
ethyl 2,5-dihydroxybenzoate (**4**)	2.04
methyl 2,5-dihydroxybenzoate (**5**)	30.32
2 - (α, α'-dimethoxymethyl) phenol (**6**)	333.13
ethyl 2-methoxybenzoate (**7**)	0.91
alcohol 2-hydroxybenzyl (**8**)	**
propyl *p*-hydroxybenzoate (**9**)	**
methyl *p*-hydroxybenzoate (**10**)	11.61

**** Cannot compute (insufficient data)**

Table 1, 2

Table 3. Mean results for ovoposition reduction (OR), of carvacrol, salicylic acid and analogues

Conc. ppm/mL	Carvacrol	Salicylic acid	carvacrol methyl ether (1)	carvacrol acetate (2)	ethyl 2,5-dihydroxybenzoate (4)	ethyl 2-methoxybenzoate (7)
250	15.71± 3.26	-1.26±3.19	26.72± 1.06	25.80±0.27	26.56±1.07	27.00±1.50
200	6.48±4.26	-1.89±0.98	23.59± 1.22	21.42±3.12	21.45±3.02	24.60±2.64
150	0.02±1.45	-11.05±5.7	20.29± 1.99	11.30±1.52	14.02±2.398	22.33±8.91
100	0.09±2.36	-8.76±2.93	16.03± 3.06	6.85±0.49	7.20±3.20	13.91±2.43
50	-2.62±1.21	-7.27±7.39	11.13± 2.39	2.34±1.57	6.20±0.2	11.21±0.68

Table 4. Mean results for hatching inhibition rate (HR), of carvacrol, salicylic acid and analogues

Conc. ppm/mL	Carvacrol	Salicylic acid	carvacrol methyl ether (1)	carvacrol acetate (2)	ethyl 2,5-dihydroxybenzoate (4)	ethyl 2-methoxybenzoate (7)
250	7.95±0.37	7.09±1.71	12.90±0.42	10.95±0.47	15.59±1.07	13.52±0.75
200	8.17±1.49	6.19±2.39	11.65±0.28	9.15±0.51	11.22±1.42	10.84±0.89
150	6.93±1.97	5.69±2.49	10.29±0.23	7.75±0.63	8.81±0.36	9.82±0.73
100	5.26±1.36	3.99±1.31	9.03±1.06	6.71±0.81	7.01±0.65	8.11±0.50
50	0.26±0.28	0.52±0.21	8.30±0.74	6.03±0.84	6.43±0.92	6.81±0.40

Table 5. Mean results for acaricidal efficacy porcentage (E), of carvacrol, salicylic acid and analogues

Conc. ppm/mL	Carvacrol	Salicylic acid	carvacrol methyl ether (1)	carvacrol acetate (2)	ethyl 2,5-dihydroxybenzoate (4)	ethyl 2-methoxybenzoate (7)
250	16.76±2.27	1.11±2.22	26.76±1.12	26.33±0.19	27.29±0.75	27.97±1.05
200	9.36±2.97	-1.31±0.68	23.33±0.85	22.50±2.17	22.77±2.10	24.21±1.84
150	0.98±1.01	-7.86±3.97	21.88±1.39	11.22±1.05	15.36±15.36	17.97±6.20
100	1.26±1.64	-6.47±2.04	18.26±2.13	7.45±0.34	6.81±2.23	15.77±1.69
50	-2.26±0.84	-8.12±5.14	11.53±1.66	2.98±1.10	6.68±0.14	11.87±0.47

Table 3, 4, 5

Keywords:
Rhipicephalus (Boophilus) microplus, Acaricidal activity, Carvacrol , Salicylic acid

P09.7
BATS AND MOSQUITO CONTROL

Riccucci M.[1], **Perrone V.*[2]**

[1]Gruppo Italiano Ricerca Chirotteri (GIRC) ~ Pisa ~ Italy, [2]Società Italiana di Medicina Veterinaria Preventiva (SIMeVeP) ~ Roma ~ Italy

INTRODUCTION:
The pursuit of more efficient and effective methods for controlling mosquitoes has long been object of study. Today Italy is by far the most heavily infested country in Europe by the Asian tiger mosquito, Aedes albopictus, vector of at least 26 arboviruses.
MATERIALS AND METHODS:
The available literature on the diet of the different species of insectivorous bats living in Italy was searched in order to obtain evidence of Culicidae. The results are briefly reported.
RESULTS:
Culicidae are found in the diet of 20 species (out of 35) of Italian bats: Rhinolophus hipposideros, Myotis bechsteinii, M.brandtii, M.capaccinii, M.daubentonii, M.emarginatus, M.mystacinus, M.nattereri, Pipistrellus kuhlii, P.nathusii, P.pipistrellus, P.pygmaeus, Nyctalus leisleri, N.noctula, Eptesicus nilssonii, E.serotinus, Barbastella barbastellus, Plecotus auritus, P.austriacus, Miniopterus schreibersii. Culicidae are a relevant part of the diet of 7 species: R.hipposideros, M.brandtii, M. capaccinii, M.mystacinus, P.nathusii, P.pygmaeus, E.nilssonii. The view that bats have no effect against Ae.albopictus has to be revised; actually the tiger mosquitoes have a bimodal cycle of biting activity but they can bite during the entire period of 24 hours, decreasing, but not completely breaking up, during night-time. Furthermore it is not uncommon to see some species of bats in daylight feeding activity, especially in cloudy weather: N.noctula, P.pygmaeus, P.kuhli, P.pipistrellus, M.mystacinus, M.nattereri M.daubentoni, E.nilssonii, N.leisleri, B.barbastellus (Becker et al., 2010, Springer Verlag, XXX-577; ECDC Technical Report (by Scholte and Schaffner), 2009, VI-45; Estrada-Franco et al., 1995, Technical Paper n.42. Pan American Health Organization, 49; Lanza, 2012, Mammalia V. Chiroptera, Calderini, XIII-786; Medlock et al., 2012, Vector-Borne and Zoonotic Dis, 12: 435-47; Paupy et al., 2009, Microbes and Infection, 11:1177-85; Riccucci, 2011, Argomenti, 3:72-9; Romi et al., 2009, Istituto Superiore di Sanità, III-52)
CONCLUSIONS:
Mosquitoes (more than 3500 species worldwide) live everywhere in every continent; they have a strong genetic plasticity and a high degree of adaptation, hence several tropical species are able to colonize new habitats in temperate zones. Environmental methods and biological control are valid and effective alternatives to chemical control and are key components of the integrated strategy. Bats alone are not “the” solution to the problem of mosquitoes, as no single method can be successful, but they can be an useful integration and support reducing mosquito populations with no pollution effects. We want to remind the words of the late entomologist Giorgio Celli: «Cerchiamo di rivalutare anche i pipistrelli ... Alcune specie sono dei grandi mangiatori di zanzare. ... Non devono essere considerati determinanti, però tutte queste cose vanno integrate».



Fig.1



Fig.2

BIBLIOGRAPHY
Becker et al., 2010, Springer Verlag, XXX-577.
ECDC Technical Report (by Scholte and Schaffner), 2009, VI-45.
Estrada-Franco et al., 1995, Technical Paper n.42. Pan American Health Organization, 49.
Lanza, 2012, Mammalia V. Chiroptera, Calderini, XIII-786.
Medlock et al., 2012, Vector-Borne and Zoonotic Diseases, 12(6): 435-47.
Paupy et al., 2009, Microbes and Infection, 11: 1177-1185.
Riccucci, 2011, Argomenti, 3: 72-79. (with a detailed list of references on bats-mosquitoes interactions)
Romi et al., 2009, Istituto Superiore di Sanità, III-52.

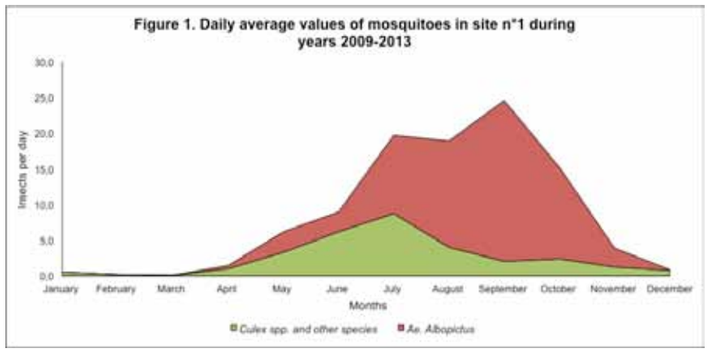
Keywords:
Chiroptera, Aedes albopictus, Culicidae

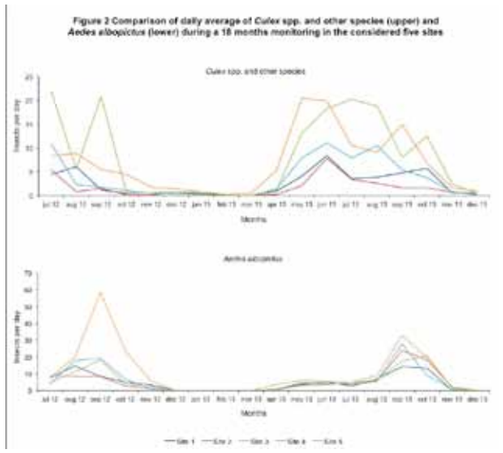
P09.8
ABUNDANCE AND SEASONAL DISTRIBUTION OF AEDES ALBOPICTUS IN AN URBAN CONTEST

Torina A.*[1], Blanda M.C.[1], Randazzo K.[1], La Russa F.[1], Scimeca S.[1], Lelli R.[1]

[1]Istituto Zooprofilattico Sperimentale della Sicilia ~ Palermo ~ Italia

INTRODUCTION:
Mosquitoes constitute a group of insects of great health interest. They are bloodsucking Diptera, cosmopolitan, with a great ability of adapting to the environment. Aedes albopictus is a competent vector of many virus transmitted diseases, including Dengue fever, Eastern equine encephalitis and Chikungunya. Ae. albopictus is a daytime, outdoor mosquito, that bite preferably on man. Ae. albopictus is the important Asian vector mosquito introduced in Italy in September 1990 in Genoa. Ae. albopictus presence was noticed in the urban area of Palermo, Sicily, in the autumn 2004 and the first monitoring was carried out in 2005 in Palermo (Torina et al., 2006, Parassitologia, 48: 166). Many studies analyzed mosquitoes distribution and abundance over wide areas, in this research we investigate the seasonal pattern of Ae. albopictus in a circumscribed area of Palermo, Italy.
MATERIALS AND METHODS:
BG sentinel traps with BG-Lure® (BioQuip) chemical attractant were used. One trap was positioned by the Laboratory of Entomology (site n. 1) at the Institute Zooprofilattico of Sicily from July 2009 to December 2013, near a little well maintained garden without water stagnation. From 2012 additional traps were located at other four different sites, covering an area of 2 hectares for a period of 18 months. The site n. 2 has similar characteristics to the site n.1, while the sites n. 3 e n. 4 were placed at the end of an access ramp to buildings, in shady and cemented areas. Site n. 5 is located in a small green area, near a plant nursery. Traps worked uninterruptedly and catches were collected three times a week. Collected insects were killed at -80°C and mosquitoes were counted and identified using the stereomicroscope, according to the morphological features. Mosquito daily average was estimated to evaluate the abundance.
RESULTS:
In site n. 1, mosquito daily average distribution was uniform over the four years, presenting a greater abundance from June to October (with a maximum of an average of 33 exemplars per day in September 2010) and a significant decrease between January and March. Ae. albopictus was present from June to October, with an abundance of about 22 insects per day in September, while it was almost absent from December to March (Graphic 1). The other species, mainly represented by Culex pipiens, had a peak in June and July with about 9 individuals per day. A similar trend was observed in sites with similar environmental features (Site 1-2 and 3-4). Site n. 3 showed the highest number of Ae. Albopictus, with 58 insects per day in September 2012, while the site n. 5 presented a higher number of the other species, greater than 20 insects per day in some months (Graphic 2).





CONCLUSIONS:
Observed seasonal and spatial differences showed that Ae. Albopictus prefers urban areas, while C. pipiens and the other species are more frequently found in low maintained green areas and for these mosquitoes vegetation is very important.
This study showed that even in an urban restricted contest it is possible to achieve ecological considerations in relation to the abundance, species presence and microhabitats preferences that could direct the choice of sites for monitoring plans on a large scale.

Research supported by The Italian Ministry of Health (IZS PLV 04/11). Authors thank to Rosa Filippi and Pippo Bono for their technical support.

Keywords:
mosquitoes, urban area, Aedes albopictus

P09.9
IS AEDES ALBOPICTUS DEVELOPMENT INFLUENCED BY DENSITY DEPENDENT CONSTRAINTS?

Usai F.[1], Sori F.[1], Roncada P.[1], **Stancampiano L.*[1]**

[1]Dipartimento di Scienze Mediche Veterinarie - Università di Bologna ~ Ozzano dell'Emilia (BO) ~ Italy

INTRODUCTION:
Aedes albopictus is a competent vector of at least 22 arboviruses, including dengue and chikungunya viruses (Gratz, 2004, Med. Vet. Entomol., 18:215-227). The potential for pathogen transmission by Ae. albopictus has increased the need to understand its ecology and population dynamics. Previous studies have shown that density-dependence can affect larval development (Lord, 1998, J. Med. Entomol., 35:825-829). Density-dependent competition for the available food resources has often been argued as the factor influencing larval development (Walsh et al., 2012, Plos One, 7:1-6).
Aim of the study was to assess if larval development time is influenced by the density of laid eggs and the possible consequence of development delay on the efficacy of the larvicidal compound diflubenzuron (DFB), extensively used on a monthly basis in the national territory for tiger mosquitoes control.
MATERIALS AND METHODS:
Ovitraps were positioned in August 2013 for the collection of Ae. albopictus eggs; eggs were counted and 48 ovitraps (24 with high density of eggs: mean 51.6, range 30-93; 24 with low density of eggs: mean 11.9; range 9-15) were placed in tanks with 0.5 liter of water. Within each group, 3 subgroups consisting of 8 ovitraps were formed: 1 control without DFB and 2 with DFB at the concentration of 0.5 ppm and 0.25 ppm, respectively. Eight (4+4) controls without eggs were also added to assess the degradation over time of both DFB concentrations in absence of larvae. The level of water was maintained constant for the duration of the trial. The larvae were fed ad libitum with dried Gammarus pulex.
Hatchings, development times, mortality and emerging adults were monitored for 8 weeks. In this period, DFB concentration was assessed every ten days by HPLC UV-DAD method after liquid-liquid extraction with diethyl ether.
RESULTS:
The number of eggs is significantly related with the last-hatching time and the time of last adult emergence (Negative binomial regression, $p<0.01$).
DFB showed powerful larvicidal action and long persistence. In treated tanks adult emergence occurred 2 months later in only three tanks with 0.25 ppm (9 adults overall, 8 of them from tanks with high egg density). In 18 tanks DFB was still detectable after 50 days from the contamination with concentrations higher than the DFB LC90 for Ae. albopictus (Arshad et al, 1995, J. Am. Mosquito Contr., 11:72-76). Larval density did not influence significantly DFB degradation. After an initial sharp reduction of DFB concentration it remained almost stable, until a final intense reduction in the last two weeks. However, in the controls with no larvae the latter reduction did not occur and the concentration remained stable for a longer period.
CONCLUSIONS:
The presence of high egg densities determines a slowed down development whose duration potentially exceeds the period of effectiveness of DFB. Ae. albopictus seems to undergo density-dependent self-regulatory mechanisms independent from food resource availability that can stabilize mosquito population dynamics and possibly affect control measures.

Keywords:
Aedes albopictus, population dynamics, control

NOTES

POSTER 10

PARASSITI DELLA FAUNA SELVATICA

P10.1
FINDING OF ALOUATTAMYIA BAERI (DIPTERA CUTEREBRIDAE) FROM MONKEY ALOUATTA PIGRA LAWRENCE 1933 FROM THE STATE OF YUCATÁN MEXICO

Quintero Martinez M.T.*[1], Islas Donde G.[2]

[1]Maria Teresa Quintero M, ~ Depto. de Parasitologia, FMVZ-UNAM ~ Mexico, [2]Guillermo Islas Donde ~ Fac. de Ciencias, UNAM ~ Mexico

INTRODUCTION:
The objective of this paper is communicate the presence of Alouattamyia baeri on Alouatta pigra. A. pigra is known as black howler monkey, is found distributed in Tabasco, Chiapas and Yucatan states in Mexico and Central America: Belize, Guatemala, Panama, Costa Rica French Guiana and northern Brazil, is banned in Mexico marketing so is subject to forfeiture, in this monkey will have studied various parasites including ectoparasites
MATERIALS AND METHODS:
In the course of a confiscation of a monkey A. pigra provenance Yucatan, Mexico (this monkey has a geographical distribution covering the southeastern Mexico to Argentina) (Milton, 1993, Sci Am., 269:86-93); two larvae as cause of myiasis were isolated in A. pigra; larvae were isolated and placed in a jar of alcohol then sent to the Entomology Laboratory of the Department of Parasitology, Faculty of Veterinary Medicine of the National Autonomous University of Mexico for determination. In the laboratory these larvae were observed under the stereomicroscope following Alva Peterson key 1960.
RESULTS:
The larvae was determined as Alouattamyia baeri larvae III; this fly was firstly described by Shannon and Greene 1926. The name of the fly genus is due to the name of the genus Alouatta monkey. The morphological characteristics of the larvae are: the orange brown to black color with characteristic scales.
CONCLUSIONS:
In Mexico has reported the presence of these fly larvae on monkeys at different dates (Arroyo et al, 2008; Azkarate et al, 2012) on Alouatta palliata from Los Tuxtlas, Mexico, but there is not publication of the presence of these fly larvae on A. pigra.
The find consisted in larvae isolated from the body of a monkey A. pigra of confiscation monkey from the state of Yucatan, the monkey had furuncular injuries which larvae were removed, the larvae isolated were two showed a orange brown color, measuring 1.5 cm to observe under the microscope it was possible to see the typical scales of these larvae as determined by their morphology Alouattamyia baeri.

Keywords:
Alouatta pigra, Alouattamyia

P10.2
TOXOPLASMA GONDII IN SYMPATRIC WILD HERBIVORES AND CARNIVORES: EPIDEMIOLOGY OF INFECTION IN PIEDMONT

Zanet S.[1], Bosio F.[1], Trisciuoglio A.[1], Bruno S.[1], **Ferroglio E.*[1]**

[1]University of Turin, Dept. Scienze Veterinarie ~ Grugliasco ~ Italy

INTRODUCTION:
Toxoplasma gondii is an apicomplexan parasite able to infect almost all warm-blooded animals that has, in Europe, the domestic cat as main definitive host. Worldwide, 6 billion people are infected with this parasite. The goal of our research is to evaluate the prevalence of T. gondii infection in wild animals from a previously unsampled area in Northern Italy where 0.1% of women seroconvert during pregnancy each year.
MATERIALS AND METHODS:
We sampled and tested by PCR skeletal muscle and central nervous system of 355 wild animals (n= 121 roe deer Capreolus capreolus, n= 105 wild boar Sus scrofa, n= 94 red fox Vulpes vulpes, n=22 chamois Rupicapra rupicapra, n= 13 red deer Cervus elaphus).
RESULTS:
The overall prevalence of infection with T. gondii was 10.99% (IC 95% 8.14%-14.67%). A higher rate of infection was recorded in carnivores and omnivores (red fox 20.21%, IC 95% 13.34%-29.43%; wild boar 16.19%, IC 95% 10.36%-24.41%) compared to ruminants (2.48%, IC 95% 0.85%-7.04% in roe deer; 0.00%, IC 95% 0.00%-22.81% in red deer, and 0.00% Alpine chamois IC 95% 0.00%-14.87%) confirming the importance of tissue cysts in transmitting infection.
CONCLUSIONS:
The relatively high prevalence of T. gondii DNA in highly consumed game species (wild boar and roe deer) give valuable insights into T. gondii epidemiology and may contribute to improve prevention and control of foodborne toxoplasmosis in humans.

Keywords:
Toxoplasma gondii, wildlife, PCR

P10.3
NASOPHARYNGEAL BOT FLY (CEPHENEMYIA STIMULATOR) IN ROE DEER (CAPREOLUS CAPREOLUS) IN NW SPAIN

Arias M.S.[1], Pajares G.[1], Paz Silva A.[1], Diez Baños N.[1], Suarez J.L.[1], Sánchez Andrade R.[1], Morrondo P.[1], **Diez Baños P.*[1]**

[1]University of Santiago de Compostela ~ Lugo ~ Spain

INTRODUCTION:
Cephenemyia species are specific to cervid hosts. In Europe, larvae of C. stimulator cause obligated myiasis in roe deer. Nasopharyngeal bot infestation in roe deer (Capreolus capreolus) was investigated in NW Spain.
MATERIALS AND METHODS:
During 2013 and 2014, 24 bucks and 24 does (7 were roe adults, >3 yrs) were culled. During the necropsy, the skin of the ventral side of the neck and head were removed and the oesophagi and trachea opened. The soft palate was opened to observe the oral cavity, pharynx and larynx in order to find larvae of C. stimulator. Also, head was sectioned longitudinally to examine nasal and paranasal sinuses. Second and third instars (L2 and L3) were identified on the basis of their morphological and morphometric characteristics.
RESULTS:
C. stimulator was the only bot fly found in sampled roe deer. All the three larval stages were collected. Prevalence of infestation with C. stimulator larvae was 48%. Mean intensity was 20 (ranging 2 from to 75) larvae/infested host. In our study, no significant difference of the infestation prevalence related to age was reported, but prevalence in adults (86%) was markedly higher than in fawns (42%). Regarding mean intensity, the value was higher in fawns (=24) than in adults (=8). Nevertheless, we reported mean intensity of L1 infestation was significantly higher (F=4.232; P=0.045) in does (=1.8696±0.8831) compared with bucks (=0.0833±0.4082). No clear correlations were found between gender of the host and prevalence (bucks: 67%, does: 29%) or mean intensity (bucks: =8, does: =12) when analysed.
CONCLUSIONS:
Preventing the spread of pathogens that can cause serious problems for the life of wildlife is essential. The results of this study are highly suggestive and justify the interest in this myiasis, due to the high prevalence of the infestation and the pathological consequences caused to roe deer of the study area.
Close cooperation should be should be maintained between hunters, researchers, and autorities to obtain the basic epidemiological information needed to develop future programmes to control this roe deer myiasis in NW Spain.

Work supported “Parga Pondal”-postdoctoral-research-grant- Xunta de Galicia (XUGA) to M.S. Arias a Grant (CN2012/326) of the XUGA for Consolidating and Structuring Competitive Research Groups.

Keywords:
Cephenemyia stimulator, roe deer, prevalence

P10.4
FIRST REPORT OF TRICHOSTRONGYLUS RETORTAEFORMIS IN EASTERN COTTONTAIL RABBIT SYLVILAGUS FLORIDANUS

Tizzani P.*[1], Gontero C.[1], Catalano S.[2], Zanet S.[1], Meneguz P.G.[1]

[1]Dipartimento di Scienze Veterinarie Università di Torino ~ Torino ~ Italy, [2]Department of Ecosystem and Public Health (EPH) - University of Calgary ~ Calgary ~ Canada

INTRODUCTION:
When an allochtonous species is introduced into a new environment it could introduce a wide variety of bacteria, viruses and parasites. An invasive population of Sylvilagus floridanus was introduced in northwestern Italy in 1966 (Silvano et al, 2000, Hystrix, 11: 75-78). Eastern cottontails have been vectors of allochtonous pathogens (Meneguz and Tizzani, 2002, Parassitologia, 44: 111) that were able to infect the native European brown hare (Tizzani et al, 2011, Parasitol Res, 109: 963-966). Exotic pathogens infecting autochthonous sympatric species are well documented (Tizzani et al, 2012, Proceeding XXVII Congr. Naz. SOIPA, 26-29 June, Alghero, pp. 149), less frequent is the infection of an alien species with native parasites. In this study an European autochthonous parasite (Trichostrongylus retortaeformis) is documented to infect S. floridanus.
MATERIALS AND METHODS:
The study was carried out in the protected area of “Tortona-Rivalta”, Piedmont, where L. europaeus and S. floridanus are sympatric since 1998 (Silvano et al, 2000, Hystrix, 11: 75-78). In 2007, 32 S. floridanus were culled within a project aimed to the eradication of alien species. The 32 eastern cottontails were collected throughout an entire year: 6 in spring, 9 in summer, 6 and 11 in autumn and winter respectively. We examined the small intestine to detect the presence of the most common hare parasites: Trichostrongylus retortaeformis. In each positive intestine, a minimum number of 15 males was examined and identified following the identification keys from Skrjabin et al (1954, Essential of nematodology, Trichostrongylids of animals and man, 3: 526). Epidemiological indexes of prevalence, abundance and intensity are reported.
RESULTS:
We found 2 species of nematodes: Trichostrongylus calcaratus (T.C.) and Trichostrongylus retortaeformis (T.R.) (Figure 1). Prevalence, abundance and intensity of the nematodes are reported in Table 1.

	Prevalence		Abundance		Intensity	
	T.C.	T.R.	T.C.	T.R.	T.C.	T.R.
Spring	100	16.7	1058.3	0.17	1058.3	1
Summer	100	0	761.1	0.33	761.1	1.5
Autumn	83.3	16.7	138.3	1	166	3
Winter	100	0	30	0.18	36.7	2

Table 1. Prevalence, abundance and intensity of T. retortaeformis (T.R.) and T. calcaratus (T.C.).



Figure 1. T. calcaratus (on the left) and T. retortaeformis (on the right) details of the spicula.

CONCLUSIONS:
T. calcaratus was already reported in Eastern cottontails in Italy by Meneguz and Tizzani (2002, Parassitologia, 44: 111). T. retortaeformis was found in 2 Eastern cottontails (P=6%). Although described in a wide variety of lagomorphs like L. europaeus (Bordes et al, 2007, J. Wildl. Dis., 43: 747-751), Lepus timidus varronis (Tenhu, 1998, Proocedings 2nd Meeting EAZWV, May 21-24, Chester, UK, pp. 183-188), Lepus timidus scoticus (Skrjabin et al, 1954, Essential of nematodology, Trichostrongylids of animals and man, 3: 526), and Oryctolagus cuniculi (Eira et al, 2007, Helminthol., 81: 239-246), this is the first reporto of T. retortaeformis in S. floridanus. The low prevalence of T. retortaeformis in our samples is in contrast with the high prevalence found in its natural hosts (Canestri-Trotti et al, 1988, Suppl. Ric. Biol. Selvaggina, 14: 317-321) T. retortaeformis may not have already fully adapted to its new host and this may negatively affect its fitness.

Keywords:
Sylvilagus floridanus, Trichostrongylus retortaeformis, Trichostrongylus calcaratus

P10.5
DESCRIPTION OF THE HELMINTH PARASITOFAUNA IN AN ISOLATED POPULATION OF WILD RABBIT (ORYCTOLAGUS CUNICULUS) IN ALESSANDRIA, ITALY

Tizzani P.[1], Ferreira Andrade D.[2], Silvestri E.[1], Ghirardi M.[1], Molinar Min A.[1], Gualtiero L.[1], Peano A.[1], Negri E.[3], **Meneguz P.G.*[1]**

[1]Dipartimento di Scienze Veterinarie, Università di Torino. ~ Torino ~ Italy, [2]Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto ~ Porto ~ Portugal, [3]Museo Civico di Storia Naturale di Stazzano ~ Tortona ~ Italy

INTRODUCTION:
Wild rabbit Oryctolagus cuniculus is a widespread lagomorph in Europe, Australia, South America and some Pacific Islands. This specie prefers a mixed habitat of Mediterranean oak savanna or scrub-forest and open areas (Ward, 2005, IUCN). There is a bibliographic blank in what concerns to the parasitofauna of the wild rabbit in Italy. Until now, most of the studies refer to the Iberian Peninsula and Macaronesia islands (Molina et al., 1999, J Helminthology, 73: 221-225; Foronda et al., 2003, J Parasitology, 89: 952-957), to the United Kingdom (Allan et al., 1999, J Helminthology, 73: 289-294). The stomach nematode Graphidium strigosum is its most common intestinal parasite (Dunsmore, 1966, Austr J Zoology, 14: 625-634). Other nematodes frequently described are Trichostrongylus retortaeformis and Passarulus ambiguus (Foronda et al., 2003, J Parasitology, 89: 952-957). One of the few Italian work on sanitary status of wild rabbit was carried out in the province of Turin, Italy (Rossi et al.,1986, Ann Ist Sup Sanità, 22: 341-344). It reported one cestode (Cysticercus pisiformis), one trematode (Fasciola hepatica) and two nematodes (G. strigosum and T. retortaeformis). The aim of this study was to increase the epidemiological data regarding the parasitofauna of the wild rabbit in Italy.
MATERIALS AND METHODS:
The gastrointestinal systems of 10 wild rabbits from the province of Alessandria (Northwestern Italy) were analysed. Usual techniques were used for analysis, collection and preservation of the nematodes (MAFF, 1986, Manual of Veterinary Diagnostic Technics, Ref Book 418). We used the identification key from Skrjabin et al (1954, Essential of nematodology, Trichostrongylids of animals and man, 3: 526).
RESULTS: Two nematodes were found: Obeliscoides cuniculi was present in 80% of the samples while Trichostrongylus calcaratus in 100% (Prevalence, Intensity and Abundance are reported in table 1). No parasites were reported from the large intestine. No one of the parasites previously described in O. cuniculi was found. The two species are commonly reported in American lagomorphs (i.e. Andrews et al, 1980, J Wildl Diseases 16: 395-401) and represent a first report for the free ranging European wild rabbit population. Previous studies have demonstrated that Sylvilagus floridanus introduced in Italy a great number of exotic parasites among which the two nematodes here reported (Tizzani et al. 2011 Paras Research, 109: 963-966).

	P	CI_L_95	CI_U_95	I	SD	A	min	max
Ob. cuniculi	80%	0.55	1.05	62.13	17.99	49.7	8	196
T. calcaratus	100%	1.00	1.00	221.4	72.63	221.4	4	870

Table 1 - Prevalence (P), Confidence Interval (CI), Intensity (I), Standard Deviation (SD), Abundance (A) and Range (min-max) of the two nematodes reported.

CONCLUSIONS:
Considering that i) these exotic nematodes are reported to infect Lepus europaeus (Tizzani et al. 2011, Paras Research, 109: 963-966) and ii) in our study area wild rabbits are in sympatry with S. floridanus (Silvano et al., 2000, Hystrix, 11: 75-78), it seems that a cross infestation between Eastern cottontail and wild rabbit occur, with an adaptation of those parasites to develop on a new host. Considering our preliminary results, an extinction of the autochtonous wild rabbit gastrointestinal parasites seems to have occurred.

Keywords:
Oryctolagus cuniculus, Obeliscoides cuniculi, Trichostrongylus calcaratus

P10.6
COCCIDIAN OOCYSTS AS STRESS INDICATORS IN RESTOCKED LEPUS EUROPAEUS FROM WESTERN EUROPE

Tizzani P.[1], Molinar Min A.[1], Silvestri E.[1], Ferreira Andrade D.[2], Carpignano M.G.[3], Bessone M.[1], Peano A.[1], Negri E.[4], **Meneguz P.G.*[1]**

[1]Dipartimento di Scienze Veterinarie, Università di Torino ~ Torino ~ Italy, [2]Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto ~ Porto ~ Portugal, [3]Dipartimento di Scienze Veterinarie, Università di Torino. ~ Torino ~ Italy, [4]Museo Civico di Storia Naturale di Stazzano ~ Tortona ~ Italy

INTRODUCTION:
Between 2012 and 2013 faecal samples were collected from hares Lepus europaeus used for restocking operations in the hunting estate CACN3 "Valli Maira e Grana" (Piedmont – Italy). The samples came from free-living hares capture in the Czech Republic. The hare used for restocking operation can remain in cages till to one week; this is extremely stressful and can affect the sanitary status and post release survival. The sanitary status of a released species is a very important parameter that can affect the efficacy of restocking operations (Black, 1991, Bird Conservation International, 1: 329-334). Coccidian load in particular is an effective indicator of stress (Chroust, 1984, Acta Vet. Brno, 53: 175-182). The aim of this study was to evaluate the welfare and survival probability of hares used in restocking operation through the evaluation of their parasite load with a special focus on coccidia oocysts.
MATERIALS AND METHODS:
A total of 96 faecal samples were collected in winter 2012 (55) and winter 2013 (41) before the released of the hares. They were analysed in the Department of Veterinary Sciences (University of Turin). Standard laboratory techniques were used to recover, collect, store the parasites and both qualitative and quantitative copro microscopic examinations were carried out (MAFF, 1986, Manual of Veterinary Diagnostic Techniques, Ref Book 418). Parasites were identified by using a morphometric approach and through the use of dichotomous keys (Aoutil et al, 2005, Parasite, 12: 131-144).
RESULTS:
Eggs of nematodes such as Trichuris sp. and Trichoistrongylus sp., as well as oocysts of Eimeria sp were found. Prevalence and abundance of the parasites are shown in table 1. Prevalence of whipworm and tricostrongilids coincide with those reported for wild populations in areas with similar environmental condition (Dubinský et al, 2010, Helminthologia, 47, 4: 219 - 225). On the contrary coccidia was found with prevalence and abundance significantly higher (Chroust, 1984, Acta Vet. Brno, 53: 175-182 ; Lukesova et al, 2012, Helminthologia, 49:159-163). We recognized 18 species of Coccidia: E. audubonii, E. cabareti, E. leporis leporis, E. l. brevis, E. europaea, E. deharroi deharroi, E. d. rotonda, E. macrosculpta, E. orbiculata, E. gresae, E. bainae, E. coquelinae, E. gantieri, E. nicolegerae, E. babatica, E. reiniai, E. stiedai, E. tailliezi.

	Trichuris sp.		***Trichostrongylu sp.***		***Eimeria sp.***	
Year	**2012**	**2013**	**2012**	**2013**	**2012**	**2013**
Prevalence	29%	39%	12%	43%	96%	100%
CI_L_95	17%	24%	3%	28%	91%	100%
CI_U_95	41%	54%	21%	58%	101%	100%
Intensity	927.9	471.9	191.4	205.6	77930.6	35402.4
Abundance	274.9	184.1	24.8	90.2	75044.2	35402.4

Table 1. Prevalence, Confidence Interval (CI), Intensity and Abundance of parasitic load in 2012 and 2013.

CONCLUSIONS:
Coccidia are index of low immune competence of the host due to stress condition (Rödel et al, 2004, Oecologia, 140: 566-576). The level of parasitic loads features a strong alteration of healthy condition. This factor could jeopardize the post release survival of the hares (Alzaga et al, 2007, Behavioral Ecology and Sociobiology, 62: 769-775).

Keywords:
Lepus europaeus, restocking operations, coccidia oocysts

P10.7
FIRST REPORT OF ALARIA ALATA IN WILD RED FOXES (VULPES VULPES) FROM EMILIA-ROMAGNA REGION, ITALY

Fiocchi A.[1], **Gustinelli A.*[1]**, Poglayen G.[1]

[1]Department of Veterinary Medical Sciences Alma Mater Studiorum University of Bologna ~ Bologna ~ Italy

INTRODUCTION:
Alaria alata is a digenean trematode of the family Diplostomatidae, which has been reported in wild red foxes (Vulpes vulpes) from several European countries (Loos-Frank et al., 1982, Z Parasitenkd., 67:99-113.; Möhl et al., 2009, Parasitol Res, 105:1-15). Although the presence of A. alata in Italy has been already reported in the past (Molin, 1854, Prodromus Faunae Helminthologicae Venetae), recent descriptions of the parasite are lacking. This report aims to update Alaria alata infection in red foxes (V. vulpes) from Emilia-Romagna Region, Italy.
According to literature, prevalence values of A. alata in the fox populations examined ranges from 0.1 % (Loos-Frank et al., 1982, l.c.) to 94.8 % (Bružinskaité-Schmidhalter et al., 2012, Parasitology, 139:120-7). We didn't find any reports of this parasite from Italy in several wide parasitological surveys (Soldati et al., 1976, Riv Parassitol, 37:329-332; Poglayen et al., 1985, Parassitologia, 27:303-11; Capelli et al., 2003, J. Mt. Ecol., 7:199-205; Di Cerbo et al., 2008, Acta Parasitologica, 53:302-311) except for a report of Alaria sp. in a red fox (Alborali et al., 2012, Collana Fond. In. Zooprof. Brescia, 91:566) and in a dog (Ferroglio et al., 2012, Mappe Parassitol., 18:160). Its complex life cycle requires a freshwater snail as first intermediate host and an amphibian as second intermediate host (Möhl et al., 2009, l.c.). Reptiles, rodents, wildboars and other vertebrates can act as paratenic hosts after feeding on infected amphibians (Wolfe et al., 2001, Vet Rec. 149:759-763). Definitive hosts, usually members of the family Canidae, become infected after ingesting mesocercariae contained in amphibians or paratenic hosts. A. alata is also a potential zoonotic agent. Humans can acquire infection after eating undercooked frog legs or raw game meat containing mesocercariae (Murphy et al., 2012, Parasitol Res., 111:283-290).
MATERIALS AND METHODS:
Between February 2013 and March 2014, we analyzed 28 red foxes and one wolf (Canis lupus) collected from hunters or found dead in the Province of Modena and Bologna (Emilia-Romagna Region, Italy). The stomach and the gut were removed during necropsy and parasites were collected using SCT (Sedimentation and Counting Technique) according to standard protocols.
RESULTS:
Only two foxes (7.1 %) out of 28 were found positive for Alaria alata (Fig.1). Both of them lived in a lowland territory, rich in humid areas and channels, a suitable environment for the life cycle development of A. alata. We found other specimens of Alaria alata in the duodena of wild red foxes from the Province of Forlì during a survey for Echinococcus granulosus. Only two (2.5%) out of 80 foxes were infected. Morphological analysis of the parasites from the four foxes confirmed our presumptive identification showing measures agreeing with the description reported by Mohl et al. (2009).



Fig. 1 - Adult specimen of A. alata

CONCLUSIONS:
The sporadic presence of A. alata in our Region might be explained by the illegal importation of game animals from Eastern Europe. Further studies on a higher number of specimens are necessary to exclude the presence of other Alaria species in Italian territory, as the importation of bullfrogs (Lithobates catesbeianus), suitable host for Alaria spp., from extra-European countries and their diffusion into the wild, could lead to a the diffusion of exotic parasites.

Keywords:
Alaria alata, Vulpes vulpes, Italy

P10.8
ANGIOSTRONGYLUS VASORUM INFECTION IN RED FOX (VULPES VULPES) IN THE CAMPANIA REGION OF SOUTHERN ITALY

Santoro M.[1], D'Alessio N.[2], Di Prisco F.[2], Gaudino M.[2], Neola B.[3], Restucci B.[3], Pagano T.B.[3], **Veneziano V.*[3]**

[1]Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome ~ Roma ~ Italy, [2]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Section of Avellino ~ Avellino ~ Italy, [3]Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Italy ~ Napoli ~ Italy

INTRODUCTION:
Angiostrongylosis is a snail-borne disease of dogs and wildlife carnivorous caused by Angiostrongylus vasorum occurring in Europe, Africa, America. The adult nematodes are found in the pulmonary arteries and right side of the heart of the definitive host where they cause verminous pneumonia and coagulopathy. In Europe, the red fox (Vulpes vulpes) is considered its natural reservoir capable of sustaining the infection without serious clinical consequences, however a recent fatal case of disseminated infection have been described in this host (Eleni et al., 2014, Parasitol Res, 113:1247-1250). In according with Koch and Willesen (2009, Vet J, 179:348-359) the prevalence of A. vasorum infection in European red fox ranges from 5 to 92.9%. In the last two decades have been documented a spreading of A. vasorum from traditional endemic foci to new geographical areas including Italy. Since the first documented cases in red fox from Tuscany (Poli et al., 1984, J Wildlife Dis 20:345-346;) several other study have been published suggesting that the Italy is a country where this parasite are actually spreading (Traversa et al., 2010, Parasites & Vectors, 3:62). Here we report on A. vasorum infection for first time in red fox from Campania Region of southern Italy; its pulmonary pathological changes are also described.
MATERIALS AND METHODS:
A total of 102 red foxes from Campania (Southern Italy), obtained between October 2012 and January 2014 were studied for A. vasorum infection. Most of foxes were collected by local hunters (n = 75) and, a few (n = 27) were road-killed specimens. Red foxes were sexed and on the basis of teeth examination, they were classified as juveniles (< 12 month) or adults (> 12 months). During necropsy, heart and pulmonary arteries were opened and examined; the nematodes were collected, counted and identified. The identification was performed with reference to CIH keys prepared by Anderson et al. (1955). Differences in prevalence between age classes and gender were assessed by the two-sided Chi-square t test. A P value of < 0.05 was considered significant. Tissue samples from lungs of red fox positive for A. vasorum were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned and stained with haematoxylin and eosin.
RESULTS:
Of the 102 red foxes studied, 43 were females (19 juveniles and 24 adults) and 59 males (14 juveniles and 45 adults). A total of 34 (33,3%) individuals were positive for A. vasorum including 19 males (15 adults and 4 juveniles) and 15 females (6 adults and 9 juveniles). Most parasites were found in lungs and just in two cases in the right heart. No significant differences were found between age classes and gender groups. Lungs of positive individuals showed varying severity of inflammatory responses. Microscopically, pulmonary tissues showed pathological changes ranging from mild to severe. Lesions consisted of granulomatous interstitial pneumonia with several eggs, larvae, and intravascular pulmonary adult nematodes.
CONCLUSIONS:
Our study represents the first study on prevalence of infection of A. vasorum and its pathological changes in red fox in Campania Region and in general in southern Italy. Prevalence of infection found suggests that the life cycle of A. vasorum is well established in the study area representing a potential infection risk for dogs. Pathological changes confirm that infection has mainly chronic course along which the infected fox may disperse parasite larvae in the environment for its life.

Keywords:
Angiostrongylus vasorum, Fox, Campania

P10.9
DESCRIPTION OF THE HELMINTH GASTROENTERIC PARASITOFAUNA OF ALECTORIS GRAECA, LAGOPUS MUTUS AND TETRAO TETRIX IN PIEDMONT ALPS. A LATITUDINAL INFLUENCE?

Tizzani P.*[1], Chiodo E.[1], Menardi G.[1], Giordano O.[2], Ficetto G.[2], Bessone M.[1], Angelo L.[1], Carpignano M.G.[3], Molinar Min A.[1], Peano A.[1], Rossi L.[1], Belleau E.[4], Meneguz P.G.[1]

[1]Dipartimento di Scienze Veterinarie, Università di Torino. ~ Torino ~ Italy, [2]Comprensorio Alpino CN2 "Valle Varaita" ~ Melle ~ Italy, [3] Comprensorio Alpino CN3 "Valle Maira" ~ Dronero ~ Italy, [4]Veterinary Practitioner ~ Barcelonnette ~ France

INTRODUCTION:
Alectoris graeca, Lagopus mutus and Tetrao tetrix are galliformes species native of the Alps. Considering the few studies available (Viganò et al., 2012, Monitoraggio dello stato di salute delle popolazioni attraverso indagini sanitarie. Analisi parassitologiche sul Fagiano di monte (Tetrao tetrix); Barera, 1986, Florio, Gamba, 1992; De Barchetti et al. 1999, La Selezione Veterinaria 8-9: 699-704; Viganò, Rotelli, 2004), our research investigated the gastrointestinal parasites of these three hosts to increase the knowledge on their sanitary status. According to the previous studies, Ascaridia sp. and Capillaria sp. are the most common parasites in Alpine Galliformes. The study aim to investigate not only the parasite diversity at a community level, but even the host-parasite relationship and the risk factors that could affect parasite presence, with a special focus on the influence of the geographic variable. For these reasons we tried to evaluate parasite presence in a wide latitudinal range, from Aosta Valley to South Piedmont.
MATERIALS AND METHODS:
The study was carried out in the Piedmont Alps in the following hunting districts (Comprensori Alpini - CA): Comprensorio Alpino Biella 1 "Biella valleys" (CABI1), Aosta Valley (AV), Comprensorio Alpino CN2 "Varaita valley" (CACN2) and Comprensorio Alpino CN3 "Maitra and Grana valleys" (CACN3). We divided the study area in "North" including CABI1 and AV and "South" including CACN2 and CACN3. We sampled a total number of 91 animals (44 A. graeca, 41 T. tetrix and 6 L. mutus). All the samples were collected at Regional Hunting Control Centers from October to November (from 2008 to 2013). The following data were collected for the culled animals: species, geographic localization, biometric parameters and date of culling. Standard laboratory techniques were used to recover, collect and store the parasites (MAFF, 1986, Manual of Veterinary Diagnostic Techniques, Ref Book 418). Parasites were identified using a morphometric approach and through the use of dichotomous keys (e.g. Skrjabin et al, 1954 Essential of nematodology, Trichostrongylids of animals and man, 3: 526).
RESULTS: Two nematodes (Ascaridia and Capillaria) and one cestode were found in A. graeca and T. tetrix. No nematodes were found in L. mutus. Prevalence, abundance and intensity of the parasites are reported in table 1.

Tetrao tetrix				*Alectoris graeca*		
Total	*North*	*South*		*Total*	*North*	*South*
41	17	24	*N samples*	44	19	25
14.63%	23.52%	8.33%	*Capillaria prevalence*	2.27%	0.00%	4.00%
14.63%	35.29%	0.00%	*Ascaridia prevalence*	27.27%	57.89%	4.00%
7.31%	11.76%	4.16%	*Cestodes prevalence*	6.81%	0.00%	12%
6.50 ± 12.51	8.75 ± 15.50	2.00 ± 1.41	*Capillaria intensity*	1.00 ± 0.00	0.00	1.00 ± 0.00
13.00 ± 12.83	13.00 ± 12.83	0.00	*Ascaridia intensity*	9.25 ± 11.82	10.00 ± 12.10	1.00 ± 0.00
1.33 ± 0.57	1.50 ± 0.70	1.00 ± 0.00	*Cestodi intensity*	2.66 ± 2.08	0.00	2.66 ± 2.08
0.95 ± 12.51	1.84 ± 15.50	0.16 ± 1.41	*Capillaria abundance*	0.02 ± 0.00	0.00	0.04 ± 0.00
1.9 ± 12.83	4.10 ± 12.83	0.00	*Ascaridia abundance*	2.52 ± 11.82	5.78 ± 12.10	0.04 ± 0.00
0.09 ± 0.57	0.15 ± 0.701	0.04 ± 0.00	*Cestodes abundance*	0.18 ± 2.08	0.00	0.32 ± 2.08

Table 1. Prevalence, Intensity and Abundance of the nematodes in Tetrao tetrix and Alectoris graeca.

CONCLUSIONS:
Ascaridia showed the highest prevalences in A. graeca while Capillaria was found with highest prevalence in T. tetrix. These findings prove a different host specificity of each nematode. Cestodes instead were reported at low prevalence in both hosts. A geographic influence on parasites distribution was found with Ascaridia prevalence in the "North" area significantly higher. This result suggests that environmental differences between the two areas could be a key factor for the diffusion of this nematode. An interesting point for a future investigation will be to evaluate the influence of parasite load on the fitness of the hosts and consequently on the population dynamic.

Keywords:
Galliformes, Ascaridia, Capillaria

P10.10
EXOTIC SMALL MAMMALS AND PARASITES

Maurelli M.P.*[1], Pepe P.[1], Noviello E.[2], D'Ovidio D.[2], Capasso M.[2], Cringoli G.[3], Rinaldi L.[3]

[1]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania) ~ Naples ~ Italy, [2]Private Practitioner ~ Naples ~ Italy, [3]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Regional Center for Monitoring Parasitic Infections (CREMOPAR, Regione Campania); Inter-University Center for Research in Parasitology (CIRPAR) ~ Naples ~ Italy

INTRODUCTION:
Exotic small mammals have become increasingly common domestic pets. As a consequence of their popularity, the interest of the scientific community in these animals has increased; however, still little information is available on the presence and prevalence of endoparasites in these animals. Exotic small mammals can harbor a wide variety of endoparasites. The aim of the present study was to investigate the occurrence of parasites in exotic small mammals in the Campania region (southern Italy).
MATERIALS AND METHODS:
A total of 100 faecal samples from 50 ferrets and 50 squirrels were collected between May and June 2013. All faecal samples were fixed with formalin 5% and subsequently examined using the FLOTAC pellet technique (Cringoli et al., 2010, Nat. Prot., 5: 503-515). This technique is performed for samples with an unknown weight of faecal material to be analyzed can be inferred by weighing the sediment in the tube (pellet) after filtration and centrifugation of faecal sample. Parasitic elements (eggs, larvae, oocysts and cysts) were counted and identified in accordance with the guidelines reported in the literature. For detection of Giardia and Cryptosporidium, the specimens were also analyzed by the Xpect® Giardia/Cryptosporidium snap test using manufacturer recommendations (Remel Thermo Scientific, USA).
RESULTS:
Out of the 100 samples examined, 24 (24.0%; 95% Confidence Interval (CI)=16.3-33.8%) showed the presence of parasitic elements (eggs, larvae, oocysts and cysts). In particular parasitic elements were found in 9/50 (18%; 95% CI= 9.0-31.9%) squirrels and 15/50 (30.0%; 95% CI=18.2-44.8%) ferrets. The prevalence found for each parasite group/genus/species are reported in table 1 and table 2 for squirrels and ferrets, respectively. None of the sample was positive for Cryptosporidium or Giardia.

Parasitic group/genus/species	Squirrels (no. examined = 50)		
	No. positive	Prevalence (%)	95% CI
Gastro-intestinal nematoda	1	2.0	0.1-12.0
Capillaria hepatica	1	2.0	0.1-12.0
Dicrocoelium dendriticum	5	10.0	3.7-22.6
Strongyloides spp.	1	2.0	0.1-12.0
Syphacia spp.	3	6.0	1.6-17.5

Table 1. Parasitic group/genus/species found in pet squirrels.

Parasitic group/genus/species	Ferrets (no. examined = 50)		
	No. positive	Prevalence (%)	95% CI
Ancylostomidae	14	28.0	16.7-42.7
Sarcocystis spp.	1	2.0	0.1-12.0

Table 2. Parasitic group/genus/species species found in pet ferrets.

CONCLUSIONS:
The present survey gave preliminary data on the parasitofauna of exotic small mammals in southern Italy and indicates that these animals may harbor intestinal parasites. Also, the results of the present study showed that the FLOTAC technique was a rapid and sensitive test to improve diagnosis and acquire new information on parasitological fauna of exotic animals as already demonstrated in reptiles (Rinaldi et al., 2012, Exp. Parasitol., 130(3): 282-284).

Keywords:
exotic small mammals, endoparasites, FLOTAC

NOTES

POSTER 11A

INFEZIONI DA MICETI

P11A.1
SURVEY ON THE YEAST FLORA IN EXTERNAL EAR CANALS OF RABBIT

Galuppi R.*[1], Dalla Torre S.[1], Tampieri M.P.[1]

[1]Dipartimento di Scienze Mediche Veterinarie - Università Di Bologna ~ Bologna ~ Italy

INTRODUCTION:
The recovery of Malassezia from rodents and lagomorphs has been rarely cited in literature, and some studies have failed to demonstrate the presence of these fastidious yeasts in rabbit (Guillot et al., 1994, J. Mycol. Med., 4: 72-79). In 2011, Cabanes & Castellà (Med. Mycol., 49: 40-48) described a novel yeast species, named Malassezia cunicoli, from the skin of two rabbit from Spain. This yeast was not able to grow on Sabouraud Dextrose Agar (SDA) and modified Dixon Agar (mDA) medium but grew slowly on Leeming & Notman agar (LNA). In the course of a survey on the fungal flora in the rabbit skin, we searched for the presence of Malassezia sp. in rabbits from Emilia Romagna and Veneto regions.
MATERIALS AND METHODS:
The screening was performed on 168 rabbits; 70 rabbits for meat and 98 dwarf rabbits. Samples were collected from both the external ear canals of each animal using swabs soaked in phosphate buffered physiological saline containing 0.1% Tween 80, according to Cabanes & Castellà (2011 l.c.). All samples were inoculated onto SDA (BBL-BD), mDA (Gueho et al., 1996, Antonie van Leeuw. 69: 337-355) and LNA (Leeming and Notman, 1987, J. Clin. Microbiol. 25: 2017-2019). These culture media included 0.05% cloramphenicol. Plates were incubated at 30°C and examined daily for 20 days. All swabs were also rolled over the surface of microscopic slides and the smears were stained with May Grunwald-Giemsa. Afterwards swabs collected from further 24 rabbits were used to inoculate 3 modified culture media with different composition and for slides.
RESULTS:
Microscopic observation of the swabs showed yeasts in one or both the external ear canals of 98 rabbit (58.3%). The yeasts were rounded, 2-4 um in diameter, with buds formed in monopolar pattern on narrow bases, like to the description of M. cunicoli according to Cabanes and Castellà (2011 l.c.). The prevalence was higher in the rabbits breeding for meat than the dwarf rabbits ($X2y = 12.33$; $p<0.01$): no statistically significant differences were observed in relation to age, gender and kind of breeding. Cultural examinations showed the presence of rare colonies of yeasts in two rabbit for meat, identified as Candida famata. All the other samples not showed any yeasts growth on all the culture media employed as screening. Also swabs from the further 24 rabbits examined, all positive at microscopic examination, were negative on the modified culture media.
CONCLUSIONS:
In the present survey we recovered, at the microscopic observation, the presence of yeasts morphologically similar to those described by Cabanes and Castellà (2011 l.c.), with a prevalence (58.3%) beyond all expectation. These authors observed M. cunicoli in only 2 out of 11 rabbits investigated (18.2%), both in cultures and in microscopic examination. Previously, only Radi (Comp. Med. 2004, 54: 434-437) observed round yeast cells by direct microscopy in 20 out of 500 (4%) rabbits affected by Sarcoptes scabiei. In the present survey, the yeasts were not able to grow on the different medium used in the screening and in the modified cultural media. This observation show how the presence of yeasts in the microflora of the rabbits' external ear canals is probably more common than you think, and that the inhibition of growth observed may be related to different nutritional requirement than the one described by Cabanes and Castellà (2011 l.c.) and Cabanes (PLOS Path. 2014, 10: e1003892).

Keywords:
yeast, Rabbit, external ear canal

P11A.2
SURVEY OF THE PRESENCE OF CRYPTOCOCCUS NEOFORMANS AND OTHER PATHOGENIC YEAST IN RACING PIGEONS HOUSES OF NORTHERN ITALY

Galuppi R.[1], Cecchetto G.[1], **Tampieri M.P.*[1]**

[1]Dipartimento di Scienze Mediche Veterinarie - Università Di Bologna ~ Bologna ~ Italy

INTRODUCTION:
Messenger pigeons are domesticated races of Columba livia, bred for they homing ability and used in the past to carry messages; nowadays these animals are basically used for sport purpose. Pigeon racing is the sport of releasing trained racing pigeons that then return to their home over a carefully measured distance. In this paper the presence of C. neformans or other potentially pathogenic yeasts and their relationship with breeding management was investigated in racing pigeon houses from Modena, Bologna and Udine provinces (Northern Italy).
MATERIALS AND METHODS:
Seventy-three samples of dry pigeon droppings collected from 21 racing pigeon house were analyzed. Mycological analyses were conducted according to Galuppi et al (2002-6° congresso Nazionale FIMUA Modena, 12-14 settembre 2002). Briefly, two grams of each specimen were suspended in 10 ml of sterile saline solution with antibiotics (50000 U penicillin, 50000 U streptomycin/100ml of saline) and stored at 5°C overnight. One hundred microliters of the suspension was then spread, in duplicate, on plates containing Niger Seed Agar (Shields & Ajello, 1996, Science, 151: 208-209). The plates were incubated at 25°C and 37°C, respectively, for 10 days and daily observed. The yeasts isolated were identified with Api 20C AUX (Biomerieux Italia S.p.a, Firenze, Italy) and Dalmau plates on Yeast Morphology Agar DIFCO (Becton Dickinson Italia S.p.a, Milano, Italy).
RESULTS:
Yeasts were isolated from 35 samples incubated at 25°C and from 22 incubated at 37°C. Cryptococcus neoformans was isolated in at least one samples from 11 pigeon houses (52.4%), Candida guilliermondii in 10 (47.6%), C. albicans in 5 (23.8%), C. famata in 6 (28.5%) and Rhodothorula sp. in 2 (9.5%). The frequency of isolation of C. neoformans was lower in farms in which disinfection treatment has been done (46.2%) than in the ones where only mechanical removal were performed (62.5%), but the differences was not statistically significant. No differences were also observed in relation to different disinfection methods (sodium hypoclorite, steramine or flame). Vice versa, the daily mechanical removal of feces was significantly related ($p<0.05$) with a lower frequency of isolation of C. neoformans, compared to the farms where the removal was performed every 7-15 days (14% vs 71%). No differences were observed related to treatment or frequencies of mechanical cleaning and the isolation of C. guilliermondii and C. albicans; instead C. famata was isolated in the 46% of the farms without daily mechanical removal and not found in the other ones (fisher exact<0.05).
CONCLUSIONS:
It is well known as the pigeon's guano is a source of infectious elements of C. neoformans and is involved in their transmission from the environment to the humans (Nielsen et al, 2007, Eukariotic cell., 6: 949-959). In the past it has been shown a high seroprevalence of antibodies against C. neoformans in pigeon fanciers (Walter et al., 1996, J Bacteriol., 92: 82-87), although studies in this regard are very scarce. In this work it is highlighted as mechanical cleaning decreases the environmental presence of this yeast, whereas the use of different disinfections methods don't seems decrease their presence. The daily cleaning and the use of a disposable filtering face mask could decrease the infection risk in the pigeon fanciers.

Keywords:
Cryptococcus neoformans, Racing pigeons, Yeasts

P11A.3
ANIMAL DERMATOPHYTOSES BY A DYSGONIC STRAIN OF MICROSPORUM CANIS.

Moretta I.[1], Agnetti F.[2], Diaferia M.[1], Danesi P.[3], Papini M.[4], Morganti G.[1], **Moretti A.*[1]**

[1]Università degli Studi di Perugia, Dip. di Medicina Veterinaria ~ Perugia ~ Italy, [2]IZS Umbria e Marche ~ Perugia ~ Italy, [3]IZS Venezie ~ Legnaro (PD) ~ Italy, [4]Università degli Studi di Perugia, Dip. di Scienze Chirurgiche e Biomediche, Clinica Dermatologica ~ Terni ~ Italy

INTRODUCTION:
Dermatophytoses are among the most frequent skin diseases in animals. Traditionally, mycological culture is considered the gold standard for the ringworm diagnosis as reliable method for phenotypic fungal identification (Chermette et al., 2008, Mycopathologia, 166:385-309), but difficulties can appear with dysgonic strains, showing no indicative conidial forms or pleomorphic colonies (English, 1978, Mycopathologia, 64:73-81).
Aim of the present study was to highlight the importance of genetic characterization of fungal strains expressing dysgonic variants, in order to achieve a precise etiological diagnosis.
MATERIALS AND METHODS:
Three stray kittens, showing alopecic lesions in the auricular area, were treated with antifungal therapy for about 20 days, until disappearance of the lesions. When they were adopted by 3 different families, lesions of Tinea corporis and Tinea capitis appeared in owners; also a puppy 5 months old present in one of the families showed typical dermatophytic lesions. The outbreak involved a total of 7 people, including a woman under chemotherapy for cancer, and a 3 years old girl.
Animals were submitted to Wood's Light. Samples obtained by hair brushing (cats) and by skin scraping (dog) were examined by direct microscopy and cultivation onto Sabouraud dextrose agar with cycloheximide and chloramphenicol (Lab. Conda S.A., Spain) at 26°C. Subsequently, the colonies were seeded on plates containing boiled polished rice grains.
Colonies identification was achieved by PCR based molecular techniques with direct sequencing of ITS1-ITS2 rRNA amplicons. Nucleotide BLAST of the sequences obtained were aligned in the CBS Knaw database.
RESULTS:
The Wood's lamp test was negative. Direct examination highlighted the presence of filamentous hyphae. Colonies on Sabouraud medium showed slow and difficult growth, only after 3 weeks, with dysgonic aspect, both macroscopically (no cottony surface and yellowish on the reverse) and microscopically (no conidial states, pleomorphic hyphae and forms similar to intercalar chlamydoconidia). Colonies with the same morphology were obtained on boiled rice. Subcultures are again under investigation in order to verify the variability of strain.
Dermatophyte species isolated from dog and cats involved in the episode were identified as Arthroderma otae (teleomorph of Microsporum canis) by molecular tools.
CONCLUSIONS:
This outbreak leads to different considerations:
- the animals with suspected dermatophytoses were subjected to a standard investigative procedure: Wood's lamp and direct examination (rapid and cheap tests) and culture. In our case, dysgonic colonies grew either on primary cultures or on boiled rice (the latter should generally stimulate the sporulation). Therefore, the molecular investigation, faster and more reliable than conventional methods, was crucial to achieve the identification of species (Sitterle et al., 2012, Med Myc, 50:305-309);
- M. canis was confirmed as species, pathogenic and aggressive in dog and humans, particularly. Thus, the girl involved never came in contact with the kittens, but has been infected by the dog; moreover, the presence of predisposing factors determine a higher susceptibility to infection, as evidenced by the involvement of a immunosuppressed woman;
- it is important to investigate the occurrence of a potential asymptomatic carriage in a pet before the adoption.

Keywords:
Dermatophytoses, dysgonic strain, Microsporum canis

P11A.4
WHITE-NOSE SYNDROME (WNS), A DEVASTATING FUNGAL BAT DISEASE

Riccucci M.[1], **Perrone V.*[2]**

[1]Gruppo Italiano Ricerca Chirotteri (GIRC) ~ Pisa ~ Italy, [2]Società Italiana di Medicina Veterinaria Preventiva (SIMeVeP) ~ Roma ~ Italy

INTRODUCTION:
White-nose syndrome (WNS) is an emerging disease responsible for the deaths of about 6 million bats in eastern North America. Since it was first observed in a New York cave during winter 2006/2007, WNS has rapidly spread south and west to 23 states and 5 Canadian provinces causing massive die-offs of hibernating bats.
MATERIALS AND METHODS:
A brief description of the disease is reported together with the observations on the presence of the pathogen fungus in Europe.
RESULTS:
Pseudogymnoascus destructans (formerly Geomyces destructans) is a psycrophilic fungus (Blehert et al., 2009, Science, 323: 227; Gargas et al., 2009, Mycotaxon, 108: 147–154). WNS affects hibernating bats; they show the white fungus on their noses and often on the other part of their bodies like the wings (Frick et al., 2010, Science, 329: 676-679; Lorch et al., 2011, Nature, 480: 376–378). Seven bat species are already identified with diagnostic symptoms of WNS: Eptesicus fuscus, Myotis leibii, M.grisescens, M.sodalis, M.lucifugus; M.septentrionalis, Perimyotis subflavus. Four more species have shown P.destructans on them but no diagnostic sign of the disease has been documented. No treatment or means of preventing transmission is known so far. Unlike North America, there has been no observed mass die-off of bats in the Old Continent, which could indicate that European bats may be resistant to the fungus. The presence of the same fungus in healthy bats suggests that P.destructans originated in Europe where local bats acquired immunity; somehow the fungus was transported in North America, causing mass mortality as happened to the Native Americans after the arrival of Christopher Columbus (Puechmaille et al., 2010, Emerg. Infect. Dis., Feb [serial on the Internet]; Puechmaille et al., 2011, PLoS ONE 6: e19167). P.destructans was confirmed in thirteen European countries: France, Belgium, the United Kingdom, the Nederlands, Germany, Switzerland, Austria, Slovakia, Poland, Hungary, Ukraine and Estonia. Extra four countries have strong photographic evidence of the presence of the fungus: Luxembourg, Denmark, Romania and the European side of Turkey. P.destructans has not been found in Italy so far. There are ten European species that have been reported with P.destructans: M.nattereri, M.mystacinus, M.myotis, M.escalerai, M.emarginatus, M.daubentonii, M.dasycneme, M.brandtii, M.blythii, M.bechsteinii. It is clear that in Europe the fungus mainly affects Myotis species. There is no known human health risk associated with WNS in bats.
CONCLUSIONS:
Bats consume billions of night-flying insects each year and help controlling bugs problematic for agriculture and forestry. "Insectivorous bat populations, adversely impacted by WNS ... may be worth billions of dollars to North American agriculture" (Boyles et al., 2011, Science, 332: 41-42); the value of bats' pest-control services in the United States ranges from $3.7 billion to $53 billion per year. There are several ongoing researches including: Oral vaccines and delivery methods for controlling disease in bats; Testing cleaning and disinfection products for efficacy against P.destructans; Identifying the European source population of North American P.destructans.

BIBLIOGRAPHY
Blehert et al., 2009, Science, 323 (5911): 227.
Boyles et al., 2011, Science, 332 (6025): 41-42.
Frick et al., 2010, Science, 329: 676-679.
Gargas et al., 2009, Mycotaxon, 108: 147–154.
Lorch et al., 2011, Nature, 480 (7377): 376–378.
Puechmaille et al. Emerg. Infect. Dis., 2010 Feb [serial on the Internet].
Puechmaille et al., 2011,PLoS ONE 6(4): e19167.

Keywords:
Pseudogymnoascus destructans , Myotis, Insectivorous bats

NOTES

POSTER 11B

IMMUNITA' E IMMUNOPATOLOGIA DELLE INFEZIONI PARASSITARIE

P11B.1

IN VIVO EVALUATION OF THE EFFICACY OF IMIQUIMOD TREATMENT OF SECONDARY HYDATID CYSTS IN GERBILS

Conchedda M.*[1], Bortoletti G.[1], Caredda A.[1], Gabriele F.[1], Seu V.[1]

[1]Dipartimento Scienze della Vita e dell'Ambiente Università di Cagliari ~ Cagliari ~ Italy

INTRODUCTION:
In vivo studies aimed at understanding the immunobiology of the relationship between the host and the Echinococcus granulosus metacestode usually adopt the murine model of experimental secondary infection, as it avoids handling the oncospheres. One feature of the developmental biology of E.granulosus is in fact the remarkable ability of protoscoleces (PSC) to re-differentiate in the intermediate host to produce secondary cysts, as a result of the accidental rupture of a primary cyst or by spillage during surgery. Infection by E. granulosus evokes cellular and humoral responses but these are not able to eliminate the parasite. The parasite in fact usually produces long-lasting infections in immunocompetent hosts and modulation of the host's immune response has been proposed as a strategy for ensuring parasite survival. It is assumed that subversion of the immunomodulation may in turn result in an advantage to the host. The purpose of this study was to investigate the effect of imiquimod, a low molecular weight imidazoquinolinamine compound and immune response modifier, during experimental secondary infection with CE of G1 strain in gerbils, a very susceptible experimental animal for "in vivo" investigation of cystic echinococcosis of ovine origin.
MATERIALS AND METHODS:
Imiquimod is a potent synthetic TLR7/8 agonist that induces Th-1-mediated immune responses (IFN and IL-12) as opposed to Th-2 responses (IL-4, IL-5, IL-13 production, increased IgE, IgG1, IgA, eosinophilic infiltrates). Meriones unguiculatus Mongolian gerbils infected by intraperitoneal injection with 100 or 2000 E. granulosus PSC have been treated with Imiquimod (1 mg/Kg/die) for 6 days, starting from day -1, and for 5 cycles of 6 biweekly treatments starting 80 days p.i. Animals together with controls were then euthanized 18 months p.i. and size and weight of the cysts were measured and their morpho-structural changes examined.
RESULTS:
Results showed that early treatment with Imiquimod reduces, in a statistically significant way, the number of cysts that develop in gerbils compared to controls (0.25+ 0.5 cysts vs 7+5 in light infection, 11+13 vs 39+47 in heavy infection). In addition a strong decrease in parasite load (obtained by multiplying for each animal the diameter of each cyst in mm by the number of cysts) was observed in both experiments, with a high frequency of collapsed cysts/total. This agrees with the results of previous studies using BALB/c mice as experimental hosts, where analysis of cytokine produced during in vitro spleen cell cultures showed a precocious Th0/Th1 response. IFN-γ production peaks during the first weeks p.i., when the great majority of PSC are destroyed by the host response, especially by IFN-γ-activated macrophages, producing toxic nitrogen oxide metabolites.
CONCLUSIONS:
In the secondary echinococcosis gerbil model the observed inhibition of cyst development and growth confirms the protective effect of immunomodulation when Th1 bias occurs. The possible inhibition of this response using regulatory cytokine IL-10 warrants further investigation.

Keywords:
cystic echinococcosis, experimental secondary infection, gerbils

XXVIII Congresso Nazionale SoIPa, Roma 24-27 giugno 2014

NOTES

NOTES

POSTER 12

PARASSITI DEGLI ANIMALI DA COMPAGNIA E RISCHIO ZOONOSICO

P12.1
CONTAMINATION BY CANINE GEO-HELMINTHS IN GREEN PUBLIC AREAS OF PADUA, ROME AND TERAMO, AND PEOPLE'S PERCEPTION OF THE RELATED HEALTH RISKS

Simonato G.*[1], Traversa D.[2], La Torre F.[3], Frangipane Di Regalbono A.[1], Carlin S.[4], Meloni S.[6], Boscolo S.[5], Tessarin C.[1], Pietrobelli M.[1]

[1]Dipartimento di Medicina Animale, Produzioni e Salute, Università degli Studi di Padova ~ Padova ~ Italy, [2]Facoltà di Medicina Veterinaria, Università di Teramo ~ Teramo ~ Italy, [3]Novartis Animal Health ~ Origgio (Varese) ~ Italy, [4]Istituto Zooprofilattico Sperimentale delle Venezie ~ Legnaro (Padova) ~ Italy, [5]Practitioner ~ Chioggia (Venezia) ~ Italy, [6]Practitioner ~ Roma ~ Italy

INTRODUCTION:
Term "zoonoses" has been recently extended as "any detriment to the health and/or quality of human life deriving from direct or indirect relationships with (other) vertebrates" (Mantovani, 2001, Inf. Circ.–WHO Mediterr. Zoon. Control Cent., 51). The environmental contamination with infective parasite elements can be included in this new definition, given that human syndromes caused by pet geo-helminths may lead to different syndromes of varying severity (Traversa, 2012, Parasit. Vectors, 5:91).
This survey estimated the environmental contamination by canine geo-helminths in green public areas of Padua, Rome and Teramo (Veneto, Lazio and Abruzzo regions, respectively), along with an evaluation of the people's perception of the related health risk.
MATERIALS AND METHODS:
Canine faecal samples (n=677) were collected from June to September 2012 in 13, 6 and 5 green public areas of Padua (P, n=270), Rome (R, n=209) and Teramo (T, n=198), respectively. At the same time, pet and non-pet owners in P and R were interviewed for awareness on the potential transmission of parasites via animal faeces. Samples were copromicroscopically analyzed with floatation using a sodium nitrate solution (density 1.3). Differences in parasites with high prevalence were statistically evaluated with the Chi-squared test (p<0,05) using an interactive calculation software (Preacher, 2001, http://quantpsy.org). The same statistical test was used to compare the proportion of pet and non-pet owners aware of the health risk associated with canine faecal pollution.
RESULTS:
The overall parasite prevalence (40/677) was 5.9% (Table 1). Trichuris vulpis and Toxocara canis showed lower prevalence values in P than in R and T (Table 1). A total of 469 interviews were administered and only the 10.9% (51/469) of respondents gave correct answers, with no significant differences (χ^2=1.436; p=0.231) between pet (n=311) and non-pet (n=158) owners.

Parasites	Overall prevalence n=677 (%)	Padua n=270 (%)	Rome n=209 (%)	Teramo n=198 (%)	χ^2	p value
Trichuris vulpis	30 (4.4)	4 (1.5)	16 (7.7)	10 (5.0)	10.857	p<0.005
Toxocara canis	13 (1.9)	2(0.7)	4 (1.9)	7 (3.5)	4.737	p<0.01
Ancylostoma caninum	3 (0.4)	0	3 (1.4)	0	-	-
Eucoleus aerophilus	2 (0.3)	0	1 (0.5)	1 (0.5)	-	-

Table 1. Results of copromicroscopic and statistical analyses

CONCLUSIONS:
This survey highlights the role of canine nematodes as a source of infection for dogs and humans in green urban areas. The most prevalent parasites were T. vulpis and T. canis, in agreement with previous studies (Traversa et al., 2014, Parasit. Vectors, 7:67). The lower prevalence in P is probably due to the common use of chemopreventives for Dirofilaria immitis, which is endemic in the region. The questionnaires revealed a low level of knowledge on the potential role of dogs in the transmission of zoonoses, thus suggesting the importance of a continuing education to enhance awareness of pet owners and general public.

This work was supported by Novartis Animal Health.

Keywords:
Helminths, zoonoses, urban areas

P12.2
COMPARISON OF THREE SEROLOGICAL TEST KITS FOR RAPID IN-CLINIC DETECTION OF DIROFILARIA IMMITIS (NEMATODA: FILARIOIDEA) ANTIGENS

Frangipane Di Regalbono A.*[1], Cassini R.[1], Signorini M.[1], Simonato G.[1], Rinaldi M.[2], Ciocchetta S.[3], Pietrobelli M.[1]

[1]Dipartimento di Medicina Animale, Produzioni e Salute, Università degli Studi di Padova ~ Padova ~ Italy, [2]OR SELL srl ~ Carpi (Modena) ~ Italy, [3]a) QIMR Berghofer Medical Research Institute - b) Istituto Zooprofilattico Sperimentale delle Venezie ~ a) Brisbane, Queensland, Australia - b) Legnaro, Padova, Italy ~ Italy

INTRODUCTION:
Procedures to detect microfilariae (e.g. Modified Knott's test-MKt) are widely used for diagnosis of Dirofilaria immitis infections. Nevertheless, the scant effectiveness of this approach in single sex or occult infections induced the development of immunological assays to detect heartworm antigens (Fuller et al., 1987, Vet. Immunol. Immunop., 17:345-356). This study provided sound scientific evidences on the quality performance of the modified Knott's test and three commercial test kits for rapid in-clinic use.
MATERIALS AND METHODS:
Whole blood samples were collected from May to October 2012 on 140 dogs from Northeastern Italy and with different epidemiological history, i.e. 33 dogs (Group G1) born and always lived in D. immitis free areas of the Belluno province; 17 microfilaraemic dogs (G2) aged between 2 and 5 years, living in D. immitis endemic areas of Padua and Verona provinces, and never subjected to chemoprophylaxis or filaricidal treatments; 90 dogs (G3) older than 2 years, and randomly chosen in endemic areas of Padua and Rovigo provinces. Samples were analysed by MKt, serological test kits (CHW AG 2.0, BIONOTE, Korea; WITNESS, Synbiotics Corp., USA; SNAP HTWM, IDEXX Lab., USA), and subjected to DNA extraction and PCR amplifications of the 5S ribosomal spacer using S2-S16 primers. Concordance between tests was evaluated by k statistic. Performance Indexes-PIs (Accuracy=AC; Sensitivity=SE; Specificity=SP; Positive=PPV and Negative=NPV Predictive Values) were calculated for each serological test using the dataset (n=50) involving negative (G1) and positive (G2) samples. The software used were SPSS for Windows (version 16.0) and Win Episcope 2.0, choosing a 95% confidence interval (CI).
RESULTS:
Out of 90 samples from G3, 12(13.3%), 11(12.2%), and 8(8.9%) were positive at SNAP HTWM, CHW AG 2.0, and WITNESS tests, respectively. Considering 138 samples tested with each serological kit, the strength of agreement was very good (k=0.827) comparing CHW AG 2.0 with SNAP HTWM and WITNESS, slightly declined to a good level (k=0.806) between SNAP HTWM and WITNESS. Both CHW AG 2.0 and SNAP HTWM showed all PIs=100%, whereas lower values of SE (76.5%; CI=56.3-96.6) and NPV (89.2%; CI=79.2-99.2) were detected for WITNESS. In G3, occult infections were detected in 5 samples by both CHW AG 2.0 and SNAP HTWM, and in 3 samples by WITNESS. SNAP HTWM and CHW AG 2.0 showed a good strength of agreement (0.666≤k≤0.760) with MKt and PCR, meanwhile moderate k values were observed comparing WITNESS with MKt and PCR (0.464 and 0.538, respectively). No PCR positive samples were detected in G1, while PCR confirmed D. immitis infection in G2 and G3.
CONCLUSIONS:
Although Knott's test is suggested as a valid first step to detect D. immitis infections (ESCCAP Guideline, 2nd Ed, 2010), false negative results are expected, and serological assessments are required to detect occult infections. In this regard, CHW AG 2.0 performance indexes seem to be equivalent to those of the SNAP HTWM, and better than those detected for WITNESS.

Keywords:
Dirofilaria immitis, immunodiagnosis, Knott test

P12.3
TOXOPLASMOSIS AND RISK BEHAVIOURS IN MANAGEMENT OF URBAN FERAL CATS IN OSTIA - ROME

Malandrucco L.*[1], Mazzocchini P.[1], Spaziani A.[1], Bacci F.[1], Ciccone G.[1], Felici F.[1], Mazzarelli F.[1], Mundo V.[1], Pizzuto M.[1], Pirico' S.[1], Tomassetti I.[1], Fantini C.[1]

[1]ASL ROMA D ~ ROMA ~ Italy

INTRODUCTION:
Toxoplasmosis is a zoonosis spread worldwide. In Europe 50-80% of human beings are estimated to be infected (EFSA Journal, 2014, 12: 3547). The Public Veterinary Service ASL Roma D in Ostia is mainly involved in the health management of stray cats (cat colonies). The population is estimated to be about 4,000 cats living on the Roman coast and care taken by around 453 citizens. Zoonoses prevention and control is a priority for Public Veterinary Services.The aim of this study was to detect the seroprevalence of Toxoplasma gondii in cats anesthetized for neutering surgery and to find out the Toxoplasmosis knowledge level of cat caretakers and their risk behaviors
MATERIALS AND METHODS:
From April 2012 to May 2013, 210 blood samples of cats, admitted for neutering surgery to the Public Veterinary Service Asl Roma D in Ostia, were collected and sent to the reference laboratory for Toxoplasma gondii Immunoglobulin G and Immunoglobulin M detection. A questionnaire was created to identify the risk behavior in the management of cat colonies and administered to the cat caretakers (N=37) of the tested cats, investigating the following items: personal particulars, methods of the catch, hygiene behaviors observed by the cat caretakers, knowledge on Toxoplasmosis and its transmission, risk perception, human test for Toxoplasmosis performed and its result. Data entry and analysis was done by software EpiInfo vers.3.5.3.
RESULTS:
28% of 210 had the Toxoplasma gondii antibodies (only 5% IgM). 61% of these were females, the average age was 24 months (range5-144) and European breed. By multivariate analysis, Toxoplasmosis positive sieroprevalence shows an association versus cats over 20 months of age (Odds Ratio 0,29, 95%; C.I. 0,15–0,59; p=0.0005). According to the questionnaire data, it was found that 84% interviewed were women (range 34-81 years, mean 55 years); the women were housewives (43%) compared with men (68%) retired and older (range 49-76, mean 62 years). Education wise, 50% of the group had a high school certificate and only 11% were graduates. 86% said that they knew about Toxoplasmosis and 70% correctly indicated it infectious disease and transmitted orally. Almost all interviewed personally took precautions when the animal was captured and (86%) said that they were rarely or never in contact with the feces (60 %). 84% exhibited proper hygienic behavior in the management of the colony; 97% believed that it is important to perform correct hygienic behavior in order to reduce the risk of infection. Only 30% of the group was subject to a Toxoplasma gondii test resulting negative (82%). We have defined two new variables performed by recoding others variables: 1) risk-class (22% yes) and 2) good knowledge of Toxoplasmosis (65% good). Multivariate analysis shows association between "good knowledge" and "sex" (females versus males Odds Ratio 18,6; C.I. 95% 2.3–148; p=0.005).
CONCLUSIONS:
Findings of the study were likely good. In fact the hygiene behaviours kept by the cat caretakers are mostly correct and they seem to have good knowledge about the disease. Probably, women are more informed for the successful and continuous education made by physicians;but we must not underestimate 35% in which the survey has highlighted a lack of knowledge. We should plan information activities, in order to promote accurate information, resulting probably in a decrease of risk exposure to this infection and other zoonoses transmitted by cats.

Keywords:
Toxoplasmosis, zoonoses, cats

P12.4
TICKS INFESTING DOMESTIC AND WILD ANIMALS IN SARDINIA, ITALY

Foxi C.*[1], Cabras P.[1], Chisu V.[1], Lecca V.[1], Masala G.[1], Satta G.[1]

[1]Istituto Zooprofilattico Sperimentale della sardegna ~ Sassari ~ Italy

INTRODUCTION:
It has been known that ticks can transmit a great variety of pathogens that cause severe diseases on their vertebrate hosts. The main parasites involved are viruses, bacteria, protozoa and filarial nematodes (Dantas-Torres, 2008, Vet. Parasit., 152: 173-185). In the last decades, tick-borne diseases are on the rise worldwide (Parola and Raoult, 2001, Clin. Infect. Dis., 7: 80-83). In this emerging scenario more information regarding tick distribution, ecology and host preferences are needed to better understand the risk these arthropods pose to domestic and wild animals and human population. Aim of this study was investigate the tick fauna and determine the prevalence of tick infestation in domestic and wild animals in Sardinia island.
MATERIALS AND METHODS:
Ticks were collected from naturally infested vertebrate hosts by practitioner veterinary in 36 collection areas of Sardinia Island during the years 2011 and 2012. Ticks were removed from their hosts with tweezers, placed in vials and stored at room temperature The species were identified based on morphological characteristics (Manilla, 1998, Acari Ixodidae, pp 280).
RESULTS:
A total of 775 hard ticks were identified to the species level. Moreover, 10 damaged ticks were identified to the genus level only, as Hyalomma spp. (6 specimens) and Ixodes spp. (4). No soft ticks were collected. Rhipicephalus turanicus (309 specimens) was the most common species, followed by Rh. sanguineus (186), Rh. bursa (165), Dermacentor marginatus (37), Haemaphysalis punctata (31), Hae. sulcata (15), Rh. pusillus (15), Hyalomma marginatum marginatum (10), Hy. lusitanicum (7). Rh. turanicus ticks were removed from goat, mouflon, marten, wild boar, pig, sheep, hare and fox. Rh. sanguineus ticks were collected from dog, marten, wild boar and hare. Rh. bursa was found in mouflon, goat, sheep and cattle while Rh. pusillus occurred on marten, pig and goat. Specimens of D. marginatus were removed from dog, pig, mouflon, wild boar and deer. All Hae. punctata ticks were collected from mouflon while specimens of Hae. sulcata come from goat, sheep and mouflon. Hy. m. marginatum was detected in wild boar and pig and Hy. lusitanicum in wild boar. The damaged ticks belonging to Hyalomma genus were collected from marten and hedgehog. The samples identified as Ixodes spp. were, instead, removed from partridge and buzzard.
CONCLUSIONS:
In this study we report the results of 2 years survey with the aim of evaluating the species diversity and the host preference of free-living ticks. Rhipicephalus was confirmed to be the most represented genus in Sardinia (Satta et al., 2011, J. Med. Microbiol., 60: 63-68). Rh. turanicus and Rh. bursa are the major ectoparasites of livestock but are frequently found on wild vertebrates. Rh. sanguineus is a dog-associated species that is well widespread throughout Sardinia; other hosts are usually infested when dogs are present in the area. Only a few specimens of Ixodes ticks were found. As previously reported by other authors, this is a tick genus uncommon in Sardinia (Alberti et al., 2005, Emerg. Infect. Dis., 11:1322-1324). Our findings increase the knowledge of the association ticks and vertebrate hosts in Sardinia. The data could provide a basis to create a map illustrating tick distribution and provide a useful contribution to understanding the epidemiology of tick-borne diseases.

Keywords:
ticks, hosts, Sardinia

P12.5
ORNITHONYSSUS BACOTI INFESTATION IN PET CAMPBELL'S DWARF HAMSTERS (PHODOPUS CAMPBELLI) AND RAT MITE DERMATITIS IN THE OWNERS

Lanza A.[1], **Perrucci S.*[2]**

[1]Centro Veterinario Gianicolense ~ Roma ~ Italy, [2]Dipartimento di Scienze Veterinarie-University of Pisa ~ Pisa ~ Italy

INTRODUCTION:
Ornithonyssus bacoti, known as tropical rat mite, is a blood-feeding ectoparasitic arthropod belonging to the family Macronyssidae. At night the parasites search for their hosts and seek dark hiding places during the daytime. O. bacoti primarily infests rodents, but it has been reported also in other small mammals and in humans if suitable preferential hosts are unavailable or in the case of close contact with infected animals and environments (Beck, 2008, Travel Med & Inf Dis, 6: 245–249). In humans the mite is responsible for dermatologic lesions known as rat mite dermatitis. This study reports O. bacoti infestation in pet Campbell's dwarf hamsters and in the owner family
MATERIALS AND METHODS:
In the month of December 2012 a pet Campbell's dwarf hamster with dermatological problems was presented. The subject, a female about 1 year old, was housed in a hamster cage with two other females of the same age. All hamsters had been purchased from the same pet shop, at different times between October and December 2012. After about two weeks from the introduction of the third hamster, the owners noticed a gradual increase in itching in one of the two hamsters previously owned. At the same time, one of the six members of the family, a boy of 11 years in age, showed dermatologic symptoms including pruritus, papules and scabs on the trunk, arms and legs diagnosed as a mite dermatitis by the physician. The boy lived in a room where the hamster cage was located. After about two more weeks, two other members of the family showed the same clinical signs. For mite collection, on the infested pet the Scotch test was performed, while the cage was inspected and mites collected with a needle. Isolated mites were preserved in 80% ethanol, mounted by using Hoyer's medium application according to conventional techniques, and microscopically observed for species identification.
RESULTS:
At the scotch test, the hamster was positive for mites. From the bottom of the cage and the litter, a large number of mites were collected. Mites were highly variable in size and in color, from reddish to dark brown. All isolated mites were adult females ranging from 937 to 1339 µm in length X 453.2-957.9 µm in width (mean 1033.8 µm X 556.2 µm). On the basis of morphology and chaetotaxy, isolated mites were identified with the species O. bacoti.
CONCLUSIONS:
Hamsters were treated with an acaricide, the cage was thoroughly cleaned and disinfested with an acaricide containing permethrin. The home was also disinfested and a professional exterminator was recommended. In conclusion, mite identification allowed to advise the pet owners on eliminating the origin of the infestation.

Keywords:
Ornithonyssus bacoti, Phodopus campbelli, rat mite dermatitis

P12.6
CAT-SCRATCH DISEASE DIAGNOSED BY PCR DETECTION OF BARTONELLA HENSELAE DNA IN HUMANS

Paglia M.G.*[1], Vulcano A.[1], Toffoletti A.[1], Di Caro A.[1], Pane S.[1]

[1]INMI Lazzaro Spallanzani ~ Roma ~ Italy

INTRODUCTION:
The cat-scratch disease (CSD) is a zoonosis caused mainly by Bartonella henselae (Bh), an emerging, or reemerging, human pathogen and, occasionally, by other Bartonella species, such as B. bacilliformis, B. clarridgeiae, B. elizabethae, B. quintana and B. vinsonii. Cats are the reservoir for these bacteria and fleas play an important role in transmission among cats. Animals become infected during the flea blood meal or by ingestion of infected fleas and flea feces. Usually, CSD manifests as a subacute regional lymphadenitis following a cat scratch or bite. The characteristic disease begins with a primary lesion at the site of inoculation, which develops into a papule or pustule, and, generally, 7 to 14 days after infection, regional lymphadenopathy occurs proximal to the primary lesion. This self-limiting disease resolves spontaneously within 1-2 months without any antimicrobial therapy. Complicated cases can occur with infections of the liver, spleen, heart, lungs, joints, bones, brain or a lingering high fever without other symptoms. All of these complications usually resolve without any lasting illness. Laboratory diagnosis is based on culture isolation, serology, histopathology, and molecular detection mainly in lymph node biopsies and aspirates, and more rarely in peripheral blood samples. We aimed to perform the molecular detection of Bh in clinical specimens of suspected CSD patients using an "in-house" Bh nested-PCR, targeting the 60-kDa heat shock protein gene.
MATERIALS AND METHODS:
From January 2002 to December 2013, 329 samples (peripheral blood, lymph node biopsies, skin and granulomatous lesions) were collected and analyzed for the presence of Bh DNA. Only a single sample was taken for each patient.
DNA was extracted using QIAamp® DNA Mini Kit (Qiagen). For formalin-fixed and paraffin-embedded (FFPE) tissues, 5-10 mm slices were deparaffinated by treatment once with xylol and twice with ethanol. After drying of the tissue, manufacturer's protocol was followed. A 220 base pair DNA fragment was generated with an "in-house" Bh nested-PCR (Boaz et al., 1997, J Cl Microbiol, 35:1924–1930). DNA purification was controlled by amplification of human HLA DQalfa DNA.
When available, sera from Bh DNA positive patients were tested for the presence of specific antibodies using a commercial IFA assay (Euroimmun). Serum samples were considered to be positive when a titer ≥1:360 and ≥1:100 for antibodies of class IgG and IgM respectively were obtained.
RESULTS:
All investigated specimens showed a positive signal in the HLA DQalfa PCR, confirming that the DNA extraction was successful and excluding general inhibitory effects for PCR. Among the 329 samples, 3.6% (5 FFPE lymph node biopsies, 2 fresh lymph node biopsies, 4 fine-needle lymph node aspirates and 1 pustule fragment) were positive by Bh nested-PCR. Both IgG and IgM responses were observed in 2 patients (16.6%), while the detection of IgG alone was found in 3 patients (25%).
CONCLUSIONS:
In this study, we showed that PCR method can be used as additional means to help make the diagnosis of CSD from different source of samples and can offer a supplementary method for patients where the clinical interpretation of Bh serology is difficult for both low sensitivity and specificity.

Keywords:
Bartonella henselae, Cat-scratch disease, PCR

NOTES

AUTHOR INDEX

NOTES

A

Abay S.M. 54, 55, 153

Abdeddaim A. 285

Abramo F. 101, 121

Accardi C. 326, 338

Acerra V. 169

Addis P. 164

Agnetti F. 352, 386

Aguiar Figueredo L. 243

Aguilar R. 57

Aiello A. 56

Alaimo A. 265

Alano P. 52, 53

Albonico F. 330

Albonico M. 93, 94, 124

Alessandrino P.E. 195

Alonso Díaz M.A. 299

Alvarez Garcia G. 188

Alvarez Mercado J.M. 299

Ambía Medina J. 298

Amprimo V. 171

Anastasi A. 169

Angelo L. 380

Angheben A. 83, 145

Anile S. 226

Annoscia G. 97, 121, 176

Antognini E. 304

Aprile S. 135

Aquilino V. 134

Arcà B. 60, 180, 331

Arcangeli G. 312

Arese P. 57

Argentini C. 287

Arias M.S. 374

Arnoldi D. 238

Arzese A. 258

Avellino P. 251

Averaimo D. 350

Avila Acevedo G. 299

B

Babiker A.M.A. 149, 177, 190, 328

Bacchetta R. 333

Bacci C. 301

Bacci F. 396

Badiali D. 197

Baiocchini A. 285

Banchi A. 105

Bandi C. 113, 182, 183, 215, 333, 360

Barchiesi F. 304

Barda B. 93, 94

Bardelli M. 112

Bardoni A. 113

Barrera V. 57

Bartolini R. 134, 261, 278, 284, 350

Bartoloni A. 114, 199, 266, 270

Barzanti P. 264, 353

Basilico N. 53, 57, 355

Bassini A. 295

Basso A. 125

Bassole I.H.N. 59

Bazzocchi C. 113, 182, 301, 333, 360

Bellagamba R. 354

Belleau E. 380

Bellisario B. 169

Benedetti E. 157, 158, 308

Benedict M.Q. 152

Beraldo P. 127, 225, 258

Bernardoni E. 316

Berrilli F. 89, 172, 198, 200, 232, 326, 347
Bertolini S. 364
Berto M. 328
Berton V. 163
Bertuccini L. 179
Besognet B. 127
Bessone M. 377, 380
Bianchi R. 205
Biasci A. 363
Biasion L. 222
Biasutto D. 197
Biggeri A. 141
Bisoffi Z. 145, 196
Bisseye C. 251
Bizzarro T. 329
Blackman M.J. 51
Blanda M.C. 368
Blanda V. 220, 265
Boari A. 137
Boasso A. 279
Boccolini D. 157, 158, 308
Bokzic J. 61
Boldrini R. 319
Bonafini S. 196
Bona R. 52
Bongiorno G. 204, 205, 287
Bongiovanni L. 99
Bonilauri P. 116, 361
Bortoletti G. 297, 390
Bosco A. 104, 130, 144
Boscolo S. 394
Bosi G. 165
Bosio F. 373
Bougouma E.C. 251
Bousema T. 60
Bozic J. 183, 184, 185, 213
Bracaglia G. 319, 327, 338, 339, 341, 346
Bragoni R. 119
Branchini B.C. 52
Brandão Filho S.P. 150, 151
Braun G. 134
Breitschwerdt E. 117
Brianti E. 64, 67, 97, 117, 121, 135, 139, 226, 257, 263, 362
Broccatelli S. 189
Brownlow A. 318
Brúderová T. 41
Brunetti E. 273
Bruni A. 211
Bruno B. 264
Bruno S. 373
Brun R. 51
Bruschi F. 75, 250, 252
Bucci P. 157, 158, 287, 308
Burioni R. 94
Buscemi M.D. 321

C

Cabanelas E. 227
Cabras P. 283, 397
Cacciò S.M. 197
Caddori A. 297
Cadeddu R. 263
Cafarchia C. 214, 240, 241, 242, 243, 244
Caffara M. 76, 313, 314, 316, 320
Cafiero M.A. 305, 307
Cafiso A. 182, 360
Cagetti M. 297
Calabretta M. 52
Calderini P. 274

Callea F. 319, 340, 346
Calzetta M. 212
Calzolari M. 116, 361
Camarda G. 52, 53
Cammà C. 191, 356
Cammilleri G. 321, 322
Campbell B.E. 97, 176, 216
Camuffo S. 306
Cancrini G. 114, 199, 266, 270, 274, 282
Candusso S. 258
Canonico C. 304
Capasso M. 381
Capelli G. 45, 115, 118, 154, 156, 158, 177, 190, 206, 210, 222, 223, 238, 244, 306, 312
Capitani O. 239
Capolongo C. 200
Capone A. 61, 183, 184, 185, 213
Cappelli A. 61, 183, 184, 185, 213
Cappelli G. 144
Caputo B. 211, 335
Cara A. 52
Caracappa G. 171
Caracappa S. 220, 265, 315
Cardoso L. 101, 176
Caredda A. 297, 390
Carlin L. 357
Carlin Sara 156, 210, 306
Carlin Silvia 394
Carpignano M.G. 377, 380
Casalone C. 112, 364
Cassini R. 99, 110, 149, 177, 190, 259, 395
Castro L.R. 282
Casulli A. 223, 271, 272
Catalano S. 375
Catani M. 351
Catelan D. 141
Cattoli G. 177
Cavallero S. 96, 171, 230
Cavazza G. 314, 316
Cazzin S. 118, 154, 156, 222, 223, 312
Ceballos L. 112
Cebrián-Torrejón G. 56
Cecarini V. 213
Cecchetti S. 178
Cecchetto G. 385
Centaro E. 100
Cerroni L. 319
Cersini A. 330
Cevenini L. 52, 53
Charlier J. 104
Cherchi S. 329
Chianese G. 55, 57
Chiavacci L. 364
Chiodo E. 380
Chisu V. 283, 397
Chovancová B. 230
Ciadamidaro S. 41
Ciccone G. 396
Cimmino R. 294, 295
Cincinelli G. 148
Cinquepalmi V. 200
Ciocchetta S. 156, 206, 306, 395
Ciociola T. 242
Cioli D. 125
Cipriani P. 169, 318
Citterio C. 223, 233
Ciufolini M.G. 47, 157, 158, 205, 287, 308
Claps S. 295

AUTHOR INDEX

Cogliati M. 244
Coiante V. 172
Coles G. 130
Colizzi V. 253
Collins M.C. 152
Colnago G. 138
Colombo A.A. 195
Colombo M. 330
Columba M. 207
Coluzzi M. 128, 290
Comandatore F. 360
Concato C. 340
Conchedda M. 297, 390
Conforti M. 34
Conti S. 242
Coppellotti O. 209
Corbellini G. 35
Corbett Y. 53, 57, 355
Cordovani E. 189
Corradi M. 361
Corsi N. 330
Cortes H. 101, 176
Cosentino V. 128, 290
Costa A. 171, 315, 321, 322
Costantini C. 59
Costarelli S. 189
Craig A. 51
Cringoli G. 87, 93, 94, 104, 130, 141, 144, 262, 381
Crippa A. 208
Crisafi B. 250
Crisanti A. 152
Crisi P.E. 137
Croft S. 51
Crucitti D. 220
Cuéllar C. 249
Culurgioni J. 164
Currà C. 179
Currò V. 171, 315

D

Dabire R. 209
D'Agostino R. 265
Dahiya N. 54, 55, 153
D'alessandro S. 53, 355
D'Alessio N. 221, 379
D'Alfonso R. 198, 200, 326, 347
Dalla Torre S. 384
Dal Pont M. 328
D'Amelio S. 96, 171, 230, 232, 315
Damiani C. 61, 183, 184, 185, 213, 215
Damiano S. 209
Danesi P. 154, 173, 222, 238, 244, 312, 328, 386
D'aniello F. 56
D'Anna N. 102
Dantas Torres F. 43, 67, 97, 101, 102, 111, 117, 121, 150, 151, 176, 214, 216, 243, 362
Da Rold G. 110, 118, 238, 312, 328
Daschner A. 74, 249
Da Silva F.J. 150
Da Silva K.G. 150, 151
Davalos D. 57
D'Avino N. 189
Davison N. 318
De Berardinis A. 261
De Bernart F. 296
Defilippo F. 116, 361
De Francesco I. 98
Degani M. 196
De Gennaro M. 252

Delai N. 328
Del Chierico F. 319, 326, 338, 340, 346
Del Giudice F. 257
De Liberato C. 99, 148, 155, 232
Deligianni E. 179
D'elios M.M. 75
Dellamaria D. 223
Della Torre A. 59, 211, 212, 335
Del Nonno F. 285
Del Prete L. 262
De Maio L. 327
Demanche C. 238
De Maria C. 220
Dembo E. 54, 153
Demeneghi D. 234
De Morais R.C.S. 151
De Moura M.R.A. 150
De Paiva Cavalcanti M. 151
Deplazes P. 223
Desiato R. 364
Dettori A. 189
Diabate A. 209
Diaferia M. 115, 136, 386
Diarra A. 251
Diaz Alcazar E. 234
Díaz P. 103, 227, 228
Di Caro A. 354, 399
Di Cave D. 172, 198, 200, 326, 347
Di Cerbo A.R. 131
Di Cesare A. 64, 99, 100, 127, 134, 136, 137, 224, 261, 278, 284, 350
Di Costanzo R. 137
Di Cristanziano V. 198, 347
Di Domenico M. 155, 191, 356
Diez Baños N. 374
Díez Baños P. 103, 227, 228, 347
Di Francesco G. 99, 191
Di Giuli C. 120
Di Giulio E. 284
Di Loria A. 294
Di Luca M. 157, 158, 308
Di Matteo A.M. 195
Di Matteo S. 329
Di Mattia T. 356
Dimitri C.F. 137
Di Muccio T. 204, 221
Dinis J. 335
Dini V. 188
Diociaiuti A. 319
Di Paola G. 242
Di Pasquale C. 220
Di Prisco F. 379
Di Provvido A. 191
Disclafani R. 315
Djurković-Djaković O. 72
D'Offizi G. 285
Doménech-Carbó A. 56
Dore F. 142, 257, 263, 276, 277, 279, 336
Dori G. 54
Dottori M. 116, 361
Dougnac C. 318
D'Ovidio D. 381
Drago A. 154, 156
Drake J. 134
Drakeley C. 60
Drigo M. 110
Dubey J. 329, 337
D'Urso V. 204
Dutto M. 96

E

Egidi S. 86

Eleni C. 99

El Hachem M. 319

Epis S. 113, 119, 182, 183, 185, 215, 333, 360

Esposito Abate R. 329

Esposito F. 54, 55, 153

F

Fabbi M. 119

Fabris C. 209

Facchinelli L. 152

Falcaro C. 190

Falsone L. 121, 135, 139, 226, 362

Fantini C. 396

Fantozzi L. 337

Fascetti R. 211

Fattorusso C. 56, 57

Fattorusso E. 57

Favia G. 61, 183, 184, 185, 213, 215

Fazii P. 250

Fedorova M.V. 158, 308

Felici A. 189

Felici F. 396

Fenu G. 277

Ferguson H.M. 212

Fernández G. 227, 228

Ferrantelli V. 321, 322

Ferrari G. 207

Ferrari N. 98, 229, 231, 233

Ferreira Andrade D. 376, 377

Ferretti F. 232

Ferroglio E. 224, 357, 373

Ficetto G. 380

Fidock D.A. 52

Figueredo L.A. 214, 241

Filippini G. 189, 352

Finelli R. 178

Fiocchi A. 378

Fioravanti M.L. 76, 313, 314, 316, 320

Fiorentino E. 221

Foglia Manzillo V. 221

Fognani G. 286

Fonti P. 98

Formato G. 330

Formenti F. 196

Formenti N. 229, 231, 233

Foroni M. 210

Fortuna C. 157, 158, 205, 287, 308

Fourie J. 208

Foxi C. 283, 397

Fracassi F. 239

Francese D.R. 112, 364

Francione E. 222, 223

Frangipane Di Regalbono A. 99, 127, 149, 154, 259, 350, 394, 395

Franke Fayard B. 179

Frantzis A. 318

Fratini F. 337

Fraulo M. 266

Frau F. 168

Frau V. 276

Frongillo F.R. 120

Fuda I. 129

Fumagalli M. 113

G

Gabriele F. 390

Gabrielli A.F. 124

Gabrielli S. 96, 114, 199, 266, 270, 274, 282

Gaffuri A. 229

Gaglio G. 121, 135, 139, 181, 226, 322, 362

Gaiarsa S. 360

Galante D. 305, 307

Galati F. 273

Galdiero M. 87

Gallo D. 139

Gallo M. 110

Gallo V. 57

Galuppi R. 239, 275, 384, 385

Gambetta B. 96, 171

Garau S. 164

García E. 317

Gargiullo C. 331

Garibaldi F. 164

Garippa G. 164, 166, 168, 188, 317

Garutti S. 239

Gatti A. 261

Gaudino M. 379

Gavaudan S. 304

Gazzonis A.L. 131, 188, 194

Gemma S. 51

Genchi C. 98

Genchi M. 93, 98, 119

Genco F. 73, 195

Gentile A. 189, 352

Geraci S. 85

Gerbelle C. 357

Geurden T. 127

Ghirardi M. 376

Ghirga P. 354

Gianchecchi M. 363

Giangaspero A. 181, 192, 193, 232

Giannelli A. 41, 67, 97, 101, 102, 111, 117, 176, 216, 226

Giannetto S. 67, 121, 135, 139, 226, 362

Giansante D. 191

Giari L. 165, 167

Gilioli S. 361

Giordano O. 380

Giovati L. 215

Giudice E. 265

Giuliato I. 206

Giusepponi V. 115

Gobbi M. 352

Goffredo M. 46

Gokbulut C. 143, 294

Gomez Morales M.A. 252

Gontero C. 375

González Fernández J. 249

González-Miguel J. 68

Goria M. 264, 353

Gottstein B. 36

Graci S. 321, 322

Gradoni L. 47, 126, 204, 221, 287

Gramegna M. 339, 341

Gramiccia M. 47, 204, 205, 221, 287

Gramolelli S. 290

Granato A. 190, 244

Grande R. 355

Grasselli A. 77

Gremo G. 57

Grimaldi C. 336

Gualtiero L. 376

Guariglia I. 144

Guelbeogo W.M. 59, 212

Guidi Alessandra 125

Guidi Alison 330

Guillot J. 238

Gustinelli A. 76, 172, 313, 314, 316, 320, 378

H

Habluetzel A. 54, 55, 140, 153, 209

Hall M.J.R. 181

Hanssen E. 179

Hassan M.M. 177

Hauffe H.C. 238

Haynes R.K. 355

I

Iacomi F. 285

Ianniello D. 94, 104

Iatta R. 214, 240, 241

Ibarra Velarde F. 298, 299, 365

Iebba V. 59, 198

Ienco A. 211

Ignjatović Ćupina A. 41, 92, 101

Immediato D. 214, 242

Imperatore C. 56

Ingravalle F. 264, 353

Interisano M. 95

Invernizzi A. 188, 194

Iori A. 282

Iorio R. 99, 137, 261, 278, 284, 350

Islas Donde G. 372

Iulini B. 112

J

Janse C. 179

Jones C. 140

Jori G. 209

K

Kabore Y. 251

Kaiser R. 347

Kamau A. 140

Keiser J. 94

Khoury C. 204, 205

Kibe L. 140

Kramer L.H. 138, 215, 301

Kreppel K. 212

Kúdela M. 41

Kumar Tiruppadiripuliyur S. 52

L

Lacasella V. 192

La Cavera E. 315

Lalle M. 178

Lanfranchi P. 229, 231, 233

Lanza A. 398

Lanzoni O. 182

Lara Y. 114

La Rosa G. 95

La Russa F. 368

La Torre F. 134, 259, 284, 394

Latrofa M.S. 43, 97, 111

Lecca V. 397

Lees R.S. 152

Lelli R. 265, 368

Lepri E. 342

Levecke B. 93, 104

Lia R.P. 102, 117, 127, 214

Lisi O. 204

Loiacono M. 330

Lombardo F. 60, 180, 331

Lombardo M. 57

Longhini A. 138

Lonigro A. 192

López C. 103, 227, 228

Lorenzoni M. 167

Loria G.R. 253

Losito L. 327

Lucantoni L. 54, 55, 153, 209

Luchi S. 252

Luciana R. 252

Luciani M. 191

Luciano P. 56

Luini M.V. 188

Lupidi G. 54, 55, 153, 209

M

Macchioni F. 114, 199, 266, 363

Maccioni V. 277

Macías D. 164

Maggioni A. 188

Maggiora M. 188

Magi M. 363

Magliano A. 148, 155

Magnino S. 93

Maioli G. 116, 361

Maiolini B. 41

Malandrucco L. 396

Malatesta D. 99

Mallia E. 226

Manca M. 363

Mancianti F. 286, 332

Mancini E. 212, 335

Mancini G. 305, 307

Mancini M.V. 61, 183, 184

Mandara M.T. 342

Mandola M.L. 112

Mandrioli L. 313

Manfredi M.T. 131, 188, 194

Manfredini A. 119

Manfrin A. 163, 312

Mangano V. 60, 251

Mangili P. 352

Manica M. 211

Manjarrez A. N. 365

Mannelli A. 112

Manuali E. 352

Manuguerra B. 138

Manunta M.L. 263

Marangi M. 181, 192, 193, 232

Marangon S. 118, 206, 223

Marani I. 232

Marceca M. 82

Marcer F. 173, 260, 312, 318

Marchi A. 205, 287

Marchiori E. 173

Maresca C. 189, 352

Mariani U. 143, 294

Mariano A. 285

Mariconti M. 113, 182, 273, 333, 360

Marigo A. 106

Marino F. 220

Maroli M. 47, 204, 287

Marrocco T. 57

Marruchella G. 99

Marti Gimenez M. 84

Martin E. 333

Martínez Sernández V. 103

Martini M. 110

Martini S. 154, 210

Martin M. 207

Martin R. 51

Marucci G. 95

Masala G. 283, 397

Masala S. 166, 168

Massetti A.P. 88

Massimo M. 225

Massirio I. 116

AUTHOR INDEX

Matia M. 105
Mattei R. 252
Mattiucci S. 169, 170, 250, 270, 318
Maurella C. 364
Maurelli M.P. 262, 381
Mazzarelli F. 396
Mazzocchini P. 396
Mazzotta P. 164
Mazzucato M. 206
Mbogo C. 140
Mcgovern B. 318
Mcgrath S. 207
Mele S. 164, 317
Meloni S. 394
Menardi G. 380
Menconi V. 76, 314, 316, 320
Menegon M. 129
Meneguz P.G. 375, 376, 377, 380
Menna M. 56
Mercado R. 349
Mereghetti V. 333
Merella P. 164, 168, 317
Meroni V. 73, 195, 273
Messina V. 329
Meyer W. 244
Michelini E. 52, 53
Mignone W. 264
Mingotto F. 258
Miotti C. 100
Miotto L. 295
Miranda D.E.D.O. 150, 151
Misiano P. 53
Moascatelli F. 304
Modiano D. 60, 251
Mogliani W. 242
Molinar Min A. 376, 377, 380
Molineri G. 194
Molini U. 356
Mondin A. 110
Moni A. 148
Monno R. 200
Montagna M.T. 240
Montalbano Di Filippo M. 200
Montarsi F. 118, 154, 155, 156, 190, 206, 210, 306, 308, 328
Montero F.E. 164, 317
Montesano C. 253
Montesi F. 163
Montresor A. 93, 94, 124
Morandi B. 275, 351
Morán Gálvez J.T. 317
Morchón R. 68
Moresi M. 304
Moretta I. 194, 351, 386
Moretti A. 115, 194, 386
Morgan E. 130
Morganti G. 65, 115, 136, 224, 342, 386
Morgoglione M.E. 144
Mori Ubaldini F. 250
Morrondo P. 103, 227, 228, 374
Mortarino M. 330
Mosca A. 364
Moura A.C.D.A. 150, 151
Muglia S. 168
Mugnaini L. 286, 332
Mundo V. 396
Munoz Calabuig E. 84
Muntoni S. 142, 277, 279
Mura A. 283

AUTHOR INDEX

Muraca M. 326, 327, 338, 339, 340, 341, 346
Musella V. 141, 262
Mutafchiev Y. 101
Mutarello M. 259
Mutinelli F. 306

N

Napoli E. 121, 135, 139, 226, 362
Narcisi V. 191
Nardi V. 169
Nardoni S. 286, 332
Nascetti G. 169, 170, 318
Natalie N. 249
Nebié I. 60, 251
Neglia G. 295
Negri E. 376, 377
Neola B. 143, 221, 294, 379
Nicolardi L. 210
Nicoletti G.J. 273
Nicoletti L. 158
Nocita E. 334
Noviello E. 262, 381

O

Ochoa Galvan P. 298
Ogboi J.S. 153
Ogboi S. 54, 55
Oliva C. 152
Oliva G. 126, 221
Oliveira Arruda De Abreu G. 243
Olivieri A. 179
Omodeo S.G. 206, 222, 259
Ortega C. L. 365
Ortega Mora L. 188
Osuna A. 84
Otaševic S. 92
Otranto D. 41, 43, 67, 97, 101, 102, 111, 117, 121, 127, 150, 151, 176, 214, 216, 226, 240, 241, 242, 243, 362
Ouédraogo J.B. 54, 209
Ouédraogo K.R. 54
Ouedraogo R. 209

P

Paco B. 319
Pagano T.B. 379
Paganotti G.M. 128, 290
Paglia M.G. 285, 354, 399
Pajares G. 227, 228, 374
Pajoro M. 360
Palacio Abella J. 164
Palazzo L. 305
Palei M. 118, 206
Palma G. 169
Palumbo M. 120
Pampurini F. 100
Panadero R. 103, 227, 228
Pane S. 399
Paoletti B. 134, 261, 278, 284, 350
Paoletti M. 170, 318
Papini M. 386
Papini R. 286, 296, 332
Parada C. 84
Parapini S. 53, 56, 57, 355
Parigi M. 275, 351
Parrilla M. 130
Parvez M.A.K. 355
Pascotto E. 225
Pascucci I. 191, 356
Passamonti F. 342
Passeri B. 301
Paul D R. 181

Paule L. 230
Pau M. 336
Pautasso A. 112, 364
Pavone S. 352
Paz Silva A. 374
Peano A. 244, 376, 377, 380
Pedrotti L. 229
Peletto S. 112
Peña S. 349
Pennacchio S. 262
Pennisi M. 139
Pepe P. 104, 262, 381
Perandin F. 196
Pérez A. 103, 227, 228
Perez Del Olmo A. 317
Pérez Morales H. 365
Perrone V. 77, 96, 367, 387
Perrucci S. 105, 398
Persampieri T. 152
Persico M. 56, 57
Petrarca V. 211, 335
Petric D. 101
Petrić D. 41
Petroni G. 182
Pfister H. 347
Pham V. 180
Piantedosi D. 221, 294
Piazza C. 98
Piazza M. 263
Pica Mattoccia L. 125
Piccolo S. 328
Pichler V. 335
Piergili Fioretti D. 115, 120, 194, 224, 351
Pietra M. 275
Pietrobelli M. 149, 190, 222, 259, 260, 306, 394, 395
Pini A. 356
Pinna M. 116
Pinto Brandão Filho S. 243
Pinto J. 335
Pintore M.D. 112, 364
Pintus D. 172
Pipia A.P. 142, 257, 263, 275, 276, 277, 279, 336
Piras M.C. 166, 168
Piredda M. 283
Pirico' S. 396
Pironi F. 167
Pitzalis F. 263
Pizzi E. 50, 337
Pizzocaro M.L. 110
Pizzuto M. 396
Plantard O. 113, 360
Platonov A.E. 158, 308
Podaliri Vulpiani M. 191
Poglayen G. 226, 275, 351, 378
Polonelli L. 215, 242
Pombi M. 59, 180, 212, 335
Ponzi M. 50, 179, 337
Poppi L. 173
Porcellato E. 177, 328
Possenti A. 329, 337
Pozio E. 95, 178, 197, 252, 273, 329, 334, 337
Prati P. 119
Prearo M. 76, 316, 364
Pretto T. 163, 312
Prieto A. 227, 228
Prudente C. 135
Putignani L. 232, 319, 326, 327, 338, 339, 340, 341, 346

Q

Quaglio F. 316
Quaranta V. 305
Quintavalla A. 57
Quintero Martinez M.T. 372

R

Radaelli M.C. 364
Radicchi G. 121
Raele D.A. 305, 307
Ragionieri M. 357
Ramalho M. 150
Rambozzi L. 234
Ramírez Lubianos C. 365
Ramos R.A.N. 67, 97, 117, 214, 216
Randazzo K. 220, 368
Ranno S. 319, 327, 338, 339, 341, 346
Ranson H. 212
Ravagnan S. 115, 118, 156, 177, 190, 206, 260, 328
Reddel S. 340
Remoli M.E. 157, 158, 205, 287, 308
Restucci B. 379
Rezanezhad H. 129
Rianda A. 285
Ribeiro J. 180
Ricci I. 61, 183, 184, 185, 213, 215
Riccucci M. 367, 387
Righetti M. 316
Righi C. 189, 352
Rinaldi F. 273
Rinaldi L. 94, 104, 130, 141, 144, 262, 381
Rinaldi M. 395
Rinnovati R. 106
Ripamonti F. 195
Rivosecchi L. 41
Rizzo C. 60
Rizzo F. 112
Rizzoli A. 44, 238
Rocchigiani G. 286, 332
Roda A. 52, 53
Rodrigues A. 335
Rodrigues Garcia M. 84
Rodríguez Llanos J. 164
Rojas Gonzales P. 199, 266
Rojas P. 114
Romano A. 264, 353
Romano R. 128, 290
Romanucci M.R. 99
Romi R. 157, 158, 308
Roncada P. 369
Ronca R. 60
Ronci L. 172
Roncoroni C. 143
Rosà R. 211
Roselli M. 114, 199, 266
Rossi G. 253
Rossi L. 188, 234, 380
Rossi Paolo 61, 183, 184, 185
Rossi Patrizia 273
Rufrano D. 295
Ruschioni S. 304
Russo F. 118, 206, 210
Russo G. 128, 290

S

Sacchi L. 119
Sagnon N. 59, 212
Salgado Zamora H. 365
Salis F. 277, 336
Salvatore S. 336
Salviato A. 118

XXVIII Congresso Nazionale SoIPa, Roma 24-27 giugno 2014

AUTHOR INDEX

Salvini R. 113
Sánchez Andrade R. 374
Sánchez Morales K. 298
Sangueza O. 319
Sanna G. 142, 257, 263, 276, 277, 279, 336
Sanna N. 164
Sanou A. 212
Santangelo F. 59, 198
Santaniello M. 144
Santolamazza F. 251
Santoni D. 50
Santori D. 137
Santoro MARIO 379
Santoro Mariastella 200, 232, 319, 326, 327, 338, 339, 341, 346
Sartorelli P. 233
Sassera D. 113, 182, 333, 360
Satta G. 397
Savioli L. 124
Sawadogo S. 209
Sayyaf Dezfuli B. 165, 167
Scacchia M. 191, 356
Scala A. 142, 263, 276, 277, 279, 336
Scala F. 257
Scalone A. 221
Scaramozzino P. 148
Scarpona S. 253
Schaper R. 136
Schippa S. 59, 198
Schwarzer E. 57
Scimeca S. 368
Sciortino S. 322
Scoccia E. 352
Sconza S. 278
Scremin M. 210, 306
Sebastianelli M. 189
Seghetti M. 137
Segundo E. 266
Sepulveda N. 251
Serra C. 257
Serra V. 360
Setini A. 172
Seu S. 336
Seu V. 297, 390
Severini C. 129
Severini F. 157, 158, 204, 308
Sferra G. 50, 180
Sgorbini M. 296
Siciliano G. 52, 53
Siden Kiamos I. 179
Signore M. 52
Signorini M. 110, 149, 177, 210, 395
Silvestri E. 376, 377
Silvestrini F. 52
Silvestri P. 148
Simonato G. 222, 223, 260, 394, 395
Simón F. 68
Simón L. 68
Sini A. 279
Sinisi A. 54
Sirima B. 60
Sirima S. 251
Sirri R. 313
Skorokhod O. 57
Smaldone G. 143, 169
Snabel V. 230
Sori F. 369
Spadari A. 106
Spano F. 329, 337

AUTHOR INDEX

Spanu V. 168
Spaziani A. 396
Speich B. 94
Spina S. 264
Stancampiano L. 106, 369
Stanneck D. 117
Stansgaard A.S. 149
Stefanelli S. 105
Stefani F. 286
Stensgaard A.S. 149
Struchiner C. 180
Suarez J.L. 374
Sulcis D. 276
Suniga F. 114
Suniga Rios F. 199
Superti F. 179, 287
Sylla L. 352

T

Tabacchi F. 128, 290
Taglialatela-Scafati O. 54, 55, 57
Tagliapietra V. 238
Tamarozzi F. 273
Tampieri M.P. 239, 384, 385
Tamponi Chiara 275
Tamponi Claudia 142, 257, 263, 276, 277, 279, 336
Tancredi F. 274
Tarallo V.D. 117, 362
Taramelli D. 51, 53, 56, 57, 355
Tarantino V. 220
Tarantola M. 233
Tchidjou H. 346
Terregino C. 163
Tessarin C. 110, 173, 260, 328, 394
Timmen Wego M. 347
Tinari A. 287
Tinti M. 329
Tizzani P. 375, 376, 377, 380
Toffoletti A. 354, 399
Toma L. 157, 158, 308
Tomassetti I. 396
Tomassone L. 112
Tomeo A. 361
Tonanzi D. 95
Torina A. 112, 220, 265, 315, 368
Tosciri G. 263
Tosi F. 163
Tosini F. 197, 334
Totino V. 59, 114, 198, 199, 266, 270
Tozzi S. 120
Tramontin S. 77
Tranquillo V. 188
Traoré A. 212
Traversa D. 64, 99, 100, 102, 127, 134, 136, 137, 259, 260, 261, 278, 284, 350, 394
Trevisiol K. 223
Trisciuoglio A. 357, 373
Trogu T. 229, 231, 232, 233
Trombini C. 57
Turner L. 339

U

Ubeira F. 103
Ugonino P. 357
Ulissi U. 213
Usai F. 369
Utzinger J. 93, 94
Uyoga S. 57

V

Vaccalluzzo V. 204

Valerio L.	152
Valzano M.	61, 183, 184, 185, 213
Varcasia A.	142, 257, 263, 276, 277, 279, 336
Varloud M.	207, 208
Varricchio E.	295
Venco L.	66, 100
Veneziano V.	143, 221, 294, 295, 379
Ventura L.	341
Vera Montenegro Y.	298, 299
Vercruysse J.	93, 104
Verna F.	364
Veronesi F.	65, 115, 136, 194, 224, 342
Verra F.	251
Vicari D.	315
Vicari N.	119
Vicente J.	335
Vichova B.	230
Victoriano Llopis I.	234
Viganò R.	229, 231
Viglietti A.	98
Viglio S.	113
Villora Montero M.	317
Vincenzi L.	285
Visco S.	276
Vismarra A.	301
Vitale F.	263
Vitale N.	364
Vivas M.	317
Vulcano A.	354, 399
W	
Wall R.	40, 216
Warin S.	207
Webb S.	318
Weetman D.	335
Whittle A.	216
Y	
Yerbanga R.	54
Yerbanga S.	209
Z	
Zaccarelli M.	285
Zanardello C.	259, 260
Zanatta R.	264, 353
Zanet S.	357, 373, 375
Zannini F.	222
Zanzani S.A.	131, 188, 194
Zidda A.	279

NOTES

NOTES

XXVIII Congresso Nazionale SoIPa, Roma 24-27 giugno 2014

NOTES

NOTES

XXVIII Congresso Nazionale SoIPa, Roma 24-27 giugno 2014

NOTES

Finito di stampare
nel mese di Giugno 2014
da Litografia La Ducale srl
PARMA

CON IL PATROCINIO DI

Policlinico Umberto 1°
Fondazione PTV Policlinico Tor Vergata
Ordine provinciale di Roma dei Medici Chirurghi e degli Odontoiatri (OMCEO)
Ordine dei Medici Veterinari della Provincia di Roma
Ordine Nazionale dei Biologi (ONB)
Federazione Nazionale degli Ordini Veterinari Italiani (FNOVI)
Roma Capitale

MAJOR SPONSORS

ALTRI SPONSORS

EDILMON

MERIDIAN BIOSCIENCE EUROPE

TECNOCHIMICA MODERNA