XXX Congresso Nazionale

SoIPa

Società Italiana di parassitologia

MUTAMENTI AMBIENTALI e PARASSITI

Università degli Studi di Milano

26 · 29 giugno 2018
MILANO

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Carissimi,

i Dipartimenti di Medicina Veterinaria, Bioscienze, Scienze Biomediche e Cliniche "Luigi Sacco", Scienze Biomediche Chirurgiche ed Odontoiatriche e di Scienze Farmacologiche e Biomolecolari dell'Università degli Studi di Milano sono lieti di ospitare nella città di Milano il XXX Congresso Nazionale della Società Italiana di Parassitologia (SoIPa).

La forte connotazione interdisciplinare del Comitato Organizzatore, che include competenze biologiche, mediche e veterinarie, rispecchia una peculiarità della SoIPa, per certi aspetti unica nel panorama scientifico non solo nazionale. Infatti la SoIPa, che tra i suoi soci ha ricercatori provenienti da diversi ambiti scientifici, basa su questa peculiarità il suo punto di forza e dimostra la capacità di sapersi rapportare alle nuove realtà emergenti.

Il XXX Congresso Nazionale SoIPa, che si apre con una sessione plenaria dedicata al "Cambio d'uso del territorio alpino", si articola in 7 tavole rotonde e 15 sessioni scientifiche con 173 contributi su argomenti che spaziano dalle nuove frontiere a livello di ricerca alle più attuali realtà di campo.

La tematica del Congresso "Mutamenti ambientali e Parassiti" non vuole essere uno slogan, ma piuttosto sollecitare una doverosa presa di coscienza sulle problematiche a livello epidemiologico e socio-economico rispetto a fenomeni epocali che riguardano non soltanto l'ambito scientifico ma anche il vivere quotidiano. In effetti, i nuovi scenari che si vanno delineando in termini di salute umana, animale ed ambientale, impongono una sempre maggior attenzione e la Parassitologia ne è fortemente coinvolta.

L'auspicio è quello di offrire una panoramica esaustiva sulle problematiche attuali e di sviluppare una stimolante discussione a partire dal contributo di oltre 1000 colleghi, tra autori e coautori. Peraltro va sottolineato, come da tradizione dei Congressi SoIPa e a conferma della vivacità della nostra Società, la partecipazione sia di relatori di indiscussa fama internazionale che di numerosi "giovani ricercatori".

Il Comitato Organizzatore, infine, ringrazia vivamente i componenti del Consiglio Direttivo SoIPa, per il loro invito ad organizzare nella città di Milano il XXX Congresso e per il loro costante supporto nelle diverse fasi organizzative.

Si ringraziano inoltre i moderatori delle tavole rotonde e delle sessioni scientifiche e gli Enti e le Aziende che hanno contribuito all'organizzazione di questo importante Evento.

Il Presidente del Comitato Organizzatore

Paolo Lanfranchi

UNIVERSITÀ DEGLI STUDI DI MILANO

Dipartimento di Medicina Veterinaria
Dipartimento di Bioscienze
Dipartimento di Scienze Biomediche e Cliniche "Luigi Sacco"
Dipartimento di Scienze Biomediche Chirurgiche ed Odontoiatriche
Dipartimento di Scienze Farmacologiche e Biomolecolari

COMITATO ORGANIZZATORE

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COMITATO SCIENTIFICO

Comitato Organizzatore, Consiglio Direttivo SoIPa e Moderatori delle tavole rotonde e delle sessioni scientifiche

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Laura Rinaldi (Università di Napoli "Federico II")
Antonio Scala (Università di Sassari)

CON IL PATROCINIO DI

Federazione Regionale degli Ordini dei Medici Veterinari della Lombardia

PROGRAMMA IN SINTESI

	Martedì, 26/06/2018	
	ORE 15.00-18.00	
Registrazione (Aula Magna)		
	ORE 18.00-20.00	
Cerimonia inaugurale (Aula Magna)		
	ORE 20.00-22.00	
	Apericena (Loggiato del Rettorato)	
	Mercoledì, 27/06/2018	
	ORE 9.00-13.00	
Plenaria: Cambio d'uso del territorio alpino (Aula Magna)		
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	0RE 14.00-16.00	
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	ORE 16.00-16.20	
	COFFEE BREAK	
	ORE 16.20-16.40	
Claudio Genchi: Subcutaneous dirofilariosis [*Dirofilaria repens*]: a zoonotic infection spreading throughout Europe (Aula Magna)		
	ORE 16.40-18.00	
Sessione 3: Endo ed ectoparassiti degli animali da compagnia (Aula Magna).	**Sessione 4**: Diagnosi delle malattie parassitarie 2 (Aula 102)	**Sessione 5**: Biologia molecolare e filogenesi in parassitologia 2 (Aula 111)
	ORE 18.00-20.00	
Assemplea dei Soci (Aula Magna)		
	ORE 20.45-23.00	
parasEATaly (Cortile del 700)		

	Giovedì, 28/06/2018	
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	ORE 10.30-11.00	
	COFFEE BREAK	
	ORE 11.00-13.00	
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	ORE 13.00-14.00	
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	ORE 14.00-14.20	
	Guadalupe Mirò: "Post-Authorization Safety Assessment of Letifend® Revaccination in the Field: Pilot Study in Spain" (Aula 102)	
	ORE 14.20-16.00	
Tavola rotonda 5: La strada verso l'eradicazione della malaria: sfide e successi (Aula Magna)	**Sessione 8**: Parassitosi trasmesse da artropodi (Aula 102)	**Sessione 9**: Epidemiologia delle malattie parassitarie 2 (Aula 111)
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	COFFEE BREAK	
	ORE 16.20-18.00	
Tavola rotonda 6: Parassiti del cane in Italia, cos'è cambiato? (Aula Magna)	**Sessione 10**: Malattie trasmesse da artropodi vettori all'uomo (Aula 102)	**Sessione 11**: Parassiti della fauna acquatica (Aula 111)
	ORE 20.30	
	Cena sociale (Osteria Del Binari, Milano)	
	Venerdì, 29/06/2018	
	ORE 8.30-10.30	
Tavola rotonda 7: Parassitosi in apicoltura: attualità epidemiologiche e prospettive per il controllo (Aula Magna)	**Sessione 12**: Controllo e monitoraggio dei vettori (Aula 102)	**Sessione 13A**: Alimenti e parassiti (Aula 111). **Sessione 13B**: Entomologia medica e veterinaria (Aula 111)
	ORE 10.30-11.00	
	COFFEE BREAK	
	ORE 11.00-12.30	
Sessione 14: Parassitosi degli animali da reddito (Aula Magna)	**Sessione 15**: Terapia e farmacoresistenza (Aula 102)	
	ORE 12.30-13.00	
Saluti e arrivederci ai XXXI Congresso SOIPA (Aula Magna)		

PROGRAMMA DETTAGLIATO

MARTEDI' 26 Giugno 2018

ORE 15.00-18.00

Registrazione (Aula Magna)

ORE 18.00-20.00

Cerimonia inaugurale (Aula Magna)

Saluti delle Autorità
Saluti del Presidente del Comitato organizzatore del XXX Congresso SoIPa, Prof. **Paolo Lanfranchi**
Saluti del Presidente della Società Italiana di Parassitologia SoIPa, **Prof. Fabrizio Bruschi**
Lezione magistrale: "La peste, ieri e oggi" **Massimo Galli** (Università degli Studi di Milano).

ORE 20.00-22.00

Apericena (Loggiato del Rettorato)

MERCOLEDI' 27 Giugno 2018

ORE 9.00-13.00

Sessione plenaria: Cambio d'uso del territorio alpino (Aula Magna)
Moderatori: Paolo Lanfranchi, Michele Mortarino

"La rapida degradazione della criosfera alpina, il sintomo più evidente dei cambiamenti climatici" **Claudio Smiraglia** (Università degli Studi di Milano).

"Comunità microbiche dei ghiacciai e cambiamenti climatici" **Roberto Ambrosini** (Università degli Studi di Milano), **Andrea Franzetti** (Università degli Studi di Milano-Bicocca, Italia).

"Conseguenze biotiche del ritiro dei ghiacciai: dinamica delle comunità e funzionamento degli ecosistemi" **Francesco Ficetola** (Università degli Studi di Milano).

"Parassiti e global change: lezioni dalle comunità biotiche montane". **Luca Rossi** (Università degli Studi di Torino).

ORE 13.00-14.00

Pranzo

ORE 14.00-16.00

Tavola rotonda 1: Cambiamenti ambientali e scenari epidemiologici: previsioni e modelli (Aula Magna)
Moderatori: Luca Rossi, Gioia Capelli

"Co-infection, an old challenge in a changing world" **Emmanuel Serrano** (Universitat Autònoma de Barcelona, Spain).

"Geospatial Health, global changes and new parasitological scenarios" **Laura Rinaldi** (Università degli Studi di Napoli, Federico II).

"Predicting parasite spread in an era of rapid climate change: model limitations and new ways forward" **Eric Morgan** (Queen's University Belfast, UK).

Sessione 1: Diagnosi delle malattie parassitarie 1 (Aula 102)
Moderatori: Fabrizio Bruschi, Stefano D'Amelio, Romualdo Grande

A. Cafiso, V. Serra, C. Romeo, D. Sassera, E. Olivieri, O. Plantard, C. Bandi, C. Bazzocchi. *Midichloria mitochondrii* transmitted to the vertebrate host by *Ixodes ricinus*: a transient passenger or an infectious agent?

V. Mangano, F. Perandin, F. Verra, F. Migliaccio, M. Prato, S. Romano, L. Bargagna, M. Degani, S. Tais, Z. Bisoffi, F. Bruschi. Risk of transfusion transmitted malaria: evaluation of commercial ELISA kits for the detection of anti-*Plasmodium* antibodies in candidate blood donors.

A. Vola, F. Tamarozzi, R. Noordin, A. De Silvestri, T. Manciulli, E. Brunetti, M. Mariconti. Serodiagnosis of human cystic echinococcosis: comparison of two rapid diagnostic tests.

S. Gabrielli, L. Fontanelli Sulekova, F. Furzi, G. Taliani, S. Mattiucci. Preliminary results on the occurrence of *Blastocystis* subtypes and correlation with faecal microbiota in HIV patients referred to University Hospital "Umberto I" in Rome.

D. Di Cave, M. Montalbano Di Filippo, F. Berrilli. *Blastocystis* sp. in patients from the Polyclinic of Rome Tor Vergata.

F. Genco, E. Antoniazzi, S. Scarrone, M. Prestia, M. Suzani, V. Meroni. Diagnosis of toxoplasmic chorioretinitis: moving towards standardised assays.
S. Cavallero, A. Martini, G. Migliara, C. De Vito, S. D'Amelio. Anisakiasis in Italy. Analysis of hospital discharge records in the decade 2005-2015

Sessione 2: **Biologia molecolare e filogenesi in parassitologia 1** (Aula 111)
Moderatori: Claudio Bandi, Davide Sassera

B. Arcà, A. Colantoni, C. Fiorillo, F. Severini, B. Haase, M. Di Luca, R.A. Calogero, F. Lombardo. Salivary microRNAs from anopheline mosquitoes: additional players in vector-host-pathogen interactions?

F. Lombardo, G. Bevivino, C. Gargiullo, B. Arcà. Discovery of novel antimicrobial peptides in the salivary glands of the malaria mosquito *Anopheles gambiae.*

A. Olivieri, M. Chaand, F. Fratini, V. Mangano, E. Pizzi, F. Celani, S. Mochi, C. Birago, V. Tirelli, D. Modiano, M.T. Duraisingh, M. Ponzi. A specific class of erythrocyte membrane microdomains is involved in *Plasmodium falciparum* invasion of the host cell.

M. Castelli, A.M. Floriano, C. Bandi, G Petroni, D. Sassera. Endless interactions, most beautiful: genomics of novel Rickettsiales provides insight into the evolution of the order.

V. Serra, A. Cafiso, S. Epis, A. Negri, D. Rubolini, C. Bandi, C. Bazzocchi. Detection and quantification of a novel bacterium of the genus *Midichloria* (family *Midichloriaceae*, order Rickettsiales) in the hard tick *Hyalomma marginatum*.

F. Comandatore, D. Sassera, G. Radaelli, S. Epis, C. Bazzocchi, S. Montante, D. Di Carlo, M. Brilli, V. Serra, M. Perini, E. Clementi, L. Sacchi, C. Bandi. *Midichloria mitochondrii* life-cycle: what can mathematical analyses tell us?

ORE 16.00-16.20

Coffee break

ORE 16.20-16.40

"Subcutaneous dirofilariosis [*Dirofilaria repens*]: a zoonotic infection spreading throughout Europe" **Claudio Genchi** (Università degli Studi di Milano) (Aula Magna).

ORE 16.40-18.00

Sessione 3: Endo ed ectoparassiti degli animali da compagnia (Aula Magna)
Moderatori: Claudio Genchi, Salvatore Giannetto

M. Mainiero, L. Ellse, R. Wall. The toxic effect of essential oils on mites.

V.D. Tarallo, V. Colella, M.A. Cavalera, G. Deak, C.M. Gherman, A.D. Mihalca, D. Otranto. Larval development of *Angiostrongylus chabaudi*, the causative agent of feline angiostrongylosis, in the snail *Cornu aspersum*.
E. Napoli, A. Sfacteria, C. Rifici, G. Mazzullo, S. Giannetto, E. Brianti. Interaction of *Cornu aspersum* immune-system against developmental stages of *Aelurostrongylus abstrusus*.

L. Nguyen-Viet, F. Dantas-Torres, K.L. Bui, D. Otranto. Ticks infesting dogs in Vietnam: preliminary data.

P. Pepe, M.P. Maurelli, L. Colombo, R. Armstrong, E. Battisti, M.E. Morgoglione, D. Counturis, L. Rinaldi, G. Cringoli, E. Ferroglio, S. Zanet. A national survey of Ixodidae tick distribution in owned dogs in Italy.

A.L. Gazzonis, M. Marangi, S.A. Zanzani, L. Villa, A. Giangaspero, M.T. Manfredi. Epidemiology and genetic diversity of *Blastocystis* sp. in dogs housed in sanitary and rescue shelters.

Sessione 4: Diagnosi delle malattie parassitarie 2 (Aula 102)
Moderatori: Giovanni Poglayen, David Di Cave

J.M. Abbate, F. Arfuso, G. Gaglio, E. Napoli, S. Giannetto, E. Brianti. Does cat litter interfere on *Aelurostrongylus abstrusus* L1s survival?

M. Caffara, A. Gustinelli, O. Palenzuela, C. Székely, G. Cech, M.L. Fioravanti. Molecular tools for identification of zoonotic metacercariae in freshwater fish.

M.S. Latrofa, G. Palmisano, G. Annoscia, D. Otranto. A proteomic approach to identify candidate antigens for serodiagnosis of canine onchocercosis.

G. Annoscia, M.S. Latrofa, V. Colella, M.A. Cavalera, C. Maia, C. Martin, J. Šlapeta, D. Otranto. Real time-PCR for the detection of the zoonotic *Onchocerca lupi*.

R. Iatta, D. Buonfrate, P. Paradies, M.A. Cavalera, A. Capogna, F. Iarussi, J. Šlapeta, G. Giorli, P. Trerotoli, Z. Bisoffi, D. Otranto. Occurrence, diagnosis and follow-up of canine strongyloidiasis in naturally infected shelter dogs.

A. Bosco, M.P. Maurelli, A. Amadesi, C, Chartier, N. Ravinet, G. Cringoli, L. Rinaldi. Pooling faecal samples in cattle for the assessment of gastrointestinal nematode infection intensity and anthelmintic drug efficacy using Mini-FLOTAC.

Sessione 5: **Biologia molecolare e filogenesi in parassitologia 2** (Aula 111)
Moderatori: Bruno Arca', Simone Cacciò

S. Mattiucci, E. Bello, M. Paoletti, A. Levsen, S. Webb, J.T. Timi, G. Nascetti. Next-Generation development of microsatellite markers in the three species of the *Anisakis simplex* (s.l.) complex (Nematoda: Anisakidae).

S. Cavallero, F. Lombardo, M. Salvemini, C. Cantacessi, S. D'Amelio. Transcriptomic analyses of non-pathogenic marine ascaridoid *Hysterothylacium aduncum* and pathogenic *Anisakis simplex* sl larvae.

M. Montalbano Di Filippo, S. Cavallero, R. Meoli, C. Eleni, C. De Liberato, F. Berrilli. Molecular identification of *Mesocestoides* sp. metacestodes in a captive gold-handed tamarin (*Saguinus midas*).

M. Montalbano Di Filippo, A. Novelletto, D. Di Cave, F. Berrilli. Detection and genetic variation of *Vermamoeba vermiformis* from different water sources in Italy.

G. Marucci, L. Bertuccini, S. Cecchetti, C. Wylezich, M. Lalle. In deep analysis of different Giardiavirus (GLV) in naturally infected *Giardia duodenalis* trophozoites.

D. Di Cave, M. Montalbano Di Filippo, F. Berrilli. Diversity of *Pneumocystis jirovecii* across Europe: a multicentre observational study. The Italian contribution.

ORE 18.00-20.00

Assemblea dei Soci (Aula Magna)

ORE 20.45-23.00

ParasEATaly (Cortile del 700)

GIOVEDI' 28 Giugno 2018

ORE 8.30-10.30

Tavola rotonda 2: Malattie trasmesse da vettori del cane e del gatto: dalla biologia al controllo (Aula Magna)
Moderatori: Annunziata Giangaspero, Emanuele Brianti

"Future shock: changing threats from ticks and tick borne disease in Europe" **Richard Wall** (University of Bristol, UK).

"Tick borne pathogen transmission times: the importance to act fast!" **Domenico Otranto** (Università degli Studi di Bari).

"The control of canine and feline leishmaniosis in Europe: where do we stand?" **Gioia Capelli** (Istituto Zooprofilattico Sperimentale delle Venezie, Padova).

Sessione 6: Epidemiologia delle malattie parassitarie 1 (Aula 102)
Moderatori: Giovanni Garippa, Edoardo Pozio

R. Cassini, G. Simonato, P. Mulatti, S. Ravagnan, M. Pietrobelli, G. Capelli. Cystic Echinococcosis surveillance: a 10-years experience in a hypo-endemic area.
A. Casulli, M. Siles-Lucas, C.M. Cretu, K. Vutova, O. Akhan, G. Vural, A. Cortés, F. Tamarozzi, E. Brunetti. The international impact of HERACLES collaborative project on cystic echinococcosis.

P. Rossi, F. Tamarozzi, F. Galati, E. Brunetti, O. Akhan, C. Cretu, K. Vutova, A. Casulli, Heracles Extended Network. The European Register of Cystic Echinococcosis (ERCE): where are we and where to go (HERACLES project).

A. Casulli, F. Tamarozzi, O. Akhan, C.M. Cretu, K. Vutova, D. Akinci, R. Chipeva, T. Ciftci, C.M. Constantin, M. Fabiani, B. Golemanov, D. Janta, P. Mihailescu, M. Muhtarov, Serra Orsten, M. Petrutescu, P. Pezzotti, A.C. Popa, L.G. Popa, M.I. Popa, V. Velev, M. Siles-Lucas, E. Brunetti. The prevalence of abdominal cystic echinococcosis in rural Bulgaria, Romania and Turkey: results from cross-sectional ultrasound population-based surveys (HERACLES project).

R. Romano, F. Tabacchi, G. Russo, G.M. Paganotti. *Plasmodium falciparum* malaria and Human *Herpes virus* 8 (HHV8): co-infection in Ugandan children.

A.R. Sannella, Y. Suputtamongkol, E. Wongsawat, S.M. Cacciò. Cryptosporidiosis among HIV patients from Thailand: zoonotic species and high genetic variability in *Cryptosporidium hominis* and *C. meleagridis*.

F. Berrilli, M. Montalbano Di Filippo, D. Di Cave, C. De Liberato. Identification and spatial distribution based on 18S and gdh genetic variability of *Giardia* spp. from human and animals in Italy.

S. Gabrielli, L. Fontanelli Šuleková, G. Ceccarelli, M. Pombi, R. Esvan, M. Lopalco, S. Vita, S. Mattiucci. Human migration and parasites transmission: is there really a risk?

E. Perugini, M. Pombi, W.M. Guelbeogo, M. Calzetta, H. Ranson, N. Sagnon, A. Della Torre. Malaria entomological inoculation rate in a village of Burkina Faso reveals high transmission risk both indoors and outdoors despite the high LLIN coverage.

Sessione 7: Parassiti della fauna selvatica a vita libera (Aula 111)
Moderatori: Nicola Ferrari, Pier Giuseppe Meneguz

A. Michelutti, V. Cagnin, S. Pasqualotto, D. Vio, D. Dellamaria, K. Trevisiol, F. Obber, C. Citterio, P. Danesi. *Trichinella britovi* and wildlife: epidemiological situation in north-east Italy.

A. Di Blasio, S. Robetto, S. Zoppi, S. Gallina, E. Ferroglio, R. Orusa, L. Rossi. The long-term prevalence trend of sylvatic trichinellosis in NW Italy.
C. Romeo, A. Cafiso, E. Fesce, F.J. Martinez-Rondan, P. Lanfranchi, N. Ferrari. Disease threats and invasive species: helminths infecting raccoons introduced to Italy.

L. Di Renzo, G. Di Francesco, E. Marchiori, C.E. Di Francesco, V. Olivieri, A. Cocco, C. Tessarin, F. Marcer, I. Pascucci. Severe case of spirorchidiasis in a loggerhead sea turtle (*Caretta caretta*) from Adriatic Sea.

T. Trogu, N. Formenti, N. Ferrari, S. Bellometti, L. Pedrotti, L. Corlatti, P. Lanfranchi. Parasitological community of red deer (*Cervus elaphus*): effects on population and reproduction.

N. Formenti, T. Trogu, S. Bellometti, A. Gugiatti, L. Pedrotti, A. Gaffuri, P. Lanfranchi, N. Ferrari. *Toxoplasma gondii* in naturally infected red deer (*Cervus elaphus*): spread, infection dynamics and effects on host behavior.

G. Grandi, L. Chitimia-Dobler, P. Wilhelmsson, P.E. Lindgren, B. Olsen. Ticks on migratory birds: results from seven bird stations in Sweden.

M. Salvetti, M. Marangi, A. Bianchi, I. Bertoletti, L. Roy, A. Giangaspero. Microscopy and molecular investigation on *Lipoptena* (Diptera: Hippoboscidae) circulating in wild animal species in Italy.

E. Kariuki, L. Kariuki, M. Maloba, H. Kutima, D. Masiga, L. Musila, M. Montagna, S. Alali, D. Sassera, I. Horak. Ixodid ticks (Acari: Ixodidae) of rhinoceroses in Kenya: new tick-host associations and updated ecological distribution.

ORE 10.30-11.00

Coffee break

ORE 11.00-13.00

Tavola rotonda 3: Parassiti e microbiota (Aula Magna)
Moderatori: Bandi C., Matteo Brilli

"Parasites - Friends or Foes of a Healthy Immune System?" **Henry McSorley** (University of Edinburgh, UK).

"The hygiene hypothesis and the microbiota-diabetes connection" **Paolo Fiorina** (Università degli Studi di Milano, Ospedale Fatebenefratelli-Sacco-Melloni, Milano).

"Parasitome going into microbiome" **Lorenza Putignani** (Ospedale Pediatrico Bambin Gesù, Roma).

Tavola rotonda 4: Parassiti, benessere e produzioni animali (Aula 102)
Moderatori: Laura Rinaldi, Mario Pietrobelli

"How can we stop parasites from becoming a major welfare issue on tomorrow's farms?" **Eric Morgan** (Queen's University Belfast, UK).

"How parasites can influence the welfare and production of ruminants?" **Antonio Scala e Salvatore Naitana** (Università degli Studi di Sassari).

"Socio-economic aspects of parasite control in livestock" **Edwin Claerebout** (Ghent University, Belgium).

"How gastro intestinal nematodes can influence the quantitative and qualitative milk production in small ruminants?" **Giuseppe Cringoli** (Università degli Studi di Napoli, Federico II).

ORE 13.00-14.00

Pranzo

ORE 14.00-14.20

"Post-Authorization Safety Assessment of Letifend® Revaccination in the Field: Pilot Study in Spain" **Guadalupe Mirò** (Università Complutense di Madrid, Spain) (Aula 102).

ORE 14.20-16.00

Tavola rotonda 5: La strada verso l'eradicazione della malaria: sfide e successi (Aula Magna)
Moderatori: Donatella Taramelli, Sara Epis, Sarah D'Alessandro

"Malaria eradication versus drug resistance evolution: which one wins?" **Tim Anderson** (Biomedical Research Institute, San Antonio, Texas, USA).

"New tasks and tools to block the human-to-mosquito transmission of *Plasmodium falciparum*" **Pietro Alano** (Istituto Superiore di Sanità, Roma).

"The challenge of malaria eradication in real life" **Francesco Castelli** (Università degli Studi di Brescia).

Sessione 8: Parassitosi trasmesse da artropodi (Aula 102)
Moderatori: Domenico Otranto, Guadalupe Mirò

F. Veronesi, A. Santoro, T. Di Muccio, V. Stefanetti, F. Passamonti, M. Diaferia, M. Gramiccia. Emerging feline vector-borne infections in central Italy.

E. Battisti, S. Zanet, B. Hertel, A. Trisciuoglio, S. Bruno, E. Ferroglio. Prevalence of vector-borne pathogens in wild carnivores from Piedmont region.

L. Ciuca, F. Simon, R. Morchon, L. Kramer, M. Genchi, D. Acatrinei, C. Roman, M.P. Maurelli, G. Cringoli, L. Rinaldi. Dirofilariosis: prevalence and clinical relevance in dogs and humans in Eastern Europe.

S.A. Zanzani, E. Olivieri, E. Pintore, G. Garippa, A.L. Gazzonis, L. Villa, V. Melosu, A. Scanu, N. Columbano, E. Sanna Passino, M.T. Manfredi. A serological study of exposure to tick-borne pathogens in donkeys from Asinara island.

L. Villa, A.L. Gazzonis, C. De Maria, M.F. Persichetti, G. Caracappa, S. Caracappa, F. Vitale, S.A. Zanzani, E. Olivieri, M.T. Manfredi. Seroprevalence of selected equine vector-borne diseases in horses reared in Northern Italy.

S. Zanet, M. Blanc, E. Battisti, A. Trisciuoglio, C. Trentin, M. Ragionieri, E. Ferroglio. A region-wide survey in Aosta Valley for ticks and tick-borne pathogens.
S. Buezo Montero, P. Gabrieli, F. Severini, L. Picci, M. Di Luca, F. Forneris, L. Facchinelli, M. Ponzi, F. Lombardo, B. Arcà. Toward the development of serological markers of human exposure to *Aedes* mosquitoes: analysis of *Aedes albopictus* salivary antigens in a murine model.

E. Martin, I. Varotto Boccazzi, Y. Corbett, L. Sacchi, G. Bongiorno, N. Ferrari, L. Gradoni, I. Ricci, C. Bandi, S. Epis. The association between the killer yeast *Wickerhamomyces anomalus* and the sand fly *Phlebotomus perniciosus*: a potential tool for the control of leishmaniasis.

Sessione 9: Epidemiologia delle malattie parassitarie 2 (Aula 111)
Moderatori: Laura Kramer, Vincenzo Veneziano

B. Morandi, G. Conboy, G. Poglayen, J. Vanleeuwen. Risk factors associated to endoparasites in dogs and cats at Prince Edward Island (Canada).

S. Morelli, E. Grillotti, I. Russi, S. Manzocchi, P. Beraldo, A. Viglietti, P.E. Crisi, C. Pezzuto, C. De Tommaso, F. Pampurini, D. Traversa. Large scale survey on the occurrence of canine and feline extra-intestinal nematodes in Italy.

V. Colella, C. Maia, A. Pereira, L. Cardoso, I. Scandale, F. Dantas-Torres, D. Otranto. Research needs to tackle zoonotic onchocercosis caused by *Onchocerca lupi*.
G. Dessi', C. Tamponi, A. Varcasia, A.P. Pipia, F. Barraqueddu, S. Visco, G.P. Sedda, S. Carta, A. Scala. Sero-epidemiological survey on canine leishmaniosis in Sardinia, Italy.

C. Tamponi, G. Dessi', A. Varcasia, S. Pinna, G.P. Sedda, S. Carta, A. Scala. Seroepidemiology of Toxoplasmosis in sheep and goats cohabiting in Sardinia (Italy).

L. Guardone, F. Lion, G. Terracciano, M. Carminati, M.O. Endidi, M. Maaroufsidatt, M.A.O. Barka, S. Di Lello, M. Scacchia, R. Cassini. Epidemiological study on endoparasites of one-humped camels, sheep and goats in the Tiris-Zemmour region, Mauritania.

C. De Maria, M.F. Persichetti, V. Blanda, G. Caracappa, A. Torina, S. Caracappa. Seroprevalence of equid piroplasmosis in Italy.

S.A. Zanzani, A.L. Gazzonis, V. Lippolis, L. Villa, M.T. Manfredi. Seasonal variation and survival of *Parascaris equorum* eggs in soil.

ORE 16.00-16.20

Coffee break

ORE 16.20-18.00

Tavola rotonda 6: Parassiti del cane in Italia, cos'è cambiato? (Aula Magna)
Moderatori: Domenico Otranto, Maria Teresa Manfredi

"Indagine sulle parassitosi interne del cane in Italia: un gioco di squadra" **Emanuele Brianti** (Università degli Studi di Messina).

"Parassitosi intestinali" **Laura Rinaldi** (Università degli Studi di Napoli, Federico II).

"Parassitosi extra-intestinali" **Angela Di Cesare** (Università degli Studi di Teramo).

"Analisi degli schemi terapeutici e di profilassi: possiamo fare di meglio?" **Ezio Ferroglio** (Università degli Studi di Torino).

Sessione 10: Malattie trasmesse da artropodi vettori all'uomo (Aula 102)
Moderatori: Nicoletta Basilico, Spinello Antinori, Chiara Bazzocchi

S. Ravagnan, F. Toniolo, G. Da Rold, E. Porcellato, C. Falcaro, P. Danesi, F. Montarsi, G. Capelli. A retrospective analysis on the occurrence of *Borrelia miyamotoi* in *Ixodes ricinus* ticks in North-East Italy, 2007-2017.

G. Bongiorno, T. Di Muccio, R. Bianchi, M. Gramiccia, L. Gradoni. Laboratory transmission of a central Asian *Leishmania tropica* by the bite of the western European sand fly *Phlebotomus perniciosus*.

D. Scaccabarozzi, I. Varotto-Boccazzi, E. Martin, S. Villani, S. Zava, L. Cavicchini, S. Delbue, I. Colombo, D. Taramelli, S. Epis, N. Basilico, Y. Corbett. *Leishmania tropica* infection induces immune responses through NOD2 pathway.

I. Varotto Boccazzi, Y. Corbett, R. Nodari, M. Perini, L. Gradoni, C. Bandi, S. Epis. *Asaia* bacteria engineered to express the *Wolbachia* surface protein induce a Th1 polarization. Implications for the control of leishmaniasis.

S. D'Alessandro, N. Basilico, V. Messina, R. Nodari, F. Silvestrini, D. Taramelli, Y. Corbett. A cellular method to measure phagocytosis of *Plasmodium falciparum* gametocytes by bone marrow macrophages.

S. Paone, M. Chaand, S. D'Alessandro, S. Parapini, F. Celani, M. Pourshaban, V. Tirelli, M. Ponzi, M. T. Duraisingh, A. Olivieri. The human GTPase Rac1 plays an important role in *Plasmodium falciparum* invasion and growth inside human erythrocytes.
R. Nodari, Y. Corbett, A. Negri, I. Varotto Boccazzi, N. Basilico, S. Parapini, D. Taramelli, S. Epis, C. Bandi. Use of efflux pump inhibitors in *Plasmodium falciparum*, to increase drug efficacy.

N. Basilico, J. Konstantinović, S. D'Alessandro, D. Scaccabarozzi, M. Videnović, K. Bogojević, N.T. Jovanović, S. Orsini, L. Gradoni, B.A. Šolaja. Activity of novel aminoquinoline derivatives against *Leishmania infantum*.

Sessione 11: Parassiti della fauna acquatica (Aula 111)
Moderatori: Letizia Fioravanti, Simonetta Mattiucci

P. Merella, G. Garippa, C. Burreddu, S. Mele, U. Luzzana, A. Born-Torrijos, J.F. Palacios-Abella, G.S. Van Beest, J.A. Raga, F.E. Montero. Trematode infections in cultured gilthead seabream *Sparus aurata* L. (Sparidae) from the Mediterranean Sea: pathways and associated threats for aquaculture.

E.V. Dmitrieva, D. Sanna, M.C. Piras, G. Garippa, P. Merella. Combining morphology and genetics to resolve the status of a monogenean from the taxonomic "waste-basket" *Haliotrema* Johnston & Tiegs (Ancyrocephalidae).

M. Palomba, A. Colantoni, M. Paoletti, G.L. Sbaraglia, G. Nascetti, S. Mattiucci. Expression profiles of three relevant genes in *Anisakis pegreffii* larvae (Nematoda: Anisakidae) cultured in vitro, and from experimentally infected fish.

A. Gustinelli, M. Caffara, V. Menconi, M.L. Fioravanti. Risk assessment on the presence of zoonotic parasites in freshwater and marine fish farmed in Italy.

S. Mattiucci, G.L. Sbaraglia, S. Filippi, P. Cipriani, G. Nascetti. Insights into the life-cycle of the two sibling species of the *Contracaecum rudolphii* Hartwich,1964 (sensu lato) complex (Nematoda: Anisakidae) from Central Italy.

L. Giulietti, A. Levsen. M. Paoletti, D.H. Grevskott, M. Bao, P. Cipriani, S. Mattiucci. First molecular identification of the fish myoliquefactive parasite *Kudoa thyrsites* (Myxosporea, Multivalvulida) in *Lepidopus caudatus* from the Alboran Sea (Mediterranean Sea).

ORE 20.30

Cena sociale (Osteria Del Binari - Milano)

VENERDI' 29 Giugno 2018

ORE 8.30-10.30

Tavola rotonda 7: Parassitosi in apicoltura: attualità epidemiologiche e prospettive per il controllo (Aula Magna)
Moderatori: Michele Mortarino, Ezio Ferroglio

"The five Ws about *Nosema ceranae*" **Aranzazu Meana** (Universidad Complutense de Madrid, Spain).

"Identification and quantification of *Lotmaria passim* (Trypanosomatidae) in investigation of its prevalence, annual dynamics and relationship with *Nosema ceranae* (Microsporidia)" **Jevrosima Stevanovic** (University of Belgrade, Serbia).

"How to enhance performances of organic acids against *Varroa destructor* through application of Good Beekeeping Practices" **Giovanni Formato** (Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Roma).

Sessione 12: Controllo e monitoraggio dei vettori (Aula 102)
Moderatori: Luigi Gradoni, Fabrizio Montarsi

L. Tawe, P. Ramatlho, T. Kgoroebutswe, K. Waniwa, C.W. Muthoga, D.S. Ntebela, M. Pombi, N.N Makate, G.M. Paganotti. Preliminary data on *Anopheles* species distribution in Botswana.

F. Gradoni, S. Carlin, G. Da Rold, S. Ravagnan, F. Russo, M. Palei, S. Martini, M. Di Luca, G. Capelli, F. Montarsi. Occurrence of potential malaria vectors in north-east Italy.

S. Carta, L. Cavallo, V.D. Tarallo, C. Tamponi, S. Visco, G. Tosciri, A.P. Pipia, P.A. Cabras, G. Dessì, C. Gai, A. Varcasia, A. Scala. Updates on Phlebotomine sand flies in Sardinia (Italy).

E. Napoli, F. Arfuso, G. Gaglio, S. Giannetto, E. Brianti. Are dogs living on different level floors equally exposed to sand fly bites?

B. Caputo, M. Manica, F. Filipponi, P. Cobre, C.M. De Marco, L. Iesu, V. Petrella, M. Blangiardo, R. Rosa', C. Bianchi, M. Salvemini, A. Della Torre. Pilot validation of mosquito nuisance assessment by ZanzaMapp, a mobile application to involve citizen in mosquito monitoring.

G. Gaglio, E. Napoli, F. Arfuso, J. Abbate, S. Giannetto, E. Brianti. Attractiveness of different coloured LEDs for phlebotomine sand fly monitoring.

V. Pichler, C. Malandruccolo, R. Bellini, D. Arnoldi, A. Rizzoli, F. Severini, L. Toma, M. Di Luca, R.P. Lia, D. Otranto, F. Montarsi, S. Carlin, M. Ballardini, A. Pautasso, G. Triglia, P. Serini, A. Della Torre, B. Caputo. Pyrethroid susceptibility status of *Aedes albopictus* and *Culex pipiens* populations across Italy.

F. Montarsi, P. Visentin, A. Drago, S. Carlin, A. Della Torre, G. Capelli, M. Pombi. Comparative testing of two sticky traps to monitor resting *Aedes albopictus* and *Aedes koreicus* (Diptera; Culicidae) in Italy.

A. Negri, M. Ferrari, R. Nodari, I. Varotto Boccazzi, E. Martin, V. Mastrantonio, S. Urbanelli, D. Porretta, C. Bandi, S. Epis. Gene silencing in the malaria vector *Anopheles stephensi* to increase insecticide susceptibility.

Sessione 13A: Alimenti e parassiti (Aula 111)
Moderatori: Antonio Frangipane di Rigalbono, Sergio Zanzani, Stefania Perrucci

M.A. Gomez Morales, G. Della Casa, E. Licata, G. Merialdi, A. Amati, G. Rugna, S. Cherchi, D. Tonanzi, M. Ramini, G. Marucci, M. Interisano, A. Ludovisi, V. Faeti, E. Pozio. Long term study on *Trichinella* muscle larvae and circulating IgG in pigs.

L. Guardone, N. Rosellini, D. Nucera, L. Tinacci, P. L. Acutis, A. Guidi, A. Armani. Occurrence of *Anisakis* spp. larvae in products made of herring (*Clupea harengus*).

M. Marangi, R. Papini, M. Cereda, F. Ferrara, G. Normanno, A. Giangaspero. Lab-on-chip molecular integrated platform to detect *Toxoplasma gondii* from several matrixes.

A.L. Gazzonis, A.M. Marino, R. Giunta, R. Conti, G. Garippa, L. Rossi, W. Mignone, L. Villa, S.A. Zanzani, M.T. Manfredi. Seroprevalence of *Toxoplasma gondii* infection in beef cattle raised in Italy: a multicenter study.

Sessione 13B: Entomologia medica e veterinaria (Aula 111)

R. Gherbi, M. Bounechada, M.S. Latrofa, G. Annoscia, V.D. Tarallo, F. Dantas-Torres, D. Otranto Monitoring sand fly populations in Setif: a new focus of cutaneous leishmaniasis in Algeria.

A. M. Floriano, E. Olivieri, A. Cafiso, E. Kariuki, D. Di Carlo, M. Pajoro, R. Matteri, S. Montanaro, C. Bazzocchi, D. Sassera. Molecular screening of pathogenic and symbiotic bacterial species in African ticks.

E. Olivieri, I. Varotto Boccazzi, C. Romeo, A. Desirò, A. Cafiso, V. Serra, A.M. Floriano, S. Epis, D. Sassera. *Midichloria mitochondrii* localization and quantification in the organs of the hard tick *Ixodes ricinus*.

M. Pombi, R.P. Lia, S. Latrofa, S. Manzi, R. Panarese, F. Beugnet, J. Fourie, D. Otranto. Field survey on *Phortica variegate* and the high infection rate of *Thelazia callipaeda* in Lazio and Basilicata regions.

G. Da Rold, S. Ravagnan, S. Carlin, B. Flaminio, T. Lilja, C. Silaghi, S. Ormelli, G. Capelli, F. Montarsi. Creation of a reference sample-collection for morphological and biomolecular identification of arthropods of medical and veterinary importance.

ORE 10.30-11.00

Coffee break

ORE 11.00-12.30

Sessione 14: Parassitosi degli animali da reddito (Aula Magna)
Moderatori: Giuseppe Cringoli, Antonio Scala

R.P. Lia, A. Sazmand, M. Mirzaei, Y. Ghahvei, M. Golchin, E. Lefoulon, C. Martin, D. Otranto. *Onchocerca fasciata* (Filariodea: Onchocercidae) in camels (*Camelus dromedarius*) from Iran: morphological and molecular characterization.

L. Villa, A.L. Gazzonis, S. Mazzola, S.A. Zanzani, C. Perlotti, G. Sironi, M.T. Manfredi. Investigating on *Besnoitia besnoiti* (Apicomplexa, Sarcocystidae) in naturally infected dairy cattle by an integrated approach.

A. Habluetzel, F. Pagliacci, L. Pacifici, S. Casabianca, A. Vantini, A. Cudini, M. Russo, C. Bisci, S. Pallotti, E. Prenna, F. Esposito. Impact of the 2016 earthquake in Central Italy on livestock farms and effects of tensile emergency shelters on animal health.

L. Rossi, L. Rambozzi, F. Chiesa, S. Rubiola, V. Dermauw, R. Piovano, P. Dorny. Multi-test monitoring of a “Cysticercosis storm”: a case report.

B. Paoletti, D. Traversa, R. Cassini, A. Frangipane Di Regalbono, I. Moretta, A. Di Cesare, A. Mauti, F. La Torre, E. De Angelis, F. Veronesi. Endoparasites of livestock in central italy.

F. Castagna, D. Britti, A. Bosco, A. Poerio, M. De Alcubierre, G. Cringoli, V. Musella. Use of *Punica granatum* extract for the control of gastrointestinal nematodes in sheep.

Sessione 15: Terapia e farmacoresistenza (Aula 102)
Moderatori: Donato Traversa, Fabrizia Veronesi

F. Buono, C. Roncoroni, L. Pacifico, D. Piantedosi, B. Neola, A. Fagiolo, V. Veneziano. Efficacy of major anthelmintics against *Cyathostominae* in donkeys.

S. Carta, A. Varcasia, C. Tamponi, A. Corda, F. Nonnis, L. Tilocca, M.P. Meloni, G. Dessì, A. Scala. Chronic lethal peritonitis secondary to Mesocestodiasis in a dog from Sardinia (Italy).

M. Marangi, K. Bartley, H. Wright, A. Giangaspero, L. Roy, A. Nisbet. Towards the molecular characterization of voltage gene sodium channel in *Dermanyssus gallinae* isolates.

D. Traversa, E. Grillotti, C. De Tommaso, S. Morelli, P.E. Crisi, E. Di Giulio, C. Pezzuto, L. Venco, F. Pampurini. Field efficacy of Advocate® (Bayer Animal Health) in the treatment of dogs naturally infected with *Angiostrongylus vasorum*.

E. Facchini, M. Zetti, L. Colombari, M.E. Andreis, M. Di Giancamillo, R. Rizzi, M. Mortarino. Exploring tolerability and efficacy of formic acid for the control of *Varroa destructor* in honeybees: standard and alternative approaches.

ORE 12.30-13.00

Saluti e arrivederci al XXXI Congresso SOIPA (Aula Magna)

ELENCO POSTER

P1 E. Battisti, D. Ranucci, F. Chiesa, S. Zanet, E. Ferroglio, F. Veronesi. *Toxoplasma gondii* DNA in ewe's milk from Umbria region.

P2 G. Cammilleri, A. Costa, S. Graci, M.D. Buscemi, R. Collura, G. Giangrosso, V. Ferrantelli. First survey on the presence of anisakid parasites in farmed European sea bass and gilthead sea bream produced and marketed in Sicily

P3 G. Vinchesi, B. Pinto, C. Mangia, F. Bruschi, S. Piaggi. Glutathione trasferase omega -1 (GSTO-1) and *Toxoplasma gondii* infection.

P4 C. Lucchetti. Synergism of ML/doxycycline adulticide effect: an in vitro study

P5 S. Gaiarsa, A. Cafiso, L. Baker, G. Capron, R. Daveu, G. Batisti Biffignandi, O. Plantard, C. Bazzocchi, A.R. Jex, D. Sassera. Digging deep into intramitochondrial symbiosis: dual transcriptomics of the hard tick *Ixodes ricinus* and its bacterial symbiont *Midichloria mitochondrii*

P6 F. Spairani, L. De Marco, S. Epis, A. Capone, G. Chiappa, J. Bozic, E. Crotti, M. Perini, C. Bandi, I. Ricci, D. Sassera. Phylogenomic analysis of members of the *Meyerozyma guilliermondii* species complex.

P7 G. Barbieri, C. Ferrari, E. Ursino, P. Gabrieli, S. Mamberti. G. Radaelli, E. Clementi, L. Sacchi, G. Gasperi, D. Sassera, A.M. Albertini. Identification of a novel *Brevibacillus laterosporus* strain with insecticidal activity against *Aedes albopictus* larvae.

P8 B. Flaminio, F. Montarsi, M. Barbujani, M. Mazzucato, N. Ferrè', A. Granato, F. Mutinelli Assessing the presence and spread of *Vespa velutina* in north-east Italy through a surveillance program.

P9 F. Fois, P. A. Cabras, J. Culurgioni, D. Scaravelli, P. Orrù, G. Garippa, P. Merella, D. Cillo, S. Rolesu, S. Cappai. Preliminary data on the presence of *Hippobosca longipennis* Fabricius, 1805 (Diptera: Hippoboscidae) in Sardinia.

P10 P. Masini, S. Zampetti, F. Biancolini, G. Miñón Llera, I. Moretta. Preventive inspections in a bus company with detection dog units trained to detect bedbugs (*Cimex lectularius*)

P11 S. Zampetti, P. Masini, F. Biancolini, A.G. Miñón Llera. A case of infestation of tropical rat mites *Ornithonyssus bacoti* (Acari: Macronyssidae) in Rome, Italy.

P12 M. Pombi, A. Giacomi, G. La Marca, M.S. Latrofa, T. Di Muccio, M. Gramiccia, D. Otranto, S. Gabrielli. Unexpected transmission patterns of *Leishmania infantum* and *L. tarentolae* involving *Sergentomyia minuta* and humans in Lazio region.

P13 M. Calzetta, M. Pombi, S. Fidati, W.M. Guelbeogo, H. Ranson, N. Sagnon, A. Della Torre. *Anopheles funestus* exophilic behaviour associated to high sporozoite rates following LLIN distribution in a village of Burkina Faso.

P14 S. Parapini, R. Grande, M.R. Gismondo, D. Taramelli, N. Basilico. Cultivation, gametocytes production and drug sensitivity of *Plasmodium falciparum* isolates from malaria patients.

P15 M. Di Luca, L. Toma, A. Amendola, M.E. Remoli, F. Severini, D. Boccolini, R. Romi, G. Rezza, G. Venturi, C. Fortuna. *Chikungunya virus* ECSA strains, with and without the E1:A226V mutation: study of vector competence of *Aedes albopictus*.

P16 G. Angeloni, Z. Pasquini, B. Canovari, F. Barchiesi, M. Agostini, D. Boccolini, M. Menegon, L. Gradoni, R. Romi, C. Severini, M. Conquista, S. Gavaudan, E. Antognini. An autochthonous malaria case due to *Plasmodium ovale curtisi* in Central Italy.

P17 M. Pajoro, D. Pistone, N. Vicari, S. Peli, S. Rigamonti, R. Viganò, L. Colombo, F. Riccardi. Survey on ticks and bacterial tick-borne pathogens: Ossola valley, North-West Italy, (2010-2017).

P18 M. Ferreira-da-Cruz, L. Rodrigues Gomes, A. Lavigne, C. Leonel Peterka, P. Brasil, D. Ménard, C.T. Daniel-Ribeiro. Absence of K13 polymorphism in *Plasmodium falciparum* parasites from brazilian endemic area.

P19 L. Amato, C. Tessarin, G. Lynen, G. Di Giulio, R. Cassini. Epidemiological investigation on bovine piroplasmoses in Tanga Region, Tanzania.

P20 M.P. Maurelli, L. Rinaldi, V. Caruso, V. Foglia Manzillo, G. Oliva, G. Cringoli, M. Gizzarelli. Canine leishmaniosis and filariosis in the Molise region, southern Italy.

P21 V. Mangano, A. Marvelli, L. Bargagna, G. Moscato, F. Bruschi. Risk of transfusion transmitted Chagas disease: screening of candidate blood donors at Pisa University Hospital.

P22 D. Ianniello, M.P. Maurelli, A. Bosco, P. Pepe, R. Vascone, A. Amadesi, G. Cringoli, L. Rinaldi. Mini-FLOTAC is able to detect lungworm larvae in cats, dogs, hedgehogs and sheep.

P23 M. Santoro, F. Berrilli, C. Glé, D. Di Cave, V. Di Cristanziano, R. D'Alfonso. Role of *Entamoeba* spp. in enteric infections in Côte d'Ivoire.

P24 S. Gabrielli, V. Mangano, R. Poscia, P. Fazii, F. Bruschi S. Mattiucci. Molecular identification of three cases of human dirofilariosis due to *Dirofilaria repens* from Central Italy.

P25 S. Pane, L. Romano, G. Foglietta, C. Severini, M. Menegon, S. Bernardi, T. K. Hyppolite, P. Palma, A. Onetti Muda, L. Putignani. Diagnosis of perinatal malaria in the globalization era.

P26 A. Zbriger, A. Gavazza, G. Fichi, V. Marchetti, G. Rocchigiani, F. Mancianti, S. Perrucci. Intestinal parasites in dogs affected by oncological diseases.

P27 F. Sauda, L. Malandrucco, C. De Liberato, S. Perrucci. Prevalence of gastrointestinal parasites in shelter cats of central Italy.

P28 G. Simonato, A. Frangipane Di Regalbono, G. Dotto, P. Danesi, C. Tessarin, D. Pasotto, M. Pietrobelli. Surveillance of zoonotic parasites in assisted therapy animals.

P29 M.C. Guerrero M., M.C. Martínez Ortiz De M., J.C. Morales L. Case report: presence of *Dermanyssus gallinae* in Mexican canaries (*Serinus canaria*).

P30 M. Parigi, F. Ardolino, M. Falletta, D. Petrini, S. Rota, L. Rinaldi, L. Dipineto, P. Massi. Detection of *Giardia duodenalis* in pets in Italy.

P31 M.P. Maurelli, L. Rinaldi, A. Bosco, M.E. Morgoglione, M. Santaniello, D. Ianniello, A. Amadesi, G. Cringoli. Helminth infections in hunting, stray and sheep dogs in the Molise region, southern Italy.

P32 D. Traversa, L. Della Salda, A. Diakou, C. Sforzato, M. Romanucci, S. Morelli, A. Frangipane Di Regalbono, R. Cassini, G. Simonato, R. Iorio, V. Colaberardino, A. Di Cesare. Fatal patent troglostrongylosis in a litter of kittens.

P33 S. Gabrielli, E. Brianti, F. Furzi, G. Gaglio, E. Napoli, S. Mattiucci. Molecular epidemiology of *Blastocystis* in domestic and farmed animals in Italy: preliminary results.

P34 B. Morandi, G. Conboy, G. Poglayen, J. Vanleeuwen. VTH (Veterinary Teaching Hospital), AVC (Atlantic Veterinary College), Prince Edward Island (PEI), Canada: an overview of eighteen years of parasitology traffic.

P35 D. Boccolini, M. Menegon, M. Di Luca, L. Toma, F. Severini, M. L'Episcopia, A. Caraglia, S. D'Amato, F.P. Maraglino, R. Romi, L. Gradoni, C. Severini. Malaria surveillance in Italy: a public health topic of relevance.

P36 G. Iraci Sareri, L. Chiarenza, R. Galuppi, G. Poglayen. Parasites in cage and aviary birds: an update?

P37 I. Pulitano', G. Poglayen, M.P. Tampieri, R. Galuppi. Canine faecal contamination and parasitic risk in the city of Bologna.

P38 D. Traversa, A. Di Cesare, E. Gueldner, F. Veronesi, S. Morelli, P.E. Crisi, F. Pampurini, M. Schnyder. Seroprevalence of the cat lungworm *Aelurostrongylus abstrusus* in central Italy.

P39 A.L. Gazzonis, S.A. Zanzani, L. Villa, A. Vetere, M.T. Manfredi. Parasitic contamination in off-leash fenced dog areas soil: an underestimated zoonotic risk?

P40 R. Galuppi, L. Balboni, M.P. Tampieri, G. Poglayen. Keratinophylic fungi in off-leash areas soil of Bologna (Northern Italy) **.**

P41 R. Galuppi, G. Neretti, A.C. Ricchiuto, C.A. Borghi, G. Poglayen, B. Morandi. The fourth state of matter chapter II: biocidal effect of plasma against yeast.

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SESSIONE PLENARIA

CAMBIO D'USO DEL TERRITORIO ALPINO

The accelerated down wasting of the Alpine glaciers. A clear sign of climate change

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The worldwide retreat of glaciers in the course of the last few decades is frequently considered as a clear and unambiguous sign of climate change and global warming. The glaciers of the European Alps are showing general mass loss and shrinkage since the end of the Little Ice Age (around 1850). During the 1850-1980 period most of Alpine glaciers lost about one third of their area and since 1980 until today more than another one third of their ice has melted. This severe melting rate is driven by important changes occurring in mid-tropospheric conditions such as the rapid temperature increase during the last few decades and cannot be explained only by natural climate variability. Therefore glacier variations in geometry could be really recognized as key features for detection of the so called "climate change" and of its impacts on the environment. Glaciers not only represent meaningful indicators of climate changes, they are also precious landscape elements (a landscape very rich in geodiversity and in biodiversity, as well) and valuable freshwater, energy and tourist resources. Accelerated glacier down wasting is well appreciable from the results of recent data analysis, based on mass balance surveys (from field and remote sensing measurements). More than one-fifth of the Alpine glaciation is located on the Italian side of the Alps (an area of 368 km^2 according to the 2015 New Italian Glacier Inventory); a total non-negligible value compared to the Alps as a whole (about 2000 km^2). The comparison with previous data is often limited by the different sources, methodologies, techniques applied in compiling data records; anyway, the general trend emerges clearly and makes it possible to draw a general picture of the changes in Italian glaciers that have taken place in the past decades. According to the inventory of the Italian Glaciological Committee (1959-1962), the total surface of the Italian glaciers reached 527 km^2. The comparison with the total datum of the new inventory suggests a surface reduction of about 30% from the half of the last century. The glacier shrinkage on the Italian Alps (not only!) is accelerating in the last years and is going to change deeply the mountain landscape. In the next decades the Alps are expected first to show features and forms now visible for example on the Pyrenees, where the glaciation is today the relic of the previous one and is only formed by small cirque glaciers; in a second phase the Alps could resemble the Apennines, where no real glacier now can be found. In other words the Italian Alps are undergoing a very strong and rapid transition from a glacial system to a paraglacial system, more accelerated respect to the other Alps sides, due to their limited glacier dimension (average surface 0,40 km^2) and glacier types (mainly mountain glaciers and glacierets). It means that the areas, where in the recent past the main landscape shaping factors were glaciers, are now subject to the action of melting waters, slope evolution, periglacial processes. With such a rapid evolution of mountain slopes, the surviving glacier surfaces are hosting a wider and thicker debris cover and the glacier forefields are strongly enlarging. It follows that glaciers and glacier forefields are showing rapid and strong evolution, not only from the abiotic components (glacial and periglacial morphologies), but also from the biotic components (plants, vegetation, arthropod and bacterial communities, among the others). Regarding for instance the debris covered glaciers (glaciers with the ablation zone covered by a debris layer, usually capable to descend below the climatic tree line), they could be considered potential warm-stage refugia for cold-adapted biotic features (plant and arthropod communities). Furthermore, in the glacier forefield the abiotic evidences of climate change (the strong ice mass loss) seem related with change in vegetation, which indicates an acceleration of colonization rates.

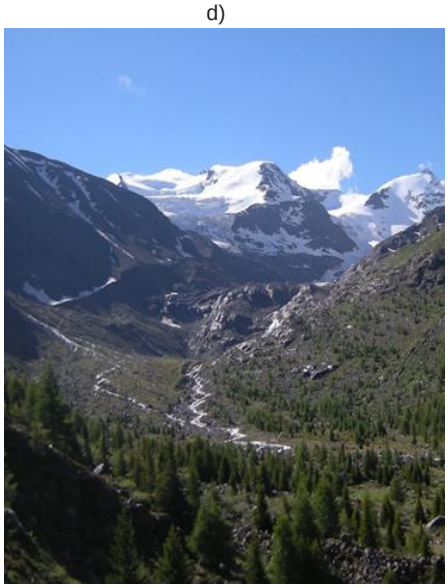
Fig. 1: The shrinkage of the Forni Glacier (Alta Valtellina), one of the largest ice body of the Italian Alps: a) 1890 (photo by V. Sella; b) 1929 (photo by G. Mentasti; c) 1947 (photo by A. Desio); d) 2017 (photo by C. Smiraglia)

a)

b)

c)

d)



Microbial communities of glaciers and climate change

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Keywords: cryoconite holes, chlorpyrifos, Forni Glacier, metagenome

INTRODUCTION. Glaciers and ice sheets have been recently recognized as an independent terrestrial biome that hosts ecosystems dominated by microorganisms (Anesio and Laybourn-Parry, 2012, Trends Ecol. Evol. 27:219–225). In these environments, peculiar structures, called 'cryoconite holes', form where a fine-grained wind-borne sediment accumulates in small depressions and locally decreases the albedo promoting the underlying ice melting. These structures are considered hot spots of biodiversity in glacier environments because the ponds are filled by the melt water and host metabolically active ecological communities that can include bacteria, algae, viruses, rotifers, tardigrades, nematodes and collembola (Cook et al. 2016, Prog. Phys. Geogr. 40:66–111). The global shrinkage of the cryosphere due to global warming is affecting the extent of these communities in ways that have only started to be investigated. In particular, the response of ice microbial communities can trigger feedback mechanisms that can improve ice melting and therefore exacerbate the impact of climate change on ice environments. For instance, increased growth of ice microbes promoted by enhanced ice melting may darken the ice surface, reduce albedo and promote further ice melting (Tedstone et al., 2017, Cryosph., 11:2491-2506).

Improved ice melting can also determine an increased release from the glaciers of substances that have been scavenged from the atmosphere during snow deposition. This process raises particular concern when enhanced glacier melting determines increased release of contaminants stocked in the ice for decades (Villa et al., 2003, J. Atmos. Chem., 46:295–311). Bacterial communities of ice environments, particularly those of cryoconite holes, affect also these processes because of their metabolic versatility, which allows them to metabolize even the most recalcitrant substances.

The studies we present aim at investigating the bacterial communities of cryoconite holes of the Forni Glacier (Italian Alps), focusing on their seasonal variability and their metabolic activity, with a particular focus on the metabolic pathways that may allow them to act as a "biofilter" for organic pollutants on glaciers by accumulating them and promoting their biodegradation, thus significantly contributing to their removal.

MATERIALS AND METHODS. Samples used for the analyses of the temporal variation of the cryoconite microbial communities were collected aseptically on Forni Glacier (46°24'00'' N, 10°35'30'' E; elevation 2600-3670 m a.s.l.) during the ablation seasons (July-September) of 2012, 2013, 2015 and 2016. Sample collection, storage and processing were performed according to the procedures described in Franzetti et al. (2017, Environ. Microbiol. Rep. 9:71–78). Briefly for the description of the structure of the bacteria communities, Illumina sequencing of 16S rRNA gene was carried.

To investigate the metabolic functions of the microbial communities in cryoconite, we used whole metagenomic sequencing of cryoconite samples collected in 2014 as described in Franzetti et al. (2016, ISME J., 10:2984–2988). We focused, in particular, on marker genes for photosynthesis, use of inorganic and organic compounds, including pollutants, as energy source, and autotrophy/heterotrophy.

Degradation of pollutants was investigated by installing *in situ* microcosms on Forni glacier as described in Ferrario et al. (2017, Environ. Pollut. 230:919–926). In particular, we focused on the degradation the organophosphorus insecticide chlorpyrifos (CPF), a xenobiotic tracer that

accumulates on glaciers after atmospheric medium- and long-range transport. Experiments were performed in 2015 on the Forni Glacier. Microcosms were prepared in pyrex bottles with local cryoconite and meltwater under four experimental conditions ("light biotic", "dark biotic", "light sterile", "dark sterile") resulting from combining the following treatments: 1) bottles covered with tinfoil ("dark" condition) in order to exclude the contribution of photolysis processes or kept transparent ("light" condition) and 2) sterilizing ("sterile" condition) or not ("biotic" condition) bottles in a pressure-cooker. Bottles were then left on the glacier surface and collected at different times to assess CPF concentrations by gas chromatography (Agilent Technologies, Santa Clara, CA).

Detailed meteorological data were recorded by an automatic weather station (AWS) located on the surface of the Forni Glacier at 2660 m. a.s.l., 400 m apart from the area where we collected cryoconite and used to define the beginning and the end of the melting season of each year and a Temperature Index (TI) indicating whether each day of the melting season was warmer (positive TI values) or colder (negative TI values) than expected for that period of the year.

Data were analysed by means of principal component analysis (PCA) and redundancy analysis (RDA) based on Hellinger distance, linear models (LMs) and generalized linear models (GLMs). P-values of multiple tests were corrected using the FDR procedure when appropriate. Analyses were performed with R 3.4.2.

RESULTS AND DISCUSSION. In the study of the temporal variation of the structure of cryoconite hole bacterial communities we found that, overall, the most abundant taxa (Cyanobacteria, Sphingobacteriales and Burkholderiales) were the same in all years. These taxa have been described as typical of cryoconite holes worldwide (Cook et al. 2016, Prog. Phys. Geogr. 40:66–111). A RDA including only July samples showed significant variations in the structure of bacterial communities between years ($F_{3,88} = 34.34$, $P = 0.001$) with significant differences between all pairs of years ($F_{1,49} \geq 15.10$, $P_{FDR} \leq 0.002$). The structure of the bacterial communities therefore differed between years already at the beginning of the melting season. We then focused on data collected in 2013 and 2016, when samples were collected along the whole melting season, and found that the structure of cryoconite bacterial communities differed between years ($F_{1,116} = 22.75$, $P = 0.001$), changed along the melting season ($F_{1,116} = 2.85$, $P = 0.001$) and varied according to TI ($F_{1,116} = 8.22$, $P = 0.001$).

The structure of bacterial communities therefore changes temporally both within and between ablation seasons. Autotrophs (namely Cyanobacteria), in particular, decreased, and heterotrophs (namely Burkholderiales and Sphingomonadales) increased in both years ($F_{1,116} \leq 5.263$, $P_{FDR} \geq 0.085$). The ecological processes that affected these trends seemed mainly driven by temperature. Indeed, abundance of no taxon changed with day of melting season ($F_{1,116} \leq 3.778$, $P_{FDR} \geq 0.594$), while abundance of Cyanobacteria decreased at increasing values of TI, while Sphingobacteriales and Burkholderiales increased ($F_{2,116} \geq 12.254$, $P_{FDR} \leq 0.004$). Thus, seasonal gradients seem to occur, which drove communities toward an increase in heterotrophic taxa and a decrease in autotrophic ones.

The metagenomic study of the metabolic functions of the microbial communities in cryoconite holes showed that oxygenic photosynthesis was among the dominant metabolisms in cryoconite holes. CO_2 fixation was also widespread and almost completely achieved through Calvin-Benson cycle. However, high *puf*LM (photosynthetic reaction center L and M subunits) gene coverage, mostly affiliated to Proteobacteria, was found, suggesting that aerobic anoxygenic phototrophs (AAPs) may contribute to energy input of the ecosystem. Results also provided evidences that CO-oxidizers occurred and were fed by photochemically-produced CO, probably thank to high light intensity and abundant organic matter in cryoconite. We can therefore

hypothesize that higher CO_2 concentration could increase autotrophic metabolisms, including oxygenic photosynthesis and promote AAPs growth. This expected growth of microbes could darken ice surface as it is known that dark particles and pigmented microorganisms (phototrophes and algae) that live at the ice surface contribute to decrease ice albedo and enhance ice melt, thus generating a positive feedback for glacier shrinkage (bioalbedo effect).
The microcosm experiment showed that CPF decay rates differed significantly among the four experimental groups ($F_{3,26} = 9.771$, $P < 0.001$) and post-hoc comparisons indicated that decay rates were significantly faster under light-biotic conditions than under other conditions ($t_{26} \geq 3.055$, $P \leq 0.026$), which, in turn, did not differ significantly to one another ($|t_{26}| \leq 2.044$, $P \geq 0.198$). These results indicate that biodegradation contributed to the removal of CPF from the glacier surface more than photo- and chemical degradation. The high concentration of CPF (2-3 µg g^{-1} w.w.) detected in cryoconite holes and the estimated half-life of this compound (35–69 days in glacier environment) indicated that biodegradation can significantly reduce CPF concentrations on glaciers and its runoff to downstream ecosystems by degrading up 10% of the absorbed CPF released to surrounding freshwaters.
A further analysis of the metagenomic data of cryoconite holes allowed for the reconstruction and annotation of 65 partial bacterial genomes and the detection of genes involved in CPF biodegradation. In particular, we could reconstruct one abundant genome related to Burkholderiales that harboured the *mpd* (methyl parathion hydrolase) gene known to be involved in CPF metabolism. This genome also harboured genes coding for aerobic anoxygenic phototrophy, carbon monoxide oxidation and biodegradation of aromatic hydrocarbons, which, overall, suggest a very high metabolic versatility.
Overall, these results suggest that ice environments host active metabolic communities, which change temporally according to climatic conditions, and have large metabolic versatility. The occurrence of AAPs, in particular, suggest that the carbon budget of ice environments, which overall cover about 10% of land surface area if the Earth, should be revised by integrating these alternative metabolisms that have been overlooked so far. Finally, the ability of microbial populations to degrade contaminant occurring on glaciers disclose the possibility that they can acts as “biofilters” of pollutants thus reducing the impact of glacier melting to down valley ecosystems.

Biotic consequences of glacier retreat: community dynamics and ecosystem functioning

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Keywords: glacial retreat, environmental DNA, metabarcoding, community ecology

INTRODUCTION. Glaciers show a pattern of retreat at the global scale. Increasing areas are exposed and colonized by multiple organisms, but lack of global studies hampers a complete understanding of the future of recently deglaciated terrains. What will be the fate of these areas? How do animals, plants and microorganisms colonize them? How do they interact to perform successful colonization? Which are the climatic, geological and biogeographical processes determining colonization patterns? How does ecosystem functioning evolves through time? Until now, the complete reconstruction of soil communities was hampered by the complexity of identification of organisms, thus analyses at broad geographical and taxonomic scale have been so far impossible. In our research we combine innovative methods and a global approach to boost our understanding of the evolution of ecosystems in recently deglaciated areas.

MATERIALS AND METHODS. We are investigating chronosequences in glacier forelands ranging from recently deglaciated terrains to late successional stages of soil pedogenesis. Investigated chronosequences include retreating glaciers from the Alps, the Andes, Canada, Himalaya and the Caucasus. Trough environmental DNA metabarcoding, we are identifying species from multiple taxonomic groups, to obtain a complete reconstruction of biotic communities along glacier forelands over multiple mountain areas across the globe. This allows measuring the rate of colonization at an unprecedented detail. Information on assemblages is then combined with analyses of soil, landscape and climate to identify the drivers of community changes.

RESULTS AND CONCLUSION. Soil features show strong modifications, with a consistent increase of carbon stock and organic contents. Environmental DNA allows retrieving reliable information on all main components of soil biodiversity. Species richness tends to increase with age after glacier retreat, but the pattern is not consistent across taxonomic groups. Species richness shows a steady increase for plants and animals, while for protists very high richness is also found a few years after deglaciation. For animals, bacterivorous and decompositors dominate early stages, while more functional groups are present in late successional stages. Very few taxa are in common between early and late successional stages, indicating strong turnover of communities. Overall, the complexity of food webs strongly increases with time. Our study shows that environmental DNA allows an all-inclusive community ecology, which reveals how complex biotic interactions arise through time, and will help to predict the impacts of climate change on the whole ecosystems.

PARASITES AND GLOBAL CHANGE: LESSONS FROM THE MOUNTAIN BIOTIC COMMUNITIES

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Keywords: mountain biomes, global warming, parasites, conservation issues

The global climate warming is not uniform worldwide, but presents asymmetries among the different regions of the Earth, in which warming is occurring at different rates. On the Alps, the climate warming appears to be stronger than the average global signal and, similarly to the Artic, alpine environment can be seen as hot spot and early indicator of climate change. In the Alps, major shifts from previous meteorological data series have been recorded for temperature and the duration and height of the snow cover, and to a minor extent for winter and summer precipitations. As largely known, all these parameters may have direct and/or indirect impact on the dynamics of parasitic taxa affecting (in a complex game) humans, domestic animals and wildlife. Unfortunately, trends to climatic change in the Alps are expected to exacerbate during current century.

The study of parasitism in mountain wildlife and sympatric outdoor raised livestock under a global worming scenario is particularly stimulating, since ecological variables to consider are (relatively) less complex than in lower altitude biomes. In spite of this, the number of host/parasite models which have captured the interest of researchers in Europe and North America is quite limited, suggesting (with few exceptions) suboptimal allocation of resources and maybe a diminished attitude to uncertain and time consuming fieldwork in the new generations. In this presentation, while reviewing relevant research carried out in mountain biomes worldwide, I will also try to: i) address major knowledge gaps; ii) eventually raise interest on so far poorly exploited models; iii) stress the importance of old data series and the need to have them available soon to the benefit of new generation scientists.

That said, it is common knowledge that the spread of vectors and vector borne infections in the mountain biomes have been amongst the earliest signs of climate change worldwide. In the Alps, well known examples are Canine Leishmaniasis, now endemic in several xeric inner-alpine valley, a range of tick borne zoonotic and not zoonotic infections, the midge transmitted Blue Tongue and Schmallenberg virus and the black fly transmitted Nodular Onchocercosis by *Onchocerca jakutensis* in red deer. Similarly, liver fluke (*Fasciola hepatica*) infection has been observed in mountain-dwelling *Rupicapra pyrenaica* in the Eastern Pyrenees. Other significant contributing factors to these emergencies have been also highlighted in recent literature, which are rather related to modified human attitudes towards land use, wildlife management, the frequency and type of outdoor activities, animal welfare issues, etc. Since only few (if any) of these human-driven factors are liable to management in a realistic preventive perspective, effective policies to raise awareness and enhance individual and social resilience are definitely needed. Their fine tuning will most probably represent the bulk of our ethical commitment towards society in the upcoming decades.

In a conservation perspective, shifts in the geographic distribution of (previously) “lowland” parasites and the foreseen larger abundance and/or longer transmission period of “traditional” parasites already represent a matter of concern for mountain wildlife and the sympatric extensively raised livestock managers. Outbreaks have been predicted in the case of the former “new” ones, favored by limited if nul herd immunity in the potential exposed hosts, whereas new demographic equilibria to the host detriment will be likely outcome for the second ones. However, parasites themselves are at risk in a global warming scenario, with special fragility for

the “specialists” coevolved with the so called glacial relicts. It is the responsibility of our community to help institutional sponsors and resource management agencies to realize (after centuries of prejudices) that parasites are major components of the diversity of mountain biomes and that actions should be taken to know, monitor and preserve them with similar dignity as their hosts.

TAVOLA ROTONDA 1

CAMBIAMENTI AMBIENTALI E SCENARI EPIDEMIOLOGICI: PREVISIONI E MODELLI

Co-infection, an old challenge in a changing world

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Keywords**:** Community ecology, Concomitant infections**,** Diseases ecology, Poliparasitism

What the American zoologist Victor Ernest Shelford (1887 - 1968) stated over a century ago is more important than ever for a modern parasitologist. In words of professor Shelford: “a study of the relations of a single species to the environment conceived without reference to communities and, in the end, unrelated to the natural phenomena of its habitat and community associations is not properly included in the field of ecology” (Shelford, 1919, cited in Morin, 2000, Community Ecology, Wiley-Blackwell, 407 pp). This integrative vision of nature has driven the thought of ecologists for many years who understood the value of considering communities instead of single species to evaluate the impact of global changes (Walther, 2000. Philos. Trans. R. Soc. Lond. B. Biol. Sci, 365: 2019-2024).

The same paradigm change has happened in the field of parasitology. In fact, in the mid-70s, parasitologists felt the need of evaluating whether the concepts of community ecology had place in its field (Hair and Homes, 1975). The first works in this line tried to understand the processes driving helminth parasite assemblages within hosts (See Sousa 1994 for a revision and Hair and Holmes, 1975. Act. Parasitol. Pol, 23: 253-269, for a study case). In other words, whether the presence of one parasite species influence each other’s abundance and distribution at infrapopulation (e.g., within a host), metapopulation (between hosts) and suprapopulation (between host communities) scales. The consequences of those interactions between parasites have been updated in a clear and didactic manner in Johnson et al., 2015 (Science, 349 (6252): 1259504).

This new framework, however, was more concerned in understanding rules behind parasite assemblages than in its implications for human or animal health. Two decades later, parasitologist around the world accepted that most parasite species were distributed in structured communities (Poulin, 1995, Ecol. Monograph, 65 (3): 283-302) and underlined the role of the immune system on the processes of interspecific competition or facilitation among parasite species (Poulin, 2001, Parasitology, 122: 3-11). That idea was envisaged many years before by experimental parasitologists (Addel-Wahab et al., 1974, Am. Soc. Trop. Hyg, 23 (5): 915-918, revised in Cox, 2001, Parasitology, 122: 23-38), but kept aloof from pathologist and epidemiologist until recent times. In consequence the birth of a more holistic view of the causes and consequences of parasite communities needed more years to come to light (Fig. 1).

The excellent work of Pedersen and Fenton (2007, Trends. Ecol. Evol, 22 (3): 133-139) may have marked the beginning of a new era for co-infection science. Both researchers suggested that the same bottom-up and top-down processes governing ecosystems also worked in the host ecosystem. According to their theories, for example, the local disruption of abomasal physiology (bottom-up process) caused by *Ostertagia* infection may explain the enhanced resistance of lambs to a *Haemochus* infection (Dobson and Barnes, 1995, J. Parasitol, 25 (4): 495 - 501). However, in the same host model, a top-down regulation through immune suppression caused by *Haemonchus* may facilitate the establishment of *Trichostrongylus* nematodes (Lello et al., 2004, Nature, 428: 840 - 844). These bottom-up and down regulations will run in many other co-infections e.g., virus-bacteria, bacteria-helminths, protozoa-helminths, causing a wide range of outcomes not only for the pathogen community but also for the host’s health (Cox, 2011, Parasitology, 122: 23-38).

In fact, rather than a curious collection of intra and interspecific interactions among pathogens, the implication of co-infection for our daily lives is far from being fully understood. Co-infection is the norm rather than the exception in all living beings. In humans, for example, prevalence of co-infection exceeds one-sixth of the global population (Pullan and Brooker, 2008, Parasitology, 135: 783 - 794). In fact, the three major infectious diseases of humans (human immunodeficiency virus infection (HIV), malaria and tuberculosis (TB) are generally diagnosed in co-infection with helminths (Salgame et al., 2013, Nat. Immunol., 14: 1118 - 1126). Co-infection is associated with host susceptibility to virulent pathogens, extend infection duration, rise transmission rates, health impairment and reduced treatment efficacy (Vaumouring et al., 2015, Parasit. Vectors, 8: 585). Moreover, the effectiveness of vaccination campaigns is also affected by co-infection with nematodes. To avoid being eliminated, these parasites actively dampen the immune response of their hosts suppressing the effect of vaccines (Haben et al., 2014, Plos. Negl. Trop. Dis, 8 (9): e3170). Similarly, disease testing is also affected by co-infections. One of the well-known examples is the ability of *Fasciola hepatica* for impairing the tuberculin test used to diagnose bovine tuberculosis in cattle (Claridge et al., 2012, Nat. Comm., 3: 853). As result, the prevalence of bovine TB is increasing exponentially in cases year-on-year. Along the same lines, trichiuriasis and giardiasis have been associated with a higher likelihood of false negative skin tuberculin test among refugees (Watts et al., 2017, Travel. Med. Infec. Dis.,16: 35-40).

However, the standard parasitological books, particularly in veterinary parasitology, have long been silent on the magnitude and consequences of co-infections (Cox, 2011, Parasitology, 122: 23-38). Nonetheless, the multispecies nature of the main infectious diseases demands a more holistic approach to complement traditional biomedical treatments. Today we are facing an urgent need to move beyond the “one pathogen-one disease” paradigm not only to better understand infection dynamics of malaria or tuberculosis, but also to advance our ability to manage infections.

Figure 1. This imaginary wild boar evokes de idea of a host as an ecosystem for parasites. From the skin to its inner organs, a host constitute a wide variety of habitats which conform a complex ecosystem as suggested by Pérez et al (2006, Biodiv. Conserv, 15: 2033 - 2047). Interactions between parasites and between parasites and the microbiome alter host susceptibility to new infections, extend infection duration, rise transmission rates, hamper disease testing, worse clinical symptoms and consequently treatment and prevention strategies. Designed by M. H. Pongiluppi.

Glossary

Parasite assemblage: Group of parasites that can be grouped together for a reason. Whether parasite species in an assemblage are or not truly structured communities depends on the existence of statistical associations among species that allow predictable patterns of species co-occurrence.
Bottom-up regulation: It is a kind of population density regulation which happens naturally through interspecific completions for food supply.
Ecological community: Group of species occupying the same place in a particular time and interact in various ways e.g., competition, predation parasitism, mutualism or commensalism.
Co-infection: Also called mixed infection or concomitant infection, refers to a situation in which more than one infectious agent coexists in the same host. This term is applied not only to concomitant infections with different parasite species but also with members of the same species but different strain. This definition can be applied to infections with any kind of pathogen combination e.g., virus-virus, virus-bacteria, bacteria-helminths and so one.
Top-down regulation: It is a kind of population density regulation which happens naturally through or due to the action of predators.

Geospatial Health, global changes and new parasitological scenarios

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Keywords**:** Mapping, Livestock, Helminths

Geospatial health is now a firmly established approach for geo-positioning, collating, exploring, visualizing and analyzing parasitological data in a spatially explicit manner at various scales (local, regional and area-wide scales). In the era of global changes and industry 4.0, a new wave of technological innovation is underway in veterinary and human parasitology (Vercruysse et al., 2018, Parasitology, in press). Furthermore, advances in epidemiology (e.g. geographical information science – GIS) and diagnostics (e.g. automated systems) in the last decades have been used to better guide surveillance and control programmes of parasite infections. The scientific breakthrough includes a plethora of technologies, methods, software, models, tools and web-based platforms available for scientists, decision-makers, stakeholders and end-users working in the field of epidemiology of parasitic infections. Use of these new technologies supported by mobile-, visual- and electronic-based (m-, v- and e-health) approaches are considered research priorities to strengthen the use of geospatial tools for monitoring and modeling parasites of veterinary and public health importance. Intuitive technologies, such as Google Earth enable scientists around the world to share data and maps in a readily understandable fashion without the need for much technical assistance (Rinaldi and Cringoli, 2014, Parasitology, 141:1803-1810) and now even drone imageries may been used to map parasite and vector habitats at very fine scales (De Roeck et al., 2014, Geospat Health 8:S671-83.

Maps and models can be shared in real time not only with decision-makers, but also with the individual researchers who may continually extend the evidence-based patterns of parasitic infections of public health relevance as, for example, echinococcosis (Deplazes et al., 2017, Adv. Parasitol, 95:315-493). GIS have much more to offer than digital cartography functions and could act as powerful tools for early problem detection and solving in order to: inform and educate; empower decision-making at all levels; help in planning and in predicting outcomes before making any financial commitments; change practices; monitor and analyze global changes and new parasitological scenarios.

In this context, geospatial health has been the *leitmotiv* of recent multidisciplinary and inter-sectoral EU research programmes (e.g., COST Action CAPARA, EU FP6 PARASOL, EU FP7 GLOWORM, COST Action COMBAR) whose scientific and practical deliverables were risk maps and models (at regional/country levels as well as at pan-European scale) for ruminant helminths (e.g. gastrointestinal nematodes, liver fluke) taking into consideration weather and environmental predictors as well as farm management factors (Rinaldi et al., 2015, Geospat Health. 9:257-9). Mapping and modeling are also among the research priorities of the recently formed Livestock Helminth Research Alliance (LiHRA) aimed at responding to global changes that impact on livestock, farming practices and helminth infections and identify areas for future research (www.lihra.eu).

Despite its outstanding potentiality, geospatial health is prone to several weaknesses and challenges and effective health responses depend upon the ability of quality data and sustainable parasitological systems to provide accurate and timely information for action. SWOT (Strengths, Weaknesses, Opportunities, and Threats) analysis and integrated recommendations are needed to promote best practice for the best use of geospatial tools in the era of climatic and global changes and new parasitological scenarios (Bergquist et al., 2018, Geospat. Health, 13:699).

Undoubtedly, we are still far from knowing the relative contributions of climate, environmental, management and socio-economic influences to the changing parasitological scenarios. Thus, there is a clear need to integrate medical, veterinary, biological, statistic, agricultural and social sciences to better understand how these factors are affected by climatic changes. Crucial for this task will be investment not only in systematic surveillance of livestock parasites for immediate animal and human health purposes, but also commitment to long-term surveillance, including data collection to identify and track impacts of climatic changes on various helminth parasites.

Predicting parasite spread in an era of rapid climate change: model limitations and new ways forward

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Keywords**:** Climate change, modelling, uncertainty

Climatic and other environmental changes are threatening parasite control by altering epidemiological patterns and making disease threats increasingly unpredictable (Cable et al. 2017 Phil. Trans. R. Soc. 372, 1719, 2160088). Widespread anthelmintic drug use is masking the consequences but is becoming less effective (Rose et al. 2015 Vet. Rec. 176, 21). Therefore, better prediction of risk periods is important to support well-timed and effective interventions. In this presentation I describe some recent advances in mechanistic modelling of parasite transmission, especially for gastrointestinal nematodes of grazing ruminants, and argue that these models are a necessary complement to statistical (empirical) models if we are to anticipate future epidemiological changes under global warming. Thus, the spring scour worm of sheep in the UK, *Nematodirus battus*, overwinters on pasture and hatching dates in spring can vary by more than six weeks year-to-year, yet treatment times are traditionally constant. Empirical models based on March soil temperature have been overtaken by climate change and are of no use in many years. A mechanistic model based on air temperature and calibrated using data from laboratory experiments of parasite hatching (Van Dijk and Morgan 2008 Parasitology 135, 269) performed well and is now used to provide real-time hazard maps each spring. Farmers report increased ability to anticipate high risk periods, and associated improvements in health parameters.

Extending the approach used for *N. battus* to other parasite species is more challenging. Often, parameter uncertainty compromises attempts to build strong models, especially when complex processes such as host immunity are included (Verschave et al. 2016 Trends Parasitol. 32, 724; Verschave et al. 2016 Vet. Parasitol. 223, 111). In many cases, factors other than climate have a dominant role in epidemiology, such that small changes in farm management outweigh large impacts of climate change on parasite life cycles. For example, predicted increases in blowfly strike in sheep can be greatly attenuated by altering shearing times (Wall and Morgan 2009 Trends Parasitol. 25, 308). Moreover, linking climate-driven hazard to disease risk necessitates information on exposure, and hence management detail such as movement between pastures and treatment times, and capturing this easily on farms can be difficult (Morgan 2013 An. Health Res. Rev. 14, 138). A major frontier for applying predictive models of parasite epidemiology in a meaningful way on farms is the automation of data collection and processing so that model outcomes are tailored to specific situations.

Modelling has an important role to play in predictive parasitic disease risks to companion animals and not only farm animals. Thus, a very robust and successful model exists for *Dirofilaria immitis* transmission risk in dogs in Europe (Genchi, Rinaldi et al. 2009 Vet. Parasitol. 163, 286), which uses temperature requirements for development in the mosquito intermediate host to predict areas at risk of parasite expansion. Similar approaches could be used, for example, for other emerging parasites of pets such as the lungworm *Angiostrongylus vasorum*, advancing from existing, largely empirical models (Morgan et al. 2009 Parasitol. Int. 58, 406).

Ultimately, predictive models of parasite epidemiology under climate change depend on thorough calibration from empirical data, and validation using field data. By linking models and data closely together in a continuous cycle of improvement, our ability to predict and deal with changing epidemiology is constantly being upgraded.

TAVOLA ROTONDA 2

MALATTIE TRASMESSE DA VETTORI DEL CANE E DEL GATTO: DALLA BIOLOGIA AL CONTROLLO

Future shock: changing threats from ticks and tick-borne disease in Europe

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In recent decades, many arthropods and arthropod mediated infections have shown striking changes in distribution and prevalence in Europe and these are expected to change further as a result of factors linked to habitat modification, changes in land use and climate change. Furthermore, global trade and the greater movement of people and pets have increased the potential for the introduction and establishment of novel vector species not previously present in some areas, as well as novel pathogens. Many of these issues are particularly well highlighted by the changing patterns of the distribution, abundance and phenology of ticks in Europe. The abundant tick species, *Ixodes ricinus*, is highly sensitive to climate variation. In northern latitudes, *I. ricinus* is typically active in spring and early summer, quiescent in mid-summer and usually has a secondary peak of activity in later summer or autumn. However, studies have shown that *I. ricinus*, are increasingly able to survive and quest throughout the winter where previously the winter temperatures would have been too cold to support tick activity, resulting in year-round risk periods for tick bites and associated pathogen transmission. There is also considerable evidence for the altitudinal and latitudinal expansion of its range and, in some areas, large long-term increases in abundance in regions where previously ticks had been rare. Associated with these changes, there has been an increase in recorded cases of tick borne diseases such as Lyme borreliosis, caused by the spirochaete *Borrelia burgdorferi* s.l., and TBE in parts of central Europe where infection risk was previously thought to be low and outbreaks of tick-born disease, such as *Babesia canis*, in areas where previously they were absent. However, it is often difficult to differentiate climate-mediated changes in vector distributions from underlying changes in host abundance; it can be equally difficult to disentangle range expansions and shifts due to changes in the distribution of suitable habitat from colonisation of habitat that was previously suitable for parasite persistence but remained free of disease for reasons such as isolation or national biosecurity. For example, over the last 20-30 years populations of roe deer, *Capreolus capreolus*, have also expanded in areas of increased tick abundance. Nevertheless, recent studies provide compelling evidence that *I. ricinus* populations are increasing independently of changes in wildlife abundance, most probably associates with changes in climate. Hence, general predictions of the likely changes in the prevalence of of ticks and tick borne disease are complex and are modulated by many factors. These include biological processes such as host immunity and even the behaviour of pet owners and their responses to perceived changing threat.

Tick borne pathogen transmission times: the importance to act fast!

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Keywords: ticks, transmission time, vectors, tick-borne pathogens, dog, cat, control

Tick-borne pathogens have developed a close relationship with ticks, which has resulted in a long evolved balance with their biology, ecology and blood feeding habits, instrumentally to the infection of several animal species, including humans (Otranto D., 2018, Vet Parasitol., 15;251:68-77). Beside the peculiar case of tick ingestion (*Hepatozoon* spp.) blood feeding represents the main way of pathogen transmission, being the time frame in which pathogens are transmitted to any animal host governed by a large number of biological variables related to the vectors, the pathogens, the host and environmental factors. Ticks can swallow blood for 200–600 times their unfed bodyweight with the largest amount of imbibed blood ever reported (i.e., 8.856 ml) in a *Hyalomma asiaticium* individual tick. Pathogen transmission times are governed by a large number of biological variables related to the vectors, the pathogens and the host immune responses though pathogen introduction / inoculation into a host does not imply to any instance that it shall succeed in establishing, through its multiplication (infection) and, eventually, causing disease. Overall, the length of feeding periods is shorter (from seconds to a few minutes) in blood feeding insects (e.g., sand flies, mosquitoes, black flies), and longer (from days to weeks) in many slow feeders, such as ixodid ticks (e.g., *Rhipicephalus sanguineus* sensu lato). Agents transmitted by Ixodidae (hard ticks) undergo up to several days of development until they infect the host whereas those transmitted by Argasidae (soft ticks) are ready for transmission immediately after the feeding starts. Therefore, *Borrelia* spirochetes causing Lyme disease are transmitted by hard ticks several days after attachment, whereas those causing relapsing fever, vectored by soft ticks, usually are transmitted within minutes after attachment. Ixodid ticks (but not argasid) feed only before moulting to the next stage and usually pathogens are transmitted transtadially (e.g., *Ehrlichia*) or transovarially (e.g., *Babesia* spp., *B. burgdorgeri* sensu lato). In both cases, pathogens must survive in ticks during moult, even for months, until the next host is available, and they may also require some length of reactivation time at the next blood meal. Pathogen transmission is influenced by environmental determinants including seasonality. An important process occurring before almost all tick-transmitted pathogens (but not viruses) are inoculated to a new receptive host is their pre-activation, which occurs in about 24-48h since the tick attachment. This event relates to the biology of pathogens themselves (e.g., their migration to or multiplication in the salivary glands), and of the tick vector. Indeed, in most cases, pathogens, such as *Anaplasma* spp., *Babesia* spp., *B. burgdorferi* sensu lato, *Rickettsia* spp. are not harboured in the tick mouthparts ready to be immediately inoculated into the host at the moment of tick attachment. Although the determinants affecting pre-activation processes are not completely understood, multiple signals trigger pathogen multiplication and transmission in the ticks, such as the increase in temperature following the contact with the vertebrate host and the process of blood engorgement. The production of tick salivary anticoagulants and other active compounds that modulate host cutaneous immunity and inflammation and enhance vasodilation represent other drivers triggering the reactivation and the pathogen transmission. The detection of *Rickettsia* spp. and *Anaplasma phagocytophilum*-like organisms in the salivary glands of experimentally infected and unfed ticks suggested that these pathogens could be transmitted quickly to the host. However, information inferred by the retrieval of pathogens in the salivary glands at different time points do not lead to any definitive conclusions about their transmission times to the hosts, as other studies

indicate that the same pathogens may need longer times (i.e., from 10 h for *R. conorii* and *R. rickettsii* to 24-48 h for *A. phagocytophilum* after feeding).

Another important variant impacting the pathogen transmission time is represented by the feeding status of ticks before attachment on the host, with *R. rickettsii* being transmitted by unfed *Amblyomma aureolatum* ticks after >10 hours, but within 10 minutes in fed ticks. The latter event is typical of interrupted feeding, which may occur under field conditions, mostly when more infected animals are co-housed in the same place. This is the case of the brown dog tick *Rh. sanguineus* s. l., which, in turn, is vector of a large number of pathogens to animals and humans. Male ticks previously attached to one dog can move onto another co-housed dog and resume feeding, possibly reducing pathogen transmission times. In the case of massive tick infestation, tick co-feeding is a common event and intrastadial pathogen transmission may also occur, with inpredictable consequences on pathogen transmission times (*E. canis* by *Rh. sanguineus* s.l.). These observations emphasize the importance of controlling arthropod vectors through their removal, or using repellent or lethal compounds to ideally avoid attachment and consequent pathogen transmission.

Blocking pathogen transmission, and thus preventing the infection of dogs and cats, may be achievable by the use of chemical compounds if they are characterised by a fast onset of killing activity or repellence against arthropods. The fast speed of kill exerted by systemic isoxazoline, as well as the repellent effect of pyrethroids have renewed the interest of the scientific community and pharmaceutical companies towards reducing the burden of vector-borne diseases under field conditions. However, endosymbionts and vaccines targeting ticks or pathogen antigens should be further investigated as alternative strategies towards the goal of achieving an effective integrated control of tick-borne diseases.

The control of canine and feline leishmaniosis in Europe: where do we stand?

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In Europe *Leishmania infantum* is transmitted to vertebrate hosts through the bite of an infected sandfly of the genus Phlebotomus. The prevention and control of a vector-borne disease include the use of compounds on hosts that avoid transmission by the vector (anti-feeding products and systemic ectoparasiticides) or that enhance the immune response of the host (not only but mainly vaccines). Other systems to control canine leishmaniosis, as treatment of infected animals, control of the vector and culling of dogs will not be addressed in this note.
Anti-feeding products are repellents, meaning that they avoid the landing of the vector on the host and their attachment for the blood meal, while systemic ectoparasiticides permit the attachment of the vector, which is killed by the molecule ingested during the blood meal. Systemic ectoparasiticides can prevent transmission of pathogens if the vector is killed before the transmission process is started.
The prevention of Leishmania transmission to the host requires the use of a repellent product.

Leishmania infection prevention: why a repellent?
The blood meal of the sandfly takes few minutes. For instance the average time of feeding of *Phlebotomus neglectus*, a proved vector of *L. infantum*, ranged from 3 min 46 s to 5 min 26 s, in laboratory studies (Ivović et al., 2010). Also, sandflies probe repeatedly before full engorgement and it's documented that they can transmit Leishmania by probing alone without engorgement by the inoculation of infective free-swimming promastigotes from the proboscis into the wound (Killick-Kendrick et al. 1979).
Thus, transmission of the parasite is a very quick event that can be blocked only by the use of a repellent, i.e an anti-feeding effect.
Repellent insecticides that are licensed in Europe are listed in table 1. Pet owners have a wide choice of products in different formulations (spray, spot-on and collars), with various onset and length of activity which cover the different need of protection in endemic and non-endemic areas for leishmaniosis.

Table 1 – Repellent activity of insecticides licensed in Europe against Phlebotomus sandfly (modified from Miró et al., 2017)

Ingredients	Brand name (Company)	Type	Onset	Duration	% of anti-feeding effect	Refs
Permethrin Indoxacarb	Activyl® Plus (MSD)	Spot-on	24–48 h	3–4 weeks	99-84	Frenais et al. 2014
Permethrin Imidacloprid	Advantix® (BAYER)	Spot-on	24–48 h	3–4 weeks	96.7-74 ≥88.1-≥92.2	Miró et al., 2007 Boushira et al., 2018
Permethrin Piriproxyfen	Duowin®(VIRBAC)	Spray	Instant	2–3 days	> 90	Molina et al., 2006
Permethrin Fipronil	Effitix® (VIRBAC)	Spot-on	24–48 h	4 weeks	95.8-86.8	Franc et al., 2015
Permethrin	Exspot® (MSD)	Spot-on	24–48 h	2 weeks	>90 99-67.6	Molina et al., 2001 Molina et al., 2012
Permethrin Fipronil	Frontline Tri-Act® (MERIAL)	Spot-on	24–48 h	4 weeks	>80	Dumont et al., 2015
Deltamethrin	Scalibor® (MSD)	Collar	7 days	6 months	96	Killick-Kendrick et al.,1997
Flumethrin Imidacloprid*	Seresto® (BAYER)	Collar	**	6-8 months	--	--
Dinotefuran Permethrin Piriproxyfen	Vectra 3D® (CEVA)	Spot-on	24–48 h	4 weeks	87 71.4	Lienard et al., 2013 Molina et al., 2006

* for this formulation there are no studies of direct repellency for phlebotomine. The collar proved to be repellent against sandflies indirectly by showing protection of dogs and cats against Leishmaniosis in highly endemic areas (see table 2)
** immediate protection against fleas and with repellent protection against ticks within 48 hours

Their anti-feeding effect in laboratory studies is always very high but never 100%, meaning that some sandflies succeed to land and attach before to (usually) dye or escape. Since not all the sandflies are infected even in very high endemic areas, the use of repellents often translates in very high protection against Leishmania infection in the field, reaching also 100% in dogs (table 2). However, the rate of protection is variable depending on the device used (table 2) and, in practice, depending also on the compliance of the dog owners, who should strictly follow the indications of the company on the correct use of the repellent. Indeed, almost all the studies show that the repellent efficacy decreases over time and therefore it's pivotal to replace (collar) or re-applicate (spot-on) the compound in time.

Table 2 – Field studies on the efficacy of repellent insecticides in the prevention of canine and feline leishmaniosis in endemic areas (modified from Otranto and Dantas-Torres, 2013)

Active ingredient	Brand name (Company)	Type	Species	Protection	Refs
Permethrin-Imidacloprid	Advantix® (BAYER)	spot-on	dog	88.9-100%	Otranto et al., 2007
			dog	100%	Otranto et al., 2010
Permethrin	Exspot® (MSD)	spot-on	dog	50%	Giffoni et al., 2002
			dog	84%	Ferroglio et al., 2008
Permethrin-Fipronil	Frontline Tri-Act® (MERIAL)	spot-on	dog	100%	Papadopoulos et al., 2017
Deltamethrin	Scalibor® (MSD)	collar	dog	50%	Reithinger et al., 2004
			dog	50.8%	Foglia Manzillo et al., 2006
			dog	84%	Ferroglio et al., 2008
			dog	61.8%	Brianti et al., 2016
			dog	88%	Papadopoulos et al., 2017
Flumethrin- Imidacloprid	Seresto® (BAYER)	collar	dog	100%	Otranto et al., 2013
			dog	93.4%	Brianti et al., 2014
			dog	88.3%	Brianti et al., 2016
			cat	75%	Brianti et al., 2017

Leishmania vaccine: a road full of obstacles

Due to the difficulties in diagnosis, treatment and control of canine leishmaniosis a vaccine is highly desirable. Among a wide range of potential Leishmania antigen candidates only three vaccines have been registered as canine vaccines: two consisting of parasite purified fractions with saponin adjuvants (Leishmune® in Brazil, CaniLeish® in Europe) and one based on recombinant chimeric protein Q (Letifend® in Europe). These vaccines showed to confer significant protection against disease and death under natural conditions (Oliva et al., 2014; Gradoni, 2017; Fernández Cotrina et al., 2018).

CaniLeish was tested in a randomized, double-blind, controlled trial by exposing Beagle dogs to natural *L. infantum* infection in endemic areas of the Mediterranean basin, and showed an efficacy of 68.4% and decreased the risk of progression to symptomatic active infection 3.8-fold (Oliva et al., 2014). The vaccine stimulates an appropriate Th1 cell-mediated immune response (Moreno et al., 2012), which is still active one year later (Moreno et al., 2014; Martin et al, 2014). The vaccine does not prevent but reduce the intensity of infection in *Ph. perniciosus* fed on *L. infantum* infected dogs (Bongiorno et al., 2013).

However, in a recent field study in a highly endemic area of southern Italy, no statistical difference was detected in *L. infantum* positive dogs for bone marrow PCR and/or cytology between the CaniLeish (15.4%) and non-treated control dogs (10.0%) and no difference was even recorded in the frequency of active infections between dogs vaccinated and control dogs (Brianti et al., 2016). Another complication is that serological tests may not distinguish between naturally infected and vaccinated dogs with the risk of vaccination of infected animals (Solano-Gallego et al., 2017).

The efficacy of Letifend, assessed in 549 dogs living in France and Spain naturally exposed to two *L. infantum* transmission seasons, was 72% (Fernández Cotrina et al., 2018). As for the previous one also Letifend reduced the incidence of clinical signs related to leishmaniosis. Re-

vaccination of seropositive dogs demonstrated to be safe and not to worsen the course of the disease.

Recent reviews on Leishmania vaccine agree on the need of more adequately powered randomized clinical field studies on vaccine efficacy and its impact on parasite transmission (Gradoni, 2014, Wylie et al., 2014).

Conclusions

The availability and efficacy of repellent products which prevent the sandflies bites and Leishmania infection is greatly increased in the last decades for dog, while for cats there is still a need for discovery and testing of new compounds. The use of these products significantly reduced the risk of *L. infantum* infection in dogs and cats. Vaccines, licensed only for dogs, showed to be able to reduce the onset of clinical signs and the severity of the disease, but are still far from the efficacy of bacterial and viral vaccines.

Therefore, the protection of pets in highly endemic areas is still a challenge, which requires the simultaneous commitment of researchers, companies, veterinarians and pet owners.

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TAVOLA ROTONDA 3

PARASSITI E MICROBIOTA

Parasites - Friends or Foes of a Healthy Immune System?

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Key words: Helminth, immunomodulation, IL-33, TGF-β

INTRODUCTION. Infection with helminth parasites is associated with suppression of immune responsiveness. This can have useful side-effects (reduced allergy, autoimmunity and inflammation) and deleterious consequences (increased parasite burden, decreased efficacy of vaccines). We study how helminth parasites mediate their immunomodulatory effects, through study of their secreted products.

MATERIALS AND METHODS. The secreted products of the murine intestinal helminth *Heligmosomoides polygyrus* are collected, and immunomodulatory effects identified. Through proteomic analysis candidate proteins are selected and recombinantly expressed. These molecules are tested in *in vitro* immunological assays, in *in vivo* models of immune-mediated disease, and in biochemical studies of their mechanism of action.

RESULTS. We have identified several immunomodulatory proteins from the products of *H. polygyrus*, including HpTGM (a mimic of host TGF-β) and HpARI (a suppressor of IL-33 responses). HpTGM ligates the host TGF-β receptor through a novel binding mechanism, potently inducing regulatory T cell responses in murine and human cells. HpARI binds murine and human IL-33, and secondarily binds to host DNA, tethering IL-33 within necrotic epithelial cells, preventing its release and inhibiting its effects.

CONCLUSIONS. Through analysis of the products of a murine intestinal parasite we have found several new immunosuppressive molecules, which could shed light on the variety of ways in which helminth parasites modulate host immune responses. Furthermore, development of these identified products could lead to new treatments for diseases mediated by inappropriate immune responses.

The hygiene hypothesis and the microbiota-diabetes connection

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During the last decade, it became evident that the gut microbiota located in the gastrointestinal tract provides important health benefits to its host. Most of the benefits come through a regulation of immune homeostasis contributing to self-tolerance and to Treg and B-cell development. Several results have been published supporting these evidences, including those in which it is demonstrated that IgA production is strongly regulated by intestinal microbes and that mice deficient in B cells or in IgA have commensal bacteria altered. Therefore, it is obvious that modifications of these gut microbial communities can determine immune dysregulation, leading to the development of autoimmune disorders, including type 1 diabetes (T1D). The first connection between microbiota and T1D has been observed in 2008 when Went and colleagues showed that the presence of a microbiota is important for diabetes reduction in a NOD mice. Moreover, besides results obtained in preclinical models, longitudinal studies carried out in humans revealed that bacteria diversity decreases after seroconversion in children that develop T1D and increased before seroconversion in these subjects. Given the importance of gut microbiota in diabetes, here we will discuss microbiome involvement in the development of T1D.

TAVOLA ROTONDA 4

PARASSITI, BENESSERE E PRODUZIONI ANIMALI

How can we stop parasites from becoming a major welfare issue on tomorrow's farms?

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Keywords**:** freedom, transmission, management systems, optimization, animal health

Parasites are frequently transmitted via the environment, and richer environments often provide more possibilities for infection. Internal parasites in particular are commonly transmitted by the faeco-oral route, and intensification of production that involves housing of animals relies on breaking this route through sanitary measures. More recently, trends away from intensive housing and towards more 'wholesome' outdoor production have re-opened parasite infection routes, especially in poultry and pigs. Meanwhile, in grazing ruminants, intensive outdoor grazing is only possible where parasite (especially helminth) control is good, and so these management systems are utterly dependent on failing antiparasitic drugs.

This presentation will address the trade-offs between welfare and parasite infection risk in farmed animals, and query whether these can be truly optimized in the absence of a common currency. Thus, welfare is often measured in terms of animal behavior, while disease is measured through clinical and subclinical indicators. The welfare costs of disease are not quantified in welfare assessments, even though they can be large, especially for endemic infections such as parasites, which affect very large numbers of animals. At the same time, new thinking in welfare science considers stress and negative mood to be in some sense adaptive (Mendl et al. 2010 Proc. R. Soc. B 277, 2895), so that judgements of the welfare costs of systems based on such indicators might be limited. Similar arguments have been made for humans, for whom some stress might be better than none, or excess. Recent developments in assessing impacts of parasites on human welfare, for example using quality adjusted life years (Payne et al. 2009 Trends Parasitol. 25, 393) might pave the way for more holistic assessments in animals, which might be suited to balance welfare and disease risks in future systems. This, however, would seem impossible without anthropomorphizing. Thus, it would be difficult to equate the negative individual impact of heat stress on a dairy cow with that of parasitic gastritis, so future management under climate warming cannot be optimized without making subjective judgements (Skuce et al. 2013 Animal 7, 333).

In the absence of formal mechanisms for optimizing management systems across (parasitic) disease risk and traditional measures of welfare, it would seem wise to integrate both into measures that are also meaningful for production. Non-specific indicators of poor performance can help to identify problems at individual and herd levels, and provoke further investigation and intervention. In this respect, emphasis within veterinary parasitology on identifying the most affected individuals could help inform wider on-farm strategies for health monitoring (Charlier et al. 2014 Vet. Rec. 175, 250). For example, choosing anemic goats for antiparasitic treatment can achieve improved productivity and health at modest cost, even in African smallholder systems with little farmer training (Walker et al. 2015 Vet. Parasitol. 214, 80). The production benefits in turn encourage wider engagement, and monitoring systems can then be expanded to address other problems such as lameness, once farmers are habituated to frequently inspect their animals. For larger herds, automated monitoring of behavior might hold the key to early detection of health problems that affect both welfare and production, and there is great emerging interest in deployment of enabling technology on farms. Using imaging and sensors to provide such early warning is already established in more intensive production systems such as poultry

(Ben Sassi et al. 2016 Animals 6, 62). In grazing ruminants, parasites are the main production-limiting disease (and, we might argue, the main welfare risk), and systems to monitor parasitic infection and disease could provide the foundation for integrated health monitoring technology. A great deal of additional research is needed in parallel with technology development, to characterize impacts of parasitism on animal behavior, welfare and production, and this should proceed simultaneously from empirical and mechanistic directions.

How can parasites influence the welfare and production of ruminants?

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Keywords: parasites, welfare, production, ruminants

INTRODUCTION. Over the last few years the general public has been made aware of the subtle relationship between animal welfare and production as well as the importance of understanding the complete production process, starting from the primary production on livestock farms.
Access to EU funding for rural development now requires respect for animal welfare. In addition, animal welfare is indispensable for obtaining certification models focused on the reality of production systems and respectful of consumer rights. These models require products that are obtained from animals reared following ethical methods.
Several research groups have also focused on animal welfare and various concepts have been defined such as the “five freedoms” which refer to the physical and mental state of animals, as well as “welfare quality projects”, which refer to the absence of disease/pain as a welfare criterion when investigating the effect of parasites.
While respect for animal welfare is often stressed intensive farming, and in the field of parasitic diseases in ruminants, there are also problems in extensive and semi-extensive breedings.
Parasitosis in ruminants can also lead to a state of stress, which represents a modification in the normal physiological state of the animal, adopted to cope with unfavorable stimuli (Fraser and Broom,1990, Farm Animal Behaviour and Welfare. 3rd edition. Bailliere Tindall, London, England).
This report considers the general pathogenic aspects (i.e. stress) and those related to animal welfare and the production of widespread parasitosis, such as gastro-intestinal nematodes (GIN), myiasis (hypodermosis, oestrosis, cutaneous myiasis); and sheep metacestodosis, such as coenurosis by *Taenia multiceps* and Cystic Echinococcosis (CE).
The aim of the present work is therefore to highlight the importance of controlling parasites for the enhancement of animal welfare also from an economic-productive perspective. The work also identifies specific indicators related to animal welfare in parasitic infections which can be routinely used in a national and international environment.
RESULTS AND CONCLUSIONS. During their lifetime all vertebrate species need energy for physiological maintenance - defined as a nutritive equilibrium. Physiological maintenance entails a continuous adaptation to several situations including genetics, production and breeding systems, nutritional balance, environmental interaction and climate change (allostasis). Therefore, according to the *resource allocation theory* the resources available to an organism are limited and consequently must be conserved. If not, body structures and basal metabolic processes will be weakened and will no longer support the metabolic performance sufficiently, with a consequent high risk of disease. This implies that high-productivity farm animals do not provide sufficient resources to adequately cope with stressful conditions (Huber, 2018, Animal, 12:528-536).
Livestock selection has largely been based on production traits such as milk production or growth rate, but as these productions are increased through one biological process affecting other functions such as disease resistance. Parasites can greatly affect the amount of resources available to their host both through direct competition and the physiological costs caused by the immune responses. This involves a reallocation of resources and thus changes the response in the expression of other traits.

During parasite infection, the organism releases glucocorticoids and thus mobilizes resources for immune competence to parasite defense. The stress responses result from its ability to support adaptation, however this catabolic activity is exerted at the expense of the anabolism-based production potential.

Helminth control strategies worldwide are based almost entirely on the frequent use of anthelmintic drugs, which are increasingly regarded as unsustainable given the emergence of multiple drug-resistant parasites. In developed countries, there is a demand for animal products derived from ethical, green and clean breeding programmes.

Rauw et al., 2012 (Front Genet., 14:263-267) reviewed the negative side effects of selection for high production and concluded that high productivity in livestock may mean that there are insufficient resources to adequately cope with stress factors, and hence poor welfare whenever resources are limited.

The acquisition and expression of immunity against parasites is genetically controlled by varying the breeds and the individuals of the same breed (McManus et al., 2014, 21:56). The implementation of breeding programmes is one of the most important factors as the host-parasite interaction occurs at several levels. Selection schemes can confer resistance or resilience to parasite infection. Of course, where the objective is to prevent the spread of the disease to other animals (zoonosis), disease resistance is more of a requisite.

Many farmers have already begun to adapt their farming practices and strategies in response to the indications of European Food Safety Authority in terms of animal welfare. The options include production adjustments, diversification, intensification, changing land-use, and altering the timing of operations. In relation to adaptation strategies and their impacts, there are important differences between extensive and intensive systems for livestock production.

The future sustainability of the livestock industry, must employ farming techniques that protect the environment, public health and animal welfare, while enabling farmers to derive adequate financial rewards.

As regards the pathogenetic aspects linked to a single parasitosis, possible indicators of the status of infestations on farms will be examined and evaluated through a pathogenetic analysis. Specific parameters of infestation will be analyzed, such as diarrhea, serum pepsinogen, FEC (faecal worm egg counts) values for GIN, evaluation of the nasal discharge score for *Oestrus ovis*, subcutaneous nodule count from *Hypoderma* spp. in cattle along with an assessment of antibody rates against the larvae of these dipterans. We also propose a mapping of sheep farms through the registration of data acquired at the slaughterhouse or through ultrasonography on parasite diffusion. This would enable farms to be certified in relation to this metacestodosis of zoonotic importance. The mapping could include cystic echinococcosis and also coenurosis and cysticercosis metacestodosis which are often underestimated from an economic-productive aspect.

Research in this area could lead to the development of rational policy guidelines for their possible application for safeguarding animal welfare and their production.

In fact, the proposed measures to date have not always been applicable and proposed appropriately. It is worth mentioning here the measure for the control of animal welfare in cattle by the regional government of Sardinia (RDP 2014/2020, Reg (EU) No. 1305/2013 - Measure 14 - Animal welfare). This measure referred to monitoring which was supposed to be carried out four times a year in at least 10% of the animals bred for the general analysis of ectoparasites. A scotch tape test was proposed, whose interpretation, reading and effectiveness were poorly applicable in the field.

A rational monitoring and control of parasitosis, also in terms of rural / social development, will also depend on the capacity to involve the operators of the sector, whose should be trained in the importance of carrying out measures for the control of parasites and animal welfare, regardless of the possible governmental rewards.
The integration of specific parasitological indicators that certify the status of animal welfare in breeding could also have positive economic repercussions for all of the production chain.

Socio-economic aspects of parasite control in livestock

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Keywords: livestock, helminths, economics, communication

Essentially all herds/flocks in a grass-based production system are affected by helminth infections. Helminth infections of livestock affect productivity in all age classes, and are amongst the most important production-limiting diseases of grazing ruminants.
Over the last decade, there has been a shift in focus in the diagnosis of these infections from merely detecting presence/absence of infection towards detecting the impact of infection on production. This has been facilitated by different epidemiological studies observing consistent negative correlations between helminth diagnostic test results and measures of productivity. These established relationships can be used to indicate helminth-induced production losses associated with a test result of a specific farm. However, the economic impact of helminth infections and helminth control measures also depends on multiple other factors, such as farm-specific input and output prices and local regulations. Animal health decisions have a significant impact on production efficiency, but are also subject to resource scarcity and budget constraints. Hence, economic evaluation frameworks are needed that can be integrated in decision making. Such economic models of animal diseases are important because they contribute to balance expenditures on disease control with the actual disease costs and to evaluate the economic attractiveness of animal health interventions compared to other investment opportunities.
Moreover, it is now well understood that farmer's decisions about their enterprises are not solely based on financial and business criteria. Farmer's motives are rooted in deeply held values and are influenced by attitudes, beliefs and social norms. Understanding all the values that drive farmer behaviour requires socio-psychological research, aimed to lead to increased understanding of a farmer's rationality and more effective advisory interventions. Behavioural frameworks can be constructed, based on theories in the fields of behavioural and health psychology and validated with data from quantitative (surveys) and qualitative (focus group meetings, in-depth interviews) socio-psychological research, in order to identify factors with significant influence on farmers' behaviour intention. Identified barriers and benefits for sustainable worm control can then be tested using interventional communication experiments. In addition to these cognitive factors, intuition is also an important driver of human behaviour. Intuitive, unconscious farmer behaviour can be influenced by discrete stimuli (nudges). Together with factors from the cognitive behavioural frameworks mentioned above, these nudges can be used to steer farmers behaviour towards more sustainable parasite control.
In this presentation, we will review recent insights in the farm-specific economic impact of helminth infections on (dairy) cattle farms as well as in farmer attitudes and behaviour regarding helminth control in dairy and sheep farms. Combining better economic assessments of helminth infections on a farm together with a deeper understanding of the non-economic factors that drive a farmer's animal health decisions should result in more effective control strategies, a higher farmer's compliance and increased farmer satisfaction.

How gastrointestinal nematodes can influence the quantitative and qualitative milk production in small ruminants?

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Keywords: Small ruminants, gastrointestinal nematodes, milk production

Small ruminant farming plays a *significant role* in the sustainability of rural communities around the world, as well as being socially, economically and politically highly significant at national and international levels (Morgan et al., 2013, Agriculture, 3, 484-502). Efficient small ruminant livestock production is also crucial to meet the increasing demands of meat and dairy products, especially in areas in which land is unsuitable for growing crops (Chiotti et al., 1995, J Rural Stud, 11, 335-350).

Small ruminant dairying is particularly important to the agricultural economy of the Mediterranean region, which produces 66% of the world's sheep milk and 18% of the world's goat milk (Pandya et al., 2007, Small Rum, Res, 68, 193-206). Among the factors that negatively affect the livestock production, infections with parasites and in particular gastrointestinal nematodes (GINs) continue to represent a serious challenge to the health, welfare, reproduction and productivity of grazing ruminants throughout the world. These pathogens can cause severe disease and are amongst the most important production-limiting infections of grazing ruminants in Europe and globally (e.g. www.discontools.eu) as recently reviewed by member of the Livestock Helminth Research Alliance – LiHRA (Charlier et al., 2017, Transbound Emerg Dis, in press).

The impact of GINs on productivity depends on the host species, its geographical location and physiological status and will further be largely dependent on the degree to which a farmer can counteract infection-induced energetic losses by the provision of protein-rich diets (Kyriazakis & Houdijk, 2006, Small Rum Res, 62, 79-82).

Essentially, all flocks in a grass-based production system are affected and the major economic impact is due to sub-clinical infections causing reduced growth and milk/wool production (Morgan et al., 2013, Agriculture, 3, 484-502). A recent comprehensive study, funded by the EU-GLOWORM project, investigated the economic burden of helminth parasitism on the EU livestock industry. Results indicated a staggering annual loss of €2.3 B in dairy cattle and €1.1 B in meat production from sheep caused by GINs alone.

Anthelmintic resistance and climate change is likely to alter the geographical distribution of these parasites and their impact on production animals, thus increasing the need for a clear understanding of the cost of parasitism in order to develop sustainable control strategies (Mavrot et al., 2015, Parasit Vectors, 8-557).

In dairy goats, Hoste et al. (2005, Small Rumin Res, 60, 141-151) showed that subclinical parasitism caused a decrease in milk yield ranging from 2.5% to 10%. Studies conducted in Italy involving untreated naturally infected animals and anthelminthic-treated animals showed that milk production was significantly higher in treated goats than in the untreated animals and treatment led to a persistent increase in milk yield, ranging from 7.4% to 18.5% compared to control values. In addition, GIN infection might also produce a deteriorating effect on milk quality, since milk from the untreated group showed 29.9% lower fat content, 23.3% lower protein content and 19.6% lower lactose content than milk from the control group (Rinaldi et al., 2007, Trans R Soc Trop Med Hyg, 101, 745–746). In a study in Argentina by Suarez et al., (2017, Onderstepoort J Vet Res, 2219-0635), differences between groups were even higher than the studies by Rinaldi et al. (2007), with a mean treatment response of treated group (41.8 %) higher

than untreated group milk yield.

In sheep, strategic treatments of grazing animals with anthelmintics has positively influenced not only the growth of lambs (Fthenakis et al., 2005, Vet. Med A: Physiol Pathol Clin Med, 52, 78-82) but also the wool production (Cobon and O'Sullivan, 1992, J. Agric Sci, 118, 245–248) as well as milk yield (Cringoli et al., 2009, Vet Parasitol, 164, 36-43). Studies conducted in sheep farms in southern Italy showed that milk productions of the treated groups were significantly higher than those of the control groups with values between 18.9 % and 44.2% (Cringoli et al., 2008, Vet Parasitol, 156, 340-345). Furthermore, in dairy sheep, experimental studies conducted on infected lactating ewes showed a significant effect of nematode infections on production. In two similar studies, sheep infected with larvae of *Teladorsagia circumcincta* showed a 17% or 11.9% reduction, respectively, in milk production compared with helminth-free controls (Cruz Rojo et al., 2012, Vet Parasitol, 185: 194-200). Likewise, a study comparing milk yield in ewes orally infected with *Haemonchus contortus* larvae weekly during pregnancy and lactation reported a marked weight loss and reduction of 23% in milk yield (Thomas & Ali, 1983, Int J Parasitol Parasites, 13:393-8).

In Europe sheep and goat milk are mainly used for a production of fine cheese varieties and it has been know that one third of the world's production is from Mediterranean countries. Therefore, it is important to measure the effects of GIN infections on milk composition, which effects the quality of cheese. In this context low level of GIN infection revealed better body condition of sheep and a higher milk production and protein which improve the cheese yield of milk (Cruz-Rojo et al., 2012, Vet Parasitol, 185, 194–200).

These results highlight the need for an agreement among stake-holders and end-users on sustainable and integrated control strategies for preventing milk production losses in small ruminants.

In conclusion, we acknowledge that studies explicitly examining the impact of GIN infections on milk production in small ruminants remain comparatively few. This highlights the fact that we are still far from knowing the relative contributions of burden, co-infections, environmental, management and socio-economic influences to the impact of GINs on livestock production. Thus, there is a clear need to integrate veterinary, agricultural and social sciences to better understand the real production and economic costs of GIN infections.

TAVOLA ROTONDA 5

LA STRADA VERSO L'ERADICAZIONE DELLA MALARIA: SFIDE E SUCCESSI

Malaria eradication versus drug resistance evolution: which one wins?

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Control of microbial pathogens follows a repetitive and depressing cycle: a new drug is introduced and works well for a while, until drug resistant pathogens arise and spread. This is bad news for the people infected, but provides an excellent opportunity to study recent selective events or those that are still ongoing. How many times does drug resistance arise in microbial populations? What determines whether particular drug resistance alleles spread? How many genes are involved? Answering these questions is critical if we are to develop sensible “evolution proof” strategies for controlling malaria and other pathogens. Resistance to a succession of antimalarial drugs has arisen over the past half century and invariably arises in SE Asia: my lab uses molecular data and an evolutionary framework to better understand the dynamics of drug resistance evolution in the face of strong drug selection.

New tasks and tools to block the human-to-mosquito transmission of *Plasmodium falciparum*

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Keywords: Malaria, parasite transmission, *P. falciparum* gametocytes, transmission-blocking drugs

INTRODUCTION. Despite the significant success of reducing the incidence and the morbidity of malaria in the past 15 years, the global burden of this disease, with 215 million cases and 415,000 deaths per year in 97 endemic Countries, mainly in Africa, is still unbearable. Besides sustainable malaria control programs, research is critical to address the challenges posed by the objective to radically eliminate this disease and to eradicate the malaria parasites.
Epidemiological studies are highlighting the important role of asymptomatic and adult individuals as an infectious reservoir for *Plasmodium falciparum* transmission (Gonçalves et al., 2017 Nat Commun. 8:1133), indicating that the parasite stages responsible for the human-to-mosquito transmission, the gametocytes, are key targets for interventions to eliminate transmission and eventually eradicate Plasmodium.
P. falciparum gametocytes develop from blood stage asexual parasites in ten days, in which intraerythrocytic sexual differentiation progress from stage I to the mature stage V. Although this process is reproducible *in vitro*, making it possible to identify compounds able to kill these parasite stages, the non-dividing nature of gametocytes has posed significant constraints in the development of specific, sensitive, robust and scalable cell based assays to identify anti-gametocyte compounds. This also impaired quantitative analyses with specific inhibitory compounds to characterize gametocyte metabolism for the identification of suitable parasite biochemical drug targets.
MATERIALS AND METHODS. The unsurpassed sensitivity of luciferase reporter-based assays was chosen as a main research avenue to develop *P. falciparum* gametocyte cell based assays suitable to be scaled up for drug high-throughput screening (HTS). Potent luciferases, including luciferase natural variant or *in vitro* mutagenized enzymes characterized by a red or a green light emission shift were introduced as reporter genes in *P. falciparum* transgenic lines (Cevenini et al., 2014 Anal. Chem. 86: 8814-21). Cloning of appropriate parasite gene promoters to drive expression of the luciferase reporters was used to generate different *P. falciparum* lines in which the bioluminescent signal was specific for all gametocyte stages (D'Alessandro et al., 2016 J. Antimic. Chemother. 71: 1148-58) or only for stage V, mature gametocytes (Siciliano et al, 2017, Mol. Microbiol. 104: 306-318).
To further address the need to develop a cell based assay specific for the stage V, mature gametocytes, a phenotypic assay was also developed. This assay was designed to quantitate the efficiency of mature gametocytes to undergo the first step of gamete activation, in which the elongated *P. falciparum* gametocyte becomes spherical ("rounding up"). Use of a fluorescent dye to stain gametocytes and the quantification of "rounding up" by an automated image recognition algorithm enabled development of a HTS assay in which effect of compounds could be readily measured in a phenotypic assay on mature gametocytes, which, importantly, was applicable also to non-transgenic, wild parasites (Lucantoni et al., 2015 Sci. Rep. 5: 16414).
RESULTS AND CONCLUSIONS.
Towards a screening pipeline for drugs to block P. falciparum *transmission.*
The development of luciferase reporter lines producing stage specific bioluminescent signals enabled the implementation of HTS screening for compounds able to kill gametocytes at all stages of development or specifically at the stage of mature gametocytes. In addition, the use

in these assays of a novel luciferase substrate formulation, free of exogenous ATP and of luciferase stabilizing factors typically present in commercial kits, resulted in a bioluminescent signal more reliably reflecting gametocyte viability (Cevenini et al., 2014 Anal. Chem. 86: 8814-21).

In view of the need to compare the activity of hits obtained with the above luciferase reporter assays on wild type gametocytes, the readout from these assays was systematically compared with that of a parasite Lactate Dehydrogenase based assay that had been adapted to test compound activity on gametocytes (D'Alessandro et al, 2013, J. Antimic. Chemother. 68: 2048-2058). Results showed that the two assays produce highly comparable results, with correlation coefficient ~0.9 (D'Alessandro et al., 2016 J. Antimic. Chemother. 71: 1148-58), indicating that gametocyte luciferase assays can be used as a frontline HTS assays to explore large unbiased compound libraries to interrogate chemical diversity, whereas pLDH and additional phenotypic assays can be used as orthogonal assays in a first tier of hit identification and validation (Birkholtz et al., 2016, Trends Parasitol. 32: 669-81).

Gametocyte assays to identify metabolic pathways targetable in the parasite transmission stages

Easy, quantitative, reliable and sensitive gametocyte cell based assays opened the route to assess effects of drugs and compounds on gametocyte viability with an unprecedented robustness and accuracy. An analysis was conducted to test the effect on gametocytes of the combination of compounds described for unbalancing redox equilibrium in *P. falciparum* asexual stages used in combination with methylene blue, a drug described to efficiently kill all gametocyte stages. An isobologram analysis based on a mature gametocyte luciferase assay reaveled a potent synergy between methylene blue and a specific inhibitor of the parasite PfGluPho, the enzyme responsible for most of the NADPH production in the parasitized erythrocyte (Siciliano et al, 2017, Mol. Microbiol. 104: 306-318). This result revealed for the first time a synergistic effect of gametocyte killing compounds and unveiled an unexpected exquisite sensitivity of stage V gametocytes to treatments unbalancing the redox equilibrium and depleting the parasite intracellular reducing power. This results opens the route to targeting this metabolic pathway and, in the immediate future, to potentiate activity of methylene blue, a drug already showing in clinical trials a promising *P. falciparum* transmission blocking activity (Dicko et al., 2018 Lancet Infect Dis. S1473-3099(18)30044-6).

From measuring gametocyte sensitivity in vitro *to predicting mosquito infectiousness*

So far, several HTS exercises to identify anti-gametocyte drugs have produced several candidate compounds that could be developed into parasite transmission blocking drugs, fulfilling one of the recently revised Target Candidate Profiles for malaria eradication (TCP5) proposed by Medicines for Malaria Venture (Burrows et al., 2017 Malar J. 16:26). Since their appearance, gametocyte cell based assays have been challenged by the question of what level of accuracy was their readouts predictive of compound ability to block parasite transmission through mosquitoes. To answer this question no HTS assay is presently available to substitute the gold standard technique to measure gametocyte infectiousness to mosquitoes, which is the technically complex, labour intensive and relatively unsafe xenodiagnosis, i.e. the analysis of experimental infections of insectory reared mosquitoes fed with cultivated gametocytes. A key challenge in the development of tools to identify and validate transmission blocking drugs, but also to conveniently measure the efficacy of control measures affecting human-to-mosquito transmission, or to readily map the *P. falciparum* transmission reservoir in epidemiological surveys, is therefore the development of *in vitro* and *ex-vivo* simple, sensitive and reliable

assays able to functionally predict whether an individual is, at the moment of sampling, a malaria parasite transmitter.

Challenges of malaria eradication in real life

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According to the World Health Organization (WHO, 2016), "***Malaria elimination*** *is the interruption of local transmission (i.e. reducing the rate of malaria cases to zero) of a specified malaria parasite in a defined geographic area. Continued measures are required to prevent reestablishment of transmission.* ***Malaria eradication*** *is defined as the permanent reduction to zero of the worldwide incidence of infection caused by human malaria parasites as a result of deliberate efforts*".

The United Nations Agenda for Development (2030 Agenda, Goal 3, Target 3), declared in September 2015, aims at ending the epidemics of tuberculosis, HIV and malaria by the year 2030. In particular, the 2030 Agenda confirmed the vision of a world free of malaria set out in the Global Technical Strategy (GTS) for Malaria 2016-2030 whose principal targets for the year 2030 are (WHO, 2016):

1. Reducing malaria case incidence by at least 90%
2. Reducing malaria mortality by at least 90%
3. Eliminating malaria in at least 35 countries
4. Preventing the re-establishment of malaria in all countries that are malaria-free

Are these targets within the reach of humankind by the year 2030 with the control and therapeutic tools nowadays available? The topic is complex and the answer unknown.

As a matter of fact, since 2010 the incidence of malaria cases and deaths have been reducing steadily from 237 million cases and 591.000 deaths to 216 million cases and 445.000 deaths in 2016. From the year 2000 to 2016, 18 countries have declared 0 local cases for three years or more, and six among them (Morocco, Armenia, Turkmenistan, Sri Lanka, United Arab Emirates and Kyrgyzstan) have been officially declared malaria free by WHO (WHO, 2017).

These great achievements are the result of a global strategy combining the use of rapid diagnostic tests (RDTs), the adoption of artemisinin combination treatment (ACT) policies and the use of impregnated bed-nets (ITN), indoors residual spraying (IRS) activities and chemoprophylaxis of pregnant women and children. These activities have been supported by an intense funding effort of the international community, mainly channeled by the Global Fund.

However, after the initial global reduction in the incidence of malaria cases and deaths, the 2017 World Malaria Report (WHO, 2017) shows a progressive slowing down of the decrease, with stagnating (and somehow slightly increasing) figures in some countries. The sub-saharan African region remains the hard core of malaria with 91% of the total number of cases. Fourteen of the 15 countries where 80% of the global burden of malaria is recorded are in the African region, except India.

What are the factors that endanger the achievement of ending malaria by 2030? Those factors are complex and multifaceted, at both global and local levels.

The yearly global funding to fight malaria has remained quite stable around 2.5 US$ billion since 2010, which represents almost 40% of the 6.5 US$ billion needed to reach the targets. The global economic crisis occurred in 2009 has virtually hindered the needed fund increase.

As a consequence, the number of people sleeping under impregnated bed nets has not increased as planned in the most endemic areas of Africa. Similarly, the number of RTDs distributed in 2016 fell by 26 million (from 247 million in 2015 to 221 million in 2016) mainly in

Africa (WHO, 2017). The availability of ACT treatments has also increased, but the dreadful phenomenon of fake or under dosed drugs is out of control in many low - resource countries.
The ACT resistance reported in South-East Asia has not been identified yet in Africa and the resistance of Plasmodia to pyretroids, despite being reported in as many as 81% of reporting malaria endemic countries, has not been associated to the burden of malaria morbidity and mortality (Malaria Atlas Project. 2017)
In a multicentric survey in Africa, the proportion of feverish children in malaria endemic areas who do not receive medical attention was reported to be still unacceptably high (median 39%) (WHO, 2017), probably due to limited access to skilled health workers.
The available tools to fight malaria have definitely improved during the last 15 years. Rapid diagnostic tests have increased the rate of the correct diagnosis of malaria, preventing dangerous over and under treatments. The artemisinin combination therapies have offered an effective
treatment for all species of plasmodia, with low potential risk for the occurrence of drug resistance, despite the need for accurate drug susceptibility surveillance. Additionally, impregnated bed nets and indoor residual sprays are still good tools for vector control.
However, despite the tremendous efforts and extraordinary results obtained, too many children still dye of malaria, mainly in Africa. Many challenges remains unaddressed.
In particular, in well-structured health systems the complete access to effective care remains the true challenge to fight malaria, as well as tuberculosis, HIV and any other poverty-related diseases. One among the unaddressed challenges is the high rate of clinical malaria in women (Roll Back Malaria, 2007), possibly related to poorly investigated social factors (gender malaria) that require urgent attention.
Additionally, the increased human mobility to and from malaria endemic areas – both *P. falciparum* and *P. vivax* – is posing a serious threat to malaria elimination and eradication strategies, mainly in low incidence countries.
The final way to malaria eradication will probably be paved by a future effective vaccine, as difficult as it will be. The currently available vaccine, despite providing only a modest and temporary level of protection for clinical and severe malaria, is now being tested on a large scale in 3 high-malaria burden African countries (Ghana, Kenya and Malawi).

* The Authors are responsible for the views contained in this article and for opinion expressed therein, which are not necessarily those of UNESCO and do not commit the Organization

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TAVOLA ROTONDA 6

PARASSITI DEL CANE IN ITALIA: COS'E' CAMBIATO?

Italian nationwide survey on endoparasites of dogs

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INTRODUCTION. Endoparasite infections are common conditions of dogs worldwide that in many cases are not managed properly. In addition, many nematode parasites of dogs, such as *Toxocara canis*, *Ancylostoma caninum* and *Dirofilaria* spp. display marked zoonotic potential as infected dogs serve as reservoirs for humans. Although several surveys indicated that the prevalence of infection by gastrointestinal (GI) nematodes has decreased over the time due to improved veterinary care, recent studies throughout Europe have shown that intestinal helminths are a common occurrence in dogs. In addition, some species of cardiopulmonary nematodes, i.e. *Dirofilaria immitis* and *Angiostrongylus vasorum*, have been also increasingly reported and are apparently spreading into previously non-endemic areas of Italy (Otranto et al., 2009; Parasit Vectors., 2 Suppl 1: S2; Giangaspero et al., 2013; Parasito Res 24(2):1357-1361; Traversa et al., 2013, Parasitol Res, 112(7): 2473-2480; Rinaldi et al., 2014, BMC Vet. Res., 10:236; Olivieri et al., 2017, BCM Vet. Res. 13(1): 165). The reasons for the overall spreading of endoparasites of dogs are not completely understood, though climatic, environmental and ecological factors (e.g., growth of fox populations and the increased sympatry between companion and wild animals) seem to play a key role. As a matter of facts, the increased awareness of cardiopulmonary nematodes coupled with the availability of more sensitive diagnostic tests, including improved copromicroscopical techniques, molecular and serological assays, have significantly facilitated diagnosis and have led to an increase of reports. In the last years, the occurrence of canine intestinal and cardiopulmonary parasites has been investigated in a number of surveys in Italy (Di Cesare et al., 2011, Parasitol. Res. 109 Suppl 1: S87-96; Guardone et al., 2013, Vet Parasitol., 192(1-3)192-198; Pipia et al., 2014, Parasitol. Res. 113:1505-1509; Del Prete et al., 2015, BMC Vet. Res., 11:306; Simonato et al., 2015, Parasitol Res., 114(5): 1963-1970; Magi et al., 2016, J Helminthol, 90(1): 121-124; Traversa et al., 2017, Comp. Immunol. Microbiol. Infect. Dis., 51:69-75; Sauda et al., 2018 Parasite, 25:2). However, these studies have been conducted patchily across the peninsula and have used different diagnostic tools leading to results which are difficult to compare. Therefore, given the veterinary and public health importance of internal parasites of dogs and the recent changes in the epidemiology of some species of cardiopulmonary nematodes, a nationwide survey on these parasitic infections in dogs is needed.

In this study, we assessed the prevalence and the geographical distribution of internal parasites of dogs in Italy. Data on treatments and preventive regimes of sampled animals were also recorded and analyzed.

MATERIAL AND METHODS. Thirteen regional units, five in northern regions and four in both central and southern regions, were involved in the study; the sampling areas were selected by each unit in order to have a wide and as much as possible homogeneous geographical coverage of the region. The study population was composed of dogs older than 6 months with constant or regular access to the outdoors. Animals treated with drugs active against internal worms in

the month before sampling or under treatment against canine heartworm disease (*D. immitis*) were excluded from the study. Dogs were enrolled in the study following the collection of the signed informed consent by owner; dogs were identified using an alphanumerical ID code, physically examined and sampled for blood (~5 mL) and faeces (~20 g). Anamnestic data and information on treatments and prophylaxis against parasites were also recorded at the time of sampling.

Samples were transported to the reference unit and analysed within 2 days. Briefly, faeces, were tested by Mini-FLOTAC (Cringoli et al., 2017, Nat. Protoc. 12:1723-1732) using saturated sodium chloride flotation solution (s.g. 1.200) for detecting helminth eggs and by modified Baermann technique for metastrongyloid larvae. The blood samples were examined for blood microfilariae by Knott's test, and the sera analysed with a rapid serological assay for the detection of *A. vasorum* circulating antigens (Angio Detect®). Animal data and laboratory results were entered into an electronic data capture system (Castor Electronic Data Capture, Ciwit BV, Amsterdam, The Netherlands, 2018) and submitted to quality control and validation process.

RESULTS AND CONCLUSIONS. A total of 1748 dogs were included in the study. Sample was gender-balanced (50.6% females and 49.4% males) and uniformly distributed across the country, being 31.8% of the dogs from northern, 29.2% from central and 39.1% from southern regions. Over forty percent (44.2%) of the animals were kept in households, while 55.8% were housed in kennels. The age of the animals ranged from 6 months to 17 years (mean: 58.2 months); 45.4% of the subjects were hunting dogs, 41.8% kept as companion dogs and 12.8% had different uses (guard = 8.9%, rescue = 2.1% and sport = 1.8%). The majority of the enrolled dogs were living outdoors (64.2%) and cohabitating with other dogs (83.3%), cats (24.4%) or other domestic animals (10.1%). As regards to breeds, 60.8% of the dogs were purebred while 39.2% were crossbred.

Five-hundred-fifty-six faecal samples (31.8%) tested positive for one or more helminth species with the Mini-FLOTAC technique. *Trichuris vulpis* was the most frequent parasite species retrieved, being detected in 13.5% of the samples, followed by Ancylostomatidae (11.6%), *T. canis* (9.0%), *Capillaria aerophila* (4.8%), *Capillaria bohemi* (1.9%) and *Toxascaris leonina* (1.0%). The total number of dogs infected by gastrointestinal nematodes (i.e., *T. canis, T. leonina, T. vulpis*, and hookworms) was 490 out of 1748 (28.0%). Eggs of *Dipylidium caninum* (0.3%*),* eggs of taeniids other than *D. caninum* (0.1%), larvae of *A. vasorum* (0.2%) and/or protozoa cysts such as *Cystoisospora* spp. (0.7%) and *Giardia duodenalis* (0.8%), were also found in a few samples. Thirty-five faecal samples (2.0%) out of the 1748 examined scored positive at Baermann technique; detected species were *A. vasorum* (1.7%), *Crenosoma vulpis* (0.3%) and *Filaroides (Oslerus) osleri* (0.1%).

Blood microfilariae were found in 52 (~3%) out of the 1748 blood samples. In particular, microfilariae of *Dirofilaria repens, D. immitis* and *Acanthocheilonema reconditum* were detected in 1.5%, 1.1% and 0.8% of the samples, respectively. The majority of animals (82.7%) was infected by a single filarial species while 17.3% with two species. Finally, the sera of 33 dogs (1.9%) scored positive at the antigenic rapid test for *A. vasorum* leading to an overall positivity for *A. vasorum* assessed by copromicroscopy (Mini-FLOTAC and Baermann) and serology of 2.1% (37/1748). In particular, four dogs were positive to all the techniques, 21 to both Baermann and serology, while 8 and 4 dogs scored positive only to serology or Baermann test, respectively.

This study represents the first nationwide epidemiological survey on internal parasites of owned dogs in Italy. Prevalence and species of gastrointestinal nematodes registered match with those reported in other surveys conducted throughout Europe. Though the absence of young (< 6

months) animals amongst sampled dogs, the prevalence of the three most frequently retrieved species/taxa (i.e. *T. vulpis*, Ancylostomatidae and *T. canis*) is considerably high, indicating how owned dogs may be significantly infected by intestinal parasites even if adult and apparently healthy. Only a few (30/490; 6.1%) gastrointestinal-infected dogs showed signs suggestive of gastrointestinal nematode infection (e.g., abnormalities in faecal consistency, diarrhoea, weight loss, abdominal pain and malabsorption syndrome), while the infection was apparently asymptomatic in the remaining infected dogs.

Nearly forty percent of the examined dogs received at least one product effective against gastrointestinal nematodes in the year before sampling. Despite that the use of anthelmintic drugs in animals of different ages was similar, ranging from 37.4% in adult animals to 42.6% in young animals (6 to 11 months), the percentage of dogs treated with at least one product active against gastrointestinal nematodes was higher in north and central Italy (44.9-47.0%) than in southern regions (29.3%). Rescue and sport dogs displayed the highest percentage of treatment against gastrointestinal parasites (58.3% and 45.2%, respectively), followed by companion (42.5%), hunting (38.5%) and guard (23.7%) dogs. Similarity, the percentage of dogs receiving at least four treatments per year was 19.4% in sport, 14.3% in companion, 12.2% in guard, 10.3% in hunting and 5.6% in rescue dogs. The treatment with anthelmintic products was effective in reducing gastrointestinal infections (P = 0.0386) and the frequency of infection was significantly lower in the cohort of dogs that received at least four treatments (13.6%) compared to that treated only once in the year before sampling (25.4%) (P = 0.0003).

Among lungworms, *Capillaria* spp. and *A. vasorum* were the most frequently retrieved species. Thanks to the large and well-distributed sample size and because of the combined use of copromicroscopical and serological tests, this study provides a fairly good estimation of the presence of *A. vasorum* in dogs in Italy with an overall prevalence of 2.1%. *Angiostrongylus vasorum* infection showed to be patchy distributed along the country with spots of higher prevalence (up to 7.14%) in central and southern regions. Noteworthy, despite the infection was diagnosed in animals of all ages, the frequency was twice (4.4%) in dogs of 6-12 months compared to other classes of age (0.8-2.1%) emphasising the higher risk of infection of the former group.

Clinical signs suggestive of angiostrongylosis were present in 11 (29.7%) infected animals while 26 (70.3%) were apparently healthy. Though many of the sampled dogs were living in areas where the presence of foxes (69.5%) and/or wolves/stray dogs (41.9%) had been reported, treatment with products active against respiratory nematodes was not common and only 104 (5.9%) of the examined dogs were treated with products active against lungworms. Similarly, despite nearly half (869) of the examined dogs were living in areas recognized as endemic for *D. immitis*, only 42.2% (367) was reported to be treated with anthelmintic effective for heartworm prevention. Moreover, only in 62.4% (229) of the treated dogs appropriate products were properly used, while 23.2% was treated with inadequate regimens, or with off label products (14.4%).

Results of this study showed that infections by internal parasites are a fairly common condition in owned dogs in Italy. Infection by GI nematodes are present at high prevalence and mostly sustained by species of great zoonotic concern. Respiratory nematodes are frequent and wide-dispersed as well, being *Capillaria* spp. and *A. vasorum* the most frequent species. The use of anthelmintic products, either for treatment or prevention, is still far from being correctly dispensed. This may lead to an increase of the zoonotic risk to exposed people, the recrudescence of relevant endemic parasitic infections such as canine heartworm disease or the spread of emerging ones such as angiostrongylosis. Both veterinarians and owners should

make aware of these risks and the adoption of effective control schemes as well as reliable diagnostic methods is strongly advocated.

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TAVOLA ROTONDA 7

PARASSITOSI IN APICOLTURA:

ATTUALITA' EPIDEMIOLOGICHE E PROSPETTIVE PER IL CONTROLLO

The five Ws about Nosema ceranae

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The **Five W´s** (sometimes referred to as **Five Ws + How**) are questions whose answers are considered basic in information gathering or problem solving. The "Five Ws" (and one H) were memorialized by Rudyard Kipling in his "Just So Stories" (1902):

I keep six honest serving-men
(They taught me all I knew);
Their names are What and Why and When
And How and Where and Who.

Who discovered *Nosema ceranae*?

Nosema ceranae was first detected in *Apis cerana* at the end of the XX Century by Dr Fries et al. in 1996, and then in *A. mellifera* in Spain and Taiwan during the initial years of the XXI Century by Dr Higes et al. and Huang et al. After some initial doubts, *N. ceranae* is now considered a predominant infective agent of *A. mellifera* that is related to the high losses among honey bee colonies in Mediterranean countries. Indeed, the detection of *N. ceranae* in Spain did not occur by chance but rather, it was a response to the demands of professional beekeepers. In 2004, there was a ten-fold increase in the number of samples sent to the Bee laboratory at Marchamalo due to colony losses, with well-experienced beekeepers reporting only empty hives or hives in a poor state of health. Beekeepers detected these losses especially in winter when they checked the health status of the colonies before blooming. No clinical signs of common disease were detected and consequently, it became evident that the prevalence of *N. ceranae* in these colonies was close to 90% almost all year round, from 2004 to 2006. This finding facilitated the expedition of a special license for the use of fumagillin in Spain, given the lack of official acknowledgement for its use in Europe, which produced a significant reduction in colony losses and the highest honey production of recent years.

Where is *Nosema* present?

The original host of *N. ceranae* is unknown but it is generally presumed to be Apis cerana, from which it was first isolated in 1996. The parasite was later found to have infected *A. mellifera* in preserved worker bees sampled from Los Angeles (USA) in 1975 and Asian worker bees (*A. cerana*, *A. dorsata*) from Taiwan from as early as 1968, as well as in Brazilian Africanized *A. mellifera* bees in 1979. After its initial detection in Taiwan and Spain, in 2007 *N. ceranae* was reported in the USA, Brazil, China, Vietnam, Greece, Italy, Serbia, Germany, France, Denmark, Finland and Sweden. More recently, the pandemia was verified as the pathogen crossed geographic boundaries, being detected in countries like Canada, Australia, Uruguay, Argentina, Hungary, Bosnia-Herzegovina, Croatia, Turkey and the UK, Japan and Thailand, Chile, Jordan and Saudi Arabia.

Although no population differentiation is observed among *N. ceranae* isolates from *A. mellifera*, a few specific mutations seem to be linked to particular geographical areas. While this association could be interpreted as an incipient genetic structuring of the species, this hypothesis should be regarded with some caution as it is founded on the analysis of a single locus. Similar regional clustering was previously found based on the ribosomal SSU, although the multicopy nature of this gene and the lack of molecular cloning in most of these studies raise doubts regarding their reliability. In line with the absence of clear geographic differentiation,

more than 98% polymorphism was seen to be shared among at least two of the eight *N. ceranae* genomes analysed, confirming that population structuring in *A. mellifera*, if any, is still at an extremely initial stage. Together, these data (the reduced among-isolate differentiation and the frequency spectrum of synonymous variants) support the recent worldwide invasion of Western honey bee colonies by this emergent pathogen, as previously suggested by epidemiological data.

What happen to honeybees with *Nosema*?

Since the first report of *N. ceranae* infection in *A. mellifera* bees, there has been some controversy about the consequences of such infection. However, in recent years most studies have confirmed that *N. ceranae* has a pathogenic effect in this host, expressed at least in a shortening of the (worker) honey bees' lifespan, with only some few papers failing to report this effect. A common sub-clinical sing of Nosemosis type C is the decline in honey production of about 50% reported in honey bee colonies parasitized. Similar results were obtained elsewhere, with a reduction in honey production of between 52 and 67 % in parasitized colonies.

Studies over a long periods demonstrated that *N. ceranae* can trigger premature foraging activity and shorten the lifespan of infected worker bees. Indeed, *N. ceranae* infection appears to accelerate honey bee behavioural development, and it disrupts the basic underpinnings of temporal polyethism as workers may become less flexible in their response to colony demands, leading to colony decline. Notably, decreasing the worker lifespan by 9 days, especially during the foraging phase, means that the nectar and/or pollen that would been collected by the infected bees during these days would be lost, producing a negative effect at the colony level that is consistent with earlier results. It has also been shown that infected bees take longer foraging trips and that they spend less time in the hive between successive trips, bringing back less sugar from each trip. The changes in foraging activity have a strong adverse effect on the efficiency of the colony's energetic gain, which has important implications for the individual and colony lifespan, producing a substantial demographic effect on the colony that can lead to a strong decline in population size and ultimately, to colony death. Recently it was recorded the drifting behaviour of bees parasitized by *N. ceranae* over their lifetime and also their survival, using three hives equipped with optical bee counters. It was shown that the survival of *N. ceranae* –parasitized bees was significantly lower than that of control bees, and the survival rate of parasitized bees decreased faster than the control, especially after 15 days. It has been found also that *N. ceranae* parasitism did not modify the probability of drifting but Nosema- parasitized drifters performed more but shorter drifts compared to 'healthy' drifters. Conversely, short-term studies starting with young honey bees tends to show no effects on behavior or mortality at the individual or colony level in field conditions.

Interactions between pesticides and *N. ceranae* can be expected, as both have the potential to disturb similar metabolic functions related to immunity, energetic resources and antioxidant responses.

When can nosemosis be present?

Nosema is transmitted through the ingestion of spores via contaminated water or food, through the exchange of food between bees or when bees perform their cleaning duties. A single spore can cause infection, although the average infective dose for *N. apis* has been determined to be approximately 20-90 spores per bee. When the spores enter the midgut, they extrude a polar filament through which they inject their sporoplasm into epithelial cells. Once the parasite multiplies and develops within the host-cell cytoplasm, the spores can be shed into the gut lumen, where they may be excreted or they may infect additional epithelial cells. The presence of empty spores inside the parasitized epithelium was considered evidence that autoinfection is

a common feature in the life cycle of these pathogens, causing extensive and even total destruction of the ventricular epithelial layer. Indeed, although it was thought that *N. ceranae* was only able to infect adult bees, it was recently found in pre-pupae of *A. mellifera* under laboratory conditions and in drone pupae from naturally infected apiaries, demonstrating the infectivity of this microsporidium in bee breeding) and displaying a range of pathological problems in the subsequent adults.

Once introduced into a country, the migratory movements between different climatic regions related to honey harvesting and associated to beekeeping practices enhance the potential for contact between apiaries. Thus, new colonies can easily be infected, for example through the sharing of food resources, and even through the robbery of sick hives. Royal jelly, pollen and honey may also be sources of spores. The recent report that Nosema parasites can be transmitted via insemination as a secondary mode of transmission is really striking and it means that infection by this parasite should be considered in mating stations. This probably also occurs in bumblebees, where *N. bombi* spores have previously been reported in the semen of males and where *N. ceranae* is now a common parasite in some areas.

Accurate data on colony mortality due to nosemosis type C as its main cause is hardly difficult to find in literature, principally because of the absence of clinical symptoms. Contrary, the symptoms like dysentery typical of *N. apis* infections are easy to be identified on the field. A survey in Europe carried out in 2012-2013, based nevertheless on clinical symptoms, identified nosemosis as the third disease after varroosis and AFB with a significant impact on colony mortality especially in winter. Winter mortality in colonies suffering nosemosis was 21.77% (CI 95%: 14.14-31.14) while winter mortality of colonies not suffering of nosemosis was 12.64% (CI 95%: 6.84-20.78), although significant regional differences in colony losses were observed. In the USA another survey carried out in 2014-2015 showed nosemosis affecting to 5% of the beekeepers (commercial and not commercial operations) with 53.9% of colony mortality (CI 95%: 50.0-57.8).

At present the evolution of *N. ceranae* infection in the honey bee colony and its prevalence in different regions of the world is now clearly related to environmental conditions, to the honey bee genetics and to the distinct beekeeping practices. In fact, the prevalence and virulence of this pathogen is higher in temperate areas like Southern Europe, where it has been regarded as a severe honey bee health problem, while its prevalence is lower in colder areas. The first evidence of a relationship between *N. ceranae* infection and colony loss was recorded in Spain. Subsequently, a similar link between this pathogen and honeybee colony weakness/loss was proposed in other countries with comparable temperate climatic conditions such as Greece, Israel South-east, North and Western-coast of US, Central Chile and Italy and Jordan. In colder climates like in Germany, the Balkan countries, Switzerland and Northern Greece the specific conditions (climatic and beekeeping practices) required for nosemosis C to compromise colony survival seems not to be present. This may well reflect the ability of its spores to resist high temperatures and desiccation better than low temperatures, and the ability of *N. ceranae* to complete its life cycle more efficiently at high temperatures. Thus, in temperate areas the chronic stress induced by *N. ceranae* on honey bee colonies will be more intense, ultimately favoring colony death as predicted through recent models. Nevertheless, some contradictory results obtained under field conditions might reflect the influence of the different methodologies used between studies.

The genetic diversity of the host species and its relationship to *N. ceranae* infection was also shown to be a key factor to cope infection by native or invasive parasites in any habitat. The evolution of each disease and its clinical manifestations can be also affected by specific

characteristics of the parasite agent. For example, differential virulence would explain high or low colony mortality. Different responses to N. ceranae variants from Spain and Uruguay have been observed at the molecular level in *A. mellifica iberiensis*, but not regarding infection levels that compared N. ceranae from France and Spain in the same subspecies host.

Some considerations about the epidemiology of the disease should be taken into account when selecting samples for diagnosis. As previously mentioned, foragers are the most strongly infected bees in a colony and thus, they represent the most reliable sample to detect the pathogen. However, bottom scraps and frass have been also proposed to be a reliable material to determine if a colony is infected or not. Finally, as the pathological effect of *N. ceranae* in a colony is determined by the number of bees infected, this number should be determined (percentage of infection) when attempting to diagnose the disease.

Why has not been worldly considered a pathogen?

In the past few years, the clinical manifestations of *N. ceranae* infection has become one of the most controversial aspects of beekeeping under field conditions. Years ago the Koch's postulates were described and how *N. ceranae* can cause the death of a honey bee colony by inducing chronic, although it is evident that type C nosemosis does not evolve equally around the globe, making difficult to define universal clinical signs. As previously described in veterinary medicine, a clinical sign is an objective indication of a specific event or a characteristic that can be detected either by examination or by in vivo/in vitro analysis of the subject. A disease in a group (apiary) often manifests with a spectrum of symptoms that range from unapparent to sub-signs in farm animals. Clinical features of Nosemosis type C described in Spain colonies include a longer breeding period during cold months (even when the winter break should usually occur), a higher proportion of frames containing brood relative to nurse bees during the warm months, and diminished honey production, infected colonies become clearly weakened and depleted of adult bees, and they collapse in a period of 1.5–2 years. These sub-clinical manifestations of nosemosis type C are usually not consider as they are easily confound with other causal factors. Indeed, in the absence of classical signs of nosemosis type A, the role of the nosemosis especially caused by *N. ceranae* in colony loss is usually ruled out.

The traditional method for detecting Nosema infections has been to crush adult worker abdomens in a small amount of water and examine for spores under a microscope. Always was considered that the more spores observed, the higher the level of infection. However, this seems not always to be the case and for example, *N. apis* seems to be a prolific spore producer, whereas *N. ceranae* does not produce as many spores but rather, it favors its vegetative stages (non-spore life stages). For this reason molecular analytical techniques provide a more accurate diagnosis of *N. ceranae* infection and explain incongruent results when samples came from interior bees and specie has not been identified.

How can we control Nosemosis?

Beekeeping practices may also influence the in-field evolution of nosemosis type C in the host, as well as the evolution of the pathogen. Since the queen's age is a fundamental influence on the evolution of *N. ceranae* infection and honey bee colony strength, replacement of an old queen by a younger one decreases the proportion of Nosema infected forager and house bees. This practice will maintain the overall infection rate at a level compatible with colony viability and productivity. The effect of other beekeeping practices that differ between Northern and Southern Europe on *N. ceranae* infection should also be borne in mind, such as prophylactic measures, Varroa treatments or the use of other techniques, particularly since such effects remain unknown. It was also recently shown that the therapeutic doses of oxalic acid utilized for Varroa control might inhibit the development of *N. ceranae* in laboratory and field conditions, both at

the individual and colony levels. There is also some evidence that formic acid fumigation may help to suppress Nosema. Effectivity of thymol and resveratrol against Nosema were also reported although thymol and coumaphos were suspected to increase susceptibility to infection by *N. ceranae* (and *C. mellificae*), since both products cause a significant reduction in Dscam transcription, an important element in the honey bee immune response to these parasites. The fact that the use of chemicals to treat Varroa infestation are not uniform in different regions could explain the conflicting data on the importance of *N. ceranae* on honey bee health.

Over the years of confronting Nosemosis, much effort has been invested in search of effective cure against it. So far, Bicyclohexylammonium fumagillin, an antibiotic isolated from the fungus *Aspergillus fumigatus*, is one of the few drugs known to be active against microsporidia suppressing their reproduction and multiplication at recommended concentrations. Fumagillin is extensively used to control nosema disease in apiculture for over 60 years. Its mode of action involves binding to the active site of MetAP-2 (Methionine aminopeptidase 2) enzyme, thus inhibiting its activity. Fumagillin activity is unspecific to Nosema, affecting mammalian as well as honeybee Met AP-2. In the commercial formulation Fumagillin-B®, fumagillin is present as a salt in an equimolar quantity with dicyclohexylamine (DCH). The toxicity of both components to humans caused by residues remaining in hive products is suspected. DCH contamination in the hive products is also of concern due to its stability and lipophilicity. Thus Fumagillin-B is currently not licensed in most countries of the European Union due to the side effects risk of its commercial formulation, like genotoxic and tumorigenic properties and stability in honey. Anyhow Fumagillin-B® is the only registered chemical treatment available to combat nosema disease in apiculture. The impact of this treatment on colony survival is not yet clear. In Uruguay, different winter Fumagillin treatments were able to provide temporal decrease in Nosema spores but did not affect colony survival irrespective of dose or application strategy. It has also been reported that *N. ceranae* seems to reproduce even better at lower concentrations of fumagillin, which also affects the bees' physiology, such that its use may augment the prevalence of *N. ceranae*.

Several semisynthetic and synthetic fumagillin analogues, were shown possess biological activity against *N. ceranae*-under laboratory condition but none were, however, as effective as Fumagillin-B®. The most popular alternative treatments against nosemosis in Europe are Api Herb, Nozevit, Feed Gold, Protofi l, Hive Alive and Nosestat. Other treatment such as acetylsalicylic acid with extract of *Artemisia absinthium* L and extracts of Aster scaber *and Artemisia dubia* are currently under study.

Also the effects of probiotics and prebiotics on the infection have been analyzed. A work using *Lactobacillus rhamnosus* (a commercial probiotic) and inulin (a prebiotic) showed no beneficial effect on the survival rates of honeybees infected with *N. ceranae*. Similarly, a mixture of different species belonging to *Lactobacillus, Bifidobacteria, Pediococci* and *Lactococci* species showed no advantageous effect on the infection. Another study including nutraceutical, prebiotic and probiotics showed acacia gum as the most effective prebiotic although with a high mortality as side effect, and the probiotic Protexin Concentrate© single-strain (ProtexinC1) is able to reduce the spores, increasing the bee survival.

Identification and quantification of *Lotmaria passim* (Trypanosomatidae) in investigation of its prevalence, annual dynamics and relationship with *Nosema ceranae* (Microsporidia)

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Keywords: *Apis mellifera*, honey bee parasites, trypanosomatids, microsporidians

INTRODUCTION. Trypanosomatids and microsporidians are the most abundant eukaryotic gut parasites of honey bees (Stevanovic et al., 2011, Apidologie, 41:49–58; Stevanovic et al., 2013, Apidologie, 44:522–536; Stevanovic et al., 2016, J. Invertebr. Pathol, 139:6-11; Schwarz et al., 2015, J. Eukaryot. Microbiol, 62:567–583; Vejnovic et al., 2017, J. Invertebr. Pathol, 151:76-81) and have been correlated with increased colony losses (reviewed in Schwarz et al., 2015, Curr. Opinion Insect Sci, 10:1–7; Martín-Hernández et al., 2018, Environ. Microbiol, 20:1302-1329). Among the three microsporidian species that infect *Apis mellifera*, *Nosema ceranae* has the greatest prevalence and the widest geographical distribution. Of the two tripanosomatid parasites of *A. mellifera*, *Crithidia mellificae*, which has been known for half a century, was considered prevalent until recently, when *Lotmaria passim* was first described. The latter turned out to be predominant when in-depth analyses of nucleotide sequences, accessioned as *C. mellificae* in GenBank, revealed that they all actually belong to the species *L. passim* (Schwarz et al., 2015a, J. Eukaryot. Microbiol, 62:567–583). To enable routine molecular identification of these trypanosomatids, we started to develop and validate primers capable of distinguishing between *C. mellificae* and *L. passim*. Thereafter, the prevalence of both species was investigated by analysing archival DNA samples originating from bees collected from Serbian apiaries during the nine-year period (2007–2015). This survey revealed that only one trypanosomatid, *L. passim*, parasitises Serbian honey bees, that it has been present in Serbia since at least 2007 and that the majority of colonies (60.5%) were co-infected with *L. passim* and *Nosema ceranae* (Stevanovic et al., 2016, J. Invertebr. Pathol, 139:6-11). These results have led us to continue the research with the following aims: to establish a method for quantification of *L. passim* in a real-time PCR assay, to reveal the annual dynamics of *L. passim* and *N. ceranae* co-infection and estimate their quantitative relation in naturally infected colonies.

MATERIALS AND METHODS. For identification and differentiation between two honey bee trypanosomatid species, primers were designed at polymorphic regions of the cytochrome b gene (Cytb) between validated *L. passim* (GenBank KJ684960) and *C. mellificae* (GenBank KJ684951) sequence accessions. *L. passim*: LpCytb_F1: 5'-cGAAGTgCaCATATATGCTTtAC-3' and LpCytb_R: 5'-gcCAaAcACCaATaACtGG tACt-3'. *C. mellificae*: CmCytb_F: 5'–AGTtTGAgCtGTtGGaTTTgTt–3' and CmCytb_R: 5'–AACCtATtACaGGcACaGTTGC–3'. (polymorphic positions between *L. passim* and *C. mellificae* are indicated by lower-case letters). Validation of both primer pair specificity, positive control development and the details concerning PCR and electrophoresis were described earlier (Stevanovic et al., 2016, J. Invertebr. Pathol, 139:6-11).

For the quantification of *L. passim* infection levels, specific forward primer was designed to amplify a 146 bp region of the cytochrome b gene (Cytb, GenBank KJ684960), LpCytb_F2: 5´-AGTaTGAGCaGTaGGtTTTaTTATa-3´ when used with the previously established LpCytb_R: 5´. Development and validation of positive controls and standards and the details of quantitative PCR reactions are given in Vejnovic et al., 2017, J. Invertebr. Pathol, 151:76-81. *L. passim*

infection levels were determined by calculating the number of *L. passim* individuals per bee using the equation already established (Vejnovic et al., 2017, J. Invertebr. Pathol, 151:76-81).
Identification of *Nosema* species was done by PCR-RFLP method with nos-16S-fw/rv primers (Stevanovic et al., 2011, Apidologie, 41:49–58). *Nosema* infection levels were determined by calculating the average number of spores per bee using a haemocytometer.
To reveal the annual dynamics of *N. ceranae* and *L. passim* co-infection, 10 honey bee colonies naturally infected with both species were selected from the same apiary (44°57′26.8″N, 19°17′25.0″E) in northwest part of Serbia. Samples (consisting of ~100 forager bees) from each colony were taken monthly from March 2016 to March 2017, excluding the five wintering months (October–February). A total of 80 collected samples were frozen at −20°C until analysis. Quantitative relation between the two parasite species was assessed on a monthly basis. To assess the association between parasite infection levels and ambient temperature, the average monthly temperature for the study place was recorded and checked on http://www.accuweather.com.
After normality was rejected (Kolmogorov–Smirnov test, $p<0.05$), the groups were compared using Kruskal–Wallis ANOVA followed by Dunn's multiple comparison test. The significance of differences between *N. ceranae* and *L. passim* infection levels (spore or cell equivalents/bee, respectively) was tested with Mann-Whitney U test. The Spearman correlation between *N. ceranae* and *L. passim* spore or cell equivalents/bee was tested, as well as a potential association between *N. ceranae* or *L. passim* infection levels and the average monthly ambient temperature. The statistical analysis was performed with GraphPad Prism version 6 (GraphPad, San Diego, CA, USA).
RESULTS. The *L. passim* primer pair (LpCytb$^-$F1 + LpCytb$^-$R) produced a 247 bp amplicon, while primers targeting *C. mellificae* (CmCytb_F + CmCytb_R) produced a 140 bp amplicon. Neither of these primer pairs cross-reacted with non-target honey bee trypanosomatid DNA derived from pure cell line cultures, nor did they cross-react with a related species, *C. bombi*, which infects the bumblebee. Serial dilutions of mixed-species templates that included both *L. passim* and *C. mellificae* confirmed that each primer was species-specific under disproportional scenarios, where DNA from either species predominated. Non-specific amplification did not occur under these mixed-species conditions, out to a maximum 1,000-fold difference tested (10^-1 vs. 10^-4;. Universal trypanosomatid primers confirmed that the targeted DNA was present and suitable for PCR amplification in all templates used during primer validation.
A real-time qPCR assay with primers LpCytb_F2 + LpCytb_R showed amplification efficiency in a range from 95% to 100%, enabling the specific detection and quantification of *L. passim*. A standard curve was generated with a set of serial 10-fold dilutions of recombinant plasmid DNA *L. passim* of a known concentration. Copy numbers per genome were determined based on amplicon size and preliminary evidence for 6 copies of the gene in *L. passim*.
Mann-Whitney U test revealed significantly ($p<0.001$) higher infection level of *N. ceranae* (7.04±0.36 spore/bee) in comparison to *L. passim* (5.52±0.95 cell equivalents/bee). Besides, a significant positive correlation between infection levels of these two parasite species was affirmed (Spearman correlation: Spearman $|r|=0.37$, $p<0.001$). Neither *C. mellificae* nor *N. apis* was found.
Significant differences between monthly infection levels were affirmed for both *N. ceranae* (Kruskal-Wallis test, $H=54.56$, $p<0.0001$) and *L. passim* (Kruskal-Wallis test, $H=26.19$, $p<0.001$). The correlation between temperature and infection level was negative for both *N. ceranae* (Spearman $|r|=-0.78$, $p<0.0001$) and *L. passim* (Spearman $|r|=-0.30$, $p<0.01$).

CONCLUSIONS

- The primers designed and validated for PCR identification of *L. passim* and *C. mellificae* enable reliable routine prevalence investigations and epizootiological surveys on these honey bee parasites.
- The detection of only *L. passim* in Serbia and its consistent presence since at least 2007 suggest the importance of establishing a method for simultaneous detection and real-time PCR quantification of this parasite.
- Significant positive correlation between infection levels of *L. passim* and *N. ceranae* indicates their similar annual dynamics, whilst the differences in the levels of infection between particular months point to a seasonal pattern in the incidence of both parasites.
- Both parasites, *N. ceranae* and *L. passim* reached highest loads in foragers during winter months and lowest during the summer temperature peak, in July.
- The assay which has been developed and validated creates opportunity for detailed study of *L. passim* infection kinetics and the improvement in the management practices in beekeeping related to these two parasites.

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How to enhance performances of organic acids against *Varroa destructor* through application of Good Beekeeping Practices

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Keywords: *Varroa destructor,* organic acids, low environmental impact, queen caging

INTRODUCTION. *Varroa destructor (V. destructor)* is a mite, external parasite of honey bees, responsible of varroosis. It is endemic at the global level and attacks the body of adult bees and larvae, weakening them by sucking their haemolymph and predisposing to other pathogens (e.g. viruses and microsporidia).
V. destructor is characterized by a strong adaptability to miticide treatments and is responsible of the major economic losses for beekeeping all over the world.
The fight against *V. destructor* continues to be one of the most difficult management challenges in apiculture. There are about 200 veterinary medicines registered worldwide for this parasitic disease of the bees, and around half of these are considered "low environmental impact" products.
Organic acids (e.g. oxalic acid, formic acid, lactic acid) are low environmental impact active compounds, that are authorized for their use in organic beekeeping. They have low toxicity for consumers and, so far, no varroa resistance has been reported. The negative aspect is that some of them are not always able to guarantee high and uniform miticide efficacy among treated hives and could be time-consuming. Moreover, in some conditions, they could be toxic for the honey bees (especially queen bees) or for the operators. Goals of our studies are to increase final efficacy of organic acid treatments, trying to reduce the negative aspects of the "low environmental impact" products, looking for new solutions for future varroa control strategies.
The importance of collaboration between scientists and beekeepers remain fundamental and should be always kept in mind in order to achieve the best standards for the beekeeping sector, in full respect of bees, humans and environment.
Focusing on the goal to enhance the training of veterinarians in apiculture and to enhance the collaboration between beekeepers and veterinarians, in Italy the SVETAP association (Società Scientifica Veterinaria per l'Apicoltura - Scientific Association for Veterinarians in Apiculture) was founded in 2016. The main objectives of SVETAP include the promotion of the veterinarian professional skills in beekeeping, considering: the bee health, the food safety and public health, the "One Health" view, technological upgrading and the development of innovation in beekeeping, the organization of training activities and the communication, dissemination and proper technical and scientific information on the apiculture topics, also to public opinion.
MATERIALS AND METHODS. The future of varroa control strategies is always oriented towards more sustainable, integrative, and low environmental impact methods. In this context, the "*Varroa Control Task Force*" of the "COLOSS" research group works at the international level to reach these goals: the Working Groups (WG) n. 2 "Brood Interruption", compares different techniques of brood interruption in combination with organic acid treatments to control varroa infestation; the WG n. 4 "Formic Acid Management", works to define which parameters (above all, temperature and umidity) are able to influence formic acid performances, trying to provide a baseline data for a varroa infestation prediction tool; the WG n. 5 "Assesment of New Control Methods" has the objective to test the new commercial products for controlling varroosis (e.g. *Varromed®, thermotherapy,* etc.).
Results and considerations of the strategies to control varroa obtained by the above mentioned Working Groups, together with about 15 years of field trials experiences carried out by the Apiculture Unit of Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri"

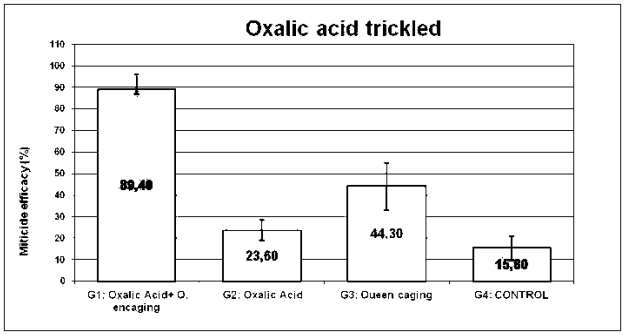
(IZSLT), will be presented. Moreover, there will be shown some examples on how to enhance final efficacy against *V. destructor* of formic acid and oxalic acid, through a proper application of Good Beekeeping Practices (GBPs).

RESULTS AND CONCLUSIONS. The oxalic acid treatment may increase significantly its efficacy in absence of brood thanks to the brood interruption techniques (e.g. queen caging for 25 days).

As examples of practical applications of integrated varroa control strategies, four studies will follow:

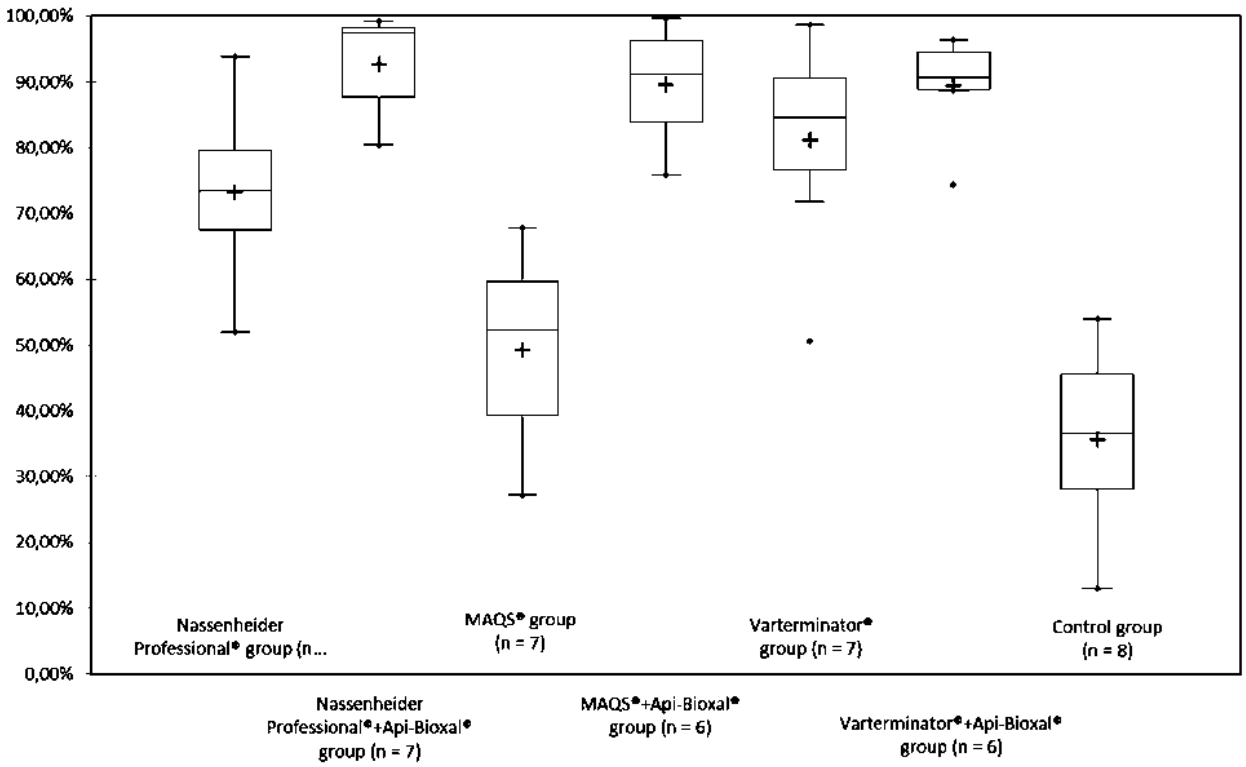
1) The importance of combining together the oxalic acid treatment in summer with this beekeeping techniques of the queen caging, getting to a final, successful (only oxalic acid: 23.6%; oxalic acid combined with queen caging: 89.4%!) treatment, is shown in figure 1.

Fig. 1 - Varroacide efficacy of oxalic acid with/without brood interruption (from: Giacomelli et al., 2011, L'Apicoltore Italiano: 2; 12-16)



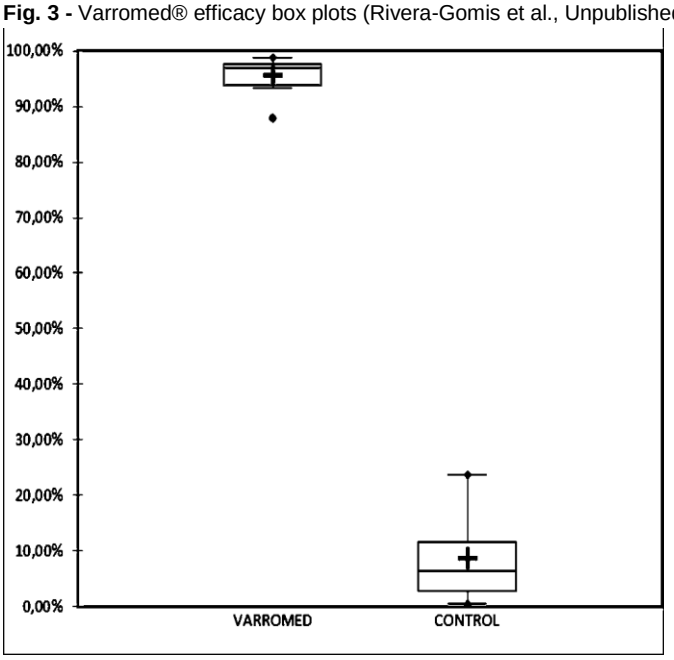
2) Regarding the performances of formic acid against *V. destructor*, we compared the efficacy of different formic acid based products (Varterminator®, Nassenheider professional® and MAQS®) in summer treatments, alone or in combination with oxalic acid (**Fig. 2**). In fact, the oxalic acid is more effective on the foretic varroa, while the formic acid is more effective on the varroa inside the cells (reproductive phase of the parasite). In combination with an oxalic acid treatment, the formic acid treatment showed a boosted efficacy, always higher than 80% for all of the three products. When this associations (formic acid + oxalic acid contemporary administration) are performed, strength of the colonies should be taken into consideration, to avoid this kind of treatments on the weaker hives.

Fig. 2 - Comparison between different formic acid treatments and their combination with oxalic acid (Pietropaoli & Formato, unpublished data)



3) In winter 2018, following the label indications, we evaluated in Central Italy the performances of Varromed®, a newly authorized EU product based on the association of oxalic and formic acids, (Fig. 3). Combining a single application of the product, in broodless conditions that we artificially obtained by caging the queen, we get a quite high and homogeneous efficacy (95,6%) among different beehives. No significant differences in mortality of honeybees between treated and control groups were observed. Without doubts, a lesser efficacy should have been observed in presence of brood.

Fig. 3 - Varromed® efficacy box plots (Rivera-Gomis et al., Unpublished data)



4) Finally, we evaluated two different brood interruption strategies in association with a subsequent oxalic acid treatment (Pietropaoli et al., 2012, Eur J Integr Med, 4(1): 93): the

“trapping comb” technique was compared with the “queen caging” using the *Var control*® cages”. Even though the final acaricide efficacy of the two beekeeping techniques was quite similar (trapping comb = 95.7%; queen caging = 91.7%), a lower infestation of adult bees could be observed in the trapping comb group (and a lower virosis impact on the colony).

SESSIONE 1

DIAGNOSI DELLE MALATTIE PARASSITARIE 1

Midichloria mitochondrii transmitted to the vertebrate host by *Ixodes ricinus*: a transient passenger or an infectious agent?

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Keywords: *Midichloria mitochondrii*, *Ixodes ricinus,* tick bite

INTRODUCTION. Ticks are important vectors for a variety of pathogens affecting humans and other animals. They also harbor numerous commensal and symbiotic microorganisms, whose role is still less investigated. *Ixodes ricinus* harbors the intracellular bacterium *Midichloria mitochondrii*, which is present in ovaries and in salivary glands (Sassera et al., 2008, Appl. Environ. Microbiol., 74:6138-6140; Epis et al., 2013, Ticks Tick Borne Dis., 4:39-45). The bacterium is vertically transmitted and shows a prevalence of 100% in wild adult females. *M. mitochondrii* prevalence reduces after some generations in laboratory conditions (Lo et al., 2007, Environ. Microbiol., 8:1280-1287). Molecular (e.g. in sheep, horse, cattle; Bazzocchi et al., 2013, Parasit. Vectors 12:350) and serological (e.g. in human, roe deer; Mariconti et al., 2012, Pathog. Glob. Health, 106:391–396; Serra et al., 2018, J. Wildl. Dis., ePub) evidences showed that *M. mitochondrii* is transmitted to the vertebrate hosts by *I. ricinus*. The aims of this work are thus: 1) understanding the seroconversion onset and the kinetic of antibody response against *M. mitochondrii* in a vertebrate model; 2) investigating the presence of circulating *M. mitochondrii* DNA in blood samples.

MATERIALS AND METHODS. Two groups of rabbits were experimentally infested respectively with wild (naturally infected with the symbiont) and with lab strain (lab; with a highly reduced amount of the symbiont) *I. ricinus* females. Blood and serum were sampled before the infestation and for eight post-infestation time points, over a 16-weeks time span. ELISA assays were performed to assess the immune response against *M. mitochondrii* flagellar protein FliD. DNA was extracted from blood samples and circulating *M. mitochondrii* DNA was detected using a nested PCR approach.

RESULTS AND CONCLUSIONS. *M. mitochondrii* presence was detected in rabbits infested by both W and LS *I. ricinus* ticks, suggesting that the transmission occurs even at low bacterial loads. Seroconversion against *M. mitochondrii* FliD antigen was observed around the first/second week post-infestation. The immune response showed O.D. values above the established cut–off around the first-fifth week post-infestation, then decreasing by the end of the experiment. Circulating DNA was detected in the blood of infested animals up to the end of the experiment, suggesting both a replication of the symbiont inside the vertebrate host and a possible true infection.

Risk of transfusion transmitted malaria: evaluation of commercial ELISA kits for the detection of anti-*Plasmodium* antibodies in candidate blood donors

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Keywords: *Plasmodium*, transfusion transmitted malaria, ELISA, IFAT

INTRODUCTION. Transfusion with blood infected with *Plasmodium* parasites represents a risk for malaria transmission. Several countries attempt to avoid such risk by testing candidate blood donors who resided in malaria endemic countries, in order to identify subjects carrying asymptomatic infection. In Italy, recent legislation (D.M. 2/11/2015) introduced the use of serological tests for the detection of anti-*Plasmodium* antibodies, and the exclusion from donation for 2 years in case of a positive result. In absence of a gold standard for malaria serology, the aim of this work was to evaluate five commercially available ELISA kits and assess their specificity, sensitivity, reproducibility and concordance.

MATERIALS AND METHODS. The five ELISA kits were used to test sera samples from malaria naïve donors (N=8), malaria naïve patients infected with parasites other than malaria (N=15), malaria patients previously tested positive by IFAT at the Centre for Tropical Diseseas in Negrar (N=56), and malaria exposed (N=43) candidate blood donors referring to Pisa University Hospital.

RESULTS AND CONCLUSIONS. The specificity of all ELISA kits was 100%, with no false positive results among malaria naïve subjects. However, sensitivity was in the range 50-60% compared to IFAT, an unsatisfactory result for a screening test. Only one kit showed optimal experimental precision (CV<20%) and inter-laboratory reproducibility (100% concordance). The results of different ELISA kits showed a concordance ranging from 51% to 95% (K_{Cohen} 0.17-0.89), raising the possibility that the same individual could be excluded from donation depending on the test used.

These preliminary results indicate how the lack of a gold standard for malaria serology must be taken into account in the application and future revision of current legislation.

Serodiagnosis of Human Cystic Echinococcosis: comparison of two Rapid Diagnostic Tests

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Keywords: *Echinococcus granulosus*, Cystic Echinococcosis, rapid diagnostic test, Serology

INTRODUCTION. Human Cystic Echinococcosis (CE) is a chronic and complex infection caused by the tapeworm *Echinococcus granulosus* (Craig et al., 2007, Trop. Med. Health., 35(4):283–92). The diagnosis of CE is based on imaging, with serology having a confirmatory role in doubtful cases. Rapid Diagnostic Tests (RDTs) are convenient to support ultrasound diagnosis in uncertain cases, especially in resource-limited settings. We evaluated the diagnostic performance of the prototype Hyd Rapid test (Universiti Sains, Malaysia) and compared it with the commercial VIRapid® HYDATIDOSIS (Vircell, Spain).

MATERIAL AND METHODS. Sera analyzed were frozen (-80°C) stored samples from patients with hepatic CE seen between 2007-2017 in the CE outpatient clinic of San Matteo Hospital Foundation. 87 serum samples, 62 from patients with CE and 25 from controls, were used for the study. Selection criteria were: presence of a single liver cyst, staged according to the WHO-IWGE (Brunetti et al, 2010, Acta Trop., 114(1):1-16). 39 sera were from patients with active cysts (CE1, CE2, CE3a, CE3b); 23 from patients with inactive cysts (CE4, CE5).

RESULTS AND CONCLUSION.

Tab. 1 Results summary

A						
	VIRapid® HYDATDOSIS		Hyd Rapid			
	Se (95% CI)	Sp (95% CI)	Se (95% CI)	Sp (95% CI)	p-value	Kappa (95% CI)
Control	-	100% (86- 100)	-	100% (86-100)	1	-
CE overall	69% (56-80)	-	64% (51-76)	-	0.18	0.885 (0.787-0.983)
Active	87% (73-96)	-	79% (64-91)	-	0.08	0.726 (0.440-1.000)
Inactive	39% (20-62)	-	39% (20-62)	-	1	0.817 (0.576-1.000)

B						
	Active				Inactive	
	CE1 (n=7) % positive	CE2 (n=8) % positive	CE3a (n=8) % positive	CE3b (n=16) % positive	CE4 (n=11) % positive	CE5 (n=12) % positive
VIRapid® HYDATIDOSIS	100%	87.5%	75%	87.5%	36.4%	41.7%
Hyd Rapid	71.4%	87.5%	62.5%	87.5%	45.5%	33.3%

A Comparison of sensitivity (Se) and specificity (Sp) **B** Tests Se by cyst stage.

The performance of Hyd Rapid test, was not statistically different from that of the VIRapid® HYDATIDOSIS test, although the former was less sensitive in the diagnosis of active cysts (borderline significant), in particular of CE1 cysts. Both RDTs had a specificity of 100%, but were equally poorly sensitive in the diagnosis of inactive cysts.

Preliminary results on the occurrence of *Blastocystis* subtypes and correlation with faecal microbiota in HIV patients referred to University Hospital "Umberto I" in Rome

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Keywords: Blastocystis, STs, microbiota

INTRODUCTION. *Blastocystis* are non-flagellated Stramenopiles found in a wide range of non-mammalian and mammalian hosts, including humans, with a prevalence from 0.5%-30% and 30-76% in industrialized and developing countries, respectively. The genus comprises distinct phylogenetic lineages, the so-called subtypes (STs); nine of them have been found in humans (Stensvold et al., 2007, Exp. Parasitol, 116:111–9). Despite numerous studies are reporting *Blastocystis* STs as implicated in different intestinal diseases and potential virulence factors have been described (Wawrzyniak et al., 2012, Parasitol. Int, 61: 437–442), the clinical significance and pathogenic potential of those STs remain unclear. Also, determining whether or not *Blastocystis* colonization is associated with gut dysbiosis would be important for understanding those pathogenic aspects. Aim of this study was to detect *Blastocystis* STs and to study the faecal microbial composition from HIV-positive and HIV-negative patients referred at the Umberto I Teaching Hospital of Rome.

MATERIALS AND METHODS. During the years 2015-2017 a cross-sectional study was conducted enrolling 160 HIV-positive and 2394 HIV-negative patients. Coproparasitological and molecular analyses were performed to detect *Blastocystis* STs. In addition, metagenomic sequencing and bioinformatics analyses were performed on a sub-sample of 24 Blastocystis-ST3-colonized (12 HIV+, 12 HIV-) and 12 *Blastocystis*-free subjects, to profile and compare their gut bacterial communities. Potential etiological factors and clinical features were also evaluated.

RESULTS. *Blastocystis* was detected in 19% and 7% of the HIV+ and HIV- subjects, respectively. It was the protozoon more frequently diagnosed in faecal samples from both patient groups. ST3 was the subtypes mainly identified followed by ST1. No significant association between immune status and the presence of *Blastocystis* was evidenced, as expected considering the high level of CD4+ observed in HIV+ patients. A higher bacterial diversity was found in *Blastocystis*-carriers, which exhibited a high abundance of *Prevotella* and *Methanobrevibacter*, whereas high percentage of Enterobacteriaceae was found in *Blastocystis*-free patients.

DISCUSSION. It is generally known that the dysbiosis of the intestinal microbiota related to metabolic or infectious diseases is typified by a reduction in the bacterial diversity and a bloom of Enterobacteriaceae. Conversely, in this study the presence of *Blastocystis* was associated with high biodiversity of bacterial flora, particularly *Prevotella*, suggesting that the colonization by *Blastocystis* would be linked with a healthy gut microbiota. Further studies including more faecal samples showing different STs are needed to contribute to the knowledge on *Blastocystis* as a pathogen or a commensal of the human gut.

Blastocystis sp. in patients from the Polyclinic of Rome Tor Vergata

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Keywords: *Blastocystis* sp., subtypes, diagnosis

INTRODUCTION. *Blastocystis* sp. is one of the most common human intestinal protozoan worldwide. This protist, genetically and phenotypically heterogeneous, is characterized by significant differences in the clinical outcome. To date nine subtypes (ST1-ST9) have been isolated in humans. However 95% of human infections sampled belong to one of just four of these subtypes (STs 1–4). So far, genetic and epidemiological data of *Blastocystis* infections in Italy are rather scarce. In order to gain further clinical and epidemiolocal data on this parasite, a microscopic and molecular identification of *Blastocystis* in faecal samples was conducted from symptomatic patients.

MATERIALS AND METHODS. Fecal samples were collected from patients enrolled consecutively from 2014 to 2017 at the Laboratory of Parasitology of the Tor Vergata University Hospital in Rome, Italy. All these specimens were collected for coproparasitological analysis as requested by the physician. Samples resulted positive at microscopy were subjected to DNA extraction, amplification and sequenced by targeting the SSU rRNA gene. Sequences were analyzed in MEGA7. The phylogenetic tree was obtained by comparing all sequences with those of homologous reference strains available in GenBank.

RESULTS AND CONCLUSIONS. Of the 2213 patients examined for microscopy, 42 patients were positive to *Blastocystis*, with a prevalence of 1.9%. No association was found between patients' gender and *Blastocystis* but being older than 67 was related with the infection. Twenty-four isolates were successfully sequenced. Five subtypes were identified by phylogenetic analysis, the majority of the samples was assigned to ST4 (12/24, 50%), followed by ST3 (5/24, 20.83%) and ST1 (5/24, 20.83%). Two samples were also attributed to the subtype ST6 (1/24, 4.16%) and the subtype ST7 (1/24, 4.16%) respectively. ST2 was not detected at all. The present study improves the knowledge of *Blastocystis* occurrence and subtypes in symptomatic patients in Italy underlying its role in human parasitic gastroenteritis.

Diagnosis of toxoplasmic chorioretinitis: moving towards standardised assays

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Keywords: *Toxoplasma gondii*, Chorioretinitis, real-time PCR, IgG/IgM Western-blot

INTRODUCTION. *Toxoplasma gondii* is a major cause of retinal infections. Clinical signs of Ocular Toxoplasmosis (OT) appear late after the infection onset and may have severe sequelae in immunocompromised hosts and children with congenital infections. First diagnosis is currently based on clinical observation and confirmed by serology PCR or immunoblotting test on acqueus/vitreus humor. The diffused use of hand-made tests lacking standardisation has been limiting the application of molecular assays in routine diagnostic.However commercial, CE marked molecular assays for DNA detection offer a new valid tool.

MATERIALS AND METHODS. Fifty three ocular fluid samples and 21 blood samples were collected between 2010 and 2017 from 55 patients affected by chorioretinitis have been retrospectively tested using *Toxoplasma* ELITe MGB Kit (ELITechGroup spa Torino Italy) after DNA extraction by EasyMAG. (Biomerieux Marcy L'Etoile France). Serum samples were tested with IgG, IgM CLIA (Diasorin Saluggia Italia) and IgG ELFA (Biomerieux Marcy L'Etoile France). In all suspected seroconversion Toxo IgM ISAGA, Toxo IgG Avidity (Biomerieux Marcy L'Etoile France), IgA Elisa (Diasorin Saluggia Italia) were performed. Comparative IgG IgM Western-Blot (LDBIO Lyon France) was performed on ocular fluid samples and serum when volumes were enough (N=12).

RESULTS. Results are summarised in the table. At the end for 27 patients OT was diagnosed. Two of them were positive only in WB. Ameliorations after treatment confirmed the diagnosis.

	RT-PCR Results n 74		**WESTERN BLOT Results n 12**	
	PCR +	**PCR -**	**WB +**	**WB -**
AQUEOUS HUMOR	23	24	7	4
VITREOUS HUMOR	2	4	1	0
BLOOD	0	21		
TOTAL	25	49	8	4

CONCLUSIONS. Our data demonstrate that the use of CE-IVD commercial assays like Toxoplasma ELIT e MGB largely contributes to the standardisation of molecular detection of *T. gondii* DNA. Improvements are expected by the introduction of integrated sample-to-result platform. In addition the concordance with the clinical outcome supports PCR relevance in the OT differential diagnosis in ocular fluid. A combined approach based on molecular testing and immunoblotting increase the diagnostic efficiency.

Anisakiasis in Italy. Analysis of hospital discharge records in the decade 2005-2015

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Keywords: Anisakiasis, hospital discharge records (HDRs), symptoms, allergy

INTRODUCTION. Anisakiasis is a fish-borne zoonotic infection caused by third-stage larvae of *Anisakis*, a parasitic anisakid nematodes with a cosmopolitan distribution. Clinical signs and symptoms are not specific and clinical signs include gastrointestinal and/or allergic symptoms as epigastralgia, diarrhea, nausea, abdominal pain and/or urticaria, rhinitis, bronco-constriction. Due to intrinsic limitations of currently available diagnostic tools and to a plethora of diverse clinical manifestations, the global prevalence of gastrointestinal and allergic anisakiasis is likely to be severely underestimated. Recently, infective larvae were found in the same localization with gastrointestinal tumors (Mineta et al., 2006, Nippon Med Sch. 73:169; Sonoda et al., 2015, Surg Today 45:1321–5). Such aspect together with the occurrence of allergic exacerbation upon secondary exposure (Baird et al., 2014) and the possible occupational exposure in specific job sectors (i.e. fish industry) and workplace activities (Audicana and Kennedy 2008, Clin. Microbiol. Rev. 21: 360-79), highlight the need to increase scientific evidences on anisakiasis. With the aim to describe the epidemiological status of Italy with respect to anisakiasis, HDRs (Hospital Discharge Records) from 2005 to 2015 were analysed, with particular attention to allergic manifestations.

MATERIALS AND METHODS. Hospital Discharge Records (HDRs) were obtained from the Ministry of Health, during a 10-year period: 2005 to 2015. HDRs included data on hospital location and on patients' characteristics, including gender, age, place of birth, educational level, primary and secondary diagnosis codes, surgical or medical procedure codes, duration of hospital stay. Patients were also evaluated based on inland or coastal residency. Multivariate analyses were performed using backward step-wise logistic regression models to assess the variables independently associated with the allergic clinical signs and symptoms.

RESULTS AND CONCLUSIONS. A total of 370 HDRs with specific code for Anisakiasis (127.1) were retrieved. Central and southern regions showed the highest number of cases reported (82.5%): almost half of HDRs originated from Apulia region (51.1%). Regarding the coastal or inland territories, 80.3% of patients were resident close to the coast (less then 25km), while 19.7% lived in the hinterland (more than 25km). A large percentage of patients (43.8%) presented allergic manifestations and 54.0% of them showed serious allergic reaction (i.e. anaphylaxis). The multivariate analyses showed that living in southern regions (OR 12.9; 95% CI 4.3-38.3) and female gender (OR 2.26; 95% CI 1.3-4.0) were independently associated with allergic manifestations, while only female gender (OR 5.1; 95% CI 1.8-14.8) was independently associated with anaphylactic episodes

SESSIONE 2

BIOLOGIA MOLECOLARE E FILOGENESI IN PARASSITOLOGIA 1

Salivary microRNAs from anopheline mosquitoes: additional players in vector-host-pathogen interactions?

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Keywords: mosquitoes, microRNA, gene expression

INTRODUCTION. Saliva of blood feeding arthropods plays a relevant role in hematophagy and vector-pathogen-host interactions. So far transcriptomic and proteomic studies clarified the complexity of mosquito saliva shedding light on the functional roles of a number of salivary proteins. miRNAs are known post-transcriptional regulators of gene expression that are not only present within cells but also in biological fluids: here they are found either in complex with Argonaute proteins or enclosed within exosomal microvesicles, which are possibly involved in cell-cell communication. miRNAs have been recently reported in the saliva of ticks and *Aedes* mosquitoes, raising the intriguing possibility that injected into the vertebrate skin during blood feeding they may play a role in host manipulation. To get insights into the salivary miRNA repertoires of anopheline mosquitoes we performed a small RNAseq analysis of *Anopheles coluzzii* saliva, salivary glands, adult males and adult females.

MATERIALS AND METHODS. Small RNAs (< 200 nt) were extracted by the miRNeasy kit (Qiagen), quality controlled on an Agilent 2100 Bioanalyzer and used for the preparation of TruSeq small RNA libraries (Illumina). Sequencing was done on an Illumina HiSeq2000 platform yielding a total of ~180 million raw reads. Standard bioinformatic tools were used for novel miRNA prediction, quality control, adaptor trimming, mapping and differential expression analysis.

RESULTS AND CONCLUSIONS. We obtained evidence of expression for at least 214 miRNAs, with 36 representing putative novel anopheline miRNAs. Differential expression analysis identified 30 female salivary gland-enriched and 68 sex-biased miRNAs. Saliva included at least 77 miRNAs and, noteworthy, part of them were asymmetrically distributed, suggesting that selected miRNAs may be preferentially directed toward the secretory pathway and saliva. Intriguingly, among the twenty more abundant salivary miRNAs, ten were essentially identical to human miRNAs targeting genes involved in host immune and inflammatory responses. This study expands the miRNA repertoires of the African malaria vectors *A. coluzzii* and *A. gambiae* providing the first evidence that some miRNAs, perhaps under the evolutionary pressure imposed by blood feeding, may be specifically conveyed to mosquito saliva. A relevant implication, with possible consequences for pathogen transmission, is that manipulation of host responses by mosquito saliva may take place not only through the biochemical and pharmacological properties of salivary proteins but also by miRNA-mediated post-transcriptional regulation of host gene expression.

Discovery of novel Antimicrobial Peptides in the salivary glands of the malaria mosquito *Anopheles gambiae*

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Keywords: *Anopheles gambiae*, salivary glands, antimicrobial peptides, feeding behaviour

INTRODUCTION. Mosquito females need a blood meal to attain nutrients for egg development. A successful blood meal relies mainly on the activity of anti-haemostatic factors in the mosquito saliva, that is also the carrier of several pathogens transmitted by mosquitoes such as *Plasmodium*. Mosquitoes initially probe under the host skin and salivate, searching for a blood vessel to pierce and to start sucking the blood. This process exposes mosquito mouthparts to microbes on host skin surface that might contaminate the ingested blood meal. Moreover, both male and female mosquitoes need plant sugar to survive, to fly and to enhance reproduction: these food sources are stored for several hours in the crop and could be similarly contaminated by microbes living on nectar and honeydew. Salivary gland transcriptomes of haematophagous arthropods proved the occurrence of salivary peptides with unknown function showing possible antimicrobial sequence features. Aim of this work is the identification of novel antimicrobial peptides (AMPs) in the salivary glands of the malaria vector *An. gambiae*.
MATERIALS AND METHODS. From salivary transcriptome we selected a list of 16 putative AMPs, based on sequence properties and expression profiles (enrichment in female glands or female/male glands). Recombinant peptides were obtained by chemical synthesis (feasible for 4 protein < 60 aminoacids) or using a cell-free *in vitro* transcription/translation system (successful for 10 out of 12 peptides) and employed in bacteria growth inhibition assays. Transcriptional regulation was also investigated by RTqPCR, challenging mosquitoes with Gram - (*Escherichia coli*) and Gram + (*Staphylococcus aureus*) bacteria. Systemic infection was induced by bacteria injection in thoraxes, while local infection was stimulated by infected sugar meals.
RESULTS AND CONCLUSIONS. Functional studies were performed employing *in vitro* growth inhibition assays and revealed antimicrobial activity for some of the recombinant peptides. Indeed, preliminary data indicate that hyp15 inhibits the growth of *E. coli* bacteria, while hyp10, hyp6.3 and hyp14.5 inhibit the growth of *S. aureus* and, finally, hyp6.2 and hyp12 reduce the growth of both strain. Transcriptional profiles of candidate genes showed a balance between systemic and local innate immune responses. Indeed, 4 genes (hyp14.5, hyp14.5-1, hyp55.3 and hyp15) were induced by bacteria injection, while 4 genes (hyp10, hyp12, sg2 and sg2a) resulted activated by bacteria feeding. Overall, this study describes the identification of novel antimicrobial activities in the salivary glands of the malaria mosquito *An. gambiae.*

A specific class of erythrocyte membrane microdomains is involved in *Plasmodium falciparum* invasion of the host cell

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Keywords: Malaria, drug, proteomic analysis

INTRODUCTION. Malaria is one of the most deadly diseases worldwide. An essential role of erythrocyte membrane microdomains in susceptibility to invasion by *P. falciparum* was suggested by the report that their disruption prevents invasion by merozoites.

MATERIALS AND METHODS. Triton-insoluble membranes were purified on sucrose gradient and analysed by label-free quantitative proteomic analysis. Functional analysis was achieved in immortalized erythrocyte precursors.

RESULTS AND CONCLUSIONS. In this study, we performed a comprehensive quantitative proteomic analysis of erythrocyte membrane microdomains. With a novel approach recently developed by the authors, we grouped the identified proteins on the basis of their buoyancy profiles. Here we propose that protein groups correspond to functional units. A 15-member group, defined “invasion-related cluster”, includes most of the host proteins characterized so far as involved in erythrocyte penetration by *P. falciparum* (semaphorin-7A, protein G subunit alpha s, basigin receptor and CD55). In immuno-localization studies we showed that 5 uncharacterized-members of the invasion-related cluster coalesce at the invasion site in proximity to the tight junction and are internalized in association with the nascent parasitophorous vacuole. A role of this class of microdomains in the invasion process has been validated by the generation of null erythroid lines for two candidate proteins, Art4 and Aquaporin I.

Parasite strains resistant to currently available drugs periodically emerge, posing a great threat to the global effort for malaria control. Here we show that a specific membrane microdomain class is involved in *P. falciparum* invasion of the host cell. We also identify two new possible malaria drug targets.

Endless interactions, most beautiful: genomics of novel Rickettsiales provides insight into the evolution of the order

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Key words: Rickettsiales, genomics, evolution

INTRODUCTION. *Rickettsiales* are a diverse bacterial order, encompassing human and animal pathogens, vector-borne agents and symbionts, some of them with remarkable and unique capabilities, but all sharing the trait of strict intracellularity. While *Rickettsiales* of medical and veterinary importance have been deeply studied, novel lineages, with unknown relationships with their host, are currently being discovered. *Midichloria mitochondrii*, for example, can be detected in the mitochondria of the ovary of the tick *Ixodes ricinus*, while several phylogenetically diverse Rickettsiales symbionts can be found in unicellular eukaryotes. Comparative studies involving such bacteria are important to enrich our understanding of the peculiar traits of specific lineages and to shed light on the evolutionary history of the order.

MATERIALS AND METHODS. Ixodid ticks were sampled from Italy, France and Australia. Ciliate protists were isolated from samples originated from multiple enviromental locations, including Italy, Denmark, Cyprus, Russia, Brazil, and India. The presence of bacterial symbionts was verified by PCR and, when possible, specific FISH assays. DNA from selected specimens was extracted and sequenced with Illumina technology. Reads derived from the *Rickettsiales* symbionts were bioinformatically extracted and selectively assembled through the blobology pipeline. The genomes were annotated with manual curation. Functional and metabolic reconstructions in relation to the host were predicted. Comparative analyses were performed through COG (clusters of orthology). Phylogenetic analyses were inferred on selected conserved ortholog genes.

RESULTS AND DISCUSSION. Multiple genomes of *Rickettsiales* symbionts of ticks and ciliates were obtained. Phylogenetic and phylogenomic analyses revealed a much wider diversity of *Rickettsiales* than previously expected. Indeed, multiple new lineages were identified, including, in particular, a completely novel family, the fourth of the order. Analysis of the genome contents show the presence of previously unknown metabolic capabilities of members of the *Rickettsiales*. In particular the presence of novel biosynthetic pathways suggests potential trends in the evolutionary history of the now four families. Our investigation may help to differentiate the origin and characteristics of *Rickettsiales* lineages, possibly opening the way for understanding their means of interaction with the hosts cells, with clear implications in the study of the pathogenicity of members of the order.

Detection and quantification of a novel bacterium of the genus *Midichloria* (family *Midichloriaceae*, order Rickettsiales) in the hard tick *Hyalomma marginatum*

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Keywords*: Midichloria*, *Hyalomma marginatum*, migratory birds

INTRODUCTION. Ticks are considered the most important vector of microbial agents maintained in nature by several reservoir hosts like birds, which contribute to the spread of microorganisms across countries and continents (Wallménius et al., 2014, Parasit. Vectors, 7:318). The detection of *Midichloria* bacteria (family *Midichloriaceae*) in *Hyalomma marginatum* ticks collected from migrating birds (Di Lecce et al., 2018, Parasit. Vectors, 11:106) contributes to define the geographic distribution of these bacteria and to increase the knowledge about their potential transmission to vertebrate hosts (as occurs for *Midichloria mitochondrii*, the best-known representative of the *Midichloriaceae* family; Serra et al., 2018, J. Wildl. Dis. Mar 16). The aims of this study are: 1) to determine the genetic variability of *Midichloria* bacteria in *H. marginatum* ticks collected from trans-Saharan migratory birds arriving in Europe from Africa in spring; 2) to quantify the amount of *Midichloria* in ticks through a novel quantitative PCR (qPCR) approach.

MATERIALS AND METHODS. *H. marginatum* nymphs were collected from three different migratory bird species (*Phoenicurus phoenicurus, Saxicola rubetra* and *Sylvia communis)* on Ventotene Island (Central Italy). DNA was extracted from all nymphs and molecular analyses (qualitative PCR) were performed in order to evaluate the presence of *Midichloria* and to determine the genetic variability of this bacterium through the amplification of four specific genes. In order to quantify *Midichloria* in *H. marginatum* samples, two sets of primers for the amplification of the gyrB (gyrase-B) gene of *Midichloria* and the cal (calreticulin) gene of *H. marginatum* were designed and used in a qPCR approach. Results were expressed as gyrB/cal copy numbers ratio.

RESULTS AND CONCLUSIONS. The molecular analyses suggest that *Midichloria* bacteria carried by *H. marginatum* ticks constitute a previously undescribed *Midichloria* strain within the *Midichloriaceae* family. Furthermore, the quantification of *Midichloria* in *H. marginatum* nymphs showed a higher bacteria load after the blood meal, similarly to what has been observed for *M. mitochondrii* in *Ixodes ricinus* ticks. Future studies should investigate the presence of this *Midichloria* bacterium in adult *H. marginatum* ticks, in order to increase the knowledge concerning its role in the biology of this tick species and its transmission mode during the tick bite.

Midichloria mitochondrii life-cycle: what can mathematical analyses tell us?

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Keywords *Midichloria mitochondrii*, *Ixodes ricinus*, life-cycle

INTRODUCTION. *Ixodes ricinus* is the most common tick in Europe and one of the most important vectors in the area. Previous studies revealed that 100% of tick females harbour the endosymbiotic bacterium *Midichloria mitochondrii*. Transmission electron microscopy (TEM) observations showed a very rare tropism for this bacterium, which is able to invade the host mitochondria rising to up to 20 bacterial cells per mitochondrial TEM section. These features resembled predatory bacteria such as *Bdellovibrio*, whose life-cycle passes through the invasion of bacterial prey cells, the replication within them and the return to the environment through lysis of the prey cell. A similar life-cycle was thus hypothesized for *M. mitochondrii*, however, the bacterium is not cultivable and thus this hypothesis can not be tested directly. In order to overcome this limitartion we designed a multidisciplinary study, including electron microscopy observations, quantitative PCR experiments and mathematical analyses.
MATERIALS AND METHODS. Seventy-nine oocyte cells from 14 different ticks were subjected to TEM observation: oocytes were classified on the basis of development status and the total number of unparasitized mitochondria, the number of mitochondria parasitized respectively by 1, 2, 3, 4, 5 or more bacteria, and the number cytoplasmatic bacteria were manually counted. In parallel, symbiont, mitochondria and tick cells were quantified through q-PCR in 30 oocytes from six different ticks, in order to estimate the total number of mitochondria and bacteria per oocyte. Collected data were then subjected to mathematical analyses using in-house R and Python scripts.
RESULTS AND CONCLUSIONS. TEM observations show that only ~10% of the mitochondria resulted parasitized by *Midichloria mitochondrii,* ~90% of which harbour a single bacterial cell. The frequency of mitochondria parasitized by single bacteria resulted negatively correlated with the frequency of cytoplasmatic bacteria, coherently with an hypothesis of continued bacterial invasion. On the other hand, the very low frequency of highly parasitized mitochondria is not coherent with a model comprising lysis of mitochondria by the bacteria. In conclusion, we gained novel informations about the life-cycle of *M. mitochondrii* within oocytes, but further studies are necessary to obtain an exhaustive description.

SESSIONE 3

ENDO ED ECTOPARASSITI DEGLI ANIMALI DA COMPAGNIA

Subcutaneous dirofilariosis [*Dirofilaria repens*]: a zoonotic infection spreading throughout Europe

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Two main *Dirofilaria* species infect dogs: *D. immitis* and *D. repens*. While *D. immitis* has a worldwide distribution, *D. repens* is currently found only in Europe, Asia and Africa. Adult *D. repens* are located in subcutaneous tissues of natural hosts where they survive for long periods of time. First stage larvae, microfilariae, circulate in the peripheral blood stream from where they are taken by the mosquito intermediate hosts. Infected mosquitoes then transmit infective L3 larvae to a new host through the blood meal. In dogs, most infections are asymptomatic, although cutaneous disorders such as pruritus, dermal swelling, subcutaneous nodules and ocular conjunctivitis can be rarely observed. At least two factors have increased the concerns about this parasitic infection 1) its spreading throughout the European countries and its prevalence in dog population that in some case has overcome that of *D. immitis* and 2) its zoonotic potential, which is much greater than that of *D. immitis*. Currently, about 4,800 cases of human *D. repens* infection have been published in European literature.
Different hypotheses can be put forward to explain these concerns. 1) climate change that has allowed more favourable conditions for survival of culicid vectors; 2) accidental hosts like humans may have a less efficient immune reaction against a parasite that is located in subcutaneous tissues, and thus less exposed to the host's immune response than, for instance, *D. immitis*. Furthermore, the lack of clinical signs in the majority of canine infections and the difficulty in diagnosing the infection (no serological tests are available and only the identification of microfilariae and differentiation from *D. immitis* can confirm the presence of the parasite) favour the further spread of this species. Finally, among the macrocyclic lactones currently used to prevent heartworm infection, only moxidectin has been found fully effective against the infective larvae transmitted by mosquitoes and partially effective (efficacy 96%) against the adult parasites in experimental studies.

The toxic effect of essential oils on mites

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Keywords: *Dermanyssus gallinae*, acaricide, essential oils

INTRODUCTION. As reports of resistance to conventional synthetic pesticides and repellents to control veterinary ectoparasites are increasing, their use is becoming increasingly problematic. Possible alternatives are the plant-derived essential oils. The objective of this study was to determine the toxic effects of essential oils and their effectiveness as acaricides on parasitic and economically important pest mites: the stored food mite *Tyrophagus longior* and the red poultry mite *Dermanyssus gallinae*.

MATERIALS AND METHODS. Seven essential oils and oil components (2-undecone, α-methyl-trans-cinnamaldehyde, methyl trans-cinnamate, ethyl cinnamate, benzyl alcohol, *Melaleuca viridiflora* and *Mentha spicata*) were used for a series of *in vitro* experiments. Contact and vapour toxicity were tested by exposing the mites to a concentration of 5%, 2.5%, 1.25% or 0.625% (V/V) in ethanol for a period of 24h. The residual activity of the oils was determined by leaving bioassays of the 5% (V/V) concentration to air-dry for 1, 2, 6, 24, 72 hours and subsequently exposing the mites to them for a 24h period. The repellency of the oils at 5%, 2.5%, 1.25% or 0.625% (V/V) concentrations was also tested, as was the synergistic effect of vanillin when added to 5% dilutions of the oils.

RESULTS AND CONCLUSIONS. Overall, the results were comparable between the two mite species, with benzyl alcohol and α-methyl-trans-cinnamaldehyde showing the most potential with a mortality rate of 80% or higher, although the toxicity was less marked with *D. gallinae*. In conclusion, these *in vitro* assays demonstrated that there is indeed some merit to this line of research, however this study is only a first step towards creating an efficient alternative to synthetic pesticides.

Larval development of *Angiostrongylus chabaudi*, the causative agent of feline angiostrongylosis, in the snail *Cornu aspersum*

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Keywords: lungworms, snails, *Cornu aspersum*

INTRODUCTION. *Angiostrongylus vasorum* and *Angiostrongylus cantonensis* may cause life-threatening diseases in several mammal species. Alongside these well-known species, *Angiostrongylus chabaudi* has been recently found in the cardiopulmonary system of domestic and wild cats from Italy (Varcasia et al., 2014, Parasit. Vectors, 7:588), Germany, Greece, Romania and Bulgaria (Giannelli et al., 2016, Vet. Par, 228:188-192). Nonetheless, significant gaps exist in the knowledge of the main ecological aspects of this parasite. This study aims to better understand the biology of *A. chabaudi* in the intermediate host and to provide a morphological description of larval stages of the nematode.

MATERIALS AND METHODS. *Cornu aspersum* (n = 30) land snails were infected with 100 first-stage larvae of *A. chabaudi* collected from a naturally infected wildcat in Romania. The nematodes were preserved in 70% ethanol, subsequently cleared, examined, drawing by compound microscope with differential interference contrast and a drawing tube. Digital images and measurements were taken using Leica LAS® AF 4.1 software.

RESULTS AND CONCLUSIONS. Larvae at different developmental stages were found in 29 (96.7%) out of 30 infected snails and a total of 282 larvae (mean 9.8 ± 3.02 larvae per each specimen) were collected from the gastropods. Here we demonstrate that *A. chabaudi* develops in snails and report *C. aspersum* as potential intermediate host for this parasitic nematode. This data, together with the in-depth description of morphological characteristics of L1, L2 and L3 will support the design of epidemiological surveys in gastropods and feline definitive hosts, and the identification of control strategies of *A. chabaudi*. Future investigations will contribute to understand the actual distribution of this parasite and whether domestic cats can sustain the lifecycle of *A. chabaudi* in the absence of the wildlife counterpart. Studies on the biology of little known parasites are fundamental before any speculative discussion on their epidemiology.

Interaction of *Cornu aspersum* immune-system against developmental stages of *Aelurostrongylus abstrusus*

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Keywords: *Aelurostrongylus abstrusus*, feline lungworm, Immune-system

INTRODUCTION. *Aelurostrongylus abstrusus* is a widespread nematode featured by an indirect life-cycle. Several species of snails have been recognised as suitable intermediate host. However, biological relationship between this parasite and its intermediate host are poorly investigated. The aim of the present study was to investigate the interaction between the snail's immune-system and the developmental stages of *A. abstrusus.*

MATERIALS AND METHODS. Twenty *Cornu aspersum* snails were randomly dived into 2 groups of 10 specimens each. Snails belonging to groups A were infected with ~ 250 first-stage larvae (L1s) of *A. abstrusus* using by-contact method, while those of group B were infected through the injection in the foot of 0.1 mL of saline solution containing ~250 L1s. Two snails from group B were sampled 4h post-infection, thereafter, two specimens from groups A and B were collected 2, 10 and 18 days post-infection (i.e. SD2, SD10 and SD18). Each collected snail was histopathologically examined.

RESULTS AND CONCLUSIONS. No difference was observed in the interaction between larvae and snail's immune-system in snails infected by the two different infection methods. In the snails of group B examined 4 h post-infection, and in those of group A and B sampled at SD2, the presence of several free L1s all around the injection site or disseminated in the innermost layer of snail's muscular foot (group A) was observed; at these time-points the snail's immune-system seems to be not yet activated as the haemocytes were not recruited and free in the tissues. At SD10, in snails of both A and B groups, some second-stage larvae (L2s) were able to escape the snail's immune-system, conversely other L2s elicited an intense reaction and were encapsulated by several haemocytes mimicking a "granulomatous-like" reaction in the internal layer of the muscular foot, leading to severe larval degeneration. On SD18 all third-stage larvae (L3s), able to overcome the host's immune barriers, were partially encapsulated by a thin layer of degenerated haemocytes. At this time-point, the L3s migrated to the outermost part of the muscular foot, near to the goblet cells, suggesting that they can be shed with the snail's mucus and continue their life-cycle.

Ticks infesting dogs in Vietnam: preliminary data

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Keywords: tick, dog, Vietnam

INTRODUCTION. In tropical areas, ticks may transmit a wide variety of pathogens to humans and animals (Otranto et al., 2009, Trends Parasitol 25:157-63). Southeast Asia provides a variety of habitats and microclimates for the survival and development of ticks (Irwin et al., 2004, Trends Parasitol 20:27-34). The most commonly retrieved tick species in dogs in Southeast Asia are *Amblyomma testudinarium, Dermacentor auratus, Haemaphysalis hystricis, Rhipicephalus haemaphysaloides, Rhipicephalus* (*Boophilus*) *microplus* and *Rhipicephalus sanguineus* sensu lato (Hoogstraal et al., 1965, J Parasitol 51:467-80; Parola et al., 2003, Clin Microbiol 1600-1608; Vongphayloth et al., 2016, Syst Appl Acarol 21:166–180). Until now, studies about ticks and tick-borne diseases in Vietnam have been limited (Petney et al., 2007, Parasitol Research 2:101). Importantly, almost all of studies ticks and tick-borne diseases were conducted long time ago (Kolonin, 1995, J Med Entomol 32:276–282; Alexander, 1972 Infect Immun 5:745–749) or have been published in national journals in Vietnamese language (Phan et al., 1977, Science and Technology press; Nguyen et al., 2014, J Science-Cantho Univ, 2:69-73) not available to the international scientific community worldwide. Therefore, we designed a study to generate more data about tick species infesting dogs in Vietnam and present preliminary results.

MATERIALS AND METHODS. Ticks (n=150) were collected from 18 dogs (out of 32 examined), being 8 from the city center of Hanoi capital and 10 from a rural area of Phutho province. Ticks were observed under stereomicroscope and identified morphologically using morphological keys (Walker et al., 2000, Cambridge University Press; Krantz and Walter, 2009, Texas Tech Univ Press).

RESULTS AND CONCLUSIONS. All ticks were identified morphologically as *R. sanguineus* s.l. (89 males, 55 females and 6 nymphs). More ticks are being collected from different provinces of Vietnam. We hope to generate genetic data regarding the tick species identified as *R. sanguineus* s.l. and also assess the circulation of tick-borne pathogens such as *Ehrlichia canis* and *Babesia vogeli*. Data generated will be useful to increase awareness among dog owners and veterinarians in Vietnam regarding the importance of ticks and tick-borne pathogens in this country.

A national survey of Ixodidae tick distribution in owned dogs in Italy

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Keywords: Ticks, Dogs, Epidemiology, Italy

INTRODUCTION. Recent changes in the distribution of ticks and the increase of the prevalence of tick-borne diseases highlight the need to develop and implement a systematic surveillance, based on comprehensive knowledge of tick species present in a geographical area (Estrada et al., 2017, Ticks Tick Borne Dis, 8:443-452). The aim of this study was to conduct, for the first time in Italy, a national survey of tick distribution in owned dogs.

MATERIALS AND METHODS. Over a period of 20 months (February 2016-September 2017) 153 veterinary practices, from to 64 different provinces (covering 17 Italian regions), were enrolled in a national-wide survey. The protocol required to examine 5 different dogs per month *at random* and to complete a questionnaire for each one. Differences in tick infestation associated with: sex, age and hair length (long and short); the dog's habitat (indoor or outdoor/kennel); and the dog's environment (urban or rural/sylvatic) were evaluated. The attachment site of ticks on the dog was also recorded. The questionnaire included also information regarding product used, date of sampling and date of last ectoparasiticide.

RESULTS AND CONCLUSIONS. A total of 3026 dogs were examined and 1383 (45.7%) were carrying at least one tick. Overall, 2439 tick samples were collected and a total of 14 species were identified, belonging to 4 genera: *Rhipicephalus* (63.8%), *Ixodes* (36.7%), *Dermacentor* (0.7%) and *Haemaphysalis* (0.2%). *Rhipicephalus sanguineus* group were the most predominant (63.6%), followed by *Ixodes ricinus* (30.6%) and *I. hexagonus* (5.6%). The most prevalent genera identified showed a clear association with climatic and environmental features of the Italian peninsula with *R. sanguineus* in the central-southern regions whereas *Ixodes* was better adapted to cold temperate of the northern regions. Twenty-four dogs had mixed tick infestations. Long-haired dogs had a higher tick infestation risk as dogs with outdoor and rural/sylvatic lifestyles. Ticks were located on the head (37.4%), the neck (28.8%), the muzzle (15.5%) and the back (15.3%) of dogs. No significant differences were found between different tick genera regarding their area of preference on the dog's body. However, a higher prevalence of *Rhipicephalus* genus ticks was found in interdigital spaces (10.8%) compared to *Ixodes* genus (0.2%). Finally, ectoparasiticide treatments were found significantly protective against tick infestation especially orally administered formulations. This study provides a comprehensive spatial coverage of the species of ticks in our country, useful to develop and plan effective control measures, as suggested by the European Scientific Counsel on Companion Animal Parasites**.**

Epidemiology and genetic diversity of *Blastocystis* sp. in dogs housed in sanitary and rescue shelters

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Keywords: *Blastocystis*, subtype, dog, kennels, molecular epidemiology

INTRODUCTION. *Blastocystis* is an intestinal protist associated with gastrointestinal disorders and affecting worldwide both humans and a wide range of animals. Up-to-now, 17 *Blastocystis* subtypes (STs) have been recognized on the basis of subunit ribosomal RNA gene (SSU-rDNA) analysis, being ST1-ST4 the most commonly found in humans. Among domestic animals, dogs seem to be involved in the spread and maintenance of the infection, naturally harboring STs in common with humans. With the aim of investigate on dogs as a potential source of *Blastocystis* infection to humans, an epidemiological survey on kennels' dogs in Lombardy was planned, to evaluate the prevalence of *Blastocystis* infection and the STs involved.

MATERIALS AND METHODS. Ninety-nine individual fecal samples were collected in six kennels, with an average of 19 animals per surveyed kennel. Samples included eight pure breeds and cross-breeds, and animals of varying age from three months to 14 years. Genomic DNA was extracted from each sample using a commercial kit; the DNA samples were subjected to PCRs in order to amplify a fragment of about 600 base pair within the 1800 bp SSU-rDNA of *Blastocystis* (Scicluna et al., 2006. Protist. 157: 77–85) and the fragments obtained purified for sequencing. STs were identified aligning obtained sequences with available published ST1-ST10 sequences. To infer the phylogenetic relationships among the sequences, a phylogenetic tree was constructed by using the neighbor-joining (NJ) method in MEGA v.6.0.6 software.

RESULTS AND CONCLUSIONS. Twenty-one fecal samples proved to be positive by PCR (21.2%), with prevalence values ranging from 18.2% to 37.5% according to the kennels; all dogs from one kennel scored negative for *Blastocystis*. Sequences obtained showed a high identity (98–100%) to homologous sequences of *Blastocystis* isolates previously reported in GenBank. The phylogenetic analysis showed that all sequences clustered with ST3 in a monophyletic group with a high bootstrap value (>95), being the first report in Italy of this ST in dogs. Previous surveys carried out in Italy showed the occurrence of ST3 as the most prevalent in human fecal samples (Mattiucci et al., 2016, Epidemiol Infect. 144:635-646. Meloni et al., 2011. Parasitol Res. 109:613-619); the results obtained in the present survey suggest dogs as possible zoonotic reservoirs for the parasite.

SESSIONE 4

DIAGNOSI DELLE MALATTIE PARASSITARIE 2

Does cat litter interfere on *Aelurostrongylus abstrusus* L1s survival?

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Keywords**:** *Aelurostrongylus abstrusus*, cat litters, survival, lungworm diagnosis

INTRODUCTION. *Aelurostrongylus abstrusus* is metastrongyloid nematode of cats responsible for cardiorespiratory disease. Direct diagnosis of metastrongyloid infections is usually performed with Baermann-Wetzel technique which relays on the viability of first-stage larvae (L1s) in the faeces. The majority of owned cats emits the faeces into cat litter and the dehydrating power of the litter may reduce the vitality of L1s and, thus, interfere with the diagnosis. The objective of this study was to assess the effect of the most commonly used cat litters on survival of *A. abstrusus* L1s at different time points.

MATERIALS AND METHODS. Four different types of cat litters were used: clumpling clay (Group A); non-clumpling clay (Group B); silica crystals (Group C); biodegradable (Group D) and a control group (Group E) without litter. First-stage larvae used in the study were obtained by Baermann-Wetzel technique from the faeces of a naturally infected cat; these L1s (~100) were injected in 20 samples of faeces (2 g each) certainly free from lungworm larvae (T_0). Thereafter, four faecal samples per each group were transferred into plastic cups containing the different types of cat litters (Groups A-D) or in empty cups (group E). The survival of L1s was assessed in each group after 3 (T_3), 6 (T_6), 12 (T_{12}) and 24 (T_{24}) hours using the Baermann-Wetzel technique.

RESULTS AND CONCLUSIONS. A decreasing trend of L1s survival was observed in all groups with statistically significant highest values at T_0 compared to T_3, T_6, T_{12} and T_{24} ($P<0.001$). At T_{24}, a significant higher number ($P<0.05$) of L1s was found in control group respect to the groups with cat litters.

Results of this study showed how the viability of *A. abstrusus* L1s is negatively influenced by the contact of faeces with the cat litter whatever is the typology of the litter. This effect is time-dependent, with a reduction of about 80% of the number of vital larvae after 3 hours and up to 100% after 24 hours according to the type of litter. Faeces exposed to cat litter may compromise aelurostongylosis diagnosis leading to false negative especially when the parasitic load is low and/or when the sample is collected many hours after the emission.

Molecular tools for identification of zoonotic metacercariae in freshwater fish

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Keywords: zoonotic metacercariae, Opisthorchioidea, multiplex PCR

INTRODUCTION. Fish-borne trematodes are commonly reported worldwide and more than 70 species are known to be zoonotic. Among others, the families Opisthorchiidae and Heterophyidae include several genera of zoonotic interest also in European Countries. Due to their small size, most of the Opisthorchioidea metacercariae cannot be detected in fish by visual inspection as provided by law. The standard procedure to detect Opisthorchiids/Heterophyids metacercariae in fish fillets is artificial digestion of fish tissue with hydrochloric-pepsin solution or by compression of muscle between two-glass slides, both followed by microscopical observation which is useless to identify them. The aim of this research, in the frame of ParaFishControl EU project, was to develop a molecular method for simultaneous identification at genus or species level of metacercariae of the most common European Opisthorchiids and Heterophyids in fish or fish products.

MATERIALS AND METHODS. For the design of a multiplex PCR for simultaneous identification of Opisthorchiids/Heterophyids metacercariae of the following parasites were used: *Opisthorchis felineus*, *Metorchis* spp., *Pseudamphistomum truncatum*, *Metagonimus* spp. and *Apophallus* spp. collected from freshwater fish in Italy and Hungary. Three molecular markers were chosen, 18S and ITS rDNA, and COI mtDNA, amplified by PCR and sequenced. Multiplex PCR primers were designed and chosen based on different fragments size in order to develop a reliable tool to discriminate the different parasites under study.

RESULTS AND CONCLUSIONS. The sequences of the 18S rDNA showed no possibility to find a region enough variable and were excluded, while the sequences of the entire ITS rDNA showed variable parts. A common reverse was designed at the beginning of the 28S rDNA, and genus specific primers were designed for *Apophallus* spp. (~1066 bp) and for *Metagonimus* spp. (~722 bp). For the other parasites the COI mtDNA was used: *Metorchis* (~500 bp), *P. truncatum* (~150 bp) and *O. felineus* (~230 bp). The development of this multiplex PCR for simultaneous identification of metacercariae of the most common European Opisthorchiids and Heterophyids in freshwater fish represents an useful tool in the field of fish product safety.

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A proteomic approach to identify candidate antigens for serodiagnosis of canine onchocercosis

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Keywords: *Onchocerca lupi*, candidate antigen, serodiagnosis, dogs

INTRODUCTION. Within the genus *Onchocerca* (Spirurida: Onchocercidae), *Onchocerca volvulus* and *Onchocerca lupi* parasitize primarily humans and carnivores, respectively. However, the zoonotic potential of *O. lupi* has been confirmed only recently and human ocular infections have been documented in different countries of Europe and USA (Otranto et al., 2011, Am. J. Trop. Med. Hyg, 84:55-58; Otranto et al., 2012, Parasit. Vectors, 5:84; Eberhard et al., 2013, Am. J. Trop. Med. Hyg, 88:601-605). To date, the diagnosis of canine onchocercosis involve surgical removal of the adult nematodes from the eye, skin snips to isolate microfilariae and PCR-based DNA assays (Otranto et al., 2013, Emerg. Infect. Dis, 19:2000-2003; Latrofa et al., 2018, PLoS Negl. Trop. Dis, 12:e0006402). Improved diagnostic methods are needed to support ongoing efforts to eliminate *O. lupi* dogs' infection. Thus, the aim of this study was to identify *O. lupi* antigens for future developing serodiagnostic tests.

MATERIALS AND METHODS. An adult female of *O. lupi* was isolated from the ocular nodule of a dog from Portugal and stored in RNA later solution at -80°C until use. The nematode specimen was subjected to protein extraction, SDS-PAGE and immunodetection by Western Blotting. Membranes were incubated with sera from uninfected and infected *O. lupi* dogs and with those from dogs positive for *Acanthocheilonema reconditum*, *Dirofilaria immitis*, *Dirofilaria repens*, *Cercopithifilaria bainae*. Dog antibodies were detected using rabbit anti-dog IgG horseradish peroxidase conjugate. The gel band corresponding to the sample positive for *O. lupi* sera was excised, the gel fragment was digested and analysed by nLC-MS/MS.

RESULTS AND CONCLUSIONS. A ~200kDa gel band reactive against *O. lupi* sera was digested using trypsin and analysed by mass spectrometry. Fourty-nine unique peptide sequences mapped onto *O. volvulus* identified five proteins, such as Actin, Calponin and Paramyosin. These antigens represent potential resource as candidate biomarkers useful for serodiagnosis of canine onchocercosis. Recombinant expression of the full protein epitope will be useful in mapping and screening dog sera infected with *O. lupi*.

Real time-PCR for the detection of the zoonotic *Onchocerca lupi*

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Key words: *Onchocerca lupi*, dogs, cats, real time-PCR, cytochrome *c* oxidase subunit 1

INTRODUCTION. *Onchocerca lupi* (Spirurida: Onchocercidae), a filarioid of zoonotic concern (Otranto et al., 2011, Am. J. Trop. Med. Hyg, 84:55-58), infects dogs and cats causing from minor to severe ocular lesions (Sreter and Szell, 2008, Vet. Parasitol, 15:1-13; Otranto et al., 2015, Parasit Vectors, 8:89; Komnenou et al., 2015, Vet. Ophthalmol, 19:245-249). The diagnosis of onchocercosis is based on microscopic examination of microfilariae from skin snip sediments and on the morphological identification of adults embedded in ocular nodules (Otranto et al., 2013, PLoS Negl. Trop. Dis, 7:e2585). However, these techniques are seldom performed, labour-intensive and requiring multiple steps to achieve a definite diagnosis. In this study a quantitative real-time PCR (qPCR) assay was standardized for the detection of *O. lupi* DNA and results were compared with microscopic examination and conventional PCR (cPCR).
MATERIALS AND METHODS. The specificity of qPCR and cPCR was assessed by processing the most common filarial nematodes infecting dogs, skin samples from *O. lupi* infected (n = 35 dogs) or uninfected animals (n = 21 dogs; n = 152 cats) and specimens of potential vector (n = 93 blackflies; n = 59 mosquitoes/midges). The analytic sensitivity of the qPCR and cPCR assays was assessed using 10-fold serial dilutions of DNA from adult and from pooled microfilariae, respectively.
RESULTS AND CONCLUSIONS. The qPCR on skin samples revealed an analytic specificity of 100% and a sensitivity up to 8 x 10^{-1} fg/2µl *O. lupi* DNA. Only 9.5% *O. lupi*-infected skin samples were positive for cPCR with a sensitivity of 8 x 10^{-1} pg/2µl of DNA. All blackflies and mosquitoes/midges tested by qPCR were negative for *O. lupi*, with the exception of eight *Simulium* spp. (n = 1 *S. erythrocephalum*; n = 1 *S. ornatum*; n = 6 *Simulium* sp.) experimentally infected. The qPCR assay herein standardized represents an important step forward in the diagnosis of *O. lupi*. This assay provides a fundamental contribution for the establishment of surveillance strategies aiming at assessing the presence of *O. lupi* in carnivores and in insect species acting as potential intermediate hosts. In addition, this test may also assist in the disease progress monitoring as well as in the diagnosis of apparently clinical healthy dogs and cats.

Occurrence, diagnosis and follow-up of canine strongyloidiasis in naturally infected shelter dogs

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Keywords: *Strongyloides stercoralis,* coprological and serological diagnosis, shelter

INTRODUCTION. *Strongyloides stercoralis* is a soil-transmitted helminth affecting several animal species globally and of potential zoonotic concern mainly in poor contexts where low hygienic conditions and humid, warm climate permit the free-living cycle of the parasite (Nutman et al., 2017, Parasitology, 144:263-273; Thamsborg et al., 2017, Parasitology, 144:274-284). Scientific knowledge on canine strongyloidiasis is hindered by the poor diagnostics currently available (Buonfrate et al., 2017, Parasitol. Res, 116:2027-2029). The study aimed to assess the occurrence of *S. stercoralis* infection in shelter dogs by comparing the sensitivity and specificity of serological, coprological and molecular methods and the larval shedding by weekly examination of faecal samples in a cohort of infected dogs.

MATERIALS AND METHODS. Faeces and blood samples from 100 shelter dogs living in southern Italy were examined through coprological techniques (i.e., Baermann, direct microscopy, Koga test and real-time PCR) and serology (i.e., IFAT and ELISA), respectively. Two composite reference standards (CRS) based on all coprological diagnostic methods alone or in combination with serological results were assessed. In addition, the faeces of 17 dogs positive to *S. stercoralis* in at least one coprological test were weekly collected for one month (T1-T4) and analyzed by parasitological and molecular tests to evaluate the larval shedding.

RESULTS AND CONCLUSIONS. Thirty-six dogs (36%) scored positive to *S. stercoralis* by coprology (22.3% to Baermann and 30% to rt-PCR). According to the CRS combined analyses the most sensitive test was IFAT (93.8%; CI: 82.8-98.7%), followed by RT-PCR (80.6%; 95% CI: 64-91.8%) and Baermann (60.6%; 95% CI: 42.1-77.1%). Of the 17 dogs scored positive to *S. stercoralis* at least at one faecal examination test and studied over a four-week period, ten (58.8%) scored negative at the Baermann at either T3 and/or T4, though RT-PCR confirmed the parasitological results in only five animals. Results indicate that the use of RT-PCR and Baermann tests is an optimal approach to detect *S. stercoralis* active infection, and therefore the reservoir dogs. Nonetheless, in the case of intermittent larval shedding misdiagnoses may occur. The IFAT assay was highly sensitive and specific with IgG titers ≥1:320. Therefore, a combination of serological and coprological tests is recommended for the diagnosis and the surveillance of *S. stercoralis* infection in dogs.

Pooling faecal samples in cattle for the assessment of gastrointestinal nematode infection intensity and anthelmintic drug efficacy using Mini-FLOTAC

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This study was performed within the Italy/France Galileo Project (2016)
Keywords**:** Cattle, Pool, Mini-FLOTAC, Anthelmintic efficacy

INTRODUCTION. Gastrointestinal nematode (GIN) infections are a serious problem for the health, welfare and productivity of cattle in Europe (Charlier et al., 2014, Trends Parasitol, 30:361-367). There is a need to improve reliable and user-friendly diagnostic tools for a rapid detection of GIN infections in cattle and for assessing efficacy of anthelmintics and anthelmintic resistance (AR). In this study a field approach was developed to validate a strategy based on pooling faecal samples in cattle for the assessment of GIN infection intensity (faecal egg count - FEC) and anthelmintic drug efficacy (FEC reduction - FECR).

MATERIALS AND METHODS. Between June and October 2017, 10 beef cattle farms were chosen in the Campania region, southern Italy. For each farm, 20 young cattle (6 to 20 months) after their first grazing season and naturally infected with GINs were selected. In 3 farms, 20 animals were divided into 2 groups of 10 animals each: one was treated with ivermectin (IVM) and one with albendazole (ALB). In other 3 farms 20 cattle were treated with IVM and in 4 farms 20 cattle were treated with ALB. Individual faecal samples were collected from cattle before (T0) and after (T14) anthelmintic treatment. In addition, the faecal samples were assembled in pools of 5 individual samples (n = 4), 10 individual samples (n = 2) and 20 individual samples (n = 1). All the individual and pooled samples were analyzed by the Mini-FLOTAC technique (Cringoli et al., 2017, Nat Protoc,12:1723-1732) with a detection limit of 5 eggs per gram (EPG) of faeces. For each farm, a composite faecal culture was performed for each group at T0 and T14. The agreement in FECs between individual samples and pooled samples was verified by a permutation test (10,000 iterations) based on Pearson correlation coefficient.

RESULTS AND CONCLUSIONS. GIN intensity (EPG) of pooled samples correlated positively with mean EPG of individual samples, with very high correlation coefficients (ranging from 0.96 to 0.99) across the 3 different pool sizes. A very high efficacy was obtained with IVM (100%) and ALB (>99%). The following GIN genera were detected by coprocoltures at T0: *Cooperia* (41.3%), *Tricostrongylus* (19.4%), *Oesophagostomum* (18.4%), *Ostertagia* (11.2%) and *Haemonchus* (9.7%). In conclusion, pooling faecal samples holds promise as a cost-saving and efficient strategy for assessing GIN nematode FEC and FECR in cattle as already showed in sheep (Kenyon et al., 2016, Vet Parasitol, 225:53-60; Rinaldi et al., 2014, Vet Parasitol, 205:216–223). Moreover absence of anthelmintic resistance was demonstrated in cattle farms from the Campania region of southern Italy.

SESSIONE 5

BIOLOGIA MOLECOLARE E FILOGENESI IN PARASSITOLOGIA 2

Next-Generation development of Microsatellite markers in the three species of the *Anisakis simplex (s.l.)* complex (Nematoda: Anisakidae)

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Keywords**:** *Anisakis,* microsatellites, population genetics

INTRODUCTION. The aim of this study was to develop and validate a panel of microsatellite loci in order to: i) provide further nuclear markers allowing to distinguish the three members of the *A. simplex (s.l.)* complex (i.e. *A. pegreffii, A. simplex* (s.s.), *A. berlandi*) (Mattiucci et al., 2014, J.Parasitol.,10:199-214), in a multilocus genotyping approach; and ii) study their population genetic structure.

MATERIALS AND METHODS. A panel of microsatellite loci were developed and validated over a large number of individuals (*N*= 1156) of those species, previously identified by other molecular/genetic markers (i.e. allozymes, mtDNA *cox2*, EF1α1nDNA). They were collected in various intermediate and definitive hosts. from allopatric and sympatric populations of the three *Anisakis* spp. The SSR-enriched library was first analyzed, by Illumina MiSeq platform, on *A. pegreffii* specimens. After assembly, 785 contigs/singlets showing a microsatellite insert with a tetra- or a trinucleotide of at least six repeat units, were considered. Seven microsatellites loci were finally selected. The allele size was determined on ABI3730, by GeneScanTM-500 LIZ Size Standard. The alleles obtained from the electropherograms were identified by Genemapper v.4.1. The seven couples of primers gave positive results also on *A. simplex* (s. s.) and *A. berlandi*. An additional microsatellite locus, i.e. AnisL7 (Mladineo et al., 2017, Int.J.Parasitol.47:215-223), was tested. Two Multiplex PCR amplifications were optimized. Hardy–Weinberg equilibrium, allele frequencies and the genotypic linkage equilibrium between each pair of loci, were tested by Arlequin3.5 (Excoffier & Lischer, 2010, Mol.Ecol.Res.,10:564-567). Population genetic analysis and instances of gene exchange between species, were performed by a Bayesian clustering algorithm, using individual multi-locus genotypes, by STRUCTURE2·3·3 (Pritchard et al., 2000, Genetics,155:945-959).

RESULTS AND DISCUSSION. All the loci were polymorphic in the three species. Most of the loci were in H-W equilibrium in the examined populations of the three species; one showed significant departure from H-W equilibrium, likely due to the presence of null alleles. The STRUCTURE analysis (K= 3), on 7 loci, (by using highest ln-probability and the delta-K optimality criteria), allowed to fully distinguish the three species, i.e. *A. pegreffii*, *A. simplex* (s. s.) and *A. berlandi*. The new nuclear loci represent robust and polymorphic markers to be used as markers, in both individual genotyping and population genetic approaches of those species.

Transcriptomic analyses of non-pathogenic marine ascaridoid *Hysterothylacium aduncum* and pathogenic *Anisakis simplex* sl larvae

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Keywords: *Anisakis simplex* sensu stricto, *Anisakis pegreffii*, *Hysterothylacium aduncum*, *de-novo* assembly

INTRODUCTION. Parasitic nematodes of the genus *Anisakis* are responsible for a fish-borne zoonosis known as anisakiasis, *via* the ingestion of raw-undercooked fish with third-stage larvae, which cause gastrointestinal illness and/or allergic reactions in humans. Prevalent in countries with large consumption of raw fish as Japan, *Anisakis* is now considered an emerging pathogen of health and economic relevance worldwide. Species responsible for anisakiasis are *A. simplex* ss (=AS) in Asia, and *A. pegreffii* (=AP), mostly in southern Europe. Despite the socioeconomic importance of anisakiasis, little is known of the biological mechanisms that determine the pathogenetic potential of various species within the Ascaridoidea. To fill this gap in knowledge, recent proteomic (Arcos et al., 2014, Proteomics 14:1547) and transcriptomic studies have investigated molecules with pathogenetic potential, such as allergens and proteolytic enzymes (Baird et al., 2016, PLoS Negl Trop Dis. 10:e0004845.; Cavallero et al., 2018, Parasit Vectors. 11:31). With the aim to identify molecules potentially involved in pathogenetic mechanisms of anisakiasis, transcriptomes of both whole larvae and pharyngeal tissues from third-stage larvae of AS and AP were sequenced, *de-novo* assembled and annotated, and compared for the first time to the transcriptome of *Hysterothylacium aduncum* (=HA), a phylogenetic related marine ascaridoid that is considered not pathogenic to humans.
MATERIALS AND METHODS. High-throughput RNA-Seq reads were obtained for AS, AP and HA (both whole larvae and dissected pharyngeal tissue), and de-novo assembed with Trinity; gene relative expression was calculated with RSEM while differential gene expression with edgeR; annotation of assembled transcripts was performed with Annocript.
RESULTS AND CONCLUSIONS. Assembled Trinity contigs were 94906, 101018 and 145023 for AS, AP and HA, respectively. Differential expression analysis was performed on identified transcripts in each species: AS, 38531, (40.6%), AP, 39579 (30.1%) and HA, 65143 (44.9%). Transcripts upregulated ($P<0.05$) in whole larvae were 282, 320 and 1817 for AS, AP and HA, respectively, while transcripts upregulated in pharyngeal-specific tissues were 612, 526 and 471 for AS, AP and HA, respectively. Annotation of HA transcriptome gave 21784 transcripts described by one more protein family motif. A large prevalence of transcripts encoding for Protein Kinase domain (pfam00069), RNA recognition motif (pfam00076) and HSP70 protein (pfam00012) were identified, corresponding to pathways mostly involved in protein modification, thus suggesting a role in host-pathogen interplay.

Molecular identification of *Mesocestoides* sp. metacestodes in a captive gold-handed tamarin *(Saguinus midas*)

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Keywords: *Mesocestoides,* metacestode, *Saguinus midas,* phylogenetic analysis

INTRODUCTION. Tapeworms of the genus *Mesocestoides* (Cestoda: Cyclophyllidea) require three hosts: one definitive and two intermediate ones, though the putative first-intermediate host has never been clearly identified. Adult worms reside in the digestive tract of many domestic and wild carnivores, birds of prey and, rarely, of humans; the metacestode larva (tetrathyridium) can be found in serous cavities of amphibians, reptiles, birds and micro-mammals. However, some isolates at the metacestode stage may asexually reproduce provoking severe systemic infections, which may occur also in definitive hosts. In the present study, a case of a peritoneal infection by *Mesocestoides* sp. occurred in a captive gold-handed tamarin (*Saguinus midas*) is described.

MATERIALS AND METHODS. In May 2016, an old male gold-handed tamarin living since 2015 in a wildlife recovery centre in Rome, after its illegal keeping in Southern Italy, was found dead after one day of malaise and anorexia. At necropsy, several nodules referable to metacestode forms were isolated, washed and stored in 70% ethanol. The samples (n=7) were photographed and measured using a ruler with at 0.001 mm accuracy. Molecular identification of the isolates (n=7) was obtained through DNA extraction and the amplification of partial fragments of cytochrome oxidase subunit 1 (CO1) and of the 12S rDNA. A phylogenetic analysis using Bayesian method, was conducted by comparison with all *Mesocestoides* sequences based on their availability in GenBank for the diverse gene partitions.

RESULTS AND CONCLUSIONS. Morphological examination allowed identifying the nodules as parasitic forms belonging to the order Cyclophyllidea, compatible with those of the genus *Mesocestoides*. Molecular analysis confirmed the identification to the *Mesocestoides* genus. Analyses of the 12S and CO1 partitions support the validity of the phylogenetic relationships within the genus previously depicted (Padgett et al., 2005, J. Parasitol., 91:1435–1443; Hrčkova et al., 2011, Parasitology, 138:638-647; Skirnisson et al., 2016, Parasitol. Res., 115:2597-2607). However, metacestode isolates from the tamarin were well separated when compared with other described *Mesocestoides* spp.. Molecular evidence may support the hypothesis of a new taxon, here genetically characterized for the first time.

Detection and genetic variation of *Vermamoeba vermiformis* from different water sources in Italy

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Keywords: *Vermamoeba vermiformis*, FLA, rDNA diversity, phylogeny

INTRODUCTION. Free-living amoebae (FLA) are ubiquitous protozoan commonly founded in natural and artificial aquatic environments. Some species, e.g. *Naegleria fowleri*, *Acanthamoeba* spp., *Balamuthia mandrillaris* and *Vermamoeba (=Hartmannella) vermiformis* can be potentially pathogenic in human and animals. Although *V. vermiformis* has been recovered from human only in a few instances compared to other FLA species, its occurrence in nature is very significant and it is recognized as important vector of pathogenic bacteria (*Legionella* spp; *Pseudomonas aeruginosa*). The purpose of this study was to (i) investigate the presence and the distribution of *V. vermiformis* in natural and artificial aquatic environments in Italy [especially geothermal springs and dental unit waterlines (DUWs)]; (ii) identify the isolates at the species level using the ribosomal DNA (18S) as molecular marker; (iii) investigate the contribution of these isolates to the diversity within *V. vermiformis* using homologous sequences retrieved from GenBank.

MATERIALS AND METHODS. The present study tested 69 samples of geothermal waters (n=36) and DUWs (n=33). Each sample was subjected to *V. vermiformis* detection by cultural method and PCR amplification of 18SrDNA region. The affinities between the sequences generated here and others reported in the literature were explored using distance based method of Neighbor Joining (MEGA7). Isolates of *V. vermiformis* were also classified by specific "allelic variations" depicted by Fuerst and collaborators (http://u.osu.edu/acanthamoeba/genetic-variation-within-v-vermiformis/: last update December 2017).

RESULTS AND CONCLUSIONS. From the 69 water samples analyzed, 37 (53.62%) [Geothermal waters = 31 (86%); DUWs = 6 (18.2%)] were positive for outgrowth of FLA based on morphological page key. Nineteen out of 37 samples (51.3%) were positive for *V. vermiformis* using molecular tools.

Phylogenetic analysis confirmed the existence of several "subgroups" already described; in particular *V. vermiformis* from DUWs were classified under the "subgroup" 1 (allelic variant = 1111); in contrast new allelic variations (4211; 7511) never described so far, were identified in all *V. vermiformis* isolated from geothermal waters, providing new data on the genetic structure within *V. vermiformis*.

In deep analysis of different Giardiavirus (GLV) in naturally infected *Giardia duodenalis* trophozoites

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Key words: *Giardia duodenalis*, *Giardiavirus*, immunolcalization, gemomics

INTRODUCTION. Giardiasis, caused by the protozoan parasite *Giardia duodenalis*, is an intestinal disease affecting almost one billion people worldwide. Infection can be asymptomatic or cause an acute and/or chronic diarrheal disease. Although clearly multifactorial, the exact pathogenic mechanisms and the factors associated with isolates virulence have not been completely identified. A small dsRNA cytoplasmic virus comprising 2 ORFs (capsid protein and capsid protein-RNA dependent RNA polymerase fusion protein), referred to as GLV (*G. lamblia* virus), family Totiviridae, has been reported in association with many human and animal isolates of *G. duodenalis*. Only two and almost identical GLV genomes have been fully sequenced, whereas extensive studies were done in 80s and 90s with the original GLV isolate. Although increased virus:parasite ratio decreases cultured trophozoites growth rate, correlation between the presence of GLV and Giardia virulence has not been investigated. In the perspective to better define the role of GLV, the characterization of three GLV strains from naturally infected *G. duodenalis* trophozoites was performed.

MATERIALS AND METHODS. GLV infected *G. duodenalis* isolates, from a human, a pig and a cat and the GLV-free WBC6 clone were used. A polyclonal antibody was raised in mouse against the N-terminus of the GLV Capsid Protein (CP) fused to a 6XHIS tag and used in western blot and CLSM immunofluorescence experiments. Intracellular localization of GLV was also assessed by TEM experiments.

RESULTS AND CONCLUSIONS. Nucleotide polymorphism among the three GLV genomes was observed by RT-PCR amplification and sequencing of the first 1500 nucleotides. Similarly, a difference in the encoded CP size was observed for the GLV from the cat isolate, supporting the existence of different translation starting codons. These genome and protein variants also correlate with a differential distribution of the viral particles in the trophozoites of the three parasite isolates, as observed by CLSM and TEM. The complete genome of the GLV from the human isolate was obtained by cloning and sequencing and by RNAseq approach, pointing on an alternative +1 slippage frameshift mechanism to regulate the translation of the CP-RdRP fusion protein. In conclusion our studies clearly highlight the existence of GLV strains that differ from those actually reported and suggest that such differences might impact virus infection biology. Complete sequencing of these and other GLV strains, controlled infection experiments in GLV-free Giardia strains and the effect of the virus in parasite-host interactions models are the next steps to evaluate GLV among factors affecting Giardia virulence.

Diversity of *Pneumocystis jirovecii* across Europe: a multicentre observational study. The Italian contribution

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Keywords: *Pneumocystis jirovecii,* Genotyping, Europe, Transmission

INTRODUCTION. *Pneumocystis jirovecii* is an airborne human-specific ascomycetous fungus responsible for *Pneumocystis* pneumonia (PCP) in immunocompromised patients, affecting >500,000 patients per year (www.gaffi.org). The understanding of its epidemiology is limited by the lack of standardised culture. Recent genotyping data suggests a limited genetic diversity of *P. jirovecii*. The objective of the study was to assess the diversity of *P. jirovecii* across European hospitals and analyse *P. jirovecii* diversity in respect to clinical data obtained from the patients.
MATERIALS AND METHODS. Genotyping was performed using six already validated short tandem repeat (STR) markers.
Sixteen European centres were asked to send 25 frozen DNA samples obtained from respiratory samples from patients with PCP over the minimum period of time. These 16 centres across Europe included five from France [FR] (Amiens, Brest, Nantes, Lyon, Paris) and one centre per country for Belgium [BE], Czech Republic [CZ], Denmark [DK], Germany [DE] Italy [IT], Poland [PL], Portugal [PT], Spain [ES], Switzerland [CH], The Netherlands [NL] and United Kingdom [UK].
RESULTS AND CONCLUSIONS. The present *P. jirovecii* genotyping study is the first one dealing with several European countries and using an Microsatellite Length Polymorphism (MLP) typing (Gits-Muselli et al., 2015). In analysing 249 cases of PCP recruited from 16 centres across 12 European countries, our main findings were the large proportion of PCP cases harbouring mixtures of *Fungal Individuals*, the limited genetic evolution of *P. jirovecii* across Europe, and the possible enrichment of genotypes at some centres, possibly linked to the underlying disease of the patient.

SESSIONE 6

EPIDEMIOLOGIA DELLE MALATTIE PARASSITARIE 1

Cystic Echinococcosis surveillance: a 10-years experience in a hypo-endemic area

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Keywords: Cystic Echinococcosis, surveillance, bovine, Italy

INTRODUCTION. The surveillance of Cystic Echinococcosis (CE) in Europe includes the detection of the disease in intermediate hosts at slaughterhouse and subsequent notification. Local health authority is supposed to investigate the farm of origin of positive animals and to treat their dogs with cestocidal drugs. However, the source of the infection is rarely demonstrated. The present study attempted to apply a new approach to the management of bovine CE cases to successfully identify the dogs at the origin of the environmental contamination with *Echinococcus granulosus* eggs.

MATERIALS AND METHODS. In the first phase of the research all cattle coming from Veneto Region farms found positive to CE at slaughterhouse in the period 2006-2010 were retrospectively analyzed to track their movements and to identify autochthonous cases. The presence of territorial cluster of bovine farms with CE cases was investigated using a spatial scan statistic. In the second phase, a similar approach was used on a 1-year time frame for 3 consecutive years (2013-2014-2015), including the area of the Region at higher risk. Targeted epidemiological investigations were conducted in all significant clusters identified during both phases, collecting fecal samples from bovine farm dogs and shepherd dogs of transhumant flocks known to pass through the same areas. The results of targeted surveys were compared with that of farms individually investigated in two control areas. All fecal samples were analyzed for taeniids eggs and DNA from positive samples were sequenced and identified.

RESULTS AND CONCLUSIONS. The study demonstrated the feasibility of a new system for CE surveillance, based on a territorial approach. The 2006-2010 retrospective survey demonstrated that 81.1% (467/576) bovines tested positive for CE were autochthonous and that the estimated prevalence among cattle of the Region was 0.33%. As regards copro-microscopic survey, out of 208 collected samples, in 6.7% (14/208; C.I.: 3.3-10.1%) it was possible to detect taeniid eggs (2 *E. granulosus*, 7 *Taenia hydatigena* and 5 samples identified at genus level as *Taenia* spp.). Twelve positive dogs were found in targeted surveys and only two in control areas. Nearly all dogs positive to *T. hydatigena* and *E. granulosus* were shepherd dogs. The 1-year retrospective and spatial analysis identified one cluster in 2014, but it missed to detect any aggregation both in 2013 and 2015. Thus, this short-time analysis is probably able to identify only areas with high contamination of *E. granulosus* eggs. In conclusion, a centralized and regular data collection allows for a constant monitoring of prevalence values among bovine resident population and pave the way for the identification of areas at risk where to conduct targeted epidemiological surveys.

The international impact of HERACLES collaborative project on cystic echinococcosis

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Keywords: Public health, cystic echinococcosis, international collaborative project

INTRODUCTION. Cystic Echinococcosis (CE) is one of the most important zoonotic diseases worldwide and was recently assigned to the list of the Neglected Tropical Diseases prioritized by the WHO. Tools for its diagnosis and treatment are currently not standardized, partly due to the complex and chronic evolution of CE and lack of funding to support prospective multicenter clinical trials, which in turn make data on this infection poorly framed and evidence supported, resulting in yet more neglect. HERACLES is a EU funded collaborative project (2013-2018) that offers for the first time a reasonable amount of funding and a real chance to break this vicious circle, promoting prospective studies on CE.

MATERIALS AND METHODS. The main goals of the HERACLES cooperative project are to: Identify the population affected by CE in Bulgaria, Romania and Turkey by ultrasound screening; create the European Register of CE (ERCE); establish the Echino-Biobank from animal and human CE patients; set-up and validate new molecular-based PoC-LoC kits based on recombinant antigens; identify cyst stage-specific biomarkers associated with CE response to therapy or lack thereof, through "omic" studies; increase drug bioavailability of benzimidazoles; train experts working in Eastern European countries, as they are crucial to fight this disease.

RESULTS AND CONCLUSIONS. Current core achievements are: 1) Creation of the HERACLES Extended Network with more than 50 centers from 30 countries (http://www.heracles-fp7.eu/interactive_map.html); 2) Completion of the biggest research-based cross-sectional study (ultrasound-based) on CE ever done (N=24,693), estimating 151,000 people infected in rural Romania, Bulgaria and Turkey; 3) Creation of the European Register as a case series for data analysis on clinical management of CE with 1,744 patients from 33 clinical centres (http://www.heracles-fp7.eu/erce.html); 4) European patent on anti-parasitic soluble drugs: "Salts of compounds having a benzimidazolic structure" (PCT/IT2016/000191); 5) creation of the Echino-Biobank repository to sustain experimental and clinical research in this field (N≈4,500 samples); 6) Worldwide collection of human cyst samples for genotyping studies (N=742); 7) First proteomic description of parasite exosomes in fertile hydatid cyst fluid; 8) Scientific papers published in peer reviewed journals: 40.

The results from HERACLES will support governments, organizations (WHO), European Commission, related European agencies (ECDC, EFSA) and the Global Burden of Disease study (IHME) to harmonize data collection, monitoring and reporting of CE. We see this as breakthrough in the current scenario of CE. The research was funded from the European Community's FP7 under the grant agreement 602051 (Project HERACLES; http://www.Heracles-fp7.eu/).

The European Register of Cystic Echinococcosis (ERCE): where are we and where to go (HERACLES project)

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Keywords: Cystic echinococcosis, Register, Public health

INTRODUCTION. Cystic echinococcosis (CE) is a neglected zoonotic infection distributed worldwide, for which the WHO advocates control efforts (WHO, 2012 Accelerating work to overcome the global impact of neglected tropical diseases – A roadmap for implementation). It is caused by the metacestode of *Echinococcus granulosus* that forms cysts mainly in the liver and secondarily in the lungs. CE is a chronic disease with proteiform, unspecific clinical manifestations ranging from complete absence of symptoms to disabling, and sometimes life-threatening conditions. CE cysts also pass through several stages, either spontaneously or as the result of treatment. Different stages respond differently to various clinical management options, however the clinical management of patients with CE still rely on expert recommendations, as the implementation of prospective clinical trials is extremely difficult (Brunetti et al, 2010, Acta Trop, 114:1-16). The European Register of Cystic Echinococcosis (ERCE; http://www.heracles-fp7.eu/erce.html) was launched in 2014 within the HERACLES project (Rossi et al., 2016, Parasit. Vectors, 9:243). ERCE is an online prospective, observational, multicentre register of patients with probable or confirmed CE, built to take into account the peculiar features of the infection. It was originally conceived as a tool to capture the number and clinical characteristics of patients with CE reaching medical attention but not official statistics, with the aim of allowing national and international authorities to acknowledge the magnitude of the problem. In December 2016, the WHO Informal Working Group on Echinococcosis that met in Geneva WHO headquarter individuated in ERCE a possible starting platform for the collection of clinical data that may be used to derive evidence on treatment recommendations from case series of patients, in the absence of prospective randomized clinical trials. In this perspective, in November 2017 a round table was held at the Istituto Superiore di Sanità in Rome with a group of active ERCE network delegates, to discuss the achievements of ERCE so far and its future development. Here we present and discuss critically an overview of data collected in ERCE to date.

MATERIALS AND METHODS. the ERCE database was searched (March 29th, 2018) and data on the number of registered patients, patients enrolled in the previous 18 months, records of follow-up visits and records of CE cyst details were analysed for each adhering centre.

RESULTS AND CONCLUSIONS. Thirty-eight centres in 13 countries (of which five extra-European) were adhering to ERCE. Of these, 29 (76%) registered patients and in turn, of these, 17 (59%) recorded at least one visit occurring within the past 18 months. A total of 1,799 patients were registered, ranging from 1 to 399 per centre. Fourteen centres (48.2% of centres ever having recorded patients in ERCE) recorded also follow-up visits. In these centres, a median of 24.7% patients also had follow-up visits recorded in ERCE. The cysts characteristics (location and stage) were recorded at least for some patients by 20 centres. In these centres, cysts

characteristics were recorded for 62% (range 12%-100%) of registered patients.

ERCE is used to harmonize data collection and reporting of CE, including data on the fundamental clinical features. The constant feedback from end-users, the monitoring of quality of data recorded and frequency of usage are key to the improvement of ERCE. The research was funded from the European Community's FP7 under the grant agreement 602051 (Project HERACLES; http://www.Heracles-fp7.eu/).

The prevalence of abdominal cystic echinococcosis in rural Bulgaria, Romania and Turkey: results from cross-sectional ultrasound population-based surveys (HERACLES project)

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Keywords: Cross-sectional study, cystic echinococcosis, ultrasound surveys, international project

INTRODUCTION. Cystic echinococcosis (CE) global prevalence is estimated at 2–3 million human cases. However, clinically diagnosed cases represent only a small proportion of the total number of real infected people. For these reasons, extended ultrasound (US) surveys on human populations are needed to quantify asymptomatic carriers and allow a more precise estimate of CE burden. Such efforts are crucial to assess, compare and prioritize interventions in limited resource settings. A cross-sectional study on abdominal prevalence of CE was undertaken in Eastern Europe under the framework of HERACLES project.

MATERIALS AND METHODS. 16 extended abdominal US population surveys were conducted in 50 villages in association with resident partners and public health centres: Hospital of Infectious and Parasitic Diseases ‘Prof. J. Kirov’ (Sofia, Bulgaria), Colentina Clinical Hospital (Bucharest, Romania), Hacettepe University Hospital (Ankara, Turkey). Ethical approvals and informed consents were obtained accordingly. Each suspected case was examined independently by 2 clinicians and patients were assigned to treatment according to WHO-IWGE (Informal Working Group on Echinococcosis) Expert Consensus.

RESULTS AND CONCLUSIONS. 24,693 people were screened during 2014 and 2015. The age and sex adjusted prevalence of abdominal CE was 0,41% (95% CI 0,29-0,58) in Bulgaria, 0,41% (95% CI 0,26-0,65) in Romania, and 0,59% (95% CI 0,19-1,85) in Turkey. Active cysts were found across all ages, including children, and in all investigated provinces. Based on the adjusted prevalence and the reference rural population size in 2015, we estimated that 7,872 (95% CI 5,520-11,220) individuals may be presently infected with abdominal CE in rural Bulgaria, 37,229 (95% CI 23,405-59,166) in rural Romania, and 106,237 (95% CI 33,829-330,751) in rural Turkey. Of these, 42,9% in Bulgaria, 40,3% in Romania, and 32,8% in Turkey may harbour cysts in active stage.

Collection of accurate epidemiological and clinical data will give a reliable picture of the burden of this disease, providing a statistically supported case series for future evaluation of efficacy and effectiveness of interventions. This is the largest US survey (research-based cross-sectional study) on CE from a single community-based study. This research received funding from the European Community’s FP7 under the grant agreement 602051 (Project HERACLES; http://www.heracles-fp7.eu/).

Plasmodium falciparum malaria and *Human herpes virus* 8 (HHV8): co-infection in Ugandan children

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Keywords: *P.falciparum* malaria, HHV-8, coinfection, children

INTRODUCTION. *Plasmodium falciparum* malaria is a priority in public health and represents one of the most important parasitic diseases in Uganda, where also *Human herpes virus* 8 (HHV8), etiological agent of Kaposi's Sarcoma (KS), is most prevalent (Romano et al., 2013, Prev. Res., 3:1-5). The malaria infection could have an impact on HHV-8 reactivation and this may influence the transmission of Kaposi Sarcoma Associated Herpes Virus (KSHV) in endemic areas. As known HHV8 establishes a long life persistent infection as result of a delicate equilibrium between viral replication and the host immune responses, that may be influenced by other pathogens such as *Plasmodium falciparum* (Romano et al., 2011, Prev. Res., 1:44-52; Romano et al., 2010, Parassitologia, 52:405-410).

MATERIALS AND METHODS. Children were enrolled during cross-sectional surveys performed in two different zones of Uganda: Kampala suburbs (Central-Southern Uganda) and in rural sites of Karamoja region (North-Eastern Uganda). Fingerpick blood samples and saliva 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. From each sample, the presence of *P. falciparum* DNA was investigated by nested PCR and the presence of HHV8 DNA was detected by Real Time PCR. Statistical analysis was performed with the application of descriptive methods and 95% confidence interval.

RESULTS AND CONCLUSIONS. We analyzed a sample of 259 children with mean age of 7.1 (1<13) years. *P. falciparum* DNA was detected in 36.7% (95% C. I. 31.0 – 42.7) of samples, while HHV8-DNA in 5.8 % (95% C.I. 9.8 – 24.4). The co-infection was detected in 8.3%. Our results lead us to speculate that the *Plasmodium falciparum* malaria, by affecting the host immune system, could represent a possible risk factor for infection or reactivation of latent HHV8. Further studies are needed investigating other Africa sub-Saharan countries where these diseases are endemic.

Cryptosporidiosis among HIV patients from Thailand: zoonotic species and high genetic variability in *Cryptosporidium hominis* and *C. meleagridis*

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Keywords: *Cryptosporidium*, HIV, Thailand, molecular typing

INTRODUCTION. Opportunistic infections still represent a serious threat for HIV-infected individuals, and *Cryptosporidium* is recognized as a leading cause of prolonged, severe diarrheal disease, accounting for up to a third of diarrhea cases in HIV patients (Marcos and Gotuzzo, 2013, Curr. Opin. Infect. Dis. 26:295-301). In this study, we investigated *Cryptosporidium* species and genotypes in archived stool samples collected during 1999-2005 from HIV-infected individuals at the Siriraj Hospital in Bangkok.

MATERIALS AND METHODS. We examined 175 patients, 97 males and 78 females (age range 15-62 years, median 34.7). For species identification, we performed PCR and sequencing of the small subunit rRNA gene DNA (Ryan et al., 2003, Appl. Environ. Microbiol. 69:4302-4307). For subtyping, we performed PCR and sequencing of the glycoprotein 60 (gp60) gene (Stensvold et al., 2014, J. Clin. Microbiol. 52:2311-2319).

RESULTS AND CONCLUSIONS. Sequencing of the SSU rRNA PCR products identified *C. hominis* as the most prevalent species (n=50), followed by *C. meleagridis* (n=23), *C. canis* (n=15), *C. felis* (n=9), *C. suis* (n=6) and *C. parvum* (n=5). Typing of *C. hominis* isolates at the gp60 locus revealed the presence of allelic families Ia, Ib, Id, Ie and If. Subtype IeA11G3T3 was the most prevalent, but allelic family Ia was the most diverse, with four subtypes. Typing of *C. meleagridis* isolates revealed the presence of allelic families IIIb, IIIe and IIIg. A single subtype, IIoA16G1, was found in *C. parvum*. The sequence of the gp60 gene for *C. canis* is reported.

This study highlights the complex epidemiology of *Cryptosporidium* infection in the highly vulnerable population of HIV-infected individuals, and confirms previous studies in Thailand showing the involvement of many parasite species. Anthroponotic transmission appears the most important route, but almost half of the cases are due to potentially zoonotic species.

Extensive genetic variability at the gp60 locus was found among *C. hominis* and *C. meleagridis* isolates, and many subtypes appear to circulate predominantly in South East Asia, pointing to specific transmission patterns in this endemic area.

Identification and spatial distribution based on 18S and gdh genetic variability of *Giardia* spp. from human and animals in Italy

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Keywords: *Giardia duodenalis*, *Giardia microti*, assemblages, philogeny

INTRODUCTION. The protozoan *Giardia* spp. is one of the most common parasite infecting humans and a large number of animal species. In captive and owner animals, including pets, *Giardia duodenalis* may represent a serious health concern for their caretakers but also for domestic animals and native wildlife populations. In Italy, no identification of other known *Giardia* species is reported, so far. Aim of the present study was to identify *Giardia* isolates from different hosts and to investigate spatial distribution based on *gdh* genetic variability among sequences of Assemblages A and B in Italy.

MATERIALS AND METHODS. Fecal samples were collected from humans and a large number of animal species. Feces resulted positive to *Giardia* cysts by microscopic investigation and by immunofluorescence were subjected to PCR and sequenced by targeting SSU rDNA and glutamate dehydrogenase (gdh) fragments gene. Phylogenetic analysis was performed by comparison of the obtained sequence with those retrieved from NCBI GenBank by MEGA7.

RESULTS AND CONCLUSIONS. *Giardia duodenalis* assemblages A, B and D were identified at the 18S locus in humans and in domestic, wild and captive animals. Moreover, sequence analyses of *Giardia* from two specimens of günther's vole (*Microtus guentheri*) unambiguously identified the isolate as belonging to *Giardia microti* species, showing 99% of identity with those available in GenBank. A well-defined cluster supported by significant bootstrap values and corresponding to *G. microti* isolates including sequence obtained from *M. guentheri* was evidenced in the NJ tree confirming species assignment. To the best of our knowledge, this data represents the first report of *Giardia microti* in Italy.

The analysis of assemblages A and B at gdh locus confirm the identification obtained by 18S for all isolates. A different A and B intra-assemblage variability among sequences generated here and all others reported in the literature from Italy was observed. The obtain data evidenced *Giardia duodenalis* transmission pathways at local scale based mainly on host specificity rather than on sympatric conditions.

Human migration and parasites transmission: is there really a risk?

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Keywords: Asylum seekers, Intestinal parasites, Migration, Screening

INTRODUCTION. Over the last decade, Europe has experienced an increasing influx of migrants undertaking the dangerous travel across the Mediterranean seeking humanitarian protection and/or improved living conditions. It represented a challenge for Italian authorities in terms of reception capacity and providing adequate medical assistance (Trovato et al., 2016. Confl. Health, 10:14). The health care of newly arrived is mostly confined to maternity and mental health issues (Bischoff et al., 2009. Eur. J. Public. Health. 19(1):59-64.). Neglected parasitic diseases (NPDs), lacking a local transmission potential, and often clinically silent, are not routinely screened. Also in case of symptoms, physicians may not be aware with the aetiological agent, possibly determining diagnostic pitfalls (Patamia et al., 2017. Neurol. Sci, 38:1105–1107). As a consequence, data on prevalence and burden of parasitic diseases among newly arriving migrants in Italy are scant. This study is aimed to evaluate the prevalence of parasites in migrants in order to improve the knowledge about the spreading of NPDs and to assess the reliable risk for their transmission in reception countries

MATERIALS AND METHODS. Newcomers asylum seekers hosted in the ASC of Castelnuovo di Porto (Rome, Italy) from March to October 2017 were screened for intestinal and urinary partasites. Socio-demographic data, medical history, risk factors for parasites were collected from each subject to perform a multivariate analysis.

RESULTS. A total of 364 migrants were enrolled (according to ethical rules) from Western, Eastern, Center and North Africa (42, 38.5, 2, 7.7%) and from West and Southern Asia (3, 7%). A surprisingly low prevalence for intestinal parasites (20.6%), which were mostly low-pathogenic protozoa, was found. No correlation between socio-economic or lifestyle factors or the travel route and the identified parasites was evidenced. However, a significant negative correlation between the time spent in travelling and the prevalence by intestinal protozoa in parasitized groups was observed.

CONCLUSIONS. This survey shows a low risk of introduction of parasitic diseases from migrants, highlighting the importance of carrying out parasitological surveys to know the parasite species in migrant populations in order to evaluate the possible risk for transmission in hosting countries**.**

Malaria entomological inoculation rate in a village of Burkina Faso reveals high transmission risk both indoors and outdoors despite the high LLIN coverage

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Keywords: *Anopheles gambiae complex*, *human landing catch, Plasmodium, bednet*

INTRODUCTION. Long lasting insecticide treated bednets (LLINs) are considered among the most effective strategies in malaria vector control, leading to the prevention of 68% of the malaria cases in Africa in 15 years (Bhatt et al., 2015, Nature 526:207-211). Despite this success, the effectiveness of LLINs in sub-Saharan Africa seems to be heterogeneous, since in some hyperendemic countries the annual incidence and the entomological infection rates are still very high (Killeen et al., 2014, Mal. J. 13:330). In Burkina Faso - despite raising of LLIN coverage from 20% to 70% led to a significant reduction of malaria prevalence - the annual malaria incidence has not been significantly affected. We here assessed the risk of malaria transmission in a LLIN-protected village of Burkina Faso where in previous surveys it has been detected a high sporozoite rate (*A. coluzzii,* 7.6% in 2011, 9.3% in 2012) despite a low rate of human blood meals (*A. coluzzii,* 20.1%).

MATERIALS AND METHODS. Host-seeking mosquitoes were collected in November 2015 by Human Landing Catch, both indoors and outdoors, in the village of Goden (Ouagadougou area). Collected mosquitoes were morphologically identified and *Anopheles gambiae* s.l. specimens were subsequently identified per species by PCR (Santolamazza et al., 2008, Mal. J. 7:163). Head+thorax of females were analysed for *Plasmodium* sporozoite presence by nested-PCR (Calzetta et al., 2018, Med Vet Entomol, in press).

RESULTS AND CONCLUSIONS. Results provided an overall estimate of 83.3 host-biting mosquitoes per person per night. Sporozoite rate among the 695 out of 1,955 *Anopheles gambiae* complex specimens analysed so far (*A. coluzzii* 55%, *A. arabiensis* 44%, *A. gambiae* 1%) was 6.9% for *A. coluzzii* and 5.1% for *A. arabiensi*), with no significant differences between indoors and outdoors collected specimens (6.6% and 5.7%, respectively; Chi-square= 0.27 P= 0.6). Based on these results the entomological inoculation rate (EIR) in the village is estimated to be 5.1 infective bites/person/day both indoors and outdoors. These results highlights that - despite the individual protection given by LLINs to the most inhabitants since several years - the mosquito population in the area is still abundant and highly infected. This leads to a high risk of malaria infection for the fraction of the human population still unprotected by bednets indoors and that exposed to mosquito bites outdoors.

SESSIONE 7

PARASSITI DELLA FAUNA SELAVATICA A VITA LIBERA

Trichinella britovi and wildlife: epidemiological situation in north-east Italy

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Keywords: zoonosis, Trichinella, wildlife

INTRODUCTION. The Regulation (EC) N. 1375/15 defines specific rules about official controls for *Trichinella* spp. in meat. The regular inspection of domestic pigs, equines and wild boars minimize the risk of human infection from the consumption of infected meat. The EFSA scientific report suggests the monitoring of epidemiological indicators as an aid in the surveillance of *Trichinella* spp. The main wildlife species covered are wild boars and bears, intended for human consumption. Other susceptible mammal species can be included depending on the relevant wildlife population of a country (EFSA J. 2011,9(10):2371). For this reason, the monitoring of wild species (mainly wild boars, red foxes and mustelids) has been implemented since 2006 in north-eastern Italy. We report the results of the wildlife monitoring for *Trichinella* spp. during 2011-2017, compared with that of the 2006-2010 period in the same regions.

MATERIALS AND METHODS. From 2011 to 2017 a total of 34,677 wild animals were tested including 24,076 wild boars, 7,418 red foxes, 3,130 mustelids (1,281 martens, 1,252 european badgers, 592 beech martens, 3 stoats, 1 weasel, 1 polecat). Muscles of wild animals were tested for *Trichinella* spp. larvae using a pepsin digestion method, according to the EC Regulation 1375/15. The species were molecularly identified by the European Reference Laboratory for Parasites (EURLP, ISS, Rome).

RESULTS AND CONCLUSIONS. *Trichinella britovi* was found in seven red foxes (0.094%). The prevalence in foxes did not change significantly ($p > 0.05$) compared to the previous period (0.05%), confirming the low endemicity of *T. britovi* in the area and the red fox as a good target for surveillance. The prevalence of *Trichinella* in wildlife in the last 30 years has dramatically decreased, considering that in 1958-60 in the same area, 31.3% of red foxes were found positive (Marazza V, 1960; Archivio Vet. It., 11(6), 507-566). This is likely due to the shift of the feeding behaviour of foxes from hunting and scavenging to food sources provided by humans settling. In those areas were human activity linked to the forest were abandoned, the increase of wild ungulate populations and their natural mortality may have contributed to a *Trichinella*-free cycle. Changing ecosystems are expected to affect wildlife populations and the pathogens they carry.

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The long-term prevalence trend of sylvatic trichinellosis in NW Italy

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Keywords: red fox, trichinellosis, prevalence, Italy

INTRODUCTION. While there is large consensus that prevalence of trichinellosis in the red fox (*Vulpes vulpes*) has obviously decreased in the North of the country since publication of a milestone survey at the end of the Fifties (Marazza, 1960, Arch.Vet.It., 11: 507-556), little information is available on the "profile" of the negative trend ever since. Understanding this unique prevalence downturn may likely contribute to the ongoing debate on epidemiology and control of this major zoonosis in Europe.

MATERIAL AND METHODS. Published and unpublished data collected since the late Eighties were retrieved from archives of the Authors' institutions (see affiliations). To prevent habitat-related biases, the origin of examined foxes was restricted to the mountain areas of Cuneo, Torino and Aosta provinces. *Trichinella* larvae were detected by means of digestion methods (either "in house" or automated). Standard descriptive statistics were used.

RESULTS AND DISCUSSION. Data are reported in the Table below. The prevalence of *Trichinella* spp. infection in foxes significantly differed between Groups (P<.00001) while no area-related difference was found in any investigated period. Apparently, the downturn in prevalence was particularly steep during the last decade of the past century. Speed of the process suggests that a major causative role was played by human attitudes towards wildlife (eg, unproper disposal of fox carcasses) which evolved during the explored time interval.

Group	Period	Province	Trichinella		Prevalence (%)	
			N.	Pos	Province	Group
1	Late 50s	CN	36	17	47.2	43.2
		TO	49	19	38.8	
		AO	26	12	46.1	
2	Late 80s Early 90s	CN	35	9	25.7	20.0
		TO	49	13	27.6	
		AO	83	11	13.2	
3	2001-2010	CN	258	16	6.2	4.1
		TO	307	7	2.3	
		AO	594	24	4.0	
4	2011-2017	CN	149	2	1.3	1.1
		TO	110	1	0.9	
		AO	384	4	1.0	

Disease threats and invasive species: helminths infecting raccoons introduced to Italy

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Keywords: zoonoses, invasive species, *Baylisascaris procyonis*, *Procyon lotor*

INTRODUCTION. Introduction of invasive species is a growing phenomenon that may cause severe alterations to parasite distribution and epidemiology (Daszak et al., 2000, Science, 287:443-449). Invading hosts may introduce to the new range alien parasites, but may also acquire local infections, potentially altering their circulation. N. American raccoons (*Procyon lotor*) have been introduced in several European countries (Beltrán-Beck et al., 2012, Eur J Wildlife Res, 58:5-15). In their native range, they act as a reservoir for rabies and harbour the roundworm *Baylisascaris procyonis*, which may cause visceral, ocular or neural larva migrans syndrome in humans (Gavin et al. 2005, Clin Microbiol Rev, 18:703-718). We investigated the helminth community of raccoons established along the Adda river (N. Italy), to determine whether they introduced to Italy zoonotic *B. procyonis,* and to detect if they harbour any other parasitic species that may represent a threat to humans, domestic animals or native wildlife.
MATERIALS AND METHODS. In 2017, 44 raccoons (16 males and 28 females) culled within an invasive species control program were surveyed for gastro-intestinal helminth infections through the examination of the whole gastro-intestinal content. All the recovered specimen were fixed in ethanol 70% and identified by a combination of morphology and molecular methods.
RESULTS AND CONCLUSIONS. 31 out of 44 raccoons (70.5%) were infected by gastro-intestinal helminths. We identified a total of 11 different parasitic taxa: the most frequently encountered helminths were the nematodes *Strongyloides procyonis* (prevalence: 25%; mean intensity: 17.4 parasites/infected host), *Capillaria putorii* (23%; 7.9) and *Porrocaecum* spp. (14%; 24.8). Overall, raccoons show an impoverished helminth community compared to their native range. A similar pattern has been observed on a several alien mammals and is considered one of the drivers underlying invasiveness (Torchin et al., 2003, Nature, 421:628-630). Our survey represents the first report of N. American *S. procyonis* in Italy, and the potential impact of this introduced parasite on native species is still unknown. Moreover, the detection of *C. putorii* and *Porrocaecum* spp., which commonly infect European mustelids and birds of prey, respectively, suggests that interspecific transmission between the invaders and native wildlife is already occurring. We found no evidence of zoonotic *B. procyonis*, but since an accurate interpretation of this negative result was crucial for public health reasons, we estimated the probability that the infection was actually absent by calculating confidence of freedom. Based on our sample size, expected prevalence and herd-level specificity and sensitivity, confidence of freedom from *B. procyonis* resulted above 99%.

Severe case of spirorchidiasis in a loggerhead sea turtle (*Caretta caretta*) from Adriatic Sea

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Keywords: loggerhead turtle, Spirorchiidae, Adriatic Sea

INTRODUCTION. Spirorchiid infections are considered the most important parasitic disease cause of stranding and mortality of sea turtles worldwide. According to Marchiori et al. (2017, Parasites&Vectors10:467), two species of spirorchiids are present in Adriatic Sea, nevertheless they seem to have not a causal effect on the death nor a strong impact on the general health status of sea turtle population in this area. This work reports a case of a severe spirorchiid infection in a specimen of loggerhead stranded along the Abruzzo coasts.

MATERIALS AND METHODS. In February 2018, an adult male of loggerhead (curved carapace length: 75 cm; weight: 44.7kg) accidentally caught was promptly hospitalized at Center Recovery treatment and rehabilitation Marine turtle (CRTM) "L. Cagnolaro". The turtle showed lethargy, listlessness, neurological compromising and penile prolapse. Twenty-four hours later, the turtle died and it was necropsied at the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZSAM). Histological and parasitological exams were carried out. Spirorchiid elements were morphologically and molecularly identified, by use of a PCR targeting the 28S gene and ITS2 spacer of rDNA.

RESULTS AND CONCLUSIONS. The turtle was thin with shells shrunken and with the central plastron area markedly depressed. The coloration of the coelomic organs was pale in respect of their normal coloration. Epididyism and deferents were bilaterally abnormal observed . Eggs of spirorchiids of both Type1 and Type 3 were observed by stereomicroscope in various organs. Histological examination showed disseminated eggs in pancreas, spleen, kidney, lung, brain, intestine, adrenal gland and thymus. In the genital tract lesions of deferens and epipdidyms were also associated to sprirochiid eggs also associated to spirorchiid eggs. Severe and diffuse multifocal granulomatous reactions surrounding numerous fluke eggs were observed. The eggs of type 1 and 3 were molecularly identified as *Hapalotrema mistroides* and *Neospirorchis* sp. Neogen 11 respectively, confirming a co-infestation. This is the first case of severe spirorchiidiasis described in free-ranging loggerhead turtles in the Adriatic Sea, similar to that recently described from Tyrrhenian Sea (Santoro et al. 2017, Dis Aquat Org 124:101-8). Much is still left to know on the epidemiology of this parasitic disease in the Mediterranean basin, including genetics of hosts, identification of intermediate hosts, risk factors. Significant organ injury was associated to multisystemic embolization of eggs in this case. Impairment of circulatory system due to disseminated granulomatous lesions could have been contributory to death in this by-caught animal.

Parasitological community of red deer (*Cervus elaphus*): effects on population and reproduction

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Keywords: Kidney fat index (KFI), gastro-intestinal helmints, pulmonar parasites, reproductive disorders

INTRODUCTION. Parasites are important indicators of biodiversity and biological parameter in wildlife as they can provide information about population health status, and about the potential interactions with other animal species and humans. Here we analyse parasite community in red deer to evauate (i) its effects on the species' health status and, by focusing on adult females, (ii) its potential impacts on reproduction. Indeed, to our knowledge no studies are available on helminths-dependent abortion or reproductive disorders in wild ungulates.

MATERIALS AND METHODS. A culling management plan within three macro-areas with different level of anthropization was conducted in Stelvio National Park in 2015, and faecal samples and abomasa were collected from 92 deer (47 adults including 32 females; 17 yearling and 28 calves). Parasitological investigations were performed in order to evaluate quantitatively parasite emission stages. Moreover, abomasal inspection and helminth morphological identification were carried out. Generalized Linear Models fitting kidney fat index (KFI), pregnancy and fetus weight as response variables and presence of pulmonar and abomasal helminths and emission of coccidia and helmintic eggs as predictors.

RESULTS AND CONCLUSIONS. An overall prevalence of 59.8% and of 40.2% emerged for *Eimeria* spp. and helminthic eggs, respectively. Lung larvae had a prevalence of 80.4%. Abomasal inspection highlighted a prevalence of 29.3% showing *Spiculopteragia spiculoptera*, *Tricostrongylus axei* and *Ostertagia leptospicularis* (dominant species) and *Rinadia mathevossiani* (co-dominant species). Statistical analyses highlighted a significant negative effect of lung larvae on KFI. None of the variables influenced the pregnancy probability, but fetus weight was significantly affected by abomasal abundance. Weight of fetus had average values of 438 g in negative females, 314 g with abomasal charge up to 40 helminths and 98 g when helminths was >40. Pulmonar parasites showed an impact on red deer health although larvae species require species indentification to discriminate their patogenicity. Conversely the recorded gastro-intestinal helmints did not affect animals. However, although parasites had no impact on pregnancy, abomasal intensity showed an indirect negative effect on fetus development. This result would suggest that helminths may influence the reproductive potential of red deer.

Toxoplasma gondii in naturally infected red deer (*Cervus elaphus*): spread, infection dynamics and effects on host behaviour

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Keywords: Toxoplasmosis, serology, manipulation hypothesis, epidemiology

INTRODUCTION. *Toxoplasma gondii*, beyond its zoonotic, economic, and conservation values, has been associated with behavioural changes in several hosts, including humans. Recent studies constitute indeed a convincing body of evidence that parasite's activity can manipulate intermediate host behaviour through neurological symptoms/alterations. In humans, loss of psychomotor performance and concentration or mental disorders were related to Toxoplasmosis, while in animals *T. gondii* may promote "risky behaviours" that may favour the parasite transmission from intermediate to other/s or to definitive hosts. As the occurrence of *T. gondii* behavioural manipulation has not be investigated in natural conditions, we focused our attention on wild red deer (*Cervus elaphus*). Therefore, we investigated (1) the spread and dynamics of *T. gondii* and (2) if the parasite may induce more risky behaviours, assuming as parameter an increase in the probability for the infected host to be culled.

MATERIALS AND METHODS. For epidemiological analysis 464 sera were collected during 2014-2017 culling plans from three areas with different level of anthropization (low, moderate, high) in Stelvio National park, while the behavioural hypothesis was tested on 81 adult females. Samples were analysed by a commercial ELISA kit. The epidemiology of the infection was analysed through a Binomial and a Gaussian Generalized Linear Models (GLM). While the behavioural hypothesis was tested by a Gaussian GLM modelling the "days from the beginning of culling" to evaluate the potential increase in the culling probability induced by *T. gondii*.

RESULTS AND CONCLUSIONS. An overall prevalence of 26.9% emerged. Adults were significantly more infected than yearlings and calves. Subjects of low anthropised area were significantly less infected than those of other anthropised ones. Deer of 2014 were significantly less infected than those of other years and seropositive subjects of this study year showed the lowest serological titres. The effect of age class and anthropization on the spread of *T. gondii* supports horizontal transmission as the main route. Seropositive adult females (49/81) had a significant higher culling probability, being culled sooner, than the seronegative ones. *T. gondii* appears to induce behavioural alterations in red deer making them more "at risk" to be culled. The supposed emerged mechanism leads to a *T. gondii* manipulation in this species that could increase its spread and transmission even to humans. Further analyses should be carried out to extend this analysis to other age classes and to preyed/found dead/roadkill animals for a wide assessment of the potential alterations induced by *T. gondii*.

Ticks on migratory birds: results from seven bird stations in Sweden

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Keywords: ticks, migratory birds, Sweden

INTRODUCTION. Transport of ixodid ticks by migratory birds is a well documented phenomenon (Hasle G.,2013, Front Cell Infect Microbiol. 3:48.). Several studies on bird-transported ticks and related tick-borne pathogens have been performed in Sweden (Olsen B. et al, 1995, Appl. Env. Microbiol. 61:3082-3087; Labbé Sandelin L. et al., 2015, PLoS ONE 10(7): e0133250). Since climate change or other factors might have been altering the circulation patterns of ticks and TBPs transported by migratory birds, a new survey was needed.

MATERIALS AND METHODS. During the season 2016, ticks were collected from migratory birds (n=557) during stop over at seven birds observatories across Sweden. Date and time of ringing, bird species, as well site of tick attachment were recorded from each tick-infested bird. Ticks were identified at stage/species level using morphological keys (Filippova N.A., 1977, Ixodid ticks (Ixodinae). Fauna USSR New Ser. 4, 1–316 (in Russian); Heylen D. et al., 2014, Ticks Tick Borne Dis. 2014 5, 693-700). Molecular analyses for the presence of TBPs are ongoing.

RESULTS AND CONCLUSIONS. A total of 1410 ticks (47%, n=668 larvae, 53% nymphs, n=741, one male) were collected. The average amount of tick/bird was 2.53 (range 1-35). Ticks were found on beak (70%), eye (28%) and ears (1.8%). *Ixodes ricinus* (93%) was the dominant species, followed by *I. arboricola* (0.93%), *I. frontalis* (0.71%) and *Haemaphysalis punctata* (0.64%). The remaining specimens could be only identified as *Ixodes* spp. One nymph of *Hyalomma* spp. was found on a marsh warbler (*Acrocephalus palustris*) from Landsort. The most represented bird species was European robin (*Erithacus rubecula*, 32%). Present results confirm that the dominant tick species is *I. ricinus*. Nevertheless, to the authors' knowledge *I. frontalis* and *H. punctata* had not been observed at Falsterbo before. As a preliminary result of analyses for TBPs, no Tick-borne Encephalitis Virus (TBEV) was found within collected ticks. Molecular species identification will be carried out for the nymph of *Hyalomma*.

Microscopy and molecular investigation on *Lipoptena* (Diptera: Hippoboscidae) circulating in wild animal species in Italy

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Kewords: *Lipoptena*, wild ungulates, identification, molecular analysis

INTRODUCTION. *Lipoptena* Nitzsch, 1818, is a blood-sucking ectoparasite of domestic and wild animals as well as, accidentally, of humans. Although unclear, they cause distress and alopecia and are suspected vectors of *Bartonella, Rickettsia* spp., *Anaplasma ovis* (Hornok et al., 2011, Vector Borne Zoon. Dis, 10:1319-1321). In Europe, four species of *Lipoptena* have been identified, i.e. *Lipoptena cervi, Lipoptena capreoli, Lipoptena couturieri* and *Lipoptena fortisetosa.* The aim of the study is to microscopically and molecularly investigate *Lipoptena* species circulating in Northern Italian areas in wild ungulates host species.

MATERIALS AND METHODS. A total of 140 specimens from *Rupicapra rupicapra* (n. 23), *Capreolus capreolus* (n.74) *and Cervus elaphus* (n.43), living in seven different areas of the Sondrio province, were collected and microscopically identified**.** For molecular confirmation, specimens were individually subjected to DNA extraction and PCRs amplification by using generic primers for COI gene (Folmer et al., 1994, Mol. Mar. Biol. Biotech. 3:294-299). PCRs positive samples were then sequenced, aligned each other and phylogenetically analyzed.

RESULTS AND CONCLUSIONS. All specimens were dentified as *Lipoptena cervi,* males (40%) and females (60%). Out of 140 collected specimens, nine, so far molecularly examined, were positive to PCRs. The percentage of identity of the obtained sequences was 98.5% with *L. cervi,* 90.8% with *L. fortisetosa,* 90.1% with *Lipoptena* sp., 87.1% with *L. depressa* and 86.6% with *L. mazamae*. Phylogenetic analysis showed that sequences cluster with/around? *L. cervi* group with a homology of 100%. The results so far obtained highlight the presence of *L. cervi* in all investigated areas and in all investigated species, including chamois, considered by literature as an uncommon host species. Once completed, the molecular investigation may help in: *i)* overcoming potentially wrong identification; *ii)* identifying new unrecorded species in Italy, such as *L. fortisetosa* (recorded in Switzerland, Germany, Poland, Czech Republic and Slovakia), *L. capreoli* (spread in the Mediterranean countries, and apparently recorded (attested?) also in Italy), and the still unconfirmed *L. couturieri* identified in Spain and France; *iii)* understanding their distribution on the Alpine area close to the borders. This is the first epidemiological study in Italy, providing a noteworthy picture of the *Lipoptena* species distribution in Italy, also in the light of their possible zoonotic vector role.

Ixodid ticks (Acari: Ixodidae) of rhinoceroses in Kenya: new tick-host associations and updated ecological distribution

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Keywords: Rhinoceroses, ixodid ticks, ecology

INTRODUCTION. The East African savannah is an important habitat for the critically endangered rhinoceroses and a wide assemblage of ticks. Ticks and rhinoceroses have co-existed for many centuries with some ticks parasitizing rhinos specifically. Although the relationship is benign, tick-borne haemoparasites have led to disease and mortality in rhinos. In a study conducted 44 years ago, twenty-one (21) ixodid tick species were reported to infest black rhinos in Kenya. However, no further tick surveys were conducted after the introduction of the white rhinos in the 1980s and 2015. The present study, conducted between 2008 and 2018, is aimed at documenting the species diversity of ixodid ticks of rhinos in Kenya.

MATERIALS AND METHODS. Ticks were collected opportunistically from 472 rhinos that were immobilized for ear notching and other management activities in sixteen independent wild Rhino populations in Kenya. Collected ticks were stored in 98% ethanol for DNA preservation. Ticks were identified at the species level using standard identification keys and by comparison with voucher specimens. The results of the tick identifications from the various rhinos were summarized and the Diversity index (Shannon, H`) calculated and analyzed with SPSS to test if there were differences in the tick diversity between the rhino subspecies and between the parks.

RESULTS AND CONCLUSIONS. Twenty-six (26) tick species were collected from 472 rhinos. *R. pulchellus* (36.4%) was the most abundant species. Black rhinos were infested with 24 tick species while white rhinos were infested with 19 species but the difference was insignificant in terms of the biodiversity index. Interestingly, significant differences in the diversity of ticks were found between the parks. Oljogi had the highest diversity (H`=1.17) while the lowest was recorded for Laikipia Nature Conservancy (H`=0.28). In addition, new host records were ascertained for six ticks namely, *Amblyomma cohaerens*, *Rhipicephalus armatus, Rhipicephalus pravus, Rhipicephalus carnivoralis, Amblyomma lepidum* and *Hyalomma truncatum*, bringing the total number of ixodid tick species associated with black and white rhinos in Kenya to 29.

SESSIONE 8

PARASSITOSI TRASMESSE DA ARTROPODI

Emerging feline vector-borne infections in central Italy

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Keywords: *Leishmania infantum*, *Cytauxzoon* spp., *Rickettsia felis*, cat

INTRODUCTION. Cats are exposed to a number of arthropods as fleas, ticks and sand flies, and thus to the pathogens they may transmit. However, epidemiology of feline vector-borne diseases (FeVBDs) is low investigated if compared to dogs, resulting in scant data available (Otranto and Dantas-Torres, 2010, Parasit. Vectors, 3:2). The present study aimed to assess the prevalence of *Leishmania infantum, Rickettsia felis and Cytauxzoon* spp. infections in cat populations living in central Italy, by molecular and serological techniques**.**

MATERIALS AND METHODS. From 2010-2016, 286 cats were randomly selected from the catteries and colonies of central Italy regions. Cats were examined for signs suggestive of FeVBDs, and peripheral blood and conjunctival swab (CS) samples were collected. Sera were analysed by IFAT to detect anti-*Leishmania* and anti-*Rickettsia felis* IgG antibodies (Ab) using commercial antigens and cut-off dilution at 1/20 and 1/10 respectively. DNA extracted from buffy coat (BC) and CS samples was submitted to a SSU-rDNA nested (n)-PCR assay for *Leishmania* (Gramiccia et al., 2010, Vet. Parasitol, 181:23-30). BC was assayed in a n-PCR assay for *Rickettsia* spp., amplifying a fragment of the *gltA* (Roux *et al*., 1997, Int. J. Syst. Bacteriol, 47: 252–261), *ompB* (Choi et al., 2005, Emerg. Infect. Dis, 11:237–244) and *ompA* (Oteo et al., 2006, J. Clin. Microbiol, 44:2669–2671) genes, and in SSU-rDNA PCR for Piroplasmida species (Olmeda et al., 1997, Acta Trop., 67: 229–234) for *Cytauxzoon* spp..

RESULTS AND CONCLUSIONS. No cats showed clinical signs of FeVBDs. Sixty-two (21.67%) cats were positive for anti-*Leishmania* IgG by IFAT, with titres ranging from 1/20 to 1/160. Forty-five animals (15.73%) were positive to *Leishmania* CS n-PCR, whereas none of the animals scored molecularly positive in BC. Considering results obtained by IFAT and CS n-PCR a slight agreement between the 2 tests was detected. The serological assay for *R. felis* revealed 23 (8.04%) positives at low titer (1/10). No *Rickettsia* neither *Cytauxzoon* DNAs were amplified using the specific PCR assays. The results of the serological and molecular survey showed a substantial prevalence of *Leishmania* exposure in the investigated cats and pointed out the limited molecular diagnostic value of BC *versus* CS samples as previously observed (Fiorentino et al., 2008, Parassitologia, 50: 159). On the contrary no evidence supports the circulation of *Cytauxzoon* spp. across domestic cats in contrast with previous detection in wild cats (*Felis silvestris silvestris*) sampled in the same monitored areas (Veronesi et al., 2016, Ticks Tick Borne Dis. 7(5):853-858). The low positive Ab titres for *R. felis* in association with no DNA amplification do not allow speculations on the exposure of feline populations to this flea-borne pathogen due the cross-reactivity existing within the transitional group.

Prevalence of vector-borne pathogens in wild carnivores from Piedmont region

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Keywords**:** vector-borne, wildlife, carnivores

INTRODUCTION. In recent years, we assisted to a marked increase of wildlife population, especially in the Alps (Meriggi et al., 2011, Ethol Ecol Evol, 23:195-210), due to wildlife conservation strategies and land use changes. At the same time, also the number and the distribution of arthropod vectors have increased for climate and habitat changes, as for the rising of wildlife population (Ferroglio et al., 2005, Emerg Infect Dis, 11(10):1618-1620). Consequently vector-borne infections, most of which with a zoonotic impact, are expected to rise. The aim of this study was to evaluate the prevalence of *Babesia spp*., *Leishmania infantum*, *Ehrlichia spp*., *Anaplasma spp*. and *Hepatozoon spp*. in carnivore sylvatic species from Piedmont region.
MATERIALS AND METHODS. An overall number of 238 animals (155 foxes, 35 wolves and 48 badgers) were analysed in this study, and spleen samples were collected. DNA was extracted and used as template for PCR, with primer sequences and thermal conditions described elsewhere for each pathogen (Gubbels et al., 1999, J Clin Microbiol, 37(6):1782-1789; Schnittger et al., 2004, Parasitol Res, 92(3):189-196; Goodman et al., 1996, N Eng J Med, 334:209-215; Ferroglio et al., 2006, Trans R Soc Trop Med Hyg, 100(7):636-641; Inokuma et al., 2002, Vet Parasitol, 106:265-271). Positive samples were sequenced, and results were compared with sequences deposited in GenBankTM. Statistical analysis with Chi-square test was performed on results by using the R software (R Development Core Team, 2015).
RESULTS AND CONCLUSIONS. The prevalence of the vector-borne pathogens showed a great difference between the analysed species. A significative higher prevalence of *Babesia spp*. was recorded in foxes and badgers than in wolves ($p<0.01$), with a prevalence of 89.66% (CI 95% 83.66-93.63%), 89.58% (CI 95% 77.83-95.47%) and 40.00% (CI 95% 25.55-56.43%), respectively. In contrast, *Hepatozoon spp*. showed a higher prevalence ($p<0.01$) in wolves [74.29% (CI 95% 57.93-85.84%)] than in foxes [5.16% (CI 95% 2.64-9.85%)], while none of the badgers tested positive for this parasite. Anaplasmataceae genus was recorded in a lesser extent in foxes and wolves than in badgers ($p<0.01$), as for *L. infantum*, whose prevalence in badgers reached 54.17% (CI 95% 40.29-67.42%). Results of the sequencing analysis showed the presence, among others, of *Hepatozoon canis* and *Babesia canis*, causes of considerable diseases in dogs. Furthermore, sequencing showed the presence of some zoonotic *Babesia* strain, like *B. venatorum*. These results, together with the high prevalence of some of these pathogens, highlighted the importance of sylvatic species in the life cycle of vector-borne parasites and the potential risk of transmission between wildlife and human/domestic animals that should not be neglected.

Dirofilariosis: prevalence and clinical relevance in dogs and humans in Eastern Europe

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Keywords*: Dirofilaria* spp., humans, dogs, Eastern Europe

INTRODUCTION. Dirofilariosis is a worldwide-distributed infection caused by *Dirofilaria immitis* and *D. repens* which affects mainly dogs and cats. Moreover, its zoonotic potential represents a constant threatening for public health, producing pulmonary and subcutaneous/ocular dirofilariosis in humans (Simón et al., 2012, Clin Microbiol Rev, 25:507-44). During the last two decades a significant spread of this infection has been observed, from the endemic areas of southern Europe towards the eastern regions. The present study was aimed to provide data regarding the prevalence and clinical relevance of dirofilariosis in dogs and humans from Romania and Moldova.

MATERIALS AND METHODS. A total of 566 blood samples from dogs hosted in shelters from eight counties of eastern part of Romania were collected and tested with serological and molecular methods for the presence of *D. immitis* and *D. repens*. A total of 450 human serum samples from eastern and southern areas of Romania and central area of Moldova were analyzed for the detection of IgG antibodies against adult somatic antigens of *D. immitis* and *D. repens*. In addition, clinical signs of a patient with ocular dirofilariosis from southern Romania were described.

RESULTS AND CONCLUSIONS. The present study showed a prevalence of *D. immitis* in dogs between seven and 12% in the eight counties, with a high value (60.1%; 95% Confidence Interval, C.I.= 55.9-64.1) in southeastern Romania; , the prevalence of *D. repens* was between 8.8% and 11.1% with the higher value (15.7%; 95% C.I.= 9.0-23.2) in northeastern part of the country. Out of 187 individuals from Romania, 7% (95% C.I.= 3.9-11.9) was positive for anti-*D. immitis* IgG, while one patient reacted against both antigens of *D. immitis* and *D. repens*. Out of the 263 individuals from Moldova, 13.7% (95% C.I.= 9.9-18.6) was positive for anti-*D. immitis* IgG while 1.1% (95% C.I.= 0.3-3.6) positive for both antigens. Only one patient was found positive for anti-*D. repens* IgG. *D. repens* worm extracted from the patient was a non-fertile female, containing only oocytes and no developing stages. In conclusion, regular parasitological surveillance and monitoring are needed in order to define the risk areas of *Dirofilaria* infection in Romania and Moldova.

A serological study of exposure to tick-borne pathogens in donkeys from Asinara island

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Keywords: tick-borne pathogens, autochthonous donkeys, *Theileria equi*, *Babesia caballi*

INTRODUCTION. The study was aimed to investigate the prevalence and risk factors associated to selected tick-borne pathogens (TBPS) of the albino and colored donkeys from the Asinara island.

MATERIALS AND METHODS. From June to November 2015, a total of 110 blood samples were collected from 40 albino and 70 colored donkeys. Serum samples were analyzed with a commercial IFAT (MegaScreen®FLUO) which detect IgG antibodies against *Theileria equi* and *Babesia caballi*. Three land cover types were defined to estimate the risk: sparse vegetation, Mediterranean shrubland, grassland. The associations between the TBPs serological positivity, the individual variables, the geographical distribution of donkey herd, and land cover types were analyzed (SPSS 20.0, Chicago, IL).

RESULTS AND CONCLUSIONS. Exposure to *T. equi* and *B. caballi* was observed in 43/110 (39%) and 26/110 (24%) donkeys, respectively; 43/110 (39%) animals presented serological positivity for one TBP, while 13/110 (12%) were tested positive for both pathogens. Serological positivity for at least one TBPs (observed in 56/110 donkeys; prevalence: 51%) was affected by coat color and land cover. The prevalence of exposure to TBPs was higher in albino donkeys when compared with colored (70% vs. 40%; p-value=0.003). Considering land cover, a highly significant predictor of TBPs exposition (p-value<0.001), the greater percentage of tested positive donkeys was found in animals grazing on pastures (86%, 24/28); lower values was observed in donkeys grazing on soil covered by sparse vegetation (42%, 20/48) or Mediterranean shrubland (32%, 12/38). These results agree with a previous study (Garippa et al., 2016, XXIX SOIPA Congress) showing that both the coat color and the type of land cover were significant predictors of the tick infestation levels in Asinara donkeys. Further, the higher prevalence of TBPs observed in albino donkeys is most likely due to higher level of ticks infestations rather than to a higher susceptibility to TBPs of the albino itself.

Research founded by Asinara National Park “Monitoraggio dello stato sanitario e dello stress da cattura negli asini dell’Asinara: screening della popolazione”

Seroprevalence of selected equine vector-borne diseases in horses reared in Northern Italy

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Keywords: Equids, IFAT, TBP, Leishmania

INTRODUCTION. Equine vector-borne disease, due to the frequent exposition of horses to tick and insects' bites, are considered an emerging problem in Europe (Beugnet and Marie, 2009, Vet. Parasitol., 163:298-305). *Anaplasma phagocitophylum* (Rickettsiales) causes granulocytic anaplasmosis, *Babesia caballi* and *Theileria equi* (Piroplasmida) are the agents of piroplasmosis. Leishmaniasis, caused by *Leishmania infantum* (Trypanosomatida) is a zoonotic disease affecting mainly dogs and occasionally other animal species, including horses. The study aimed to evaluate the seroprevalence of selected TBPs (*A. phagocitophylum*, *B. caballi*, *T. equi*) and *L. infantum* in horses reared in Northern Italy.

MATERIALS AND METHODS. Blood samples from 265 horses apparently healthy reared in Lombardy and Piedmont regions (Northern Italy) were collected. Sera were analyzed with IFAT for the detection of antibodies against the four selected pathogens. An initial screening dilution of 1.80 and 1:40 was used for TBPs and *L. infantum*, respectively; then, seropositive samples were serially diluted to determine the end-point antibody titer. Pearson chi-square statistic was performed to evaluate the correlation between seropositivity and individual and managerial data. Three age classes were considered: yearling (0-2), adult (3-9) and aged (>10 years old) horses.

RESULTS AND CONCLUSIONS. One hundred and forty-nine horses reacted positive against at least one of the considered pathogens. *A. phagocitophylum* antibodies were detected in 63 horses (23.8%), with highest prevalence in adult ones (48.1%) (p=0.002). *B. caballi* and *T. equi* were detected in 49 (8.5%) and 50 (18.9%) horses, respectively, with aged animals more exposed to *T. equi* infection (77.1%) (p=0.003). Similarly, out of 35 horses (13.2%) positive to *L. infantum*, the prevalence resulted higher in aged horses than in young and adult animals (66.7%) (p=0.035). Considering the study area, *B. caballi* (42.9%) and *T. equi* (40%) infections were mainly detected in the Southern part (Milan and Lodi provinces); on the contrary, exposition to *A. phagocitophylum* (63.5%) and *L. infantum* (45.7%) resulted higher in horses reared in the Northern area (Como, Lecco and Bergamo). Co-infections of TBPs were also recorded: three horses were positive for all pathogens, 7 for *A. phagocitophylum* and *B. caballi*, 9 for *A. phagocitophylum* and *T. equi* and 15 for *B. caballi* and *T. equi*. The study confirmed that Italian horses are exposed to TBPs and also represent reservoirs for *L. infantum* infection.

A region-wide survey in Aosta Valley for ticks and tick-borne pathogens

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Keywords: Ticks, Tick-borne pathogens, dragging, Aosta Valley

INTRODUCTION. The rapidly changing epidemiology of vector-borne diseases is becoming a global public health/veterinary concern that needs active surveillance (Mwamuye et al., 2017. Ticks Tick-borne Dis, 8(2): 208–218). The sheep tick *Ixodes ricinus* is the most common tick species in Europe and the primary vector of a broad range of disease-causing bacteria and protozoa (Heyman et al., 2010. Expert Rev Anti-Infect Ther, 8: 33–50). Mountain areas are preferential sites to study tick ecology as climatic conditions are exasperated and subject to more extreme changes. Trough environmental dragging, this study aimed to survey distribution and seasonal abundance of ticks in Aosta Valley (Northwestern Italy) and to analyze the collected ticks for the presence of *Babesia* spp., *Theileria* spp., *Anaplasma* spp., *Ehrlichia* spp. and *Borrelia burgdorferi* s.l.

MATERIALS AND METHODS. Environmental dragging was performed monthly from May 2016 to April 2017 in 34 locations of the Aosta Regional territory. Biotic and abiotic variables were recorded at each sampling site. Ticks were morphologically identified and pooled together depending on species, life-cycle stage and area of detection Species-specific PCR protocols were used on pooled samples to assess Minimum Infection Rate (MIR) of target bacteria and protozoa.

RESULTS AND CONCLUSIONS. A total of 535 ticks were collected and identified as *I. ricinus* (n=533, 70 adults, 380 nymphs, 83 larvae) and *Ixodes hexagonus* (n=2 adults). Overall, 124 tick pools were formed and analyzed. *Babesia/Theileria* spp. was detected with a MIR of 25.81% (CI95% 18.91-34.15), Anaplasma/Ehrlichia spp. with a MIR of 12.90% (CI95% 8.10-19.94) and *B. burgdorferi* s.l. with a MIR of 16.94% (CI 11.35-24.51).

Our results indicate that *I. ricinus* is the most abundant species in Aosta Valley and that it is commonly found in wooded and shadowed areas. Our findings confirm the importance of *I. ricunus* as vector of pathogenic micro-organisms. Moreover, tick's coinfection with multiple pathogens was found to occur frequently. This poses a serious challenge to diagnosis and appropriate treatment.

Toward the development of serological markers of human exposure to *Aedes* mosquitoes: analysis of *Aedes albopictus* salivary antigens in a murine model

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INTRODUCTION. The rapid spread in the European continent of *Aedes albopictus* and its involvement in recent outbreaks of chikungunya and dengue in Italy, France and Croatia highlighted the need to improve surveillance and control of *Ae. albopictus* and other *Aedes* invasive species. We previously showed that the IgG response to the *Anopheles gambiae* salivary protein gSG6 is a reliable marker to evaluate human exposure to malaria vectors (Rizzo et al., 2011 PloS ONE 6:e17980). Exploiting *Aedes*-specific salivary proteins previously identified by comparative analyses (Ribeiro et al., 2010 Insect Biochem Mol Biol 40:767-84) we plan to develop similar serological tools to assess human exposure to *Aedes* mosquitoes.

MATERIALS AND METHODS. Three groups of 4 naïve BALB/c mice were exposed to bites of either *Ae. albopictus* or *Ae. aegypti* or *An. coluzzii*. Serum samples were collected before exposure, after the 2nd and the 4th/last exposure and then 1, 2, 3, 5 months later. The IgG response to (i) salivary gland extracts (SGE), (ii) the 34 kDa salivary protein from *Ae. albopictus* (alb34k) and *Ae. aegypti* (ae34k) and (iii) *Aedes*-specific salivary peptides was measured by ELISA. Moreover, the response was analyzed in a volunteer who regularly fed an *Ae. albopictus* colony for ~3 months (L13) and then after 2 years of non-exposure (L16).

RESULTS AND CONCLUSIONS. The immunization protocol was effective, especially in the case of *Ae. albopictus* and *Ae. aegypti*, as indicated by the IgG response to SGE. Two of the four *Ae. albopictus*-exposed mice had IgG antibodies against alb34k but no one responded to ae34k. All *Ae. aegypti*-exposed mice exhibited high levels of anti-ae34k IgG but no antibodies to alb34k, confirming that despite the high identity (63%) of the two orthologous proteins there was no significant cross-reactivity in mice. No response to alb34k or ae34k was observed in mice exposed to *An. coluzzii*. Similar results were found in the single human hyperimmune serum (L13) analyzed, with high levels of anti-alb34k IgG and a negligible response to ae34k. Moreover, a significant drop of the anti-alb34k IgG levels was observed after two years of non-exposure (L16) to *Ae. albopictus*. Further validation, making use of a larger and proper set of human sera from individuals naturally exposed to *Ae. albopictus*, is certainly needed. Nevertheless, the study reported here identified some promising candidates for the development of immunoassays suitable for the assessment of human exposure to *Aedes* vectors of public health importance as *Ae. albopictus* and *Ae. aegypti*.

The association between the killer yeast *Wickerhamomyces anomalus* and the sand fly *Phlebotomus perniciosus*: a potential tool for the control of leishmaniasis

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Keywords: *Leishmania*, biocontrol, sand flies, yeasts

INTRODUCTION. Leishmaniases are parasitic vector-borne diseases endemic in 98 tropical, subtropical and temperate countries. In particular, Italy is traditionally endemic for cutaneous and visceral forms of leishmaniasis caused by the protozoan *Leishmania infantum*, whose reservoir are dogs. The containment of leishmaniases should be based on a combination of chemotherapy, animal reservoir control and integrated vector control strategies. We focused our work on the study of the mycobiota of the sand fly *Phlebotomus perniciosus*, the main vector of human and canine leishmaniasis in the Mediterranean area, with the aim of identifying microorganisms useful for the biological control of *Leishmania*. Starting from previous study on the yeast community of *P. perniciosus* (Martin et al., 2016, Med. Vet. Entomol, 30(1):101-106; Martin et al., 2018, Environ. Microbiol, 20(3):1064-1077), we investigated the potential of the yeast *Wickerhamomyces anomalus* for the development of novel tools for the control of *L. infantum*.

MATERIALS AND METHODS. We isolated, screened and molecularly identified yeast strains of the species *W. anomalus* from *P. perniciosus* specimens. The isolated strains were phylogenetically characterized and tested against selected yeast strains. Finally, in order to investigate on the potential inhibitory/killing activity of the yeast against the pathogen *Leishmania*, we tested the *in vitro* activity of toxin-producer *W. anomalus* strains against *L. infantum.*

RESULTS AND CONCLUSIONS. Results showed that the *W. anomalus* strain isolated from *P. perniciosus* inhibits the growth of sensitive yeast strains, thus displaying a typical trait of the killer yeast phenotype. Furthermore, preliminary results of *in vitro* tests of this and other killer yeast strains against *L. infantum* indicate an inhibitory activity on promastigote replication, also with evidence for *Leishmania* cell degeneration. Killer toxin-producing yeasts are thus worth of further investigations, towards the development of novel arms for the biological control of leishmaniases.

SESSIONE 9

EPIDEMIOLOGIA DELLE MALATTIE PARASSITARIE 2

Risk factors associated to endoparasites in dogs and cats at Prince Edward Island (Canada)

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Keywords: Endoparasites, Epidemiology, Risk factors, Pets

INTRODUCTION. Although many studies on the frequency of endoparasites in dogs and cats in Canada have been reported (Joffe et al., 2011, Can. Vet. J. 52:1323-1328), seasonal and/or annual patterns are often not extimated, furthermore very few and aged papers have been written about owned dogs and cats in Canada.

MATERIALS AND METHODS. The frequency of endoparasitc infections from samples of cats (2,391) and dogs (15,016) submitted to the Veterinary Teaching Hospital (VHT) of the Atlantic Veterinary College, University of Prince Edward Island-Canada was determined, using univariate and multivariate analysis. Predictors of endoparasitism, such as sex, age, geographical origin and seasonality, were also investigated through the calculation of odds ratios (OR) with 95% confidence intervals.

RESULTS AND CONCLUSIONS. Overall thirteen parasite genuses were detected, cats showed a higher frequency, with this species difference being statistically significant (χ^2=15.494; P<0.001). The most frequent genuses recovered were *Giardia* spp. (5.23%), followed by *Isospora* spp. (3.31%) and *Toxocara* spp. (3.21%). Monoparasitism was the most common in both dogs and cats, at 87.5% and 86.7%, respectively. Frequency of *Giardia* spp. was significantly higher (χ^2=8.79; P=0.03) in the dogs during fall, as well as *Toxocara* spp. (χ^2=48.5; P<0.001) and *Isospora* spp. (χ^2=31.13; P<0.001). Cats more likely of being *Isospora* spp. positive in summer (χ^2=31.27; P<0.001). Increasing the age was a protective factor in terms of parasite presence (OR=0.232; 95%CI=0.174-0.311), as well as being sterilized male (OR=0.624; 95%CI:0.419-0.931) or female (OR=0.627; 95%CI:0.419-0.938), furthermore the trend across the years showed a decreasing (OR=0.961; 95%CI: 0.931-0.991).

The apparent low frequency of endoparasites should not be interpreted too rigidly, due to the fact that our population came from a Veterinary Teaching Hospital, so some selection biases should be taken into account. For example, owners who take their pets to the veterinary clinic are more likely to follow a deworming protocol than those who do not. This study shows how the diagnosis of routine fecal examinations can be investigated, providing an appreciation of risk factors most commonly associated with endoparasitism. Future research will help to evaluate if the owners' attitudes affects the probability of parasites in pets.

LARGE SCALE SURVEY ON THE OCCURRENCE OF CANINE AND FELINE EXTRA-INTESTINAL NEMATODES IN ITALY

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Keywords: lungworms, heartworms

INTRODUCTION. Canine and feline extra-intestinal nematodes are of growing concern because of their emergence in Europe, their pathogenic role and the zoonotic potential some of them have. Thus, a continuing epidemiological monitoring is of crucial importance. This study has investigated their presence in canine and feline populations of different regions of Italy.

MATERIALS AND METHODS. Faecal and blood samples were collected from 1000 dogs in different regions of Italy, *i.e.* Abruzzo (site A, n. 218), Marche (Site B, n. 116), Molise (Site C, n. 69), Lazio (Site D, n. 171), Campania (Site E, n. 83), San Pietro Island-Sardinia (Site F, n. 54), Veneto (Site G, n. 68), Friuli Venezia-Giulia (Site H, n. 66) and Puglia (site I, n. 155). Faecal samples were also taken from 1000 cats in sites A (n. 380), B (n. 103), C (n. 111), D (n. 172), F (n. 94), G (n. 45), H (n. 32) and Piemonte region (site J, n. 63). Faecal samples were examined with appropriate techniques.

RESULTS AND CONCLUSIONS. The overall infection rates were *Aelurostrongylus abstrusus* 10.4%, *Angiostrongylus vasorum* 3.4%, *Capillaria aerophila* 3.4% (cats) and 2% (dogs), *Troglostrongylus brevior* 3.2%, *Capillaria boehmi* 1.2%, *Dirofilaria immitis* 1.8%, *Dirofilaria repens* 1.7% and *Crenosoma vulpis* 0.1%.

DOGS – *Capillaria aerophila* was found in sites A (3.7%), D (3.5%), G (2.9 %) and H (6.1%), *C. boehmi* in sites A (1.4%), D (3.5%) H (1.5%), and I (1.3%), *A. vasorum* in sites A (3.7%), C (5.8%), D (3.5%) and E (19.3%), and *C. vulpis* (0.6%) in site I. Microfilariae of *D. immitis* were found in sites F (27.8%), G (2.9%) and H (1.5%), while *D. repens* in sites A (2.8%), F (18.5%) and I (0.6%). CATS –*Capillaria aerophila* was recorded in sites A (4.7%), B (0.9%), C (5.4%), D (1.7%), F (2.1%), G (2.2%), H (6.3%) and J (1.6%), *A. abstrusus* in sites A (10%), B (3.9%), C (3.6%), D (4.7%), F (38.3%), H (3.1%) and J (20.6%), and *T. brevior* in sites A (5.8%), C (1.8%) and D (4.7%). The present data confirms that extra-intestinal nematodes of pets are endemic in Italy. Hence, they should always be included in differential diagnosis of canine and feline diseases in the presence of compatible clinical signs.

Research needs to tackle zoonotic onchocercosis caused by *Onchocerca lupi*

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Keywords: *Onchocerca lupi*, epidemiology, treatment, diagnosis

INTRODUCTION. *Onchocerca lupi* (Spirurida, Onchocercidae) is a zoonotic nematode reported in dogs and cats from Europe, the Middle East and United States (Otranto et al., 2015, Emerg. Infect. Dis, 21:868-671). In humans, *O. lupi* displays a marked neurotropism with nematodes localizing in the cervical spine of infants, children and adults. Despite severe outcomes of the infection in humans and the high prevalence in dogs from endemic countries have been recognised, proper diagnosis and treatment to tackle this parasitic infection are lacking (Otranto et al., 2015, Parasit. Vectors, 8:89). In this study, we evaluated the efficacy of oxfendazole and describe a case of imported *O. lupi* infection in Italy.

MATERIALS AND METHODS. Eleven dogs which tested positive for skin-dwelling *O. lupi* microfilariae (mfs) were enrolled in the efficacy study and treated with oxfendazole (50 mg/kg, p.o., q.d.) for 5 (G2) or 10 (G3) days or left untreated (G1). All dogs were subjected to follow-up at 30 (D30), 90 (D90) and 180 (D180) days post-treatment, via ultrasound imaging to check size of ocular nodules and skin snips to evaluate mfs reduction. In 2013, a female dog was adopted from Portugal to Italy with *O. lupi*-containing nodules on the anterior sclera, which were surgically removed (Colella et al., 2018, Transbound. Emerg. Dis, *in press*). The dog recovered without complication and lived in Italy (without further travelling abroad) until 2017, when she died. Then, skin samples were collected for genomic DNA extraction and testing for amplification and sequencing of a partial cytochrome *c* oxidase subunit 1 gene fragment.

RESULTS AND CONCLUSIONS. Percentage of reduction of mfs was 78% for G2 and 12.5% for G3 at D180, whereas the mean microfilaricidal efficacy at D30, D90 and D180 was 41%, 81% and 90%, in G2 and 40%, 65% and 70%, in G3, respectively. Percentage of reduction of ocular lesions by ultrasound examination was 50% and 47.5% in G2 and G3 at D180, respectively. Despite the decrease in ocular lesions in all treated dogs, oxfendazole was ineffective in reducing ocular lesions and skin-dwelling *O. lupi* mfs in a 6-month follow-up period. Non-encapsulated adult nematodes were recognized on the enucleated ocular globe of the imported dog. Additionally, genomic DNA from the skins tested positive for *O. lupi* at molecular testing. In this study, we discuss the need for more reliable diagnostic techniques and efficient treatment protocols to better plan future intervention strategies.

Sero-epidemiological survey on canine Leishmaniosis in Sardinia, Italy

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Keywords: leishmaniosis, dogs, IFAT, Sardinia

INTRODUCTION. *Leishmania infantum* is a protozoan parasite transmitted by arthropod vectors causing visceral and cutaneous leishmaniosis in dogs and humans in southern Europe (Otranto et al., 2013, PLoS ONE 8(2): e56374). In Italy, canine Leishamaniosis (CanL) is considered endemic in southern and central regions including Sardinia (Gramiccia M., 2011, Vet Parasitol, 181:23-30). Considering the importance of dogs in the transmission of human leishmaniosis, the aim of this study was to update the knowledge on seroprevalence of CanL in Sardinia.

MATERIALS AND METHODS. From 2013 to 2018, peripheral blood samples were collected from a total of 1046 dogs living in Sardinia region from kennels (821) and referred for routine or clinical visit at Veterinary Teaching Hospital of the University of Sassari (225). Data about sex, age, housing, size and hair length were registered for each animal. Sera were tested by an in-house Indirect Immunofluorescent Antibody Test (IFAT) to detect anti-*Leishmania* IgG antibodies, with cut-off value of 1:40 (OIE Manual of diagnostic Tests and Vaccines).

RESULTS AND CONCLUSIONS. Anti-*L. infantum* IgG antibodies were found in the 22.8% (239/1046) with titers ranging from 1:40 to 1:10,240. Dogs with antibodies titre of 1:40, between 1:80 and 1:320 and above 1:320 were respectively 7.6% (80/1046), 8.9% (93/1046) and 6.3% (66/1046).

The highest positivity rate was found in males (26.7%) than in females (19.2%), with differences statistically significative (*chi*-square test = 8.35; P = 0.0038). Examined animals were divided in 3 age classes: <1 year (16.7%), ≤1≤2 years (19%) and > 2 years (23.9%), but no statistical differences were found for the presence of anti-*Leishmania* IgG ($\chi 2$ for linear trend = 2.610; P = 0.106). No correlation was found regarding the size (small: 24.6%; medium: 20.7%; big: 25.8%) and the hair length (short: 22.6%; medium: 23.2%; long: 23.3%) of examined animals. Antibody against *Leishmania* were detected more in owned dogs than dogs from kennels (45.7% vs 16.3%)($\chi 2$ = 85.00; P < 0.00001). The higher prevalence found in owned dogs can be due to the fact that animals were sometimes referred for visit for suspected infection. The results herein reported showed that seroprevalence of CanL in Sardinia seems to be increased if compared with last survey (Pipia et al., 2014, XXVIII Congr Soipa, p 263) (22,8% *vs* 11.55%; $\chi 2$ = 27.38; P < 0.0001), and that the disease should be managed with a well-defined plan for monitoring the infection in the island.

Authors thanks Mr. F. Salis, Technician, Parasitology Lab., University of Sassari

Seroepidemiology of Toxoplasmosis in sheep and goats cohabiting in Sardinia (Italy)

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Keywords: Toxoplasma, sheep, goats, Sardinia

INTRODUCTION. Toxoplasmosis is one of the most important causes of reproductive failure and abortion in sheep and goats worldwide (Dubey, 2009, Vet Parasitol, 152: 25-80; Da Silva et al., 2013, Rev Bras Ciên Vet, 20: 179-188). The aim of this study was to investigate the presence of toxoplasmosis in sheep and goats bred together in the same flock, in order understand possible risk factors (environmental, management, feeding, etc.) and also if there is a different sensitivity to *Toxoplasma gondii* infection between sheep and goats.

MATERIALS AND METHODS. A seroepidemiological survey was performed in four different farms in the provinces of Sardinia (Nuoro, Sassari, Oristano and Cagliari), in which sheep and goats were bred together. Sampling was performed all year round and a total of 1195 blood sera were examined (626 from sheep and 569 from goats) for detection of anti-*Toxoplasma* IgG using a commercial ELISA kit (PrioCHECK ® *Toxoplasma* Ab SR, Prionics, Switzerland). The test results were interpreted by calculating, for each sample, a percentage of positivity (PP) value relative to the OD of the positive control (PP Sample = OD450 nm Sample/OD450 nm Positive Control x 100). A PP value 20 was regarded as positive, as suggested by the manufacturer.

RESULTS AND CONCLUSIONS. Prevalence rates found in sheep and goats in the four different seasons are shown in Table 1. The comparison of prevalence rates was statistically higher in sheep than in goats in all seasons, particularly in spring (51,8% vs 16,9%; P= 0,000) and winter (53,9% vs 27,1%; P= 0,000).

Our results seems to confirm that sheep is more susceptible to *T. gondii* infection compared with goats (Dubey, 2009, Vet Parasitol, 152: 25-80), even further studies are needed to better understand the dynamics of the antibody immune response among small ruminants.

Table 1. Seasonal prevalence rates and statistical analysis of results in sheep and goats

	SHEEP			GOATS				
SEASON	N°	Positive	Prevalence	N°	Positive	Prevalence	*ODDS RATIO*	χ2 test
Summer	176	63	35,8%	159	39	24,5%	1,72 (1,4<OR<2,84)	*χ2= 5,01; P= 0,025*
Autumn	157	63	40,1%	150	43	28,7%	1,67 (1,01<OR<2,76)	*χ2= 5,46; P= 0,034*
Winter	152	82	53,9%	133	36	27,1%	3,16 (1,86<OR<5,36)	*χ2= 21,12; P= 0,000*
Spring	141	73	51,8%	118	20	16,9%	5,26 (2,83<OR<9,85)	*χ2= 33,85; P= 0,000*
TOTAL	626	281	44,9%	569	138	24,3%	2,54 (1,97<OR<3,29)	*χ2= 55,74; P= 0,000*

Authors thanks Mr. F. Salis, technician, Parasitology Lab, University of Sassari for the laboratory screening of samples

Epidemiological study on endoparasites of one-humped camels, sheep and goats in the Tiris-Zemmour region, Mauritania

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Keywords: gastro-intestinal strongyles, coccidia, Mauritania, arid region, international cooperation

INTRODUCTION. The present study was conducted as part of an international cooperation project (SAL-TIZ, "Sécurité alimentaire dans la Région de Tiris-Zemmour, un défi entre développement et aide humanitaire" - N° DCIFOOD/2013/333-588) co-funded by the European Union and conducted in the arid region of Tiris Zemmour, in the north of Mauritania. Given the lack of epidemiological data for the area, the aim of this survey was to carry out the first study on endoparasites in one-humped camels (*Camelus dromedarius*), sheep (*Ovis aries*) and goats (*Capra hircus*) of the region.

MATERIALS AND METHODS. Data and samples collection was performed in two subsequent periods, May-June 2016 and September-October 2016, with a sampling plan that included the three main areas of the region: Zouerate, F'derik and Bir Moghrein. A total of 439 samples were collected (135 camels, 127 sheep, 177 goats) at slaughterhouses, livestock markets, or private premises. Fecal samples were examined by flotation and McMaster techniques in the diagnostic laboratory set up by the project in the local governmental veterinary unit. Within each host species, the presence of differences in prevalence and abundance values among age classes, sex, sampling periods and management typologies were investigated using the Chi squared and Mann-Whitney U tests, respectively.

RESULTS AND CONCLUSIONS. Prevalence and abundance values are shown in Table 1 for the three-host species. Sheep and camel parasites showed low level of infection and were not significantly influenced by the considered factors. Instead, prevalence and abundance of coccidia in goats was influenced by the management, with higher parasitic burdens in animals reared in a semi-confined system, compared to those kept with the free-range system (typical of Bir Moghrein, in the extreme north). The epidemiological values herein recorded in the three species, which are lower to the ones generally found in literature, are probably due to the particularly hostile climate of this area, with low humidity and high temperatures preventing the parasitic biological cycles. Thus, parasitic diseases do not seem to represent an animal health problem in the region, with the exception of coccidia infection in semi-confined goats.

Table 1. Results of the coprological analysis in the three-species investigated; OPG: oocysts per gram; EPG: eggs per gram.

	Camels (n=135)		**Sheep (n=127)**		**Goats (n=177)**	
	Prevalence (%)	Abundance (OPG/EPG)	Prevalence (%)	Abundance (OPG/EPG)	Prevalence (%)	Abundance (OPG/EPG)
Gastro-intestinal strongyles	18.5	41	29.1	181	5.6	9
Coccidia	14.8	27	32.3	316	60.5	1908

Seroprevalence of equid piroplasmosis in Italy

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Keywords – Equid piroplasmosis, tick borne diseases, epidemiology, Italy

INTRODUCTION. *Theileria equi* and *Babesia caballi* are tick borne protozoan parasites which cause equine piroplasmosis (EP) among equids worldwide. The present study aimed to determine seroprevalence to these pathogens in donkeys, horses, and mules from Northern, Southern, and Central Italy over a period of 6 years.

MATERIALS AND METHODS. For this purpose, some equid serum samples collected from 2012 to 2017 and analysed at the National Reference Center for *Anaplasma, Babesia, Rickettsia*, and *Theileria* (CRABaRT) and the OIE Reference Laboratory for Theileriosis and Babesiosis were included in this retrospective study. In details, 1316 specimens (887 horses, 422 donkeys, and 7 mules) were all analyzed to detect IgG antibodies against *Theileria equi* and *Babesia caballi* using a commercially available IFAT kit at dilution 1:80. The national territory was geographically differentiated into three main areas namely northern (N, n=284), central (C, n=54) and southern (S, n=978) Italy according to the origin of the samples.

The serological prevalence was compared among the 3 areas through Chi-squared test and differences were considered significant if the P-value was < 0.05.

RESULTS AND CONCLUSIONS. The overall seroprevalence of *B. caballi* and *T. equi* were 11.7% and 33.8% respectively. In details, *B. caballi* was found positive in 8.9% (n=87), 27.8% (n=15) and 18.3% (n=52) samples while the prevalence of *T. equi* was 37.3% (n=365), 40.7% (n=22) and 20.4% (n=58) respectively from Southern, central and Northern Italy. Statistically significant differences were found among the 3 areas (S,C,N) for the prevalence of *B. caballi* (χ^2= 32.961, df = 2, P< 0.0001) and *T. equi* (χ^2= 29.289, df = 2, P< 0.0001). According to previous studies (Laus et al., 2013; J Vet Med Sci.,75(6):715-20; Piantedosi et al., 2014, J Vet, 202:578-582; Bartolomé del Pino et al., 2016, Ticks Tick Borne Dis.,7:462-469), our data confirm that *T. equi* was more prevalent (>35%) in central and Southern Italy than *B. caballi* and the difference among the 3 areas was significant. Moreover, even if a higher seroprevalence of *B. caballi* in the northern area was found, the EP overall prevalence was greater (Moretti et al., 2010, Vet J. 84(3):346-350).

Seasonal variation and survival of *Parascaris equorum* eggs in soil

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Keywords: egg survival, intestinal parasite, *Parascaris* spp, horse

INTRODUCTION. *Parascaris equorum* is a large roundworm that primarily affects foals and young growing horses. *Parascaris* eggs are very resistant to environmental conditions and can determine prolonged contamination of the soil of the paddocks frequented by horses. This study aimed to monitor the dynamics of natural contamination by ascarid eggs in the soil in the absence of horses; moreover, risk factors affecting both the presence of eggs in the superficial layer of soil and the percentage of larvated eggs were evaluated.

MATERIALS AND METHODS. From November 2015 throughout December 2016, soil samples, 100 g each, from two paddocks contaminated by *Parascaris* egg were collected in a horse farm in northern Italy. The two paddocks differed for extension (1200 vs. 280 m^2) and the larger one presented a more dense grass cover. Fourteen soil samples for each paddock, were processed by previous described techniques (Roepstorff and Nansen, 1998, FAO Animal Health Manual N. 3); the final step was modified to perform quali-quantitative analysis by FLOTAC technique. The total number of eggs in each soil samples was recorded (EPS) and were corrected for the percentage of soil humidity. Eggs were divided in larvated and unlarvated and their percentage was calculated. Generalized linear mixed models (GLMM) was used to evaluate the effect of date of sampling and temperature/rainfalls in 30 days before each sampling on the total number of eggs and on the percentage of larvated eggs. Meteorological data were obtained from the nearest weather station (www.arpalombardia.it).

RESULTS AND CONCLUSIONS. The mean EPS was 57, ranging from 159 to 22 at the beginning and at the end of the study, respectively. GLMM (negative binomial distribution with log link) showed that date of sampling was a predictor of EPS in soil (p-value<0.001): the soil was still contaminated at the end of the study, but the EPS progressively decreased. High level of rainfalls in 30 days before sampling determined higher EPS counts (p-value<0.05). Mean percentage of larvated eggs was 85.5% (min-max=63.9-100%) and was positively influenced by date of sampling (p-value<0.001), mean temperature in 30 days before sampling (p-value<0.001) and mean rainfalls in 30 days before sampling (p-value<0.01) (GLMM, normal distribution). Despite the progressive decline of EPS, the increase in the percentage of larvated eggs in soil preserve the parasitological risk by *Parascaris* of contaminated paddocks for up to 14 months without further fecalization.

SESSIONE 10

MALATTIE TRASMESSE DA ARTROPODI VETTORI ALL'UOMO

A retrospective analysis on the occurrence of *Borrelia miyamotoi* in *Ixodes ricinus* ticks in north-east Italy, 2007-2017

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Keywords: *Borrelia miyamotoi, Ixodes ricinus* ticks, north-east Italy

INTRODUCTION. *Borrelia miyamotoi* is a relapsing fever-related spirochete, considered an emerging pathogen in humans, transmitted to vertebrate hosts by the same *Ixodid* tick species transmitting *Borrelia burgdorferi* s.l., the agent of Lyme disease. *B. miyamotoi* was detected for the first time in ticks in Northern Italy in 2016 (Ravagnan, Tomassone *et al.,* Parasit Vectors. 2018, 20;11(1):130). The aim of the study was to determine the occurrence and prevalence of *B. miyamotoi* in north-east Italy since 2007.

MATERIALS AND METHODS. *B. miyamotoi* was searched in 3,170 *Ixodes ricinus* ticks (279 adults; 2370 nymphs; 521 larvae) collected from 2007 to 2017 (except 2009-2010) in 60 sites of six provinces of north-east Italy. A real time PCR targeting glpQ gene (Vayssier-Taussat *et al.*, 2013, PLoS One; 8:e81439) was used to screen single adults and pooled larvae and nymphs (20 and 10 specimens maximum, respectively). Positive samples were also amplified and sequenced using a PCR targeting ~900 bp of the glpQ gene (Hovius *et al.*, 2013, Lancet 382:658). For pooled samples the minimum infection rate (MIR) was calculated (number of positive pools/number of total specimens x100). The difference of infection rates according to tick's stage, provenance and year of collection was tested using Chi-square or Fisher's exact test when appropriated.

RESULTS AND CONCLUSION. Overall, 39 samples (1.23%) were positive for *B. miyamotoi*, precisely 8 adults (2.79%), 26 nymphs (1.10%) and 5 larvae (0.96%). The pathogen was found in 13 sites (21.6%) of all the provinces monitored (range 0.33-1.96%) and in all the years (range 0.4-2.7%). All positive samples belonged phylogenetically to the European type.

No difference of infection rates was found among data recorded. In 9 sites *B. miyamotoi* co-circulated with other TBDs, i.e. *Rickettsia helvetica* (3 sites), *R. monacensis* (2), *Borrelia burgdorferi* s.s. (7), *B. afzelii* (3), *B. garinii* (3), *B. valaisiana* (2), *Cand.* Neoehrlichia mikurensis (2), *Anaplasma phagocytophilum* (3) and TBE virus (1). *B. miyamotoi* was also diagnosed in an engorged tick detached from a roe dear, in his eggs delivered in laboratory and in 10 hatched larvae. *B. miyamotoi* is confirmed to be a wide spread pathogen in north-east Italy, occurring at a low endemicity. The finding of the pathogen in questing larvae and overall in larvae hatched from a positive female, suggest the possibility of vertical transmission in *I. ricinus* ticks. *B. miyamotoi* should be considered in the differential diagnosis of febrile patients originating from Lyme borreliosis endemic regions.

Laboratory transmission of a central Asian *Leishmania tropica* by the bite of the western European sand fly *Phlebotomus perniciosus*

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Keywords: *Leishmania tropica, Phlebotomus perniciosus,* hamster, xenodiagnoses

INTRODUCTION. The competence of indigenous sand fly species to transmit non-indigenous *Leishmania* parasites is a prerequisite for the spreading of new leishmaniasis entities in a territory. *Phlebotomus perniciosus* is the proven vector of *L. infantum* in western Mediterranean, a region interested by intense human migration. Imported cutaneous leishmaniasis due to *L. tropica*, an agent of anthroponotic disease, is increasingly documented in Europe. In a previous study we found that most of the experimentally-infected *P. perniciosus* supported the initial growth of *L. tropica*, and that late-stage metacyclic infections occurred in variable proportions of infected females 7-16 days p.i. (Bongiorno et al, 2017, Proc. 6th World Congr. Leishmaniasis, 278). We report here on the successful *L. tropica* transmission to naïve hamsters by *in vivo* (footpad lesion)-infected *P. perniciosus*.

MATERIALS AND METHODS. The study was designed to mimic a natural infectious dose of amastigotes from chronic lesions, as is the case with the anthroponotic cycle. Stationary-phase cultures of *L. tropica* MHOM/IT/2016/ISS3183, recently isolated from an Afghan patient, were injected into hamster footpads. The resulting lesions, developed in about a month, were monitored for *Leishmania* infectiousness by xenodiagnosis using colonized *P. perniciosus*. When infectiousness of late lesions was considered optimal (i.e. producing about 70% infection in fed sand flies), a massive sand fly infection was performed to allow a sufficient number of potentially-infectious females to take a second blood meal. Naive hamsters were exposed to potentially-infectious bites from 8 through 19 days p.i. After the bites, whose site was recorded, individual sand flies were dissected to determine a transmissible-infection status. When skin lesions developed in the site of transmission, *Leishmania* diagnosis and monitoring over time was further performed by *P. perniciosus* xenodiagnosis.

RESULTS AND CONCLUSIONS. Two clusters of sand fly infection (on 6 infected hamsters) followed by a second blood meal (on 2 naive hamsters) involved a total of 690 females. Of these, 59 had a second blood meal - mainly on front paws and snout -, of which 37% were detected as *Leishmania* positive and 15% harboured motile metacyclics. About 2 months from *P. perniciosus* exposure, both naive hamsters showed early skin lesions in transmission sites. One month later both hamsters underwent xenodiagnosis, showing infectiousness rates of 35.5 and 7.2% respectively. Our results support the hypothesis that, in particular epidemiological situations, *P. perniciosus* may play the role of occasional *L. tropica* vector.

Leishmania tropica infection induces immune responses through NOD2 pathway

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Key words: *Leishmania tropica* infection, NOD2 pathway, innate immune responses

INTRODUCTION. *Leishmania tropica* is the etiological agent of cutaneous leishmaniasis. Pattern recognition receptors such as toll-like receptors or nucleotide oligomerization domain-like receptors (NLR) have been associated with the disease. The role in innate immune responses against *L. tropica* of NOD2, a member of the NLR family, was investigated.

MATERIALS AND METHODS. Interferon gamma (IFN-γ)-primed or unprimed immortalized mouse bone marrow macrophages (BMDM)-wild type (WT) or -NOD2$^{-/-}$ were infected with *L. tropica* at different parasite/macrophage ratios (i.e. 2.5, 5, or 10:1), for 24h. Controls, such as medium alone, muramyldipeptide (MDP), or lipopolysaccharide (LPS), were included. Levels of cytokines or nitrite released into supernatants were measured through ELISA or Griess reagents. Levels of inducible nitric oxide synthase (iNOS) mRNA and protein were retrieved through Real-Time PCR and Western blot analyses, respectively. Also, unprimed BMDM-RIP2$^{-/-}$ or -CARD9$^{-/-}$, two downstream components of NOD2 activation, were stimulated with the same ratios of *L. tropica* or controls, and the production of the pro-inflammatory cytokine TNF-α was compared with BMDM-WT.

RESULTS AND CONCLUSIONS. Data showed that *L. tropica* did not induce the production of nitric oxide in unprimed BMDM-WT. *L. tropica* induced higher levels of nitric oxide in IFN-γ-primed BMDM-WT than in unstimulated cells. Stimulation due to *L. tropica*, or to the control MDP (known to activate NOD2), was abrogated in the BMDM-NOD2$^{-/-}$, but not to LPS, as so the expression levels of inducible nitric oxide synthase (iNOS) mRNA or protein. In addition, NOD2, RIP2 or CARD9 showed to be involved in the induction of TNF-α release from BMDM.

These data suggest an involvement of NOD2 pathway in innate immune response to *L. tropica* infection.

Asaia bacteria engineered to express the *Wolbachia* surface protein induce a Th1 polarization. Implications for the control of leishmaniasis

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Keywords: immune response, leishmaniasis, *Wolbachia*

INTRODUCTION. Protective immunity against leishmaniases is generally dependent on the induction of Th1 immune responses, while an activation of the Th2-type response is associated with parasite survival and progression of the disease. *Wolbachia*, an obligate intracellular bacterium in filarial nematodes, is reported to be a Th1 inductor, as well as the *Wolbachia* surface protein (WSP), the only *Wolbachia* molecule that has been investigated in detail for its immunological properties. *Wolbachia* is not-culturable in cell-free media, as such not easily exploitable as an immune-modulating agent. For this reason, we engineered a culturable non-pathogenic bacterium, *Asaia platycodi*, to express the WSP from *Wolbachia* of a filarial nematode. The stimulation of the innate immune response by this engineered bacterium has been investigated.

MATERIAL AND METHODS. First, an immortalized macrophage cell line J774A.1 derived from BALB/c mouse was exposed to the engineered bacterium *Asaia*-WSP and to the *Asaia*-empty plasmid, *Asaia*-pHM4. Levels of Th1-cytokines, nitrite and the expression of co-stimulatory molecules were measured through ELISA, Griess and flow cytometry assays. Then, the leishmanicidal consequences of WSP production by *Asaia* was tested in co-infection experiments using the same macrophage cell line.

RESULTS AND CONCLUSION. Our results show that the engineered bacterium activate macrophages, also determining a leishmanicidal effect. In conclusion, we propose that *Wolbachia*, the WSP and the engineered *Asaia*-WSP are worth of further investigations, aimed at the development of immune-modulating adjuvants, for the development of novel types of vaccines against leishmaniasis.

A cellular method to measure phagocytosis of *Plasmodium falciparum* gametocytes by bone marrow macrophages

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Keywords: *P. falciparum* gametocytes, bone marrow macrophages, phagocytosis

INTRODUCTION. Gametocytes (GCT), the sexual blood stage of malaria parasites, develop in five morphological stages (I-V). Immature stages (I-III) are sequestered in the bone marrow, and only mature stages (V) are released back into the blood stream where they can be harvested by the mosquito vector. Few information is available about the role of innate immunity against GCT and the ability of host macrophages to phagocytize GCT. The aim of this work was to set up an *in vitro* method to study phagocytosis of *P. falciparum* (*Pf*) GCT by Murine Bone Marrow Derived Macrophages (BMDM) (Hornung et al., 2008, Nat Immunol, 9:847).
MATERIALS AND METHODS. The *Pf* transgenic strain 3D7*elo1-pfs16*-CBG99 expresses the luciferase CBG99 under the control of the gametocyte-specific promoter *pfs16.* GCT were cultured as described (D'Alessandro et al., 2016, JAC, 71:1148), and enriched by magnetic separation. BMDM were incubated with immature or mature GCT for 2/24h, in the presence or absence of the Cytochalasin D. A lysis step was performed to remove non-internalized parasites. A cell permeable solution of the luciferin substrate was added to the cells. The luminescent signal indicated the presence of parasites inside BMDM, thus phagocytosis.GCT phagocytosis by BMDM was confirmed by Giemsa staining and confocal microscopy.
RESULTS. The results indicated that both immature and mature GCT are phagocytized by BMDM. Upon pre-incubation with Cytochalasin D, which blocks phagocytosis, the luminescent signal disappeared, confirming that this method measures uptake of GCT by BMDM. Interestingly, the luminescent signal from phagocytized immature GCT was lower than that of mature GCT, suggesting a different susceptibility to BMDM activity. However, this finding need to be confirmed since the luminescent signal of immature GCT is lower than that of mature ones (D'Alessandro et al., 2016, JAC, 71:1148). The method was validated through Giemsa staining and confocal microscopy analyses, which showed the presence of parasites inside macrophages.
CONCLUSIONS. This method is suitable to measure phagocytosis of GCT from macrophages. Further experiments will confirm differences in the phagocytosis of mature vs immature GCT.

The human GTPase Rac1 plays an important role in *Plasmodium falciparum* invasion and growth inside human erythrocytes

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Keywords: Malaria, *P. falciparum,* parasite

INTRODUCTION. Malaria is one of the most deadly infectious diseases worldwide. The human protein Rac1 is a GTPase involved in actin cytoskeleton regulation and essential in invasion of the host cell by several intracellular pathogens, including *Toxoplasma gondii,* which belongs to the same phylum as *Plasmodium falciparum.* Rac1 has been extensively studied and several cell permeable inhibitors of Rac1 have already been developed.

MATERIALS AND METHODS. The role of Rac1 in *P. falciparum* invasion of human erythrocytes was tested on two different Rac1-KO erythroid cell lines and confirmed by invasion assays in the presence of Rac1 chemical inhibitors. The half-inhibitory concentration (IC_{50}) of different chemical inhibitors of Rac1 were obtained using the lactate dehydrogenase assay on *P. falciparum* cultures.

RESULTS AND CONCLUSION. We showed that Rac1 is recruited during invasion to the site of parasite entrance and co-localizes with the moving junction. We also showed that the GTPase is activated by the parasite upon invasion. In infected erythrocytes, Rac1 is further recruited to the parasitophorous vacuole membrane (PVM) in its GTP-bound form, being gradually depleted from the erythrocyte membrane. In order to investigate the role of Rac1 in malaria infection, we generated two different Rac1-KO erythroid cell lines and showed that *P. falciparum* invasion rates are significantly reduced in the absence of the GTPase. Furthermore, 11 Rac1 chemical inhibitors were able to kill the parasite in asynchronous cultures and 3 of them reduced *P. falciparum* invasion rates.

Therefore, the Rac1 GTPase has an important role in *P. falciparum* infection of human erythrocytes, both during the invasion and during the intraerythrocytic cycle. Its activation by *P. falciparum* indicates that the parasite exploits the host signal transduction machinery to invade the host erythrocyte. Rac1 could be an interesting candidate for the development of novel anti-malarial drugs to face the issue of the insurgence of malaria strains resistant to currently available drugs.

Use of efflux pump inhibitors in *Plasmodium falciparum*, to increase drug efficacy

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Keywords: doxycycline, apicoplast, drug interaction

INTRODUCTION: There is great concern regarding the rapid emergence and spread of drug resistance in *Plasmodium falciparum*. Here we present the results of *in vitro* assays on *P. falciparum*, using combinations of available drugs. We focused on doxycycline, an antibiotic that specifically targets the apicoplast of *P. falciparum,* inducing a delayed antiparasitic effect. We tested three different drugs known to inhibit efflux pumps, in combination with doxycycline, with the assumption that this could determine an increased intracellular concentration of the antibiotic, thus an increased efficacy against *P. falciparum*.

MATERIALS AND METHODS: Cultures of asexual stages of *P. falciparum,* strain D10, chloroquine-sensitive, and W2, chloroquine-resistant, were used to test combinations of doxycycline and transporter inhibitors at different concentrations. Plates were incubated at different time points at 37°C to assess the effect of the drugs after one life cycle of the parasite, and after two cycles (delayed antiparasitic effect), respectively. The IC50s were determined by measuring the activity of the parasite lactate dehydrogenase at the end of the incubation. All the experiments were performed at least three times in duplicate.

RESULTS AND CONCLUSIONS: The results indicate both synergistic and antagonistic effects with doxycycline, depending on the transporter inhibitor used. Drugs shown to act synergistically on the parasite should be further investigated, for the design of novel combined drug regimens for antimalarial treatment; similarly, further studies should be conducted on drugs acting antagonistically, in order to avoid drug combinations with reduced antimalarial effects. Our study highlights the importance of monitoring *in vitro* the drug-drug interactions, to optimize future *in vivo* protocols.

Activity of novel aminoquinoline derivatives against *Leishmania infantum*

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Keywords: *L. infantum*, antileishmanial drugs

INTRODUCTION. Leishmaniasis is a neglected disease caused by protozoa of the genus *Leishmania*. Metacyclic promastigotes are deposited into the human host's skin by the bite of phlebotomine sand flies and phagocytized by macrophages where they develop into amastigotes and multiply. *L. infantum* is the causative agent of zoonotic visceral leishmaniasis in the Mediterranean basin. Due to high cost, resistance, and toxicity to current treatments, new drugs are urgently needed. In the present study, a library of thirty 4-aminoquinoline-based compounds was tested *in vitro* and *in vivo* against *L. infantum*.

MATHERIALS AND METHODS. The *in vitro* activity against the p*romastigote stage of L. infantum* was evaluated by MTT assay. Intracellular amastigotes were obtained by infecting PMA differentiated THP-1 cells with metacyclic promastigotes from cultures and the percentage of infected macrophages in control and in drug treated cells was determined by microscopy. Cytotoxicity was evaluated by MTT assay and selectivity index was calculated. *In vivo* activity was assessed in a mouse model of visceral leishmaniasis using Balb/c mice infected intravenously with *L. infantum* amastigotes from infected hamster spleen. Relative parasite loads in control and treated mice were determined by counting parasites in the liver.

RESULTS AND CONCLUSIONS. Among the compounds tested, ten showed IC_{50} < 1 μM against *L. infantum* promastigotes. Based on the cytotoxicity data, a primary screening of the compounds against intracellular amastigotes was performed at 0.5 μM concentration. IC_{50} was calculated for the most active compounds. Two compounds (JK59, a benzothiophene derivative, and TNT224, an adamantine derivative) showed good activity against amastigotes with IC_{50}<1μM and moderate selectivity (SI between 4 and 6). These two compounds were tested in the mouse model. Both JK59 and TNT224 were able to reduce parasite load in a dose-dependent manner and were extremely active at the doses of 50 and 100 mg/kg. Two potent antileishmanial compounds with high *in vitro* and *in vivo* activity were thus identified and selected for further studies.

SESSIONE 11

PARASSITI DELLA FAUNA ACQUATICA

Trematode infections in cultured gilthead seabream *Sparus aurata* L. (Sparidae) from the Mediterranean Sea: pathways and associated threats for aquaculture

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Keywords: *Cardicola aurata*, *Cardiocephaloides longicollis*, aquaculture, Sardinia

INTRODUCTION. Parasitic diseases are important constraints to finfish aquaculture. The entry of parasites in a culture facility depends on the presence of sources of infection in the surrounding habitat (e.g. reservoirs, intermediate hosts, vectors), and on the degree of exchange with the external environment. Floating cages are open systems where high-level risks would correspond to parasites with direct life cycles and pelagic infectious stages, able to enter easily and reproduce rapidly. On the other hand, low risks seem to correspond to heteroxenous parasites as trematodes, requiring two or more hosts living close to cultured fish. However, trematodes often occur in cage facilities, and some of these parasites can be harmful to the host. The present study describes the occurrence of adult and larval trematodes in cage-reared fish from the Mediterranean Sea.

MATERIALS AND METHODS. From Spring to Autumn 2017, samples of gilthead seabream *Sparus aurata* L. (Sparidae) from two different sea cage facilities of Sardinia (western Mediterranean Sea) were examined for adult and larval trematodes. One facility (eastern) was sampled from April to July (N=116 fish, total length 23-26 cm), the other (western) in May and October (N=60, TL: 24-30 cm).

RESULTS AND CONCLUSIONS. Trematodes were only found in the eastern littoral facility: adults of the aporocotylid *Cardicola aurata* Holzer, Montero, Repullés, Sitjà-Bobadilla, Alvarez-Pellitero, Zarza & Raga, 2008 (prevalence, 3-40%; mean intensity, 1.0-1.3), located in the afferent vessel of gills; and metacercariae of the strigeid *Cardiocephaloides longicollis* (Rudolphi, 1819) Dubois, 1982 (15-43%; 1.0-1.2), located on the optical lobes of the brain. The occurrence of trematodes in cage-reared fish, the possible pathways of entrance and transmission of these parasites, as well as the potential associated threats for aquaculture are discussed.

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Combining morphology and genetics to resolve the status of a monogenean from the taxonomic "waste-basket" *Haliotrema* Johnston & Tiegs (Ancyrocephalidae)

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Keywords: Monogenea, 28S rDNA, Mediterranean Sea, Black Sea

INTRODUCTION: The genus *Haliotrema* Johnston & Tiegs, 1922 currently includes 139 nominal species parasites of a wide range of fish families. Despite the several revisions and exclusion of many species raised to the generic level, this taxon is still considered as an unnatural and polyphyletic group, defined by Klassen (1994) as taxonomic "waste-basket". Only one species, *Haliotrema cupensis* Sasal, Pages & Euzet, 1998 parasite of *Gobius cobitis* Pallas (Gobiidae), is so far known in the Mediterranean region. Recently, Merella et al. (2010) synonymised *H. cupensis* with *Ancyrocephalus cobitis* Ergens, 1963, and proposed the new combination *Haliotrema cobitis* (Ergens, 1963).
MATERIALS AND METHODS: The gills of 18 specimens of *G. cobitis* from northeastern Sardinia (western Mediterranean Sea), and of one specimen from the western Caucasus (northeastern Black Sea) were examined for the presence of ancyrocephalid monogeneans. Parasites were mounted in glycerine-jelly or stored in absolute ethanol.
RESULTS AND CONCLUSIONS: Based on an integrative taxonomic approach, combining morphology and genetics, a new genus and combination is proposed for the species *Xenoligophoroides cobitis* (Ergens, 1963) to accommodate the ancyrocephalid parasites found on the gills of *G. cobitis*. *Xenoligophoroides cobitis* does not correspond to the diagnosis of any of the known ancyrocephalid genera according to the combination of the following characters: vas deferens not looping intestinal caecum; two prostatic reservoirs; uncoiled MCO; bilobed base of MCO; accessory piece articulated with MCO through rod-shaped process; uncoiled dextral vagina; paired anchors and bars, without additional sclerites; unmodified normal distributed marginal hooks with upright thumb; parasite of Gobiidae. A molecular 28S rDNA-based phylogenetic analysis considering the examined specimens along with sequences from several closely related genera of Ancyrocephalidae, showed that all the specimens of *X. cobitis* form a well-supported clade, highly divergent from all *Haliotrema* species and other genera. Such a finding supports the establishment of the new genus for the ancyrocephalid parasites of *G. cobitis* from the western Mediterranean and northeastern Black seas.

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Expression profiles of three relevant genes in *Anisakis pegreffii* larvae (Nematoda: Anisakidae) cultured *in vitro,* and from experimentally infected fish

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Keywords: *Anisakis pegreffi*, ESPs, gene expression

INTRODUCTION. Anisakid nematodes produce and release a variety of excretory/secretory products (ESPs), which have been retained to be key players in the parasite-host adaptation and interaction. ESPs have several functions during infection, e.g. penetration of host tissues, but also able to elicit immune response both in intermediate/paratenic (fish) and accidental (humans) hosts (Mehrdana & Buchmann, 2017, Acta Vet. Scand., 59). The target parasite species of this study is *Anisakis pegreffii*, also etiological agent of human anisakiasis (Mattiucci et al., 2018, Adv. Parasitol., 99: 93-263). Aims are: *i)* to study the gene expression level of some proteins (trypsin inhibitor, glycoprotein, mioglobin) among the ESPs released by *A. pegreffii* larvae *in vitro,* and *in vivo* (in experimentally infected fish); and *ii)* to investigate if the expression levels for those ESPs are larval migration-dependent, and/or host immune response-mediated**.**
MATERIALS AND METHODS. *A. pegreffii* larvae were maintained *in vitro* culture in solid agar at the temperature of 20°C; while others were used for experimental infection of sea bass (*Dicentrarchus labrax*) under controlled conditions. RNAs from each *Anisakis* larva, collected from agar blocks and from different site of infection in the fish, was extracted and reverse-transcribed to cDNA. Specific primers were then designed to be used in real time PCR to examine the mRNA expression profiles of those target genes.
RESULTS AND CONCLUSIONS. Genes encoding for the trypsin inhibitor, a glycoprotein, and the mioglobin, were generally expressed at a low level in those *A. pegreffii* larvae "migrated" and "not-migrated" after 24h, in the solid agar; similar results were found in the larvae isolated, after 24h, from the stomach lumen of the fish, experimentally infected. Whereas, a significant increase of the expression levels of mRNA of the trypsin inhibitor, the glycoprotein and the mioglobin was registered in those larvae which reached the viscera and the liver, after 24h. The results seem to indicate a possible influence of immunological factors of the host as players in triggering the gene expression of some ESPs.

Risk assessment on the presence of zoonotic parasites in freshwater and marine fish farmed in Italy

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Keywords: Aquaculture, Italy, fish-borne zoonotic helminths

INTRODUCTION. In the last years, food fish supply for global human consumption has witnessed an increase of aquaculture as source of fish products versus capture fisheries, raising concerns about their safety, in particular the risk of transmission of zoonotic helminths to humans. Although it is generally assumed that farmed fish products have a very low prevalence of these helminths, as already assessed for Atlantic salmon (*Salmo salar*), few data are available for most of other fish species farmed in Europe. In the framework of ParaFishControl EU project, activities on "Fish product safety" are aimed to assess the risk due to zoonotic helminths in marine and freshwater fish farmed in Europe. Here the results of an extensive survey carried out in Italian aquacultured fish are reported.
MATERIALS AND METHODS. From spring 2016 to spring 2018 a total of 3822 market size fish have been examined. In particular, 1259 rainbow trout (*Oncorhynchus mykiss*) from 6 Italian freshwater trout farms have been subjected to the search of larval stages of diphyllobothriid cestodes (visual inspection and candling) and opisthorchiid digeneans (muscular compression/artificial digestion followed by microscopic examination). Furthermore, 1382 gilthead seabream (*Sparus aurata*) and 1181 European seabass (*Dicentrarchus labrax*) from 5 marine farms (4 cage systems and 1 inland farm located in Tyrrhenian and Adriatic seas) were examined for anisakid larvae by visual inspection, candling, UV-press and artificial digestion. A seasonal periodicity was applied (65 specimens/species/farm/season) to reach a statistically significant amount of fish (258) at the end of the survey for each fish farm (CL 99%, MoE 4-8%).
RESULTS AND CONCLUSIONS. No zoonotic parasites have been found in all the examined fish. The encouraging results so far obtained during these surveys seem to confirm that the risks linked to zoonotic helminths in Italian aquacultured fish species could be considered negligible, when good farming practices are applied along the production chain. Since a larva of *Hysterothylacium fabri*, not zoonotic nematode showing a food web transmission similar to *Anisakis* spp. except for having teleosts as definitive hosts, was found in one ESB from a cage-based farm, a second round survey is in progress to identify the risk factors leading to the infection and define good management practice to prevent the transmission of zoonotic helminths.

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Insights into the life-cycle of the two sibling species of the *Contracaecum rudolphii* Hartwich,1964 (*sensu lato*) complex (Nematoda: Anisakidae) from Central Italy

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Keywords: *Contracaecum rudolphii (s.l.),* sibling species, fish, cormorants

INTRODUCTION. *Contracaecum rudolphii* Hartwich,1964 (*sensu lato*) is a complex of sibling species, parasites at the adult stage, of different species of cormorants, worldwide (Mattiucci et al.,2002,Parassitologia,44:105; D'Amelio et al.,2007, Parasitology,134:1041–1051; Shamsi et al.,2009,Parasitol.Res.,105:529–538; Garbin et al.,2011, J.Parasitol.,97:476–492). The great cormorant *Phalacrocorax carbo sinensis*, is parasitized by *C. rudolphii* sp. A and *C. rudolphii* sp. B, reported from brackish and freshwater ecosystems of Central and Eastern Europe (Mattiucci et al., 2002,Parassitologia,44:105; Szostakovka & Fagerholm,2007,J Parasitol., 93:961-964). Aim of this study is to add insights into the life-cycle of the two species from basin waters of Central Italy.

MATERIALS AND METHODS. Adults of *C. rudolphii (s.l.)* (N= 583) were collected in "bolus" of 19 *Ph. carbo sinensis* from Tarquinia salt marshes, Marta river, and Orbetello lagoon between November 2016 and January 2018. *Contracaecum* sp. larvae (N=524) were obtained from fish species captured from the same areas of cormorants' sampling, as following: *Dicentrarchus labrax* (N=128), *Anguilla anguilla* (N=366), *Aphanius fasciatus* (N=5), *Atherina boyeri* (N=15), *Leuciscus cephalus* (N=10). The parasites were identified by diagnostic allozymes loci, and by mtDNA *cox2* sequences analysis (Mattiucci et al., 2008 Syst. Parasitol., 69:101–121).

RESULTS AND CONCLUSIONS. Significant differential distribution of the two species was found in the samples: *C. rudolphii* sp. B outnumbers *C. rudolphii* sp. A in cormorants wintering in freshwater ecosystem (i.e Marta river); whereas, the opposite trend was found in Tarquinia salt marsh and Orbetello coastal lagoon. Accordingly**,** larvae from *L. cephalus* fished in the Marta river resulted *C. rudolphii sp.* B*.* The last represents the first report of *C. rudolphii* sp. B larvae in fish from Italy. These results, along with reports of previous studies (Mattiucci et al., 2002 Parassitologia 44:105; Szostakovka & Fagerholm, 2007, J Parasitol. 93:961-964), seem to confirm that the life-cycle of *C. rudolphii* sp. A occurs in brackish and hyperhaline ecosystems; while *C. rudolphii* sp. B seems to have a life-cycle more adapted to freshwater environment. Relative proportions of the two parasite species in great cormorants living in those different ecosystems, seem to indicate that a differential feeding behaviour of wintering *Ph. carbo sinensis* populations in Italy would be responsible to maintain distinct life-cycles of the two sibling species of *C. rudolphii (s.l.).* In turn, these parasites could be used as "tags" to follow the geographical origin of wintering populations of migratory birds, whose composition represents a relevant aspect for their management (Frederiksen et al.,2018,JApplEcol,00:1–14).

First molecular identification of the fish myoliquefactive parasite *Kudoa thyrsites* (Myxosporea, Multivalvulida) in *Lepidopus caudatus* from the Alboran Sea (Mediterranean Sea)

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Keywords**:** *Kudoa thyrsites*, 'soft flesh', *Lepidopus caudatus,* Alboran Sea

INTRODUCTION. Myxozoans of the *Kudoa* genus infect several marine fish species, worldwide. They are of concern in the seafood industry since they may generate visible macroscopic cysts in the fish host's muscle and cause *postmortem* tissue myoliquefaction, commonly known as 'soft flesh'. One of the most economically important species is *Kudoa thyrsites*, a 'soft flesh' generating species that occurs in several wild and cultured marine fish throughout the world's oceans. The aim of this study is to identify a *Kudoa* sp. isolate causing muscle myoliquefaction in silver scabbardfish (*Lepidopus caudatus*) fished in the Alboran Sea.

MATERIALS AND METHODS. Morphometric analysis and molecular identification (nuclear small subunit 18S rDNA gene sequencing) was carried out on spores obtained from the myoliquefacted tissue of two individuals of *L. caudatus* from the Alboran Sea. *K. thyrsites* isolates obtained from Atlantic mackerel (*Scomber scombrus*) caught in the NE Atlantic Ocean (Norwegian Sea), were used for morphological and molecular comparison.

RESULTS AND CONCLUSIONS. Myxospores showed stellate appearance, with four unequal pyriform polar capsules. These morphological traits are in agreement to those reported for *K. thyrsites* isolates previously described. The (SSU) 18S rDNA sequences obtained matched 100% with those of *K. thyrsites* previously recorded in mackerel from the North Sea and Southern England, and deposited in GenBank. This represents the first molecular identification of *K. thyrsites* from fish of the Mediterranean Sea. However, its finding in the Alboran Sea - which is retained as an Atlantic oceanographic basin water - poses risk on its possible widespread in other Mediterranean fish species.

SESSIONE 12

COTROLLO E MONITORAGGIO DEI VETTORI

Preliminary data on *Anopheles* species distribution in Botswana

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Keywords: *Anopheles gambiae*, *Anopheles funestus*, Botswana, *Plasmodium falciparum*

INTRODUCTION. Botswana is one of the four front line malaria elimination countries in Southern Africa with well-coordinated malaria control operations that includes routine vector control. Past and recent studies have shown that *Anopheles arabiensis* is the only known vector of *Plasmodium* parasites in the country. This report presents a preliminary evaluation on *Anopheles* species composition in seven districts of Botswana with some inferences on their vector role

MATERIALS AND METHODS. The specimen were collected during two successive malaria seasons (2014-2015 and 2015-2016). Morphological and molecular identification of *Anopheles* were conducted to establish the species. Molecular techniques were used to identify the origin of the blood meal and the positivity to *Plasmodium falciparum*.

RESULTS. Overall 772 *Anopheles* mosquito females were collected, both from larvae collected from several breeding sites, and adults obtained from indoor pyrethrum spray catches (PSC). *An. arabiensis* accounted for the highest relative frequency in most of the districts sampled. The other species collected, among those identified, were: *An. longipalpis* type C, *An. parensis*, *An. quadriannulatus*, and *An. leesoni*. Interestingly, in the transmission season of 2015-2016, known to be affected by El Niño Southern Oscillation pattern, a significant increase of *An. arabiensis* rate was observed in all districts. PCR test for human β-globin on mosquitoes collected by PSC showed that *An. arabiensis* and *An. parensis* have bitten human hosts. Moreover *An. arabiensis* showed a non-negligible *P. falciparum* infection rate in three sites (Ngamiland, Chobe and Kweneng West districts).

CONCLUSIONS. Our results provide first time evidence of *Anopheles* diversity in several areas of Botswana. *Anopheles arabiensis* is confirmed to be widespread in all the sampled districts and to be vector of *P. falciparum*. Moreover, we report for the first time on the presence of *An. funestus* group in Botswana. Further research, entomological surveillance activities and possibly vector control programs need to be developed and implemented as well as targeting outdoors resting vectors.

Occurrence of potential malaria vectors in north-east Italy

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Keywords**:** *Anopheles*, Malaria, Entomological survey, Real-Time PCR

INTRODUCTION. Malaria is still one of the most important infectious diseases worldwide. Italy is malaria free since 1970, but in 2017 malaria cases were reported in people with no travel history in endemic areas. Following a fatal case occurred in north-eastearn Italy, an entomological survey was carried out in the areas where the patient stayed. In addition, *Anopheles* species were collected in the frame of the entomological surveillance for West Nile virus in Veneto and Friuli Venezia Giulia Regions. The aim of our study was to identify the population of *Anopheles maculipennis* complex present in north-east Italy, to assess the presence of potential malaria vectors.

MATERIALS AND METHODS. Mosquitoes were captured using CDC-CO_2 traps for West Nile surveillance (from May to October 2017) and by manual aspiration in the area of human case. In order to identify the different species of *Anopheles maculipennis* complex, a Real-Time PCR and sequencing were performed (max five mosquitoes for collection).

RESULTS AND CONCLUSIONS. Only seven *Anopheles maculipennis s.l.* were cought by aspiration in the place of human case (five *An. maculipennis s.s*. and two *An. messeae*). A total of 1,349 *Anopheles maculipennis* s.l. were collected. Of this, 252 were analyzed and identified as *An. messeae* (196; 77.7%) and *An. maculipennis s.s*. (56; 22.2%). Most of the mosquitoes were caught in Veneto, in Verona and Rovigo provinces, near farms, rivers and paddy fields that could explain the occurrence of these two zoophilic species (*An. messeae* and *An. maculipennis* s.s). According to our data, the main vectors of malaria *(An. labranchiae* and *An. sacharovi*) are absent or present at very low density. However, the traps used are not the best devices to capture anophelines and the presence of other species in the area cannot be excluded. An entomological surveillance specific for *Anopheles* is needed, in relation also to the new climatic, environmental and socio-economic changes.

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Updates on Phlebotomine sand flies in Sardinia (Italy)

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Keywords: phlebotomine, Sardinia, Leishmaniasis, sand flies

INTRODUCTION. Leishmaniasis by *Leishmania infantum* is an endemic disease in the Mediterranean area and also in Sardinia island (Italy). Parasites are transmitted by phlebotomine sandflies, and infected dogs represent the main domestic reservoir (Maroli 2008). To date, 42 *Phlebotomus* species are proven or suspected vectors of human leishmaniases in the Old World (Maroli 2013). The present research provides updated informations on the distribution and ecology of phlebotomine sand flies in Sardinia, two decades after the last survey carried out in the island by Maroli (1994).

MATERIALS AND METHODS. The was carried out from April to November 2017. Five capture sites have been placed near animal shelters or kennels once a week during the breeding season in zones with different enviroments and weather. Three capture sites were allocated in Sassari (one in the Department of Veterinary Medicine, one in a kennel and one in a periurban area), one in Tortolì (NU) and one in Decimomannu (CA). Two collection methods were used for every site: sticky traps and light traps (LaikaTrap – LaikaLab S.R.L.). All specimens captured were stored in 70% ethanol until analysis, and then observed at stereomicroscope, separated by sex and morphologically identified.

RESULTS AND CONCLUSIONS. A total of 513 Phlebotomine specimens were collected, belonging to the genus *Phlebotomus* and *Sergentomyia*. In particular the species *Phlebotomus perniciosus* (248/513, 48.3%), *Phlebotomus perfiliewi* (237/513, 46.2%), and *Sergentomiya minuta* (28/513, 5.5%). The seasonal activity of adult sand flies started at the end of May and ended in the second half of October with the highest number caught in August (*P. perniciosus* 38%, *P. perfiliewi* 45%).

Our data confirmed the presence of the three species found by the previous studies carried out in Sardinia by Bettini (1983) and Maroli (1994), even comparing data, the distribution of the species appeared modified, with the prevalence of *P. perfiliewi* increased (from 4.12% to 45%; $P < 0.0001$) compared to those found for *P. perniciosus* (from 78% to 38%; $P < 0.0001$).

Are dogs living on different level floors equally exposed to sand fly bites?

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Keywords: Phlebotomine sand fly, *Phlebotomus perniciosus*, floor, height

INTRODUCTION. The epidemiology of *Leishmania infantum* is strongly connected with that of its natural vectors, the sand flies. These insects are abundant in rural and peri-urban environments being the distribution manipulated by the biotope and environmental features. It has been suggested that height may limit the presence of sand flies because of their inability to ascendant fly. However, scant information is available on sand fly presence at different heights in urban buildings. Therefore, in this study we investigated the presence and the variety of sand fly species in the floors of a building.

MATERIALS AND METHODS. The study was conducted in a three-storey building, located in an urban area in the municipality of Messina. In the building the presence of humans and animals (i.e., dogs, cats and pigeon) was homogeneously distributed in each floor. A LED light trap (Laika 3.0 trap) was placed in the balcony of each floor (i.e., ground, 1st, 2nd, 3rd) and activated monthly (from July to September 2018) for 15 consecutive days (from 06:00 p.m. to 06:00 a.m.). Collected sand flies were stored according to floor and date and identified using morphological keys.

RESULTS AND CONCLUSIONS. Although any statistical difference was observed in the sand fly capture among floors, nearly half of the sand fly positive captures was recorded at the 3rd floor (20 out of 45), followed by ground floor (13 out of 45), 1st floor (9 out of 45) and 2nd floor (5 out of 45). A total of 67 (13 males and 54 females) sand flies were collected. Both *Phlebotomus* (11 *P. perniciosus* and 1 *P. neglectus*) and *Sergentomyia* (55 *S. minuta*) genera were trapped in all the examined floors. *Phlebotomus perniciosus* was mainly captured at the 3rd floor (i.e., 8 specimens) respect to the other floors (i.e., 1, 2 and 0 specimens at ground, 1st and 2nd floors, respectively). According to these findings, it may be speculated that the risk for sand fly bites is evenly distributed in a four-floor building, and, thus, dogs are equally exposed to *Leishmania* transmission risk regardless the floor of housing.

Pilot validation of mosquito nuisance assessment by ZanzaMapp, a mobile application to involve citizen in mosquito monitoring

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Keywords: mosquito, Aedes albopictus, monitoring, citizen science

INTRODUCTION. The global spread of invasive tropical mosquito species such as *Aedes albopictus* has expanded the risk of transmission of arbovirus (e.g. dengue and chikungunya) to previously unaffected European regions, as highlighted by the ~500 chikungunya cases reported from Italy in 2017. Wide-scale monitoring of mosquito nuisance and of risk of arbovirus transmission is difficult to implement and current monitoring schemes are limited by lack of cost-effective tools and adequate budget. To overcome these limits we developed a mobile application that allows users to report geo-localized mosquito presence by filling in a short questionnaire. In 2016 breeding seasons, we gathered >24.000 records, which represent a very encouraging feature, giving the limited effort invested in publicizing the app. We here present results from a pilot small-scale study carried out in Procida island (Naples) to validate this novel monitoring approach by comparing data obtained from citizen via Zanzamapp with data obtained by expert entomologists via Human-Landing Catches (HLCs).

MATERIALS AND METHODS. Zanzamapp data collection and daily-HLC were carried in a single week in September 2016. The relationship between the two sets of data was analysed in different spatio-temporal windows (i.e. Zanzamapp data were compared to mosquito collections at different intervals before HLC and in different buffers around HLC sites)

RESULTS AND CONCLUSIONS. Results showed a positive relationship between the weighted mean of Zanzamapp records and HLC data. The strongest correlation was found when considering Zanzamapp records obtained within 3-days from HLC and in a 100-m buffer from the HLC-site. These results support the possibility to obtain valuable information by a citizen science approach and encourage further studies to optimize the analytical approach needed to exploit Zanzamapp records for production of "real-time mosquito nuisance maps" which could complement and reinforce traditional monitoring schemes and could be applied beyond regional and national borders.

Attractiveness of different coloured LEDs for phlebotomine sand fly monitoring

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Keywords**:** Phlebotomine sand fly, *Phlebotomus perniciosus*, LED, colours

INTRODUCTION. Light traps are one of the most common tools for phlebotomine sand fly collection. Recently the use of LED lamps was proposed as an upgrade to the classical light trap because of the positive effects on trap design, weight and battery life. However, the attractiveness of different coloured LED to sand flies species of Mediterranean has not been investigated, so far. In this study, the capture performances of five light traps equipped with LED of different colour were evaluated and compared to a classical light trap model.

MATERIALS AND METHODS. From May to October 2017, a classical light trap equipped with incandescent light and 5 Laika traps 4.0 equipped with LED of different colours and wavelengths, specifically blue, UV, white, green and red LEDs were placed in a suburban area, endemic for canine leishmaniosis in Sicily. Traps were located at 50 cm and at 3 m of distance between them, and were activated biweekly for three consecutive days from 06:00 pm to 07:00 am. Captured phlebotomine sand flies were stored according to traps and date and identified at species level using morphological keys. Two-way analysis of variance (ANOVA) was applied in order to evaluate the effect of time and LED colours on sand fly species and abundance.

RESULTS AND CONCLUSIONS. Overall, 411 phlebotomine sand flies, belonging to 3 species *Phlebotomus perniciosus* (n= 298, 141 males and 157 females), *Sergentomyia minuta* (n=110, 48 males and 62 females), and *Phlebotomus neglectus* (n=3, 2 males and 1 females) were collected. The highest number of phlebotomine sand flies was captured on June ($P<0.01$) and by UV LED ($P<0.01$). As regards to species, *P. perniciosus* was mainly captured by UV LED on June ($P<0.01$). No effect of time or LED colour was recorded for *S. minuta* and *P. neglectus*. The results of the present study suggest that light trap equipped with UV LED can represent an effective alternative to classical light trap model for monitoring of phlebotomine sand fly in the Mediterranean.

Pyrethroid susceptibility status of *Aedes albopictus* and *Culex pipiens* populations across Italy

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Keywords: Insecticide resistance, vector control, *Aedes albopictus*, *Culex pipiens*

INTRODUCTION. Invasive *Aedes albopictus* and indigenous *Culex pipiens* are the most abundant mosquito species in urban areas in Italy. Both species can create considerable nuisance to citizens, and may also transmit arboviruses such as Chikungunya, Dengue (*Ae. albopictus*) and West-Nile (*Cx. pipiens*). Although insecticides represent a fundamental tool to reduce mosquito abundance and limit disease transmission, little is known on insecticide susceptibility of these species. Previous studies allowed to highlight resistance to pyrethroid insecticides commonly used to reduce adult abundance in *Ae. albopictus* populations from Ferrara (Emilia-Romagna) and Bari (Puglia) provinces. In the present study, we focused on populations of *Ae. albopictus* from Emilia-Romagna, Lazio and Calabria, where the species has been responsible for Chikungunya outbreaks in 2007 and in 2017. Furthermore, we carried out a first assessment of levels of susceptibility to pyrethroids in Italian populations of *Cx. pipiens*, in order to compare levels of resistance among the two species.

MATERIALS AND METHODS. Insecticide resistance tests were performed following WHO protocols by exposing adult mosquitoes for 1 hour to permethrin, α-cypermethrin, deltamethrin and recording mortality at 24 hours after exposure.

RESULTS AND CONCLUSIONS. Our data show a reduced susceptibility to permethrin in some *Ae. albopictus* populations from Lazio. Moreover, most *Cx. pipiens* populations analysed exhibit a clearly lower susceptibility than sympatric *Ae. albopictus*, with mortality being in few sites below 30%. Results suggest that insecticide resistance phenotypes are present in Italy, with important differences between species and sites. The lower levels of susceptibility recorded in *Cx. pipiens* populations could suggest that insecticide spraying is mostly affecting nocturnal *Cx. pipiens* rather than diurnal *Ae. albopictus*, against which they are targeted. Overall, results stress the need to carefully monitor insecticide resistance spread in Italy, also by means of molecular markers currently under investigation.

Comparative testing of two sticky traps to monitor resting *Aedes albopictus* and *Aedes koreicus* (Diptera; Culicidae) in Italy

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Keywords: Aedes albopictus, Aedes koreicus, resting trap, monitoring

INTRODUCTION. Among invasive *Aedes* species spreading in Europe, *Aedes albopictus*, *Ae. japonicus* and *Ae. koreicus* are currently present in Italy. Collection methods used for their sampling are based on ovitraps to collect eggs and BG-Sentinel to catch host-seeking mosquitoes; effective tools to collect blood-fed resting females are currently unavailable, despite the interest in studying the species feeding behaviour. We here present a comparative evaluation of the effectiveness of two sticky devices: Sticky Trap (ST) and Sticky Resting Box (SRB), to collect resting and blood-fed females of the two species. The ST has been developed and tested for sampling of *Ae. albopictus* whereas the SRB was used in outdoor sampling of *Anopheles* spp. in Africa.

MATERIALS AND METHODS. The study was performed both in the field, in an area where the two species are sympatric, and in the semi-field, i.e. in a green-house where 30 fed females of each species were contemporarily exposed to the two traps. The two traps were also compared with ovitraps by a 3x3 Latin square.

RESULTS AND CONCLUSIONS. In the field, ST collected higher number of females than SRB (mosquito/trap/day: SRB-*Ae. albopictus* 0.52; SRB-*Ae. koreicus* 0.04; ST-*Ae. albopictus* 5.74; ST-*Ae. koreicus* 0.30; Kruskal-Wallis $P < 0.0001$) and no blood-fed *Ae. koreicus* females were sampled with SRB (*Ae. albopictus* in ST=39; in SRB=1). The fed specimens sampled were all *Ae. albopictus* (ST=39; SRB=1). In the green-house, the two traps showed non-significant differences for both species (three replicates; median rates: SRB-*Ae. albopictus* 5%; SRB-*Ae. koreicus* 6%; ST-*Ae. albopictus* 17%; ST-*Ae. koreicus* 3.5%; Kruskal-Wallis $P = 0.44$). Results showed that both devices may effectively collect the two species, although the very low *Ae. koreicus* densities outdoors did not allow to definitively assess the field effectiveness of SRB. Due to the rapid expansion of *Ae. koreicus* and its establishment in new territories, this traps should be employed as additional tools to improve the surveillance of invasive mosquito species and obtain new information about its ecology.

Gene silencing in the malaria vector *Anopheles stephensi* to increase insecticide susceptibility

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Keywords: Vector-control, mosquito defensome, siRNA, Morpholinos

INTRODUCTION. The use of chemical insecticides still represents the backbone for the control of vector-borne diseases, including malaria. In the last decades, insecticide applications in treated nets and indoor spraying (IRS) have led to a drastic reduction in malaria mortality. Unfortunately, this conquer in human health is overwhelmed by heavy threats: the increase of environmental pollution and the emergence of insecticide resistance in vector populations. One of the major components of the "Defensome-machinery", involved in mosquito resistance, are efflux pumps (ABC transporters) important for processes of detoxification. Our overall aim was to assess an environmentally-safe control method, inhibiting a selected ABC transporter gene, in order to increase the susceptibility and mortality of the mosquito *Anopheles stephensi* against the insecticide.

MATERIALS AND METHODS. The inhibition of ABC gene was achieved through two different methods of species- and gene-specific post-transcriptional silencing, siRNA (RNAi method) and antisense Morpholinos (MOs), combined with the insecticide permethrin. ABC-G4, the transporter gene more closely involved in the detoxification process of *An. stephensi*, was selected as a target for both inhibition methods. Bioassays were performed on *An. stephensi* larvae which were first pre-treated with siRNA against ABC-G4, and then exposed to permethrin (control group treated with permethrin alone). The same experimental design was performed through MOs against the ABC-G4 gene. In both bioassays, mortality and relative expression of the ABC-G4 gene were analyzed at different time points (6h, 24h).

RESULTS AND CONCLUSIONS. The increased mortality in larvae treated with siRNA or with MOs confirms the role of ABC-G4 in permethrin detoxification and the potential of post-transcriptional gene silencing as a tool for the development of eco-compatible strategies for vector control. From this perspective, our results also suggest that the application of MOs could represent an innovative and effective contribution to integrated control strategies

SESSIONE 13A

ALIMENTI E PARASSITI

Long term study on *Trichinella* muscle larvae and circulating IgG in pigs

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Keywords *Trichinella*, swine, muscle larvae, circulating antibodies

INTRODUCTION. In the European Union, trichinellosis in humans is strongly reduced to less than 150 infections in 2016 (EFSA, 2017, EFSA J, 15:5077). However, pork from domestic pigs and wild boar still represents the main source of human infections worldwide (Murrell and Pozio, 2011, Em Inf Dis, 17:2194-2202). Even if parasites of the genus *Trichinella* are disappeared from most of pig farms of EU, these pathogens are still circulating among backyard and free-ranging pigs of five EU countries including Italy. To monitor *Trichinella* infections in domestic and wild swine, serology has been proposed as a cheap and fast method. However, our knowledge on circulating IgG kinetic in relation to the larval burden in muscles and age of the infection, is limited. The aim of the present study was to monitor the relationship between the IgG kinetic in serum samples and the larval burden in muscles of domestic pigs infected by *Trichinella spiralis*, *T. britovi* and *T. pseudospiralis* for a two-year-period.

MATERIALS AND METHODS. Sixty pigs of about 40 kg were infected per os with 10,000 larvae/animal of *T. spiralis* (20 animals), *T. britovi* (20 animals) and *T. pseudospiralis* (20 animals). Blood was collected one day before infection and one per month up to slaughtering. Four animals of each of the three groups were slaughtered at 2, 6 and 12 months post infection. At slaughtering, 100 g were collected from eight muscles (diaphragm pillars, tongue, masseter, intercostal, loin, shoulder, anterior and posterior leg muscles). Serum samples were tested by ELISA and Western blot for confirmation. Muscle samples were digested and larvae per gram counted.

RESULTS AND CONCLUSIONS. Infecting *T. spiralis* larvae were still present in all the eight pig muscles one year p.i. A small amount (0.2 larvae/g in the diaphragm) of *T. britovi* larvae was detected up to 6 months p.i., but no larvae were detected 12 months p.i. *T. pseudospiralis* larvae were detected in the muscles 2 months p.i., but no larvae were detected 6 months p.i. All animals seroconverted between 35 and 40 days p.i., and the highest IgG level was detected 2 months p.i. irrespective of the *Trichinella* species. One year p.i., circulating IgG were no more detectable in sera of *T. pseudospiralis* infected animals. The level of detected circulating IgG was still high (>50% ELISA index) in *T. britovi* and *T. spiralis* infected pigs, even if no larvae were detected in *T. britovi* infected pigs. The experimental study is still in progress.

Occurrence of *Anisakis* spp. larvae in products made of herring (C*lupea harengus*)

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Keywords: Anisakid larvae, artificial digestion, semi-preserved seafood products, Italy

INTRODUCTION. Herrings are the third most commercialized species in the European Union (EUMOFA report, 2017) and common hosts of third stage *Anisakis* spp. larvae (Levsen & Lunestad, 2010, Vet. Parasitol, 171:247–253). Different kind of ready to eat (RTE) herring products are available on the market. Although these products undergo technological processes able to kill viable parasites, exposure to dead larvae may cause allergic reactions (Audicana & Kennedy, 2008, Clin. Microbiol. Rev, 21:360-379). Given the scarcity of available data on anisakids in RTE herrings sold on the Italian market, the aim of this study was to assess the occurrence of *Anisakis* spp. larvae in various kind of such products.

MATERIALS AND METHODS. 120 products consisting of 50 smoked whole specimens and 70 filleted products (25 smoked, 30 smoked and marinated, 15 canned) were sampled between 2016 and 2018. In the case of whole herrings, viscera and muscle were visually inspected and separately digested using Trichineasy® (CTSV srl, Brescia). Filleted products, including marinating liquid if present, were also visually inspected and digested. Nematodes collected during visual inspection and after digestion were identified to genus level, counted and stored. A subsample (N=150) was molecularly identified targeting the *COII* gene. The positivity rate and the larval density per gram (number of larvae per g of examined tissue), both at muscle and visceral level, when present, were calculated; differences between whole and filleted products were investigated by Chi-square and Kruskal-Wallis tests.

RESULTS AND CONCLUSIONS. At least one *Anisakis* spp. larva was found in 56 products (46.7%), with a total of 1715 dead larvae collected (range 0-172 larvae/product). The majority (1559, 91%) were found in the viscera of 49 of the 50 whole herrings (98%). Interestingly, a highly significant difference ($p<0.0001$) was observed between the positivity rate and larval density of the remaining 156 larvae found at muscle level, as 149 larvae were found in the muscle of 31 whole herrings (positivity rate 62%, 0.022 larval density/g) while only 7 larvae were found in the 70 filleted products (positivity rate 10.7%, 0.001 larval density/g). Larvae were molecularly identified as *A. simplex*. Although no live larvae were found, dead visible larvae represent a defect and make the product unfit for human consumption (Reg. (EC) No 178/2002). Especially in the case of heavy infections, larvae may be evident and cause consumers' rejection. In addition, the allergenic potential of dead larvae in sensitized subjects is debated (Daschner et al., 2012, Trends Parasitol, 28:9-15). The significant difference between muscle tissue of whole and filleted herrings, likely due to differences in the production process, might result in different level of exposure depending on consumers' preferences.

Lab-on-chip molecular integrated platform to detect *Toxoplasma gondii* from several matrixes

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Keywords: *Toxoplasma gondii*, lab-on-chip, Real Time PCR

INTRODUCTION. Due to the increased widespread of food- and water-borne parasites, there is an urgent need for the development of "lab-on-chip" portable devices, which significantly makes quicker, easier and accurate the detection of parasites, and can be used in the field and on different substrates (food, water, clinical samples)**.** The efficiency of Q3 system (ST-Microelectronics^{R}), a Real Time PCR-based miniaturized platform, was previously investigated for the detection of zoonotic protozoans, including *Toxoplasma gondii* in mollusk species according to Real Time PCR protocols, previously set up in our laboratories (Giangaspero et al., 2018, Food Res Int, submitted). Being *T. gondii* successfully detected by the Q3 platform, the aim of the present work was to evaluate the performance of Q3 platform in detecting this protozoan in several food matrices and human clinical samples and to compare the results with those obtained by Real Time PCR.

MATERIALS AND METHODS. A total of 100 samples (50 previously tested positive and 50 tested negative to standard Real Time PCR - i.e., 54 pig meat, 10 Ready To Eat (RTE) salad, 18 bivalve mollusks (*Mytilus galloprovincialis*) and 18 human amniotic fluids were subjected to Q3 platform and the results were statistically evaluated.

RESULTS AND CONCLUSIONS. The prevalence of *T. gondii* DNA was 50% in the RTE salad and human samples, 66.76% in bivalve mollusks and 59.26% in pig meat samples. Compared to Real Time PCR, the sensitivity of Q3 platform was 100% for all tested substrates while the specificity was 100% for RTE salad and human samples, 81.48% for meat pig and 66.67% for bivalve mollusks.

In the light of the obtained results for vegetable and human samples, the Q3 system could be considered a valid alternative for a rapid *T. gondii*-screening test.

Future analyses (matrix purification with different techniques, more specific primers, etc.) will be carried out in order to fill the gap in the low specificity registered in samples from pig meat and bivalve mollusks, and to extend the detection also to other food- and water-borne parasites.

Seroprevalence of *Toxoplasma gondii* infection in beef cattle raised in Italy: a multicenter study

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Keywords: Serology, food-borneparasite, Toxoplasma gondii, zoonoses

INTRODUCTION. Toxoplasmosis represents an important public health issue, with the consumption of raw or undercooked meat being a major way of human infection. Among domestic animal species destined to human consumption, cattle are considered less important in the epidemiology of *Toxoplasma gondii* than small ruminants or pigs. However, considering the cultural and traditional habits of consuming products based on raw meat (e.g., roastbeef, tartare), particularly in certain countries and regions, the sanitary risk posed by *T. gondii* infection in cattle cannot be overlooked. Therefore, to update information on *T. gondii* in beef cattle reared in Italy, a multicentric seroepidemiological survey was designed in Northern regions (Lombardy, Piedmont, Trentino Alto Adige and Liguria regions) and in Sardinia.

MATERIALS AND METHODS. An overall number of 1650 individual serum samples were collected from 66 farms. Fourteen beef breeds were represented in the sampling, besides cross-breed; both young and adult animals were sampled. Collected sera were analyzed with commercial ELISAs (ID Screen®Toxoplasmosis Indirect Multi-species – ID.Vet) for the detection of anti-*T. gondii* antibodies. Individual and herd data were analyzed by means of generalized linear model (GLM), using SPSS v.19.0 (IBM, USA).

RESULTS AND CONCLUSIONS. A *T. gondii* prevalence of 11.4% was recorded, with values ranging from 5.3% in Liguria to 18.6% in Piedmont region. Both young and adult animals tested positive (p-value>0.05). Slightly lower seroprevalence values were recorded in cattle born in the sampled farms (9.4%) if compared to imported animals (12.4%); considering the latter, a higher number of sero-reactors were recorded in animals imported from abroad (13.9%) than in those originating from other Italian regions (7.5%). Noteworthy, imported animals consisted mainly in young steers from France, extensively reared until being imported in Italy and thus highly exposed to the risk of acquire *T. gondii* infection at pasture. The spread of *T. gondii* in beef cattle destined to Italian consumers is confirmed, suggesting the need of continuous monitoring of the infection also in this species.

SESSIONE 13B

ENTOMOLOGIA MEDICA E VETERINARIA

Monitoring sand fly populations in Setif: a new focus of cutaneous leishmaniasis in Algeria

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Keywords: leishmaniasis, sand flies, morphotaxonomic, Algeria

INTRODUCTION. Leishmaniases are vector-borne diseases caused by protozoan parasites of the genus *Leishmania* (Trypanosomatida: Trypanosomatidae), endemic in tropical and subtropical areas, including the Mediterranean Basin (Gramiccia and Gradoni, 2005, J. Parasitol, 35:1169-1180). To date, approximately 1.2 million cases of cutaneous leishmaniasis occur each year in countries where the disease is endemic (Alvar et al., 2012, PLoS ONE, 7:e35671). *Leishmania* parasites are transmitted to vertebrates by the bite of infected female phlebotomine sand flies. However, in spite of the scientific knowledge gained over the last decades, the understanding of the ecology of phlebotomine sand flies in some areas of Algeria is still fragmentary. The aim of the present study was to assess the phlebotomine sand fly population endemic in southern Algerian.

MATERIALS AND METHODS. From March 2016 to November 2017, 1804 phlebotomine sand flies were collected from 12 sites in the southern part of Algeria, using sticky and CDC light traps. All female specimens were identified using appropriate morphological keys (Dedet et al., 1982, Bull. Soc. Pathol. Exot, 75:588-598; Killick-Kendrick 1990, Med. Vet. Entomol, 4:1-24).

RESULTS AND CONCLUSIONS. Eight species of phlebotomine sand flies belonging to the genera *Phlebotomus* and *Sergentomyia* were identified. The most abundant species was *Phlebotomus perniciosus* (n= 1324, 73.39%), followed by *Phlebotomus papatasi* (n= 287, 17.09%), *Phlebotomus sergenti* (n= 87, 4.82%), *Sergentomiya minuta* (n= 23, 1.27 %), *Sergentomiya fallax* (n= 7, 0.39 %), *Phlebotomus bergeroti* (n= 4, 0.22*%), Phlebotomus Phlebotomus chabaudi* (n=3; 0.16 %). and *Phlebotomus longicuspis* (n=3; 0.16 %). The results herein obtained confirm the ability of sand flies to move from the arid to the semi-arid areas adapting to a new area of Algeria (Setif) . The finding of *P. perniciosus* and *P. papatasi* in Setif confirms the presence of phlebotomine sand fly vectors in an area considered to be free of leishmaniasis. This may represent a risk for human health, since *P. perniciosus* and *P. papatasi* are vectors of *L. infantum* and *L. major*, respectively.

Molecular screening of pathogenic and symbiotic bacterial species in African ticks

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Keywords: bacterial pathogens, Symbiosis, *Midichloria mitochondrii*, *Ixodes ricinus*

INTRODUCTION. As haematophagous ectoparasites, hard ticks (Acari:Ixodidae) are vectors of numerous disease-causing bacterial pathogens worldwide. In Africa, the most important tick-borne bacteria are considered those belonging to the genera *Rickettsia*, *Anaplasma*, *Ehrlichia*, *Babesia*, and *Theileria* (Jongejan & Uilenberg, 2004, Parasitology, 129:S3-S14). On the other hand, very few data on bacterial symbionts of African ticks are found in the literature. Considering that symbiotic bacteria can play a key role in the host biology, and that they may also compete with or be beneficial towards pathogens transmission and biology, we investigated the co-presence of pathogenic and symbiotic bacteria in ticks collected in Africa.

MATERIALS AND METHODS. Taxonomic identification of ticks was performed by means of both morphological (light microscopy) and molecular (PCR) techniques. The ticks were then screened for the presence of bacterial species through *ad-hoc* molecular approaches (PCR).

RESULTS AND CONCLUSION. 250 ticks collected from Kenya, Nigeria, Madagascar, Egypt, and South Africa were taxonomically identified as belonging to *Ixodes*, *Amblyomma*, *Hyalomma* , *Rhipicephalus*, *Dermacentor*, and *Haemaphysalis* genera.

Pathogen screening provides additional information on pathogens circulation in Africa, confirming the presence of *Rickettsia* spp., *Anaplasma* spp., *Borrelia* spp., *Babesia* spp. and *Theileria* spp. Furthermore, our work provides insights on the African scenario of tick-symbiont associations, laying the foundation for functional studies aimed at interaction analyses with possible implications on pest control.

Midichloria mitochondrii localization and quantification in the organs of the hard tick *Ixodes ricinus*

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Keywords: Symbiosis, *Midichloria mitochondrii*, *Ixodes ricinus*

INTRODUCTION. Symbiosis between bacteria and arthropods is considered a relevant driver of their evolution (McCutcheon and Moran, 2011, Nat. Rev. Microbiol. 10, 13–26). A wide range of tick species harbour bacterial endosymbionts in various tissues, many of them playing important roles in the fitness and biology of their hosts, in the modulation of their vectorial ability and their chemical acaricides resistance.

The females of *Ixodes ricinus* (Ixodidae), the most common hard tick in Europe and vector of tick borne zoonoses, harbour the intracellular endosymbiont *Midichloria mitochondrii* (order *Rickettsiales*) with a 100% prevalence, suggesting a mutualistic relationship. Considering that the tissue distribution of a symbiont might be indicative of its functional role in the physiology of the host, we investigated *M. mitochondrii* specific localization pattern and the relative quantification in selected organs of *I. ricinus*.

MATERIALS AND METHODS. We dissected salivary glands, gut, ovary, rostrum, tracheae and Malpighian tubules from unengorged and semi-engorged *I. ricinus* ticks. As negative control, we used the tick *Pholeoixodes hexagonus* (Ixodidae), that does not harbour *M. mitochondrii*, collected from cats in the same area. The dissected organs were then subjected to Real Time PCR (qPCR) to quantify *M. mitochondrii* (Sassera et al., 2008, Appl. Environ. Microbiol. 74, 6138–6140). *Midichloria*-rich organs were then subjected to specific immunofluorescence assay.

RESULTS AND CONCLUSION. *M. mitochondrii* was abundant in ovaries, Malpighian tubules and salivary glands of semi-engorged *I. ricinus* and in ovaries and tracheae of unengorged *I. ricinus*. Immunofluorescence performed on ovaries shows that the symbiont cells occupy most of the oocytes cytoplasm, suggesting a role of the bacterium in the host reproduction. In the Malpighian tubules, *M. mitochondrii* was detected in the proximal region of the tubules, indicating a potential role in the maintenance of homeostasis, water balance, excretion, and in the detoxification processes. Furthermore, the localization in the salivary glands is coherent with our previous hypotheses of horizontal transmission of *M. mitochondrii*. Analysis of the metabolic pathways of *M. mitochondrii* is underway to identify host supportive functions.

Field survey on *Phortica variegata* and the high infection rate of *Thelazia callipaeda* in Lazio and Basilicata regions

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Keywords: *Thelazia callipaeda*, *Phortica variegata*, eyeworm, Lazio

INTRODUCTION. The eyeworm *Thelazia callipaeda* (Spirurida, Thelaziidae) parasitizes the conjunctival sac and the nictitating membranes of domestic and wild carnivores, rabbits and humans. The fruit fly *Phortica variegata* (Diptera, Drosophilidae) is until now the only proven vector of this eyeworm species responsible for canine thelaziosis, due to the lacryphagous behaviour of male insects. Despite thelaziosis is undoubtedly an emerging zoonotic disease in Europe, the peculiar ecology of *P. variegata* associated to wild areas dominated by the presence of Turkey oak tree (*Quercus cerris*) seems to limit its local distribution to scarcely populated areas where the major reservoirs are domestic and stray dogs (Otranto et al., 2006, Med Vet Entomol 20:358-64).

MATERIALS AND METHODS. Sampling of flies was performed during September 2017 in two field sites: Oliveto Lucano (MT, Basilicata), an area known to be highly endemic for *T. callipaeda*, and Manziana (RM, Lazio), where data on this nematode in dogs and on its vector are unavailable. Flies were collected with a net: i) using a fruit bait, a white cloth bag containing fermented sliced fruit laced to the north side of an oak tree about 1m from the undergrowth; ii) using a human bait, waiting for the arrival of flies around the collector's face. The specimens were brought to laboratory, morphologically identified as *P. variegata* (Bächli et al., 2005, Fauna Entomologica Scandinava, 39:1-362) and molecularly analysed for *T. callipaeda* presence by conventional PCR (Otranto et al., 2005, Parasitology 131:847–855).

RESULTS AND CONCLUSIONS. Overall, a total of 172 *P. variegata* specimens were collected in both sites (Manziana: 74 males, 6 females; Oliveto Lucano: 91 males, 1 female). *T. callipaeda* positive specimens were found in both sites, Oliveto Lucano and Manziana (5.5% and 6.8% of males, respectively), suggesting high levels of transmission of this eyeworm by *P. variegata* males also in the latter area. Contrarily to the sampling site of Oliveto Lucano, that is an almost uninhabited wild area about 5 km far from the village, the sampling site in Manziana is a protected area enclosed into an urbanized context highly frequented by both humans and dogs. Then the epidemiological consequences of the zoonotic transmission potential of *T. callipaeda* in urbanized areas of Central Italy such as Manziana are not negligible and need to be carefully taken into account.

Creation of a reference sample-collection for morphological and biomolecular identification of arthropods of medical and veterinary importance

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Keywords: mosquito species, identification, reference specimens

INTRODUCTION. Accurate identification of mosquitoes is of paramount importance to evaluate the occurrence of pathogen-harbouring species. Morphological identification requires entomological expertise and well conserved specimens. Recently molecular methods and mass-spectrometry have become an invaluable tool for species identification. The aim of this study was to identify mosquitoes with these tools to create a species reference database.

MATERIALS AND METHODS. Twenty-four mosquitoes species (two specimens for species) belonging to six genera (*Aedes*, *Anopheles*, *Coquillettidia*, *Culex*, *Culiseta* and *Ochlerotatus*) collected in north-east Italy, Sweden and Switzerland were morphologically identified using stereo microscope and taxonomic identification keys (Severini et al., 2009 Frag. Entomol., 41(2):213-72). Each mosquito was dissected into head, thorax, legs and abdomen. The abdomen was used for molecular analysis of tree genes (ND4, COX1 and β-tubulin), while the head, thorax and legs were used to create three databases of reference spectra and to perform protein profiling analysis with MALDI-TOF-MS (Bruker Daltonic).

RESULTS AND CONCLUSION. A scientific collection of reference samples was created, with detailed photographic elements, DNA sequences and protein profiles. Methodology for DNA barcoding involves sequencing followed by comparison with sequences previously deposited into the database GenBank®. Molecular analyses showed that the COX1 gene is a suitable gene for DNA barcoding and it is highly efficient to discriminate closely related species; moreover reference sequences are well represented in GenBank®. The ND4 gene showed high efficiency for the identification of species of *Aedes*, *Ochlerotatus*, *Anopheles* and *Culiseta* genera, but it was not a phylogenetically informative marker for the species of *Culex* genus. The β-tubulin gene appears a discriminative gene, but the lack of reference sequences in GenBank® could be a limitation for its use. MALDI-TOF-MS analysis highlighted that mosquito thorax provides better identifying protein spectra, due to the higher level of protein than head and legs, that however are excellent starting matrixes for species identification. In fact, even from a single mosquito's leg it was possible to identify the mosquito species. Currently, a database including 24 mosquito species has been implemented and specimens are available upon request as reference specimens for the scientific community.

SESSIONE 14

PARASSITOSI DEGLI ANIMALI DA REDDITO

Onchocerca fasciata (Filariodea: Onchocercidae) in camels (*Camelus dromedarius*) from Iran: morphological and molecular characterization

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Keywords: *Onchocerca fasciata*, Vector-borne disease, skin nodules, dromedary

INTRODUCTION. Skin nodules caused by *Onchocerca fasciata* Railliet and Henry, 1910 (Spirurida, Onchocercidae) are a common finding in dromedary camels (El-Massry and Derbala, 2000, Vet. Parasitol, 88:305-312), though they have a minimal clinical impact. Despite the wide geographical distribution (Iran, Saudi Arabia, Jordan, Egypt, Ethiopia and Kenya) of onchocercosis in dromedaries (Sazmad and Joachim, 2017, Parasite, 24:21), the biology of this parasite, including the species of insect vectors, has not been studied in detail. Here we present a reappraisal of the morphological and morphometric description of *O. fasciata* based on light microscopy.

MATERIALS AND METHODS. A total of 76 dromedary camels aging from 1 to 12 years old and of both sexes were slaughtered in Kerman, southeastern Iran, and inspected for the presence of skin nodules. Extra tissues surrounding the nodules were removed carefully and digested by collagenase to isolate the adult worms. Individual entire and broken worms were isolated under a dissecting microscope, sex was determined and each worms' length was measured. In addition, cuticular ridges and striae were examined under light microscope while mineralized worms were identified by obliteration of cuticular ridges and vertical annulation. Sequence analysis of 12S rDNA and cytochrome *c* oxidase subunit 1 mitochondrial genes was performed.

RESULTS AND CONCLUSIONS. Twenty-four out of 76 examined camels (31.5%) harbored single or multiple skin onchocercian nodules on the neck region. From 38 isolated nodules, 23 contained viable worms (60.5%). Twenty female worms were recovered from nodules, but no males. The female body was 63-117 cm (average 90.9±19.4 cm) long and 176-210 µm (average 196.3 ±11.3 µm) wide. The identification of *O. fasciata* specimens was confirmed by sequencing analysis of two mitochondrial genes with estimation of 0.4% divergence with available *O. fasciata* sequences. Rate of infection in the present study (31.5%) was in the range previously reported in others countries. The molecular identification is coherent with the available sequences and published data. Results herein presented contribute to the knowledge on the distribution and morphology of *O. fasciata* instrumentally to elucidate the life cycle of this *Onchocerca* species.

Investigating on *Besnoitia besnoiti* (Apicomplexa, Sarcocystidae) in naturally infected dairy cattle by an integrated approach

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Keywords: Bovine Besnoitiosis, Serology, Haematology, Molecular Biology

INTRODUCTION. Bovine besnoitiosis, caused by *Besnoitia besnoiti*, is a (re)emerging disease in Europe, including Italy. However, its economic impact is scarcely considered and generally underestimated and there are still little studied aspects concerning both the parasite and the disease. Following a natural outbreak of besnoitiosis in a dairy herd, a study was planned to characterize *B. besnoiti* infection in cattle through a multidisciplinary approach.

MATERIALS AND METHODS. Suspicious abortions and clinical cases of besnoitiosis were reported in a dairy farm (September 2017, Northern Italy) housing 216 Holstein Friesian cattle. Blood samples were collected; haematological and serological analyses for *B. besnoiti* antibodies using ELISA (ID Screen® Besnoitia Indirect 2.0, IDVET) and confirmatory Western Blot were performed. Histology and molecular (endpoint PCR targeting ITS-1 region and sequencing) analyses of tissues from a slaughtered cow with chronic besnoitiosis were carried out.

RESULTS AND CONCLUSIONS. Out of 59 animals resulted positive to ELISA, 50 (23%) were confirmed by Western Blot. *B. besnoiti* prevalence was higher in cows (41%) than in calves (12%); any heifer did not result positive to the infection. Considering haematological parameters, a significant shift in the differential leucocyte formula from lymphocyte to granulocyte was recorded in infected cows (Mean±S.D.: L=46.1±18.4, G=53.9±18.4) if compared to negative animals (Student's T-test, p=0.012). This finding could be helpful in clinical diagnosis, treatment and control of besnoitiosis providing information on infection pathogenesis and subsequent immune response. Histology revealed a high load of *B. besnoiti* tissue cysts in skin, vulva, muzzle, sclera, eyelid, respiratory tract, emphasizing the possibility of parasite transmission through direct contact among animals. *B. besnoiti* was confirmed by PCR in other organs (heart, liver, aorta wall, tonsil) and especially in ovary, uterus and vulva, suggesting that the infection could affect cows' fertility and pregnancy. Parasite DNA was also found in masseters posing an important question for food security. Indeed, the presence of *B. besnoiti* in muscles has never been explored, being its impact on cattle health mainly considered restricted to the skin. Even if *B. besnoiti* is not considered zoonotic, further studies are needed to clarify if it could localize in other muscles. The study suggests that to investigate the dynamics of bovine besnoitiosis is mandatory associate clinical and various laboratory tests; the genetic characterization of the parasite and its eventual correlation with the disease outcome should also be included.

Impact of the 2016 earthquake in Central Italy on livestock farms and effects of tensile emergency shelters on animal health

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Keywords: natural disasters; livestock farms; animal health; intestinal parasites

INTRODUCTION. In August and October 2016, Central Italy was hit by a series of strong earthquakes, that led to the devastation of a large area and to the death of more than 300 persons. The situation was aggravated by an exceptionally cold winter season accompanied by heavy snowstorms. Whereas the majority of the population was displaced in hotels on the Adriatic coast, livestock farmers stayed in place in autonomously organized emergency facilities, such as campervan and mobile homes. Tensile shelters were provided to cattle and sheep farms during summer 2017. This study aimed at estimating a) earthquake damages on houses, cattle sheds, sheep pens and barns, b) earthquake effects on livestock animals and c) impact of tensile structures as livestock emergency shelters on animal health and intensity of infection with gastro-intestinal parasites in sheep and cattle.

MATERIALS AND METHODS. A semi-structured questionnaire was administered in 2017 (March –September) to 55 farmers of 11 communities of the “Alto Maceratese” area (Marche Region). An additional 24 farms were visited in 2018 (January - April) that have been provided with tensile structures for sheep and cattle. Faecal samples are being collected (April – May 2018) in 6 sheep and 6 cattle farms with ordinary shelters and tensile structures to assess whether livestock management in tensile structures may favour increased parasite burden.

RESULTS AND CONCLUSIONS. About 68% of houses and 56% of farm structures (barns, sheep and cattle shelters) of the 55 study farms, were damaged by the earthquake strokes. Sixteen of the 48 cattle breeder and 11 of the 31 sheep breeders reported damages on their animals, namely cases of deaths, miscarriages, precocious deliveries, reduced fecundity, reduced milk production and delayed growth of lambs. During the first 6 months after earthquake onset (tensile emergency structures haven’t been provided yet), various breeders (11/55) were constricted to keep their cattle and sheep in condemned shelters, outside of the damaged structures (8/55) or to accommodate them in shelters of neighbouring farms (8/55). By autumn 2017, installation of tensile shelters was completed on all livestock farms in need. Cattle and sheep farmers (n=18) interviewed after 2-4 months of use (April 2018) reported damages on the curtain-doors due to wind blasts and water tubes broken due to freezing. Concerns were expressed regarding lack of windows, extreme day-night temperature fluctuations and elevated humidity inside the shelters. Detailed results, including data on neonatal death rates of calves and lambs and density of intestinal parasites, will be presented at the congress.

MULTI-TEST MONITORING OF A "CYSTICERCOSIS STORM": A CASE REPORT

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Keywords: Bovine cysticercosis, monitoring, serology, copro-PCR

In late September 2016, the veterinarian of a traditional family-managed feedlot farm in Piedmont asked for consulting following condemnation of ten steer carcasses due to the presence of *Cysticercus bovis*. The multiple origin of condemned cattle and the epidemiological survey carried out pointed towards an internal source of infection. On face-to-face interview, two out of four family members reported (in one case with obvious reluctancy) infection with *Taenia saginata* in late 2015 and early 2016, respectively. Niclosamide treatment was prescribed but the older member refused and decided to treat herself with natural remedies. In October 2016, all cattle in the farm (N=142) were tested with the B158/B60 Ag-ELISA (Dorny et al., 2000, Vet.Parasitol, 88: 43-49) to detect current infection with cysticerci and 94 of them tested Ag positive (66.2%). In November 2016, stools of all family members were analyzed with a copro-PCR (Chiesa et al., 2010,Foodborne Pathog, Dis 7.10 1171-1175), confirmed by Sanger sequencing, showing that three of them (but not the one previously treated with niclosamide) were shedding *T. saginata* eggs. Two were apparently successfully treated while a second treatment was necessary to clear infection in the third patient. No family member was PCR positive at the end of 2016. Consecutive batches of incoming calves were serologically tested at approximately two month intervals for the whole length of the monitoring period (October 2016-September 2017). On each sampling date (8 in total), blood was collected by calves admitted since less than 3 weeks (Group 1) and between 3 weeks and 100 days (Group 2). While a single seroreactor was found in Group 1 (N=30), 71.4% of calves in Group 2 (N=105) tested seropositive until June 2017, suggesting that a significant number of animal had been infected shortly after arrival on the farm. No seroreactor was found in Group 2 calves in July and September 2017. Unexpectedly however, stool of the person previously reluctant to niclosamide treatment tested PCR-positive in June 2017. Under pressure of the relatives, she accepted to receive niclosamide treatment in our presence, and the other family members also asked to be "blind" treated (while PCR negative). Altogether, data suggest that after June 2017 (at approximately one year since the outbreak became patent) no infection occurred in incoming calves. Multi-test monitoring of the outbreak from onset to extinction provided interesting insights on (partially neglected) aspects such as poor awareness amongst farmers, trickiness of admitting infection, persisting hygienic weaknesses and (unexpected) reluctance to official treatments. It was also clear that medical and veterinary competences need better integration in a control perspective.

Endoparasites of livestock in Central Italy

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Keywords: cattle, sheep, endoparasites, central Italy

INTRODUCTION. Endoparasites of livestock may seriously impair welfare and production of ruminants. As few recent information are available on the epidemiology of these parasites in central Italy (Perrucci et al, 2007, Vet. Ital., 43:415-424; Secchioni et al, 2016, Large Anim. Rev., 22:195-201), the present study updates current knowledge on endoparasites affecting cattle and sheep reared in different areas of central Italy.

MATERIALS AND METHODS. In 2017, a total of 800 faecal samples was collected from 4 intensive dairy cattle farms from different sites of Umbria region and from 12 dairy and mix-breed sheep farms from Perugia (n. 5) and Teramo (n. 7) provinces. Samples were examined by flotation, McMaster and Baermann's techniques, and, for sheep farms, prevalence and abundance rates of endoparasites were compared, according to geographical sites using the Chi squared and Mann-Whitney U tests, respectively.

RESULTS AND CONCLUSIONS. Results are summarized in Table 1. The level of parasitism was low in cattle, whilst sheep showed high levels of parasitism, with coccidia and strongyles being the most prevalent parasites. Common mixed infections caused by lungworms, tapeworms, trichurids and/or *Strongyloides* spp. were also observed. Significant differences in prevalence rates between the two investigated Provinces were found ($p<0.05$). The prevalence of gastrointestinal and lungworms detected in ruminants was similar to those reported in previous studies in central-southern Italy, even though parasite prevalence in sheep from Teramo was higher than that previously observed (Dipineto et al, 2013, Vet. J., 197:884-885). This study confirms that endoparasites are common in sheep farms, while they are less prevalent in cattle dairy farms.

Table 1. Copromicroscopic results

Host		Cattle (n=200)		Sheep (Perugia: n=250; Teramo: n=350)					
Parasite		Umbria Prevalence (%)	Abundance (OPG/EPG)	Perugia Prevalence (%)	Teramo Prevalence (%)	sig	Perugia Abundance (OPG/EPG)	Teramo Abundance (OPG/EPG)	sig
GIN	Coccidia	56.0	88	64.0	81.4	**	140	474	**
	Trichostrongyles	18.5	1	49.6	78.9	**	68	162	**
	Strongyloides	0	0	6.0	4.0		8	1	
	Cestoda	0	0	11.6	9.7		-	-	
Lungworms	*Trichuris*	4.5	2	0.4	8.3	**	0	5	**
	Dictyocaulus	0	-	0	1.4		-	-	
	Muellerius	-	-	4.4	14.3	**	-	-	
	Protostrongylus	-	-	17.6	4.3	**	-	-	
	Cystocaulus	-	-	0	0.9		-	-	
	Neostrongylus	-	-	0	6.0	**	-	-	

OPG: oocyst per gram of faeces, EPG: eggs per gram of faeces; differences between Perugia and Teramo farms are evidenced in the column "sig": *=p<0.05; "sig": **=p<0.01; GIN: GastroIntestinal Nematodes

Use of *Punica granatum* extract for the control of gastrointestinal nematodes in sheep

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Keywords: FLOTAC, gastrointestinal nematodes, *Punica granatum*, sheep

INTRODUCTION. Gastrointestinal nematodes (GINs) are considered worldwide one of the greatest threat to the outdoor breeding of small ruminants. In recent times, the research for bioactive plants which can be used as non-conventional anthelmintic has received considerable attention because of the increasing development of resistance to chemical anthelmintics in nematodes. The aim of this study was therefore to evaluate the anthelmintic effectiveness of the natural extract rich with tannin present in peels and seeds of the pomegranate *(Punica granatum)* traditionally used for the treatment of GINs in sheep, comparing it to the efficacy of synthetic products.

MATERIALS AND METHODS. This study was conducted in sheep naturally infected by nematodes and 60 sheep were selected and divided into 3 groups (20 animals per group) homogeneous by breed, weight, physiological status and GINs eggs per gram of faeces (EPG): **TNG**, treated orally at single dose with 50 ml of pomegranate extract; **TIG,** treated with ivermectin (200 µg/Kg B/W) administered subcutaneously; **CG,** untreated. At Day 0 allocation to groups, faecal collection and examination and treatment were performed and on Days 7, 14, 21 after treatment faecal samples were collected and examined to calculate the Faecal eggs count reduction (FECR) for the evaluation of the anthelmintic efficacy. The faecal samples were examined using *Flotac double technique* (Cringoli et al., 2010, Nat. Prot. 5(3): 503-15). The formulas used to evaluate the anthelmintic efficacy are recommended by the World Association for the Advancement of Veterinary Parasitology to monitor drug efficacy against gastrointestinal nematodes in livestock based on the FECR (Coles et al., 1992, Vet. Par., 44:1992 35-44).

RESULTS AND CONCLUSIONS. The results of the epg mean FECR (%) for each group were:
TNG - D_0**:** 246 epg; D_7: 102 epg (56.1%); D_{14}: 124 epg (54.2%); D_{21}: 153 epg (45.6%).
TIG - D_0: 245 epg, D_7: 0 epg (100%); D_{14}: 0 epg (100%); D_{21}: 22 epg, (92.3%).
CG - D_0: 244 epg, D_7: CG 233 epg, D_{14}: 270 epg, D_{21}: 282 epg.
Following the guidelines provided by the World Association for the Advancement of Veterinary Parasitology (WAAVP) the extract is insufficiently active. Although the results showed a low anthelmintic efficacy, a reduction > 50% obtained with a natural extract administered in a single dose is still an encouraging result. It is therefore necessary to carry out further studies increasing the concentration and days of administration of the extract, evaluating its benefits on milk production.

SESSIONE 15

TERAPIA E FARMACO RESISTENZA

Efficacy of major anthelmintics against cyathostominae in donkeys

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Keywords: Donkeys, Small Strongyles, Egg reappearance period, Resistance

INTRODUCTION. In Italy, Cyathostominae (small strongyles), are the most common parasites in donkey farms with prevalence rate of 100% (Buono, 2018, PhD Thesis). Although in donkey massive parasitic infections are often subclinical, the impact on their health is unclear and anthelmintic treatments are the main strategy to control these internal parasites (Matthews and Burden, 2013, Equine Vet. Educ, 25(9):461-67). The aims of the present study were to evaluate the efficacy of the main broad-spectrum horse anthelmintic drugs using Faecal Egg Count Reduction Test (FECRT), and to investigate a possible development of drug resistance, determining the Egg Reappearance Period (ERP), in donkeys naturally infected by Cyathostominae.

MATERIALS AND METHODS. The trials were conducted in 2 donkey farms (A and B) located in Campania (Southern Italy) and Lazio region (Central Italy) respectively. Twenty-four animals for each farm were selected based on donkey selective therapy cut-off (FEC>300 egg per gram - EPG) and allocated to 4 treatment groups of 6 animals: Pyrantel (PYR), Fenbendazole (FBZ), Ivermectin (IVM) and Moxidectin (MOX). Donkeys were treated at horse dose rate for each tested drug. Faecal Egg Counts were performed at days -2, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77 and 84 post-treatment using the McMaster technique (detection limit of 10 EPG). At day 14, the mean efficacy of the different drugs was calculated, as Faecal Egg Count Reduction according to the formula: $FECR = \left[\left(FEC_{pre} - FEC_{post}\right)/FEC_{pre}\right]x100$ (Nielsen et al., 2013, www.aaep.org). On each sampling day, group pooled faecal samples were incubated at 27°C for 7-10 days and third stage larvae (L3) were identified using the keys proposed by the Atlas of Diagnosis of Equine Strongylidosis (Cernea et al., 2008, Editura Academic Pres).

RESULTS AND CONCLUSIONS. At 14 days post-treatment, in farm A FECRT was 100% for IVM and MOX, 99.8% for FBZ and 99.3% for PYR, suggesting that all investigated drugs were effective against Cyathostominae. For all tested drugs the ERPs were in accordance with those reported by the AAEP Guidelines. At 14 days, in farm B FECRT showed high efficacy for IVM and MOX (100%), a suspected resistance for PYR (86.3%) and resistance for FBZ (83.9%). ERPs were 8 weeks for IVM and 9 weeks for MOX, suggesting a shortened ERP rate for MOX. In all donkeys of both farms, coprocultures revealed the presence of Cyathostominae larvae. Based on our results the macrocyclic lactones were totally effective against small strongyles in donkeys, but to evaluate the anthelmintic resistance, it is crucial to associate the FECRT with the ERP value, because a shorter ERP is a precursor to the development of resistance.

Chronic lethal peritonitis secondary to Mesocestodiasis in a dog from Sardinia (Italy)

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Keywords: *Mesocestoides,* Sardinia, dog, abdominal cestodiasis, Canine Peritoneal, Larval Cestodiasis

INTRODUCTION. *Mesocestoides spp.* is a worldwide diffused Cyclophyllidea tapeworm with unique peculiarities within the Class Cestoda in many aspects of their biology, which still remains unrevealed. Its life-cycle is not still completely known but two intermediate hosts are most likely required for completion of its life cycle (Papini et al., 2010), developing the first larval stage in coprophagous arthropods, and the second one, known as tetrathyridium, in a great variety of hosts (e.g., rodents, amphibians, reptiles and birds). Tetrathyridium larvae multiply asexually by longitudinal fission penetrating the intestinal wall, invading the peritoneal cavity of hosts and eventually causing life-threatening peritonitis (Siles-Lucas and Hemphill, 2002; Boyce et al., 2011).

MATERIALS AND METHODS. A sixteen-year-old male, 30 kg, mixed-breed dog was referred to a veterinary clinic with abdominal distension, depression, hyperthermia, anorexia and dyspnoea. Complete Blood Count showed a severe leukocitosis. Abdominal ultrasonography displayed peritoneal echogenic fluid accumulation, presence of numerous cystic structures and signs of chronic peritonitis. The dog underwent surgical lavage and medical treatment with antibiotics and high doses of Praziquantel (10 mg/Kg SID for 4 days). Morphological and molecular examination of abdominal content led to the identification of *Mesocestoides spp.* After treatment, the dog showed an improvement until one year later when an ultrasonography examination revealed the presence of some cystic structures and peritonitis. Blood analysis showed anaemia, leukocitosis and hyperglobulinemia. The dog underwent a treatment with Enrofloxacin (5 mg/Kg SID for 10 days) and Fenbendazole (50 mg/Kg BID for 28 days) but he died twenty days after for multiple organ dysfunction secondary to chronic peritonitis.

RESULTS AND CONCLUSIONS. In this clinical case the treatment with high doses of Praziquantel was effective to reduce parasitic infestation and improve clinical condition of the dog, however it was not capable to eradicate the infestation and to prevent recidive. Previous studies evidenced positive results of high doses of Fenbendazole against Mesocestoides infection (Crosby 1998). This case report evidenced the need of further studies to identify the most effective therapy against Mesocestodiasis, as well as to prevent clinical forms with early diagnosis and treatments.

Towards the molecular characterization of Voltage Gene Sodium Channel in *Dermanyssus gallinae* isolates

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Keywords: *Dermanyssus gallinae*, acaricide resistance, pyrethroids, Voltage, Gene Sodium Channel

INTRODUCTION. In the last years, acaricide resistance in populations of the poultry red mite *Dermanyssus gallinae* (De Geer) (Mesostigmata: Dermanyssidae) has been recurrently suspected, especially against some pyrethroids (Marangi et al., 2009, Exp. Appl. Acarol, 48:11-18). As already reported in mite species *Tetranychus urticae*, such acaricide resistance may be due to the nucleotides variations (mutations) of the Voltage Gene Sodium Channel (VGSC), gene coding the pyrethroid's target protein (Ilias et al., 2017, Pestic. Biochem. Physiol, 135:9-14). The molecular characterization of the VGSC gene (*Deg*-VGSC) and the possible mutations involved in such resistance remain still unexplored in *D. gallinae*. The aim of this work is to obtain and molecularly characterize the full-length sequence of the *Deg*-VGSC gene using as reference sequence *Metaseilus occidentalis,* a mite species related to *D. gallinae.*

MATERIALS AND METHODS. By using *M. occidentalis* sequence as a reference, two primer pairs for the 5' and 3' end of the sequence were designed. RT-PCR reactions were performed using the PolyA-RNA purified from Scottish isolates and total RNA purified from Italian isolates as templates. The obtained PCR fragments were purified, cloned and sequenced. Conserved areas near to the 5' and 3' end of the sequences were used to design primers that span most of the gene. The obtained sequences were matched to the sequence data obtained using the MiSeq Illumina sequencing (scaffold MITE_3828) and run through the open reading frame prediction software (ORF) finder on NCBI website.

RESULTS AND CONCLUSIONS. Two fragments, confirmed by sequencing and corresponding to the start (about 1300 bp) and to the end (about 500 bp) of the *Deg*-VGSC gene were obtained. When matched with MiSeq sequences data, a full genomic sequence for the *Deg*-VGSC gene was obtained. Using ORF prediction software, an initiation codon (ATG) at base 9 with the first exon running to base 671 was obtained with a 95% identity to the reference sequence. To date, the first exon of 663bp that encodes a 220 amino acid peptide of *Deg*-VGSC coding gene sequence was obtained for the first time. Next steps will include the prediction of the remaining exons from the *Deg*-VGSC sequence, and screening of the VGSC sequences from populations of *D. gallinae* having various levels of sensitivity to pyrethroids in order to identify VGSC mutation(s) associated with pyrethroids resistance.

Field efficacy of Advocate® (Bayer animal health) in the treatment of dogs naturally infected with *Angiostrongylus vasorum*

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Keywords**:** Angiostrongylosis, Advocate®, Baermann

INTRODUCTION. *Angiostrongylus vasorum* lives in the pulmonary arteries and heart of dogs and other animals, that become infected ingesting L3s in gastropod intermediate hosts. Dog angiostrongylosis can be subclinical for a long time, but most often it causes varying clinical pictures that, if untreated, are life-threatening. Despite the clinical importance of the disease and the current geographic expansion of *A. vasorum*, few therapeutics are licensed to treat the infection. Advocate® (Bayer Animal Health) spot on has been marketed a decade ago to treat dog angiostrongylosis in single or repeated administrations. This study aimed at monitoring the efficacy of Advocate® in treating dog angiostrongylosis in clinical settings from different geographical regions of Italy.

MATERIALS AND METHODS. A total of 75 naturally infected dogs, namely 15 from Abruzzo (Site A), Lazio (Site B), Molise (Site C), and 60 from Campania (Site D) regions, were included. After a clinical examination, each dog received (day 0) a single dose of Advocate®. All dogs were clinically examined and subjected to the Baermann's test at intervals of 28 days and, when still positive, they received another Advocate® administration until negativization at the Baermann's test and at the clinical observations.

RESULTS AND CONCLUSIONS. All dogs from sites A-C tested Baermann-negative after one dose of Advocate® (efficacy on site 100%). Fifty animals from site D received two administrations, at day 0 and +28 (efficacy on site 83%), while a third dose at day +56 was necessary for the remaining 10 dogs (efficacy on site 100%). All dogs showed a complete clinical recovery in concomitance with the Baermann's negativization. Overall, the efficacy of one, two or three Advocate® topical doses was 20%, 87% and 100%. This study confirmed the efficacy of Advocate® in treating dog angiostrongylosis. As previously shown (Di Cesare *et al*., 2014, Prax. Vet, 35: 22-26; Traversa *et al*., 2013, Parasitol. Res, 112: 2473-2480; Willesen *et al*., 2007, Parasitol. Res, 147: 258-264), some cases require repeated administrations to cure the infection, especially, as for dogs from Site D, in the case of intense local parasitological pressure that may lead to severe infections with high parasite burden.

Exploring tolerability and efficacy of formic acid for the control of *Varroa destructor* in honeybees: standard and alternative approaches

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Keywords*:* Varroa destructor, formic acid treatment, evaporator

INTRODUCTION. The mite *Varroa destructor* represents one of the major threat to honeybees health, and its management is both resources and time consuming for beekeepers. Formic acid (FA) is one of the most used natural product for the treatment of varroosis, and its main feature is effectiveness both against phoretic and reproductive phases of the mite. FA can be administered through different formulations inside the hives, either as soaked gel-stripes or as a liquid poured into evaporation containers. Regarding FA efficacy and tolerability, they may greatly vary depending on FA formulation, the mode of administration and some environmental factors such as temperature and relative humidity (Pietropaoli and Formato, 2018). In the present study, we aimed to test the effects of a recently licensed 60% liquid FA formulation (Apifor60®, Chemicals Laif S.r.l) when administered with standard and alternative evaporators.
MATERIALS AND METHODS. Two field trials were performed during late spring 2016 and 2017 respectively. In 2016, FA was administered using the Nassenheider Professional® (Nassenheider e.K.) evaporator following manufacturer's instruction; the recorded effects in terms of tolerability and antivarroal efficacy were compared with a licensed in-gel FA formulation (MAQS® strips, Filozoo S.r.l.). In the 2017 field trial, an alternative approach was exploited using the Aspro-Novar Form® (Pitarresi CMA). It was inserted between brood frames, where the temperature is maintained by honeybees at ~35 °C, with FA constant evaporation. In this latter trial, the effect of liquid FA administered using Aspro-Novar Form® evaporator was compared with liquid FA administered using Nassenheider Professional® evaporator and with MAQS® FA strips. In both trials, the in-hive tolerability was assessed through the evaluation of family strength (modified Liebefeld method and monitoring of adult honeybee mortality); the antivarroal efficacy was calculated by counting the mites fallen during FA treatment and a follow-up killing of residual varroa using amitraz (Apivar® strips, Chemicals Laif S.r.l.). The environmental and in-hive temperature was recorded during the trials (iButton™ data loggers, Maxim Integrated). To explore negative effects on the capped brood, mortality and morphological defect possibly caused by FA, an MDCT-scan imaging technique was performed to produce cross-sectional images (slices) of sealed brood combs from a selected group of hives.
RESULTS AND CONCLUSIONS. Overall, the liquid FA treatment using Aspro-Novar Form® evaporator showed higher tolerability and similar efficacy compared to the Nassenheider Professional® evaporator and the in-gel FA formulation. Besides, preliminary data suggested that the MDCT-scan approach can allow monitoring the effects of FA under the cap in the honeybee brood, allowing the visualization of its inner structures.

POSTER

P1. *Toxoplasma gondii* DNA in ewe's milk from Umbria region

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Keywords: *Toxoplasma gondii*, ewes, milk, LAMP

INTRODUCTION. The apicomplexan parasite *Toxoplasma gondii* is characterized by different transmission routes (Tenter et al., 2000, Int.J.Parasitol, 30:1217-1258). Transmission of tachyzoites through unpasteurized milk has been observed, but so far only raw goat's milk has been associated to the development of acute toxoplasmosis in humans (Skinner et al., 1990, Scand J Infect Dis. 22:359-361). The aim of the present study was to evaluate the presence of *T.gondii* DNA in ewe's milk samples collected from 37 flocks in Umbria region.

MATERIALS AND METHODS. Overall, 127 samples were collected between June and September during the standard milk procedure. Samples were treated with EDTA and TE solutions (Mancianti et al., 2014, BioMed Res.Int. 7:165) to avoid the interference of casein, and then extracted DNA was used as template for LAMP reaction targeting the SAG2 gene (Trisciuoglio et al., 2015, J.Vet.Diagn.Invest. 27(6):1-4). A PCR targeting the GRA6 gene were also carried out on LAMP-positive samples. Furthermore, blood samples were collected from the same animals and IFAT or MAT were performed, in order to evaluate the presence of anti*T.gondii* antibodies.

RESULTS AND CONCLUSIONS. Results of LAMP reaction showed an overall prevalence of 14.17% (CI95% 9.16%-21.29%) [18/127], while serological tests detected anti-*T.gondii* antibodies in 39 out of 127 samples [p= 30.71% (CI95% 23.35%- 39.20%)]. PCR confirmed all LAMP-positive samples, and positivity were not associated to just one flock. Results of serological analysis showed the presence of anti-*T.gondii* antibodies in almost a third of the examined animals, and the presence of parasite DNA in milk. Since there are lots of local cheese, produced in Central Italy and exported worldwide, made from ewe's raw milk, the presence of *T.gondii* DNA in ewe's milk should highlight the potential risk of transmission to humans. However, to better understand the risk, further studies on the viability of the parasite in milk and cheese are required.

P2. First survey on the presence of anisakid parasites in farmed European sea bass and gilthead sea bream produced and marketed in Sicily

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Keywords: fish farmed, Anisakidae, food security

INTRODUCTION. The Scientific Opinion of EFSA (2010) outlines as the only fish free of health risks related to Anisakidae parasites the farmed salmon, if reared in floating cages or *on-shore* cages and fed by feed with no live parasites. Otherwise, the food operator shall verify, by means of procedures approved by the competent authority, that the fishery products do not represent a health risk regarding the presence of live parasites. This opinion was confirmed by several studies conducted on farmed salmon in Norway (Angot and Brasseur, 1993, Aquaculture, 118: 339-344; Lunestad, 2003, J Food Prot, 66:122-124). The absence of anisakid parasites in farmed fish has also been found for other marine fish species such as European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) (Penalver et al., 2010 J Food Prot, 73:1332-1334). Currently there are low evidences on the prevalence of anisakid infestation in aquaculture fish produced and marketed in Italy. In this work, a sampling plan was carried out aimed at collecting different fish samples marketed in Sicily, to verify the presence and prevalence of anisakid parasites infestation.

MATERIALS AND METHODS. A total of 143 samples of sea bass and 110 samples of sea bream from Sicilian and Greek farms were examined: all the samples were of commercial size (over 200 g). The specimens were taken and stored refrigerated then transferred to the C.Re.N.A laboratories, where visual inspection of viscera and muscle was carried out. The negative samples were subjected to chloro-peptic digestion. The larvae found were subjected to morphological identification, through optical microscopy and molecular analysis by PCR-RFLP method.

RESULTS AND CONCLUSIONS. The survey revealed the presence of two parasites belonging to the Anisakidae family, found inside the coelomatic cavity, only in a single sample of European sea bass from a single farm located in Greece, revealing a prevalence of infestation of 1.7%. The larvae were morphologically identified as belonging to the morphotype I of the genus *Anisakis*. Molecular investigations confirmed the larvae as *Anisakis pegreffii* species. No larvae were found in the samples of gilthead sea bream examined. The present work represents the first report on the presence of anisakid parasites in European sea bass. Our findings in farmed fish can be traced back to the aquaculture policies; however the prevalence of infestation in these productive realities remains very low. Furthermore, the results of this study suggest further investigations in order to have a comprehensive risk picture.

P3. Glutathione trasferase omega -1 (GSTO-1) and *Toxoplasma gondii* infection

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Keywords: *Toxoplasma gondii*, apoptosis, immune system

INTRODUCTION. The glutathione transferases omega (GSTO-1 and GSTO-2) are multifunctional enzymes involved in cellular defense and have distinct structural and peculiar functional characteristics, which differ from those of other GSTs: they lack any glutathione transferase activity, whereas they show thioltransferase, dehydroascorbate reductase and protein glutathionylation activities. Moreover GSTO-1 overexpression appears to be associated with activation of survival pathways (Akt and ERK1/2) and inhibition of apoptotic pathways (JNK1) and GSTO-1 is required for LPS-mediated signalling in macrophages (Piaggi et al., 2010, Carcinogenesis, 31:804-11; Board et al., 2016, Arch Toxicol., 90:1049-67). Our previous data showed that the GSTO-1 is overexpressed in nurse cell (NC) during *Trichinella spiralis* infection. In consideration also of the role played by GSTO1 in the activation of macrophages we aimed to evaluate if this enzyme could have a role in another type of parasitic infection i.e. that with *Toxoplasma gondii* which has the advantage to carry out i*n vitro* studies.
MATERIALS AND METHODS. U937, Jurkat and HeLa cells were infected with *T. gondii* for 24 hrs and cell number and viability were assessed. Twenty four and 72 hrs after infection of U937 and HeLa cells with *T.gondii* the cells were collected, centrifuged and the pellet was analyzed by western blotting (WB) and immunofluorescence using anti-GSTO1 antibody. Densitometric analysis of WB was performed using the Fiji Image-j program.
RESULTS. *T. gondii* infection induces a significant increase in cell proliferation in U937 and Jurkat cells compared with the control (160 % and 175% respectively) with low mortality. The infection on Hela induce only a massive cell death up to 90%. Densitometric analysis of U937 WB showed a significant overexpression of GSTO1 on both 24 and 72 hrs of infection compared with the control cells (126 and 165 % respectively). Immunofluesherence images showed nuclear translocation of GSTO-1 after 24 hrs of infection in both HeLa and U937 cells.
CONCLUSION. The *T. gondii* infection effect on cell vitality varies greatly depending on the cell type considered; a marked increase in cell proliferation is observed in U937 and jurkat cells probably due to their role as cells of the immune system, while HeLa undergo infection and die. It is known that *T.gondii* protects many cell types from apoptosis induced by various stimuli (Keller et al., 2006 FEMS Microbiol Lett., 258(2):312-9). The molecular mechanisms are not yet fully clarified but the overexpression of GSTO1 could be part of it. Our results show for the first time the nuclear translocation of the enzyme into cells infected by a protozoan. The biological significance of the GSTO-1 nuclear traslocation is unknown; the finding in *in vitro* infection with *T. gondii* opens new research fields.

P4. Synergism of ML/doxycycline adulticide effect: an *in vitro* study

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Keywords: *D. immitis*, ABC transporters, *Wolbachia*, *in vitro* assay

INTRODUCTION. Most human filarial nematode parasites and arthropods are hosts for a bacterial endosymbiont, *Wolbachia*, which, in filaria, are required for normal parasite development, fertility and survival. Due to their obligate nature in filarial parasites, *Wolbachia* have been a target for several drug discovery initiatives. Recent studies have shown that antibiotic treatment of *Dirofilaria immitis*-infected dogs can inhibit parasite embryogenesis, larval development, microfilarial production, and long-term survival of adults (Kramer and Genchi, 2014, Vet. Parasitol, 206:1-4). Moreover, Macrocyclic lactones (MLs) are antihelminthic drugs that can prevent HWD during transmission season and have also been shown to eliminate adult parasites after long-term administration (McCall et al., 2008, Adv. Parasitol, 66:193-285). Recent studies have shown that administration of doxycycline in combination with the ML ivermectin provided more rapid adulticidal and microfilaricidal effect, as well as a stronger adulticide activity, than either of the drugs administered alone (Kramer and Genchi, 2014, Vet. Parasitol, 206:1-4). It is not yet known, however, what the efficacy of the combination therapy is due to. In fact, while the mechanism of action of the MLs at therapeutic dosages is well known (i.e. inhibition of the inhibitory transmitters, causing impairment of neuromuscular function), it is still not known how antibiotics and/or *Wolbachia* depletion kill filarial worms. This obviously renders any feasible hypothesis on eventual synergism between the two drugs open to question. *In vitro* experiments have shown that ABC transporter proteins that act as efflux pumps for various drugs, including tetracyclines, are inhibited by the macrocyclic lactone ivermectin (Ballent et al., 2012, Vet. J, 192, 422-427). Thus, MLs may allow accumulation of higher concentrations of antibiotics within the nematode compared with antibiotics alone. On the other hand, it may be possible that antibiotics in some way potentiate the effects of MLs. Several compounds, including antibiotics, have recently been shown to increase intracellular concentrations of MLs such as moxidectin (Dupuy et al, 2001, J. Vet. Pharmacol. Ther, 24(3):171-7). This study proposes to develop an *in vitro* model, using adult parasites of *D. immitis*, to study the the effects of different drug treatment regimens on the ABC transporter activity.

MATERIALS AND METHODS. Adult worms of *D. immitis* were collected from infected dogs. They were treated with different combinations of the two molecules of interest. RNA and DNA were co-extracted from each treated parasite. cDNA was prepared for expression study of ABC transporters by quantitative real time PCR analysis.

RESULTS. The first steps for the optimization of the methodology were carried out. A collection of adults of *D. immitis* treated with/without doxycycline alone, ivermectin alone or a combination of the two for both 24 and 48h was obtained. Moreover, per each of the treated worms, pure RNA was co-extracted with DNA and cDNA was prepared.

CONCLUSIONS. In this study, we were able to optimize the first steps necessary for the development an *in vitro* assay aimed at elucidating the molecular mechanisms laying behind the marked filaricide effects of a combination of macrocyclic lactones (MLs) and doxycycline against *D. immitis*. Particularly, in the present research an *in vitro* model is being develop, using adult worms of *D. immitis*, to study the effects of these molecules on ABC transporter activity.

P5. Digging deep into intramitochondrial symbiosis: dual transcriptomics of the hard tick *Ixodes ricinus* and its bacterial symbiont *Midichloria mitochondrii*

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Keywords: *M. mitochondrii*, *I. ricinus*, RNA-Seq, Dual-Transcriptomics

INTRODUCTION. *Ixodes ricinus* is a hard tick, widespread in Europe and in the Mediterranean basin, that can act as vector of multiple diseases of human and veterinary importance. *Midichloria mitochondrii* is a bacterial symbiont of this tick, capable of living in the intermembrane space of mitochondria (Beninati et al., 2004, Appl. Environ. Microbiol., 70:2596–2602). The bacterium was found to be present in most tick individuals (Lo et al., 2006, Environ. Microbiol., 8:1280-1287) and to be vertically transmitted to the progeny (Sassera et al., 2008, Appl. Environ. Microbiol., 74:6138-6140). To investigate the relationship between host and symbiont we designed an *ad-hoc* protocol of dual RNA-Seq to sequence the transcriptomes of both organisms in different phases of tick engorgement.

MATERIALS AND METHODS. Ticks were collected on roe deer, cut in half and stored in RNALater on site, in order to freeze the transcription patterns in each stage. Ticks were dissected in RNALater and total RNA was extracted from ovaries and salivary glands. A custom library construction kit was designed and used in order to preserve transcripts from both organisms and to limit the prevalence of rRNA sequences in the sample (normally over 85%). The kit protocol included the use of custom designed probes for rRNA depletion. Libraries were sequenced on Illumina machines and the resulting reads were assembled to obtain a reference transcriptome to be used for downstream analyses.

RESULTS AND CONCLUSIONS. Ticks were subjected to a custom protocol for RNA sequencing which allowed to obtain an average of 40 million reads per sample and over 50% of the reads were transcripts of host or symbiont. After assembly, over 18,000 tick transcripts and over 1,200 bacterial transcripts were obtained. Differential expression analysis will be performed in the upcoming months; so far, the main result of this work is the development of a sound protocol for dual RNA-seq in this system.

P6. Phylogenomic analysis of members of the *Meyerozyma guilliermondii* species complex

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Keywords*: Meyerozyma caribbica*, Diptera, species complex, genomes

INTRODUCTION. Yeasts of the *Meyerozyma guilliermondii* species complex are widespread in nature and can be found associated with a number of sources, from environmental ones, to arthropods, to hospital patients. The species complex comprises three species, the most studied *M. guilliermondii*, *M. caribbica*, and *Candida carpophila*. Here we report the whole-genome sequencing and assembly of four *M. caribbica* isolates isolated from four Diptera species. Additionally, we employed these genomes, together with genomes obtained from public databases, to obtain a phylogenomic picture of this species complex.

MATERIALS AND METHODS. All samples derive from insect colonies maintained either at the University of Camerino or at the University of Torino. Yeasts were isolated from the mosquitoes *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*, and from the fruit fly *Drosophila suzukii*, then cultured and identified. We used Restriction Fragment Length Polymorphism (RFLP) to discriminate between the two species *M. guilliermondii* and *M. caribbica*, which are morphologically undistiguishable. We extracted whole-genome DNA from the four samples sequenced them on an Illumina HiSeq machine. We then assembled the obtained reads using the software SPAdes. Finally, we designed and employed a specific Single Copy Orthologs approach to obtain a phylogenomics of the whole species complex.

RESULTS AND DISCUSSION. The four yeast isolates from insects were identified as *M. caribbica* using the RFLP method. The four novel assemblies present reduced fragmentation and comparable metrics (genome size, gene content) to the available genomes belonging to the species complex. For what concerns the phylogenomic analysis, our results show the expected compact phylogenetic structure for the species complex and are solid, thanks to the the presence of a sizable core set of genes. Furthermore, *M. caribbica*, despite being morpohologically very similar to *M. guilliermondii*, seems to be more closely related to *C. carpophila*. Genomic analyses of yeast associated to insect could help to evaluate whether these microorganisms could be used as novel tool for insect control.

P7. Identification of a novel *Brevibacillus laterosporus* strain with insecticidal activity against *Aedes albopictus* larvae

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Keywords: *Aedes albopictus*, *Brevibacillus laterosporus*, biopesticides

INTRODUCTION. *Aedes albopictus* is a vector of viruses of public health significance, including Dengue, Zika and Chikungunya. As no vaccines or specific treatments are available, vector control is the only effective strategy for disease prevention. In the last decades, as a consequence of the restriction in the use of chemical pesticides by the European legislation, the microbial pesticides market increased and the interest in finding new entomopathogenic bacteria raised. Aim of this work was the isolation and characterization of new bacterial strains with larvicidal activity against *Ae. albopictus*.

MATERIALS AND METHODS. Forty-four soil samples were collected from different geographic areas and cultured following a specially developed protocol aimed at isolating spore-forming bacteria. Larvicidal assays were performed according to the World Health Organization guidelines: 2nd instar larvae of *Ae. albopictus* (Rimini strain) were treated with different amounts of suspensions containing a mixture of late vegetative cells and spores. Larval mortality was observed at 24h intervals during a 72h period. The obtained isolates exhibiting potent larvicidal activity were identified by 16S rRNA gene sequencing. The most promising bacterial strain was further characterized by i) dose-response curves to establish the LD50 value; ii) transmission electron microscopy and iii) whole genome sequencing, phylogenomic analysis and comparative genomic pipeline.

RESULTS AND CONCLUSIONS. Among the active isolates, one strain showing a high larvicidal activity was selected and identified as *Brevibacillus laterosporus* by 16S rRNA gene sequencing. This strain is characterized by a large, canoe shaped lamellar parasporal body and it shows a larvicidal activity significantly higher than the LMG15441 reference strain. The genome of the novel strain was obtained and phylogenomically characterized. A comparative genomic analysis to detect unique proteins, potentially involved with the larvicidal activity is currently being performed.

P8. Assessing the presence and spread of *Vespa velutina* in north-east Italy through a surveillance program

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Keywords: *Vespa velutina*, invasive species, surveillance program

INTRODUCTION. The Asian hornet *Vespa velutina* appeared in Europe in 2004, probably introduced from China. After the first detection in France, it spread to Italy in 2012, and was reported in Veneto Region (northeastern Italy) in 2016. With the aim to assess the presence and spreading of this invasive alien species in the area, a surveillance program has been activated by the Regional Agriculture Department in collaboration with the National Reference Centre for beekeeping of the Istituto Zooprofilattico Sperimentale delle Venezie.

MATERIALS AND METHODS. Geospatial data were analyzed in order to select the apiaries to be involved in the program, and a statistical analysis of their distribution was performed. In this area a cells-divided grid was defined and the number of apiaries for each cell was calculated; three classes of apiaries density per cell were defined (high, medium and low apiary density/cell). Cells with high apiary density were considered as a sampling unit, whereas medium and low apiary density cells were grouped together (two and four cells respectively) standing for a sampling unit. Overall, 229 cells were defined: 117 with high density and 112 with medium-low density grouped. In each of them, an apiary was selected as target where to place wasp traps (TapTrap®) to monitor the presence of the Asian hornet. The results were recorded in a database specifically created. Moreover, a web application was implemented with a “mobile first” approach, easy to use by smartphone and tablets, in order to quickly collect and share field and geospatial data.

RESULTS AND CONCLUSION. In total, 230 apiaries were monitored by traps and 1,728 apiary visits were recorded (on average 7.5 visits/apiary). None of the monitoring sites revealed *V. velutina*. It is clear, however, that the monitoring for the presence of this invasive alien species on the regional territory requires a protracted application beyond a single bee season in order to consolidate the result achieved. According to Regulation (EU) No.1143/2014, it is necessary to activate and maintain monitoring systems able to detect the arrival, presence and establishment of invasive alien species in Member States, such as *V. velutina*, expressly mentioned in the subsequent regulation (EU) No.2016/1141.

P9. Preliminary data on the presence of *Hippobosca longipennis* Fabricius, 1805 (Diptera: Hippoboscidae) in Sardinia

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Keywords: *Hippobosca longipennis*, dog fly, Hippoboscidae, Sardinia

INTRODUCTION. *Hippobosca longipennis* Fabricius, 1805 (= *H. capensis* Olfers, 1816), the “dog fly” is a blood-sucking louse, found mainly on dogs in the Paleartic region and on wild carnivores in Africa, including Felidae (Theodor, 1975, Diptera Pupipara, Fauna Palestina – Insecta I). It is a species with a large geographical distribution, present in Europe, Africa, Palestine, Asia Minor, China, Korea, India and various other countries (Maa, 1969. Pacific Insect Monograph 20:261-299). Bites can be painful and irritating. Although this species is considered present all around Italy, including Sardinia (Rivosecchi, 1995, in Pape *et al.*, Checklist of species of Italian Fauna, 78, Calderini, Bologna; https://fauna-eu.org), no precise data have been published on its occurrence in the island.

MATERIALS AND METHODS. Specimens were collected on hosts by tweezers, fixed in 70% ethanol and identified according to morphological keys (Theodor, 1975).

RESULTS AND CONCLUSIONS. From 2012 to 2017, fourteen specimens of *H. longipennis* were collected, in eight localities in Sardinia (Tab.1). The flies were found on four host species: dog (six specimens), cat (five), fox (two), and, unusually, human (one). In three cases co-infestation with other arthropods (Ixodida and Siphonaptera) were observed. These are the first comprehensive data on hosts, phenology and distribution of *H. longipennis* in Sardinia. With the recent report of *Ornithophila metallica* (Schiner, 1864) (Bazzato et al., 2015, Boll Ass Rom Entom, 70:137-138) and other four species (Fois et al., 2012, Mappe Parassitol, 18:108), the number of Hippoboscidae species known in Sardinia has increased to six.

Tab. 1: Specimens of *Hippobosca longipennis* collected in Sardinia.

Date	Locality	N. Specimens	Host	Co-infestation with
II/2012	Loceri	1	*Vulpes vulpes ichnusae*	
27/III/2012	Seui	1	*Vulpes vulpes ichnusae*	*Ctenocephalides canis, Pulex irritans*
VIII/2012	Dolianova	1	*Felis silvestris catus*	
16/IX/2013	Teulada	1	*Homo sapiens*	
30/VII/2014	Capoterra	1	*Canis familiaris*	*Rhipicephalus sanguineus*
4VIII/2015	Uta	5	*Canis familiaris*	*Rhipicephalus sanguineus, Ct. felis*
30/VI/2017	Carbonia	2	*Felis silvestris catus*	
11/VI/2017	Villaperuccio	2	*Felis silvestris catus*	

P10. Preventive inspections in a bus company with detection dog units trained to detect bedbugs (*Cimex lectularius*)

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Keywords: bedbugs, detection dogs, *C. lectularius*, bus

INTRODUCTION. Bedbugs (*Cimex lectularius*) are human obligated ectoparasites with cosmopolitan diffusion (Masini, 2011, Vet. Ital., 23:93-139). Their bites produce the appearance of pruritic maculopapular, erythematous lesions (Goddard et al, 2009, *JAMA*, 301:1358-1366). Bedbugs are nocturnal and gregarious insects that tend to escape from the daylight (negative phototropism) hiding in crevices where they can form aggregations of many individuals, commonly hard to see during a visual inspection (Reinhardt et al., 2007, Annu. Rev. Entomol., 52:351-374).
The economic loss derived from the infestations in bus companies (buses out of service during the pest control, reputational damage and contentious proceedings) can be significant. A prevention strategy in bus can help to reduce the costs deriving from the presence of bedbugs.
MATERIALS AND METHODS. In the city of Rome and Terni, from May 2017 to March 2018, 38 buses have been inspected with two canine detection units trained to detect bedbugs. Some buses have been inspected several times, for a total of 56 buses inspected. Our goal was to identify infestations of *C. lectularius* as early as possible in order to: reduce the out of service time of busses, perform targeted disinfestations and consequently less expensive and more effective, reduce reputational damage and contentious related to the presence of widespread infestations. The detection dogs have been previously certificated by the AICA (Associazione Italiana Cani Anti Cimici) and are provided by the company Cani Anti Cimici ® (www.canianticimici.com) (Masini et al., 2017, Int. J. Dermatol., 56:204-205).
RESULTS AND CONCLUSIONS. In 3 of the 38 inspected buses (7,9%), the canine detection units detected the presence of bedbugs that have been successively confirmed by a visual identification (true positive buses). In two busses over three, the amount of bedbugs was minimal and the disinfestation has been effective. Thanks to the dog inspections, no customer noticed the presence of bedbugs in the three buses true positive. The preventive inspections with detection dogs in bus companies have proved to be a fast and reliable strategy for the detection of infestation sources of *C. lectularius.*

P11. A case of infestation of tropical rat mites *Ornithonyssus bacoti* (Acari: Macronyssidae) in Rome, Italy

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Keywords: *Ornithonyssus bacoti*, tropical rat mite, Italy, dermatitis

INTRODUCTION. The mite *Ornithonyssus bacoti* (Acari: Macronyssidae) is a blood-feeding ectoparasite of rats and other rodents. This cosmopolitan mite also infests carnivores, birds and human. In human, their bites produce non-specific skin lesions associated with intensely pruritic (Varma, 1993, Ticks and Mites (Acari), in Lane RP, Crosskey RW, eds. Medical Insects and Arachnids, Cambridge University Press, Cambridge, 597–658).

MATERIALS AND METHODS. In this case, in an apartment in the city of Rome, a 55-year-old woman, a 54-old man and their 12-year-old son were affected by skin lesions in arms, shoulders, and upper trunk. An environmental dust sampling was performed from all the rooms in the apartment using a vacuuming cleaner, according to the method of Sercombe et al. (Sercombe et al., 2005, Allergy, 60:515-520). Microscopic examination of the dust by flotation in saturated sodium chloride solution (Sasa et al.. 1970, Japanese Journal of Esperimental Medicine, 40:367-382) revealed the presence of many mites. The mites were mounted on microscope slides one by one and immersed in Berlese's gum-chloral medium. All the mites have been correctly identified as *Ornithonyssus bacoti,* according to acarology Varma key (Varma, 1993, Ticks and Mites (Acari), in Lane RP, Crosskey RW, eds. Medical Insects and Arachnids, Cambridge University Press, Cambridge, 597–658).

RESULTS AND CONCLUSIONS. The source of the infestation was a nest of *Mus musculus* located on the roof of a veranda. The mites penetrated into the house from a roof breach produced by the nibbling of rats. The removal of the rodent nest and the sealing of the roof determined the resolution of dermatitis. There are many reports about dermatitis in human by *O. bacoti* from different part of the world. This is probably due to the large amount of population of rats and mice living near human habitat. Many rodents are infested by *O. bacoti*, for this reason they can transmit the mite to human and other animals.

P12. Unexpected transmission patterns of *Leishmania infantum* and *L. tarentolae* involving *Sergentomyia minuta* and humans in Lazio region

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Keywords: *Leishmania*, Sergentomya, PCR amplification

INTRODUCTION. The detection of potentially pathogenic new *Leishmania* species has implications in public health and highlights the importance of a deeper understanding of the circulating parasites and their vectors. This is a consequence of the geographical expansion and ecological modifications of phlebotomine vectors attributed to several environmental determinants, including, but not only, climate changes. Aside to *Phlebotomus perniciosus*, *Phlebotomus neglectus* and *Phlebotomus perfiliewi*, major vectors of *Leishmania infantum* in Italy, the sand fly fauna includes, among others, *Sergentomyia minuta*, which role in the circulation of mammalian leishmaniases has been recently discussed. Indeed, despite *S. minuta* is mainly herpetophilic and proved vector of *Leishmania* (syn. *Sauroleishmania*) *tarentolae*, records of its feeding activity on mammals, including humans are increasing, as well as the occasional detection of *L. infantum* DNA in them (Maia and Depaquit, 2016, Parasite 23:55; Pereira et al., 2016, Acta Tropica 174:45-48).

MATERIALS AND METHODS. Sand flies were collected in 2015 (July-September) by CDC light traps set outdoors (from 6 p.m. to 8 a.m.) in several rural areas of Lazio region in proximity of dogs and other domestic animals, whenever possible. Sand flies were identified per species by PCR-RFLP (Latrofa et al., 2012, Vet. Parasitol. 184:267–70). Human blood samples were collected during the same period from blood donors enrolled at the Umberto I Hospital of Rome. Detection of *Leishmania* species was performed in both samples by nested-PCR-RFLP targeting ssu-rRNA (Latrofa et al., 2018, Vet. Par. 253:39-42), and confirmed by DNA sequencing.

RESULTS AND CONCLUSIONS. A total of 344 sand fly females were collected and molecularly analysed for *Leishmania* spp. presence (Grottaferrata, RM, n=261; Arpino, FR, n=29; Anagni,FR, n=18; Maccarese, RM, n=17; Monte Compatri, RM, n=13; Roma Porta Tiburtina, n=6), of which *S. minuta* accounted for 92% of the species, followed by *P. perniciosus* (4.4%), *P. perfiliewi* (3.2%), and *P. mascittii* (0.3%). *Leishmania* species detected in *S. minuta* were *L. tarentolae* (11.3%) and *L. infantum* (2%). The human blood samples analysed were 185, in which *L. infantum* and *L. tarentolae* DNA was detected at 2.2% and 1.6%, respectively. These results indicate that, in particular circumstances, the parasitic interaction between *S. minuta* and human (or dog) could lead to unusual transmission of *Leishmania* species of which the pathogenic and epidemiological importance still needs to be elucidated.

P13. *Anopheles funestus* exophilic behaviour associated to high sporozoite rates following LLIN distribution in a village of Burkina Faso

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Keywords: *Anopheles funestus*, resting behavior, *Plasmodium*, bednet

INTRODUCTION. An increasing number of countries are moving towards control and elimination of malaria by applying strategies targeting endophagic vectors with long-lasting insecticide treated nets (LLINs) and indoor residual spraying (Killeen et al., 2014, Mal. J. 13:330). However, complete malaria elimination will be a difficult task in sub-Saharan countries where high transmission levels are maintained by genetic and behavioural plasticity of the major vectors *Anopheles gambiae* and *Anopheles funestus*. This is the case of Burkina Faso where raising of LLIN coverage from 20% to 70% between 2009 and 2014 but did not significantly affect malaria annual incidence. We here present the result of an entomological survey focused on *A. funestus* group carried out in Koubri village (Ouagadougou area) one year after a LLINs mass-distribution campaign.

MATERIALS AND METHODS. Resting mosquitoes were collected in July- December 2011 by Back-pack aspirator, PIT-shelter and Sticky Resting Box, both indoors and outdoors. Collected mosquitoes were morphologically identified and Funestus group specimens were subsequently identified per species by PCR (Koekemoer et al., 2002, J. Med. Entomol. 804-811). Head+thorax and abdomens of females were analysed by PCR for *Plasmodium* sporozoite rate (SR) and Human blood Index (HBI) respectively (Calzetta et al., 2018, Med Vet. Entomol., in press; Kent and Norris, 2005, Am. J. Trop.Med. Hyg. 73: 336-342).

RESULTS AND CONCLUSIONS. On 419 *A. funestus* group mosquitoes collected, 98.2% were found outdoors (94% *A. funestus*, 4% *A. rivulorum* and 2% *A. leesoni*). A preliminary analysis highlight an SR of 8.1% and an estimate HBI of 4%. This provides evidence for a switch of A. funestus behavior toward complete exophily, due to the indoor individual protection given by LLINs, which has not been associated to a major reduction of SR. These findings confirm the need of constant monitoring of malaria vectors, in particular outdoors, in order to obtain affordable evaluation of malaria control strategies.

P14. Cultivation, gametocytes production and drug sensitivity of *Plasmodium falciparum* isolates from malaria patients

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Keywords: *P. falciparum* gametocytes, antimalarials, pLDH

INTRODUCTION. The number of malaria cases reported in Italy is in the order of 500-600 per year (Benelli et al, 2018 Tr. Parasitol.). The Luigi Sacco Hospital is the reference infectious disease hospital of the metropolitan area of Milano. Diagnosis and *Plasmodium* species identification is based on quick tests and on malaria-positive smears examined by experienced laboratory personnel. The aim of the present work was to attempt the long term cultivation and gametocytes differentiation of the different *Plasmodium* strains isolated from patients in the Sacco Hospital independently from their geographic origin.

MATERIALS AND METHODS. During the study period, 20 blood samples from malaria patients were received. The parasites were immediately put in culture using RPMI 1640 medium, sodium bicarbonate, hypoxanthine, HEPES and glutamine, in the presence of 1% AlbuMAX II (lipid-rich bovine serum albumin). Growing and stabilized cultures were then cultivated in the presence of 10% naturally clotted heat-inactivated human serum to allow gametocyte production. Gametocytogenesis was triggered by diluting the cultures to 0.5% parasitemia, and changing medium daily without the addition of erythrocytes. When a parasitemia of 5% was obtained and the parasites were stressed by nutrient deprivation, the cultures were treated with N-acetylglucosamine to clear residual asexual parasites and gametocyte differentiation was followed for 9-10 days. Chemosensitivity tests were done on both asexual and sexual stages of parasites against a panel of known antimalarial drugs using a modified version of the pLDH method (D'Alessandro et al. 2013, JAC. 68:2048).

RESULTS AND CONCLUSIONS. Out of 20 samples, 13 *P. falciparum* isolates were well adapted to grow *in vitro* and were stored for a subsequent genotypic analysis; gametocytes were successfully produced from 9 of them. Chemosensitivity assays were performed on both asexual and sexual parasites with results comparable to those obtained with the parasite transgenic lines routinely used in drug screening assays. These results suggest that the pLDH method can be easily adapted to evaluate the chemosensitivity of field isolates to novel antimalarials, including novel transmission-blocking compounds, as requested by the ongoing global elimination/eradication efforts.

P15. Chikungunya virus ECSA strains, with and without the E1:A226V mutation: study of vector competence of *Aedes albopictus*

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Keywords: Chikungunya virus, *Aedes albopictus*, vector competence

INTRODUCTION. Chikungunya virus (CHIKV) is an enveloped virus of the genus Alphavirus, family *Togaviridae*, very widespread and transmitted to humans mainly through the bite of infected *Aedes* mosquitoes. Over the last decade, two different ECSA OIL lineages of CHIKV have been responsible for large outbreaks in Italy: the strain with an *Ae. albopictus*-adaptive mutation, in 2007 in Emilia Romagna region (Rezza et al., 2007, Lancet, 370(9602):1840-1846) and the strain without mutation in 2017 in Latium and Calabria regions (Venturi et al., 2017, Euro Surveill., 22(39)17-00646).

The aim of our study was to evaluate and compare the vector competence of a laboratory colony of *Ae. albopictus* (collected in Calabria region) for these two different strains of CHIKV.

MATERIALS AND METHODS. Experimental infections were performed in parallel with both strains, in BSL-3 cabinet using an infectious blood meal, composed of 2/3 rabbit blood and 1/3 viral seed, by using a membrane feeding apparatus. The virus strains were previously obtained by viral isolation from biological samples of patients, collected during the Italian outbreaks in 2007 and 2017. Female mosquitoes were fed and monitored after the infectious blood meal for 20 days (T=26±1°C, 70% RH, 14/10h light/dark cycle). CHIKV titer of infected mosquitoes was evaluated by quantitative Real Time PCR. Infection, dissemination and transmission rates (IR, DR, TR) were assessed by detection of the virus in abdomen, legs plus wings and saliva of fed females, respectively (Fortuna et al., 2015, Parasit. Vectors, 8:463).

RESULTS AND CONCLUSIONS. Our results indicate that both CHIKV strains disseminate in *Ae. albopictus* starting from day 3 post infection (pi) and both viruses are detected in the saliva up to 20 days pi. High vector competence of the tested mosquitoes for the two CHIKV strains was observed, showing similar values of IR, DR and TR rates (Fig.1) and confirming the important role of *Ae. albopictus* in the transmission of both CHIKV strains.

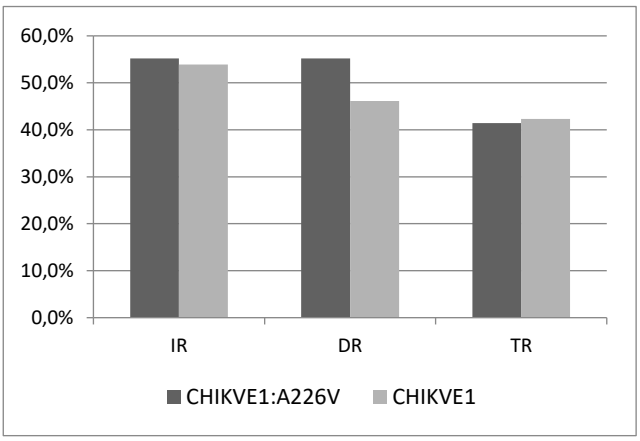

Fig. 1. Cumulative IR, DR and TR rates calculated from 3 to 20 days p.i. for CHIKV strains, with (CHIKVE1:A226V) and without (CHIKVE1) the E1:A226V mutation.

P16. An autochthonous malaria case due to *Plasmodium ovale curtisi* in Central Italy

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Keywords: Autochthonous malaria, *Plasmodium ovale curtisi*, central Italy

INTRODUCTION. In non-endemic countries malaria still represents the main imported infectious disease, however a small proportion of cases defined as "autochthonous" are also seldom recorded in Italy (7/3.633 malaria cases notified in the 2011-2015 period (0.2%); Boccolini et al, 15th SIMIT National Congress, 2016). A recent increase of such cases in summer 2017 has raised concern about the possible reintroduction of the disease; one of these was recorded on August 2017 in the Adriatic coast of Marche Region.

MATERIALS AND METHODS. A 66 year-old woman was admitted to hospital emergency room with fever and shivers that appeared every other day for one week. She had never left Italy and did not report recent contact with people who travelled abroad. In the suspect of haematological disease a blood smear was performed which unexpectedly showed malaria parasites with morphological traits common to *Plasmodium vivax* and *P.ovale*. A rapid malaria test was positive for non-falciparum malaria. The patient was treated with piperaquine/dihydroartemisinin for 3 days with excellent clinical response and full recovery. An epidemiological investigation to identify potential disease-associated risk factors including mosquito-bite exposure, was immediately conducted. *Ad hoc* entomological surveys for *Anopheles* spp. were carried out in possible risk sites targeting both adult mosquitoes (dry ice-CDC traps positioned within a radius of at least 500 m) and larvae (larval dipper in potential breeding sites).

RESULTS AND CONCLUSIONS. At the ISS malaria reference lab the blood parasite was confirmed microscopically as *P.ovale* and molecularly identified as *P.ovale curtisi* (Sutherland et al, 2010, J Infect Dis, 201:1544–1550; Fuehrer et al, 2012, J Clin Microbiol, 50:4100–4102). The epidemiological investigation confirmed that the case could not be referred to travel in endemic area; moreover none of her relative or neighbourhood have recently visited an endemic country. Overall 5 risk sites for mosquito-bite exposure and larval breeding were identified and surveyed, but none of them was positive for *Anopheles* spp. Based on the above findings, this cryptic malaria case highlights how hard is to discover a probable source of infection making also problematic the implementation of actions for vector and disease control.

P17. SURVEY ON TICKS AND BACTERIAL TICK-BORNE PATHOGENS: OSSOLA VALLEY, NORTH-WEST ITALY (2010-2017)

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Keyworkds: tick-borne pathogens, *Ixodes ricinus*, PCR

INTRODUCTION. In Italy, the availability of eco-epidemiological data on the distribution of different tick species and associated tick-borne pathogens (TBPs) is highly variable. Such information constitutes a useful guideline for the medical healthcare system, which in general has a proper awareness on the epidemiology of these vector borne diseases. However, the incidence of human tick-borne diseases in non-endemic Italian areas is probably underestimated, perhaps due to limited surveillance, asymptomatic cases, improper diagnosis (e.g. false negative in serological tests) and lack of follow up studies. Despite a recent study has provided data on *Borrelia burgdorferi sensu latu* prevalence in *Ixodes ricinus* ticks collected in Piemonte (Pintore et al., 2015, Zoonoses Public Health 62:365–374), corresponding to the increase in the number of reported Lyme disease cases in the area, this region remains one of those territories rarely investigated for the presence of ticks and TBPs.

MATERIALS AND METHODS. In our survey, a molecular screening for four important agents of zoonoses (*Rickettsia* spp.*, B. burgdorferi* sensu lato complex, *Francisella tularensis* and *Coxiella burnetii*) was performed on both questing ticks collected by dragging on vegetation in different forested areas in Ossola valley and engorged ticks collected on domestic and wild animals during the hunting seasons 2010 (Pistone et. al., 2017, Exp. Appl. Acarol. 73: 477) and 2017 (data not published).

RESULTS AND CONCLUSIONS. Two different species of *Rickettsia* (*R. helvetica* and *R. monacensis*), known to cause human illnesses and five different *Borrelia* species, proved (*B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*) or suspected (*B. valaisiana* and *B. lusitaniae*) to cause clinical manifestations of Lyme disease in humans, were found in both questing and engorged *I. ricinus*. Moreover, PCR positivity for *F. tularensis* was obtained in engorged ticks collected on two different wild ungulate species (*Capreolus capreolus* and *Cervus elaphus*). This work provided further data and broadened our knowledge on bacterial pathogens present in ticks in North-Western Italy.

P18. Absence of K13 polymorphism in *Plasmodium falciparum* parasites from Brazilian endemic Area

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Keywords: *Plasmodium falciparum*, surveillance, Malaria

INTRODUCTION. The World Health Organization (WHO) recommends efficacy monitoring for ACTs every 2 to 3 years in all endemic countries. *P. falciparum* ART-resistant parasites can be evaluated examining polymorphisms in the Kelch (PfK13) propeller domain.

MATERIALS AND METHODS. This study was performed at the Laboratório de Pesquisa em Malária, Headquarter office of the CPD-Mal of Fiocruz, located in Rio de Janeiro where malaria transmission does not occur. A total of 69 blood samples from febrile patients that attended the clinic for diagnosis and treatment for malaria at the *Ambulatório de Doenças Febris Agudas*, INI-IPEC, Fiocruz, Rio de Janeiro, Brazil, were diagnosed for malaria falciparum by microscopic and/or molecular tests. All samples were from Brazilian endemic areas of the following states: Acre (n=14), Amapá (n=15), Amazonas (n=30) and Pará (n=10).

RESULTS. *P. falciparum* DNA was successfully sequenced in all 69 isolates and after alignment with the 3D7 reference sequence, all samples were found to be wild-type.

CONCLUSIONS. The present data contributes to the ongoing surveillance of ART resistance parasites by providing baseline data on K13-propeller mutations and reinforce the pertinence of the use of ACTs in Brazilian endemic areas.

P19. Epidemiological investigation on bovine piroplasmoses in Tanga Region, Tanzania

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Keywords: piroplasmosis, cattle, East Coast Fever, Tanzania

INTRODUCTION. Tick-Borne Diseases (TBDs) are one of the major problems regarding animal health in Tanzania, a country that accounts for around 25 millions of bovines. Costs associated with TBDs include both direct losses, and costs associated with control and treatment. Babesiosis and theileriosis are more commonly known as “piroplasmoses”, and affect especially the smallholder dairy system, with *Theileria parva* (causing agent of East Coast Fever - ECF) being the major threat. However, efforts in place to control *T. parva* are to some extent obscuring the impact of other less lethal TBDs, which also have the potential to cause significant losses. The study investigated farmers’ perception and epidemiological situation of piroplasmoses in Tanga Region, Tanzania.

MATERIALS AND METHODS. The survey was carried out in July-August 2013, involving one intensive, two medium-size and five small-holders dairy farms in Muheza e Korogwe districts of Tanga Region, Tanzania. Blood samples were used to prepare blood smears, stained with Haemacolor® and microscopically examined. Moreover, Whatman® FTA Micro Card filter was used to transport samples to Italy (Parasitology Laboratory of the University of Padova) for biomolecular analyses, as previously described (Cassini et al., 2012, Vet. Parasitol., 184: 77-82). Additionally, a questionnaire was administered to farmers to gain information on herd management practices: breed of the animals, grazing system, economic impact of piroplamoses, therapeutic and preventive measures performed at farm level.

RESULTS AND CONCLUSIONS. 123 blood samples were retrieved from eight farms. Of the blood smears prepared (122), 14 (11.5%) showed single, small and circular/irregular inclusions in red blood cells at the microscope examination, whereas higher prevalence value (27/123; 22.0%) was recorded at biomolecular analysis. Within the PCR reliable positive samples, different species were identified: *T. mutans* (9), *B. bovis* (5), *T. velifera* (5), *Theileria* spp. (3), *T. parva* (1). ECF was known to all the farmers, however a true estimation of losses due to piroplasmosis was difficult to achieve. Animals were free to graze in three holdings, whereas the other five were keeping the animals in “zero-grazing”. Farmers were using acaricide once a week (5), once a month (2) or never (1). The epidemiologic situation showed a limited circulation of both *Theileria* and *Babesia*, and outbreaks of ECF or other piroplasmosis were reported by the farmers, suggesting an endemic instability state. The contact with ticks and pathogens is kept low by acaricide treatments, but the method is not economically viable and could lead to losses in case of an acaricide breakdown. Appropriate training for farmers is advisable to promote an integrated control that may also include vaccination for ECF, which seems a better option economically and epidemiologically in this area.

P20. Canine leishmaniosis and filariosis in Molise region, southern Italy

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Keywords: Dog, *Leishmania infantum*, *Dirofilaria* spp., *Acanthocheilonema reconditum*

INTRODUCTION. Dogs play an important role as hosts and reservoirs of several vector-borne infections, including zoonotic leishmaniosis and filariosis (Otranto et al., 2009, Trends Parasitol, 25:157-163). Data on these vector-borne diseases in inner areas of southern Italy are very few. Therefore, the aim of this study was to investigate the seroprevalence of *Leishmania infantum* and the presence of microfilariae (mff) of filarial worms in dogs living in Molise region, southern Italy.

MATERIALS AND METHODS. A grid-based approach within a Geographical Information System (GIS) was used in order to uniformly sample the dogs throughout the entire region (Rinaldi et al., 2006, Geospat Health, 1:33-47). For this purpose, a grid representing quadrants of 10x10 km was overlaid on the regional map within the GIS. Thus the Molise region was divided into 55 quadrants and the study was designed to sample 6 hunting, six stray and six sheep dogs in each quadrant. Laboratory analyses included an immunofluorescence antibody test (IFAT; provided by the National Reference Center for Leishmaniosis, Palermo, Italy) to detect anti-*Leishmania* antibodies in serum samples, and a Knott test to detect microfilariae of *Dirofilaria immitis*, *D. repens* and *Acanthocheilonema reconditum* in blood samples.

RESULTS AND CONCLUSIONS. A total of 752 samples were collected (347 from hunting, 180 from stray and 225 from sheep dogs) and 92 resulted positive to selected vector-borne infections (12.2%; 95% Confidence Interval, C.I.= 10.0-14.8). Specifically, 77 samples (10.2%; 95%C.I.= 8.2-12.7) showed a titer ≥ 1:160 to *L. infantum*, with a higher prevalence in hunting dogs (9.5%; 95%C.I.= 6.7-13.2). Regarding filarial infections, 10 samples (1.3%; 95%C.I.= 0.7-2.5) were positive for *A. reconditum*, 6 (0.8; 95%C.I.= 0.3-1.8) for *D. repens* and 2 (0.3%; 95%C.I.= 0.1-1.1) for *D. immitis*, with a higher prevalence in hunting dogs (1.6%; 95%C.I.= 0.9-2.9). Two samples resulted co-infected with *A. reconditum* and *D. repens*, while one sample with *A. reconditum* and *D. immitis*. Finally, two samples showed co-infection of *L. infantum* and *A. reconditum.* The present study showed that leishmaniosis and filariosis are present in dogs in Molise region. The detection of these vector-borne infections in dogs with or without clinical signs reinforces the importance of increasing the veterinary community, owners and public health authorities' awareness regarding the risk of infection.

P21. Risk of transfusion transmitted Chagas disease: screening of candidate blood donors at Pisa University Hospital

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Keywords: Chagas disease, *Trypanosoma cruzi*, serology, transfusion medicine

INTRODUCTION. Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi* and transmitted by blood-sucking triatomine bugs in Central and South American countries. In areas where the vector is absent, the most common route of transmission is transfusion with blood or transplant with organs from infected donors. Many countries are therefore adopting routine screening of donors at risk (originary from or travelers to endemic countries) for antibodies against *T. cruzi*, including Italy (D.M. 2/11/2015).

MATERIALS AND METHODS. Sera samples (N=1605) were collected from candidate blood donors at transfusional centers of North-West Tuscany (Italy) and tested at Pisa University Hospital by immunocromatography as screening assay (ICT; Chagas Quick Test, Cypress Diagnostics; employs a multi-epitope recombinant antigen) and chemiluminescence as confirmatory assay (ChL; Architect Chagas, Abbott; employs recombinant proteins FP3, FP6, FP10, TcF). Ten sera samples from Chagas patients were collected and tested by two different ELISA assays at the Centre for Tropical Diseases (Negrar, Italy; courtesy of Andrea Angheben), and tested by ICT, ChL and immunoblot (IB; Chagas IgG LineBlot, Novatec; employs the recombinant protein TcF) assays at Pisa University Hospital.

RESULTS AND CONCLUSIONS. Six candidate blood donors (0.4%) had a positive ICT result and where therefore excluded from donation for the next two years. However, all 6 subjects had a negative ChL result.

The comparison of results of ICT, ChL, ELISA and IB assays on control sera samples indicated that ICT might have a slightly lower sensitivity. We therefore suggest to substitute ICT with ELISA as screening test at Pisa University Hospital, and to introduce IB as a further confirmatory test when results of ICT and ChL are discordant.

P22. Mini-FLOTAC is able to detect lungworm larvae in cats, dogs, hedgehogs and sheep

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Keywords: Lungworms, Larvae, Diagnosis, Mini-FLOTAC

INTRODUCTION. Mini-FLOTAC technique was demonstrated to be a sensible and accurate technique to detect helminth eggs in pet, livestock and exotic animals (Cringoli et al., 2017, Nat Protoc, 12:1723-1732)**.** The aim of this study was to evaluate the performance of Mini-FLOTAC to detect lungworm larvae from cats, dogs, hedgehogs and sheep compared to reference techniques as Baermann (MAFF, 1986, 12-13) and FLOTAC (Cringoli et al., 2010, Nat Protoc, 5:503-515).

MATERIALS AND METHODS. A total of 62 fresh faecal samples were collected from 10 cats, 10 dogs, 12 hedgehogs and 30 sheep naturally infected with lungworms. Three replicates were performed for each technique. FS3 (zinc sulfate, specific gravity = 1.200) was used as flotation solution for Mini-FLOTAC (analytic sensitivity = 5 LPG) and FLOTAC (analytic sensitivity = 1 LPG).

RESULTS AND CONCLUSIONS. The results are summarized in Table 1, FLOTAC and Mini-FLOTAC were more efficient than Baermann in detecting of lungworm larvae, overcoming the limitation of time required by the Baermann test. Mini-FLOTAC can be considered a valid alternative to Baermann technique for the detection of lungworm larvae of cat, dog and hedgehog, while FLOTAC showed the best results for the detection of sheep lungworms.

Table 1. LPG values (minimum, maximum, mean±standard deviation and coefficient of variation) of lungworm larvae of cats, dogs, hedgehogs and sheep detected by Baermann, FLOTAC and Mini-FLOTAC techniques. Significant differences for different letters (a, b, c).

		BAERMANN				**FLOTAC**				**Mini-FLOTAC**			
	Parasite	**Min**	**Max**	**Mean±SD**	**CV**	**Min**	**Max**	**Mean±SD**	**CV**	**Min**	**Max**	**Mean±SD**	**CV**
Cat	*Aelurostrongylus abstrusus*	8	799	313^{b} ±39.2	0.12	10	1224	410^{b}±35.1	0.08	10	2325	854^{a}±115.7	0.13
	Troglostrongylus spp	9	270	121^{b}±21.1	0.17	22	444	223^{a}±15.3	0.07	35	560	267^{a}±32.8	0.12
Dog	*Angiostrongylus vasorum*	10	823	208^{b}±37	0.18	10	890	232^{b}±28.1	0.12	10	1310	382^{a}±34.4	0.09
Hedgehog	*Crenosoma* spp.	5	1326	400^{b}±81.6	0.2	14	1824	532^{a}±138	0.25	70	1740	754^{a}±105	0.14
Sheep	*Dictyocaulus filaria*	1	22	11^{c}±4	0.36	6	60	33^{a}±5	0.15	10	40	25^{b}±5	0.2
	Cystocaulus ocreatus	1	43	22^{c}±6	0.27	6	180	93^{a}±11	0.12	10	60	35^{b}±4	0.11
	Muellerius capillaris	5	320	162^{c}±21	0.13	12	460	236^{a}±31	0.13	10	380	195^{b}±22	0.11
	Neostrongylus linearis	4	221	112^{c}±19	0.17	12	360	186^{a}±24	0.13	10	240	125^{b}±18	0.14
	Protostrongylus rufescens	3	143	73^{b}±16	0.22	12	260	136^{a}±11	0.08	10	180	95^{a}±13	0.13

P23. Role of *Entamoeba* spp. in enteric infections in Côte d'Ivoire

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Keywords: Diarrheal diseases, intestinal co-infection, Côte d'ivoire

INTRODUCTION. Diarrhea is a common symptom of an intestinal infection sustained by bacterial, viral or parasitic etiologic agents. In endemic countries, the limited availability of specific and sensitive diagnostic techniques makes it difficult to correctly diagnose and treat gastro-intestinal diseases, especially when co-infections. Diagnostic techniques used in local health facilities may differ in their detection effectiveness and therefore influence data on the prevalence of gastro-intestinal parasites and local statistics on the general health status of the population. Among *Entamoeba* species, only *E. histolytica* is pathogenic for humans while *E. dispar* and *E. moshkovskii* have been associated with isolated symptomatic cases. In the present study, appropriate molecular approaches have been considered to improve epidemiological data on the role of different *Entamoeba* species in gastrointestinal infections in urban and rural areas endemic for enteropathies of Côte d'Ivoire.

MATERIALS AND METHODS. Between May 2013 and September 2015, 106 individual stool samples were collected from individuals of different ages in three rural villages (Assouindé, Yaou, Kimoukro) and in the urban center of Bonoua and were analyzed at the Policlinico Tor Vergata of Rome. A fragment of the 18S rRNA gene of *Entamoeba* spp. was amplified by conventional PCR and sequenced. Ninety-three of 106 samples were submitted at the Institute of Virology, University of Cologne to the xTAG® Gastrointestinal Pathogen (GPP) Luminex for the detection of intestinal pathogens most frequently responsible for diarrhea.

RESULTS AND CONCLUSIONS. Species prevalence of the 49/106 positive samples for *Entamoeba* were:1,9% *E. histolytica*, 3,8 % *E. dispar*, 15,1 % *E.coli* e 25,5 % *E. hartmanni*. The highest (39%) and the lowest (6%) prevalence was in patients from 6 to 12 and from 19 to 40 years respectively. Patients tested positive for *E. histolytica* were from the urban context and did not report any symptoms. Among patients from the rural context, none was affected by single infection, and 75% had co-infection with three or more pathogens.The present study underline the need to utilize accurate diagnostic tools to assess the scope of *E. histolytica* infection, especially in endemic areas where other *Entamoeba* species are prevalent and symptomatology in humans could be related to a large spectrum of enteropathogens.

P24. Molecular identification of three cases of human dirofilariosis due to *Dirofilaria repens* from Central Italy

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Keywords: *Dirofilaria*, humans, molecular identification, Italy

INTRODUCTION. Dirofilariosis is a vector-borne parasitic disease mainly of domestic and wild carnivores caused by the species *Dirofilaria* (*Noctiella*) *repens* - which is endemic in many countries of the Old World - and *D. immitis* - which has a worldwide distribution. In recent years, an increasing number of human cases has been reported, suggesting that dirofilariosis is an emergent zoonosis. Indeed, more than 3,500 human cases due to *D. repens* and 25 to *D. immitis,* have been reported in Europe since 1977 up to 2016 (Genchi and Kramer, 2017. Parasit. Vectors, 10:517). Here, further 3 cases of human dirofilariosis, observed in Central Italy during the year 2017, are described.

MATERIALS AND METHODS. Subcutaneous nodular painless masses were observed at the inguinal level (case 1), ocular conjunctiva (case 2) and coccyx (case 3), in patients referred at the Umberto I Policlinico, Rome (1), Pisa Hospital (2), and Pescara Hospital (3), respectively. Surgically removed nodules (cases 1 and 3) and the parasite itself (case 2) were embedded in paraffin block for histological analysis. The microscopical observation of the histological sections, revealed the presence of a nematode enclosed in the nodule. For the identification to species level, DNA was extracted from the paraffin block (Shi et al., 2002.J. Histochem. Cytochem, 50:1005–11), and the mtDNA *cox1* (about 650-bp) gene fragment was PCR-amplified using filarioid-generic primers (Casiraghi et al., 2004. Int. J. Parasitol, 34(2):191-203). Amplicons were then purified and sequenced (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS. On the basis of the morphological features, the worms were preliminary referred to *Dirofilaria* sp. The sequence analysis of the three samples showed a match of 100% with the mtDNA *cox1* sequence of *D. repens* deposited in GenBank (accession number DQ358814.1).

DISCUSSION. The occurrence of new cases of human *D. repens*-infections observed in a single year, suggests an increased spreading of these parasites in Italy. Since our Country is that with the highest number of human dirofilariosis reported cases in Europe (probably for the scientific tradition in the field), dirofilariosis should be included in the differential diagnosis in patients presenting subcutaneous nodules, and a specific serological method should be set up.

P25. Diagnosis of perinatal malaria in the globalization area

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Keywords: *Plasmodium*, diagnosis, molecular markers

INTRODUCTION. Perinatal malaria can represent an important issue for screening and diagnosis of malaria, especially for detecting and monitoring *Plasmodium* infection in early stages. The combination of affordable rapid diagnostic tests (RTD), expert laboratory microscopy, molecular and genotyping assays may address emerging atypical malaria cases.

MATERIALS AND METHODS. A 2-month-old male newborn was admitted to the Academic Department of Pediatrics of the Bambino Gesù Children's Hospital due to anemia and exposure to HIV. He was born prematurely in Italy by cesarean section at 34 weeks' gestation after a bicorial, biamniotic pregnancy with birth weight of 3,870 Kg. The mother was a 30-year-old migrant woman from Nigeria arrived in Italy at 27 weeks gestation. He was the first of non-identical twins. Combined to anemia, a spleen enlargement was revealed, therefore malaria was hypothesized. Hence, newborn's peripheral blood was collected to execute malaria diagnostic algorithm by combining *Plasmodium* spp. antigen detection, microscopy and PCR-based combined tests. The RDT, based on either *Plasmodium* spp. lactate dehydrogenase (pLDH) and *Plasmodium falciparum* histidine-rich protein II (HRP-II) antigens,
was performed by SD Malaria Ag P.f/Pan (BIOLINE, http://www.who.int/malaria/publications/atoz/9789241510035/en/). Malaria laboratory routine panel was performed on the newborn, but also on mother and other twin blood samples, by exploiting the following algorithm: *i*) RDT; *ii*) microscopy of Giemsa stained thick and thin blood smears for *Plasmodium* spp. identification and parasitemia titer; *iii*) molecular screening and typing of *Plasmodium* spp. by multiplex qualitative PCR assay based on 18S rRNA gene (Sentinel-Diagnostics). Genotyping of *Plasmodium falciparum* isolates responsible for infections of the mother and newborn was performed by amplification of a neutral microsatellite loci marker (TA109) and five highly polymorphic markers: the block 2 of merozoite surface protein 1 (*Pfmsp1*), the block 3 of merozoite surface protein 2 (*Pfmsp2*), *Pfhrp2* and *Pfhrp3 genes*. In addition, a set of specific primers were used for detecting the different families of *Pfmsp1* (K1, MAD20 and RO33) and *Pfmsp2* (FC27 and 3D7).

RESULTS AND CONCLUSIONS. The RTD mother's sample was negative, while infant's sample resulted positive; microscopy of blood smears confirmed infection with *P. falciparum* with index <1% for the mother and 1% for the son. Both mother and one twin PCR showed a positive result, confirming a *P. falciparum* infection. Six genotypic molecular markers were amplified from mother and newborn DNA samples: MS-TA109, Ta109, *Pfmsp1*, *Pfmsp2 ,Pfhrp3* and *FC27.* The markers *Pfmsp2*, FC27 (subfamily of Pfmsp2) and Pfhrp3 showed discordant genotypes between the two samples. The differences of allele composition have one plausible explanation in the multiplicity of strains for the mother during the pregnancy. Probably the mother harbored more than one isolates during the pregnancy, only or more of them transmitted to the newborn and others persisting in the mother's blood after delivery The identical allelic profiles at MS-TA109 locus level support the vertical transmission of the *P. falciparum* infection, while the possibility of a new infection, although very unlikely, it cannot be completely ruled out. A prompt diagnosis of potential congenital malaria is fundamental. Because of the increasing number of pregnant women coming from endemic areas for malaria to non-endemic countries,

the research of *Plasmodium* spp should become mandatory for all neonates and infants with fever, anemia and thrombocytopenia. A complete and accurate anamnesis of infant's mother and the inclusion of *Plasmodium* spp research into the TORCH screening for mother and infant at birth should be performed, avoiding delay in diagnosis and consequently reducing morbidity and mortality associated to the disease.

P26. Intestinal parasites in dogs affected by oncological diseases

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Keywords: dog, cancer, fecal parasites, prevalence

INTRODUCTION. Intestinal parasites are frequently recorded in dogs and may include potential zoonotic and significantly virulent species, often resulting in high dog and public health risks (Riggio et al., 2013, Vet. Parasitol., 193: 78-84; Paoletti et al., 2015, Parasitol. Res., 114: 2135–2141). Among canine intestinal parasites, helminths are generally more prevalent than protozoa (Riggio et al., 2013, Vet. Parasitol., 193: 78-84; Simonato et al., 2015, Parasitol. Res.,114: 1963-1970). In human cancer or immunocompromised patients, opportunistic protozoan infections are prevalent (Sulzyc-Bielicka et al., 2007, J. Parasitol., 93: 722-724; Marcos and Gotuzzo, 2013, Curr. Opin. Infect. Dis., 26: 295-301). Nevertheless, available data about the diffusion of intestinal parasite infections in immunocompromised or cancer canine patients are scarce. Considering this lack of data, the evaluation of the prevalence and species composition of fecal parasites in dogs affected by cancer diseases was the main aim of this study.

MATERIALS AND METHODS. Between January 2014 and June 2016, 43 privately owned dogs of different breed, sex and age, affected by different cancer diseases and living in various areas of central and northern Italy, were included in the study. More precisely, 30 dogs were affected by lymphomas and were treated with a standard 8 weeks long treatment protocol, while the remaining 13 dogs were affected by other oncological diseases. From all dogs, individual fecal samples were collected and examined for intestinal parasites by flotation test with satured NaCl solution (s.g. 1.2). A commercial rapid immune-chromatographic assay (RIDAQUICK®, R-Biopharm srl Italy) was used to detect *Giardia duodenalis* and *Cryptosporidium* spp. faecal antigens. *N. caninum* DNA was identified by PCR with species-specific primer pairs Np6+/Np21+ in a faecal sample positive for *N. caninum*-like oocysts (Müller et al., 1996, J. Clin. Microbiol., 34: 2850–2852). Statistical analysis was performed using a $\chi 2$ test with the Yates correction, when appropriate.

RESULTS AND CONCLUSIONS. An overall higher prevalence of protozoa infections (18.6%) than of helminth infections (4.6%) was observed. *G. duodenalis* (6/43; 13.95%), *Cryptosporidium* spp. (3/43; 6.47%), *Cystoisospora ohiensis*-complex (2/43; 4.65%), *Entamoeba* sp. (1/43; 2.3%), *N. caninum* (1/43; 2.3%), *Spirocerca lupi* (1/43; 2.3%), and *Toxocara canis* (1/43; 2.3%), were identified. In dogs affected by lymphomas, statistical analysis evidenced that the prevalence of protozoa was significantly higher ($P < 0.01$) than that of helminths. Moreover, the prevalence of *G. duodenalis* was significantly higher respect to that of *Entamoeba* sp. ($P < 0.01$), *N. caninum* ($P < 0.01$) and *Cystosospora* spp. ($P < 0.05$), while no statistical differences emerged between the prevalence of *G. duodenalis* and *Cryptosporidium* spp.

As previously evidenced in human cancer patients (Sulzyc-Bielicka et al., 2007, J. Parasitol. 93: 722-724), results from this study are indicative that in canine cancer patients, especially in dogs affected by lymphoma, the diffusion of intestinal protozoan infections, mainly caused by *G. duodenalis* and *Cryptosporidium*, may be high. Although further studies are needed to confirm these findings, obtained results suggest that cancer dogs should be always screened and monitored for intestinal parasites and in particular for protozoan infections.

P27. Prevalence of gastrointestinal parasites in shelter cats of central Italy

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Keywords: shelter cats, gastrointestinal parasites, prevalence, central Italy

INTRODUCTION. Intestinal parasites are widespread in cats and some species have high relevance in feline and human medicine (Riggio et al., 2013, Vet. Parasitol., 193: 78-84; Beugnet et al., 2014, Parasit. Vectors. 7: 291). In feline shelters, the limited resources and the large numbers of animals living in these facilities may facilitate parasite infections that may affect the health of animals and expose shelter workers and adoptive owners to zoonoses (Schurer et al., 2015, Can Vet J. 56: 964-70). The evaluation of the prevalence of intestinal parasites in shelter cats of Latium and Tuscany was the main aim of the present study.

MATERIALS AND METHODS. Individual fecal samples taken from 132 randomly selected cats (55/132 males and 77/132 females, 64/132 ≤18 months in age and 68/132 older than 18 months) living in public and private shelters of Latium and Tuscany were collected. Samples were macroscopically examined and then screened microscopically by fresh and Lugol stained fecal smears and by flotation test (2 g of faeces) with satured NaCl solution (s.g. 1.2). A commercial rapid immune-chromatographic assay (RIDAQUICK®, R-Biopharm srl Italy) was used to detect *Giardia duodenalis* and *Cryptosporidium* spp. faecal antigens. All animals were clinical examined in order to evaluate possible presence of compatible clinical pictures. Obtained data were statistically analysed.

RESULTS AND CONCLUSIONS. An overall prevalence of 31% (41/132) was found. A significantly higher ($p < 0.05$) prevalence of protozoan infections (18.1%, 24/132) than of helminth infections (12.8%, 17/132) was observed. Moreover, 32/132 (24%) cats were found infected by potential zoonotic species and 33/132 (25%) cats showed compatible clinical pictures. *G. duodenalis* (10.6%, 14/132), *Toxocara cati* (9%, 12/132), *Cystoisospora felis* (3%, 4/132), *Cystoisospora rivolta* (2.2%, 3/132), ancylostomatids (*Ancylostoma tubaeforme* and *Uncinaria stenocephala*) (2.2%, 3/132), *Cryptosporidium* spp. (1.6%, 2/132), *Aonchoteca putorii* (0.75%, 1/132), *Tritrichomonas foetus* (0.75%, 1/132) and *Strongyloides* sp. (0.75%, 1/132) were identified. Coinfections were found in 6% (8/132) of examined cats. At statistical analysis, investigated parasite infections were overall found significantly associated with the presence of compatible clinical pictures ($p < 0.05$). Moreover, the prevalence of protozoan infection was significantly higher ($p < 0.05$) in the shelters of Tuscany. Obtained results show high cat and public health risks in shelters from the examined area and suggest the need for more effective control measures.

P28. Surveillance of zoonotic parasites in assisted therapy animals

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Keywords: pet-therapy, parasites, zoonoses

INTRODUCTION. Animal assisted therapy is based on the pre-existing human-animal bond. The interaction between a person and a trained animal helps people recover from and/or face health problems and/or mental disorders. Since pet therapy is based on very closed animal-human interaction, the surveillance of zoonotic pathogens is mandatory for the health of both animals and humans. This study investigated for presence of ecto-parasites, intestinal parasites and dermatophythes in animals involved in several animal assisted therapy activities.
MATERIALS AND METHODS. From July 2015 until July 2017, 189 animals, including equids (n=92), dogs (n=56), cats (n= 20), birds (n=10), rabbits (n= 7), rodents (n= 3) and goat (n=1) were investigated. Faecal samples were analized by copromicroscopical procedure. Fur was examined for ectoparasites. Skin material was individually collected using Mackenzie brush technique, then was cultured on Mycobiotic agar for mycological investigations at the Parasitology Lab. – Mycology section - Istituto Zooprofilattico Sperimentale delle Venezie.
RESULTS AND CONCLUSIONS. Intestinal parasites were described in the 31.7% (60/189) of investigated animals, the 20% (12/60) of which is potentially zoonotic such as *Ancylostoma caninum* (n=1), *Eucoleus aerophilus* (n=4), *Toxocara canis* (n=2) recovered in dogs and *Giardia duodenalis* (n=5) isolated in 4 dogs and in a cat. Among dermatophytes, *Microsporum gypseum* and *Paraphyton mirabile*, potential agents of cutaneous mycosis also in humans, were isolated in a dog and in a horse, respectively. No ectoparasites were found. The close animal/human contact during pet-therapy might represent a serious risk factor for transmission of zoonotic pathogens both directly (animal-human) or via environmental contamination. It is really important to maintain an active surveillance through scheduled investigations for zoonotic pathogens. Animal screenings should be planned differently according to the risk of exposure to the pathogens linked to the environment (indoor or outdoor), to other animals and patients, during the activities.

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P29. Case report: presence of *Dermanyssus gallinae* in Mexican canaries (*Serinus canaria*)

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Keywords: red mite, *Dermanyssus gallinae,* canaries, *Serinus canaria,* México

Infestation with poultry red mite *Dermanyssus gallinae* has been reported in domestic and wild hens. This mite feeds on resting birds, mainly at night and can cause skin irritation, dermatitis and severe anemia. Red mites have a short life cycle (7 days) and spend most of their time away from the host, hiding in floors or walls cracks and crevices. Transmission occurs by direct contact between domestic birds or by means of wild birds. Mite simple were collected from body of ten canaries (*Serinus canaria*) that showed wing and leg mutilation. In addition, samples were also obtained from fabrics used to cover the canaries' cages and from the room where they were kept at night. Canaries were housed in outdoor cages, during the day and brought inside the owner's house. Mites' samples were placed in a solution of 70% ethyl alcohol and observed under stereoscopic microscope. Mites were identified as *D. gallinae* according to morphological characteristics, as e.g. the presence of three ventral shields, a curved stern with two pairs of silks and the shape of the genital region. Canaries received a treatment consisting of one drop of ivermectin (0.2-0.3 mg/kg) under the wing twice a week for two weeks. Of the ten canaries, only 3 adults survived (one male two females), while the remaining seven birds died. We hypothesized that, in this last case, red mites were transmitted by wild bird species such as turtledoves (*Columbina inca*) and pigeons (*Columa livia*) that approached the cages to obtain food during daytime. It is known that these wild birds species can carry red mites. *D. gallinae* must be controlled in pet birds since it can trigger severe skin wounds and, or in extreme cases, cause death.

P30. Detection of *Giardia duodenalis* in pets, Italy

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Keywords: *Giardia,* rabbits, reptiles, pet birds

INTRODUCTION. Rabbits, reptiles and birds are becoming popular companion animals in several countries, including Italy (Assalco, 2016). However, little is still known on their role as potential zoonotic reservoir of various pathogens. The aim of the present study was to investigate the presence of *Giardia duodenalis*, the only species within the *Giardia* genus responsible for infection of humans and other mammals, in exotic animals kept as pets in different Italian regions.

MATERIALS AND METHODS. In 2017, 99 exotic pets belonging to different species have been investigated for the coprological detection of DNA of *G. duodenalis*. Of the sampled pets, 52.5% (52/99) were small mammals (36/52 rabbits and 16/52 rodents), 27.3% (27/99) reptiles (23/27 Chelonian and 4/27 Squamata) and 20.2% (20/99) pet birds (16/20 Passeriformes, 2/20 Psittaciformes and 2/20 Columbiformes). All the animals were kept as pets and sampled individually with the exception of 15 pooled faecal samples of pet birds collected from the cages' floor. From each sample, the extracted DNA was tested with a real-time PCR targeting a portion of the *gdh* locus common to the A-H assemblages of the parasites (Yang et al*.,* 2014, Exp Parasitol 137:46-52).

RESULTS AND CONCLUSIONS. Fifteen faecal samples tested positive for the presence of *G. duodenalis* DNA (15.2%; 15/99). The highest prevalence was found in rabbits (53.3%; 8/15), followed by chelonian (39.6%; 6/15) and birds (6.6%; 1/15). Only one rabbit was referred to show gastrointestinal symptoms at the moment of the visit. The prevalence of infection found in rabbits (22.2%; 8/36) is higher than those reported in studies conducted in Europe and China (7.6% and 9.9%, in Pantchev et al., 2014, Vet Rec 175(1): and in Jiang et al., 2018 In Press, respectively). The most interesting result of this study is the positivity found in chelonian (5 tortoise and 1 turtle). To date, studies on the presence of *Giardia* in reptiles did not show evidence of the parasite in these hosts (Rinaldi et al., 2012, Parasite 19(4): 437:440), with the exception of free-living lizards sampled in Spain that tested positive for different assemblages (Reboredo-Fernández et al., 2017, Rev Bras Parasitol Vet 26(3): 395-399). The role of both reptiles and birds in the cycle of *G. duodenalis* is still unclear and further studies are needed to distinguish between actual infection and simply mechanical dissemination of cysts. Thus, the sequencing of our positive samples and the analysis of a larger number of animals are of importance in order to define the role of these animal species in the transmission and the maintenance of this zoonotic parasite the environment.

P31. Helminth infections in hunting, stray and sheep dogs in the Molise region, southern Italy

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Keywords**:** Dog, Helminths, FLOTAC, Molise region

INTRODUCTION. Helminth infections are still a problem of great health importance for dogs. Intestinal and cardiopulmonary parasites are frequently recorded in dogs causing severe clinical forms (Otranto et al., 2017, Trends Parasitol, 33:813-825). Prevalence data on these endoparasites in dogs in the Molise region is scarce. Therefore, the aim of this study was to investigate the presence of helminth infections in hunting, stray and sheep dogs living in the Molise region, southern Italy.

MATERIALS AND METHODS. A grid-based approach within a Geographical Information System (GIS) was used in order to uniformly sample the dogs throughout the entire region (Rinaldi et al., 2006, Geospat Health, 1:33-47). For this purpose, a grid representing quadrants of 10x10 km was overlaid on the regional map within the GIS. The Molise region was divided into 55 quadrants and the study designed to sample 6 hunting, 6 stray and 6 sheep dogs in each quadrant. Each faecal sample collected was examined by the *FLOTAC dual technique* (Cringoli et al., 2010, Nat Protoc, 5:503-515) using two flotation solutions: FS2 (sodium chloride; specific gravity, s.g.=1.200) and FS3 (zinc sulphate; s.g.=1.200).

RESULTS AND CONCLUSIONS. A total of 875 samples were collected (340 from hunting, 195 from stray and 340 from sheep dogs) and 54.1% (95% Confidence Interval, C.I.= 50.7-57.4) resulted positive for at least one helminth, with a higher prevalence in hunting dogs (64.1%; 95%C.I.= 58.7- 69.2); co-infections were found in 24.2% (95%CI= 21.5-27.2) of samples examined. Eight specie/groups were detected: intestinal nematodes (*Trichuris, Toxocara, Toxascaris* and Ancylostomidae) with high prevalence in hunting dogs (53.8%; 95%C.I.= 48.4-59.2) and sheep dogs (53.5%; 95%C.I.= 48.1-58.9), cardiopulmonary nematodes (*Angiostrongylus* and *Capillaria*) with high prevalence in hunting dogs (28.2%; 95%C.I.= 23.6-33.4) and cestoda (Taenidae e *Dipylidium*) with higher prevalence in sheep dogs (5.0%; 95%C.I.= 3.0-8.0).

The findings of the present study showed a high prevalence of helminths (including many zoonotic agents) in all the dog categories (hunting, stray and sheep) in the Molise region. A regular parasitological surveillance to plan effective control programs (www.escaap.org) is strongly needed to guarantee the health and welfare of pets, and to enhance the safety of people.

P32. Fatal patent troglostrongylosis in a litter of kittens

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Keywords: Troglostrongylosis, Kitten, Vertical transmission, Emodepside

INTRODUCTION. *Troglostrongylus brevior* is a snail-borne metastrongyloid nematode affecting large bronchi and bronchioles of wild and domestic felids. Its lifecycle is indirect involving gastropod intermediate and paratenic hosts. Recent reports (Brianti *et al*., 2013, Parasitology, 140: 821-824; Di Cesare *et al*., 2014, Parasitol. Res., 113: 613-618; Diakou *et al*., 2014, Parasitol. Res., 113: 3895-3898) have also suggested a vertical route of transmission leading to severe and fatal infections in kittens and young cats. This study describes a case of patent troglostrongylosis in three kittens belonging to the same litter.

MATERIALS AND METHODS. Three kittens ageing less than two weeks belonging to the same litter were referred to a private veterinary clinic, where they were housed in separate cages. Two (cats A and B) died of sudden respiratory failure after about three weeks. At necropsy adult nematodes were found in their trachea. The third kitten (cat C) showed respiratory signs few days later and scored positive for metastrongyloid L1 at the Baermann's test. Therefore, it was immediately treated by the veterinarian with a single dose of Profender® (Bayer Animal Health) containing emodepside 2.1%/praziquantel 8.6%. Two weeks after the cat was still positive and symptomatic and received a second dose of Profender®, becoming negative and clinically healthy after two weeks. Adult nematodes and lung flushing obtained at necropsy of cats A and B, and L1s from cat C were microscopically (Brianti *et al.*, 2014, Vet. Parasitol, 202:104-112) and molecularly (Di Cesare *et al.*, 2015, J. Clin. Micobiol, 53:3009-3013) examined.

RESULTS AND CONCLUSIONS. Adult nematodes found in the trachea of cats A and B and larvae shed by cat C were identified as *T. brevior*. Animals were referred at about 2 weeks of age, thus they acquired troglostrongylosis before their arrival in the clinic. The presence of developing eggs and L1s of *T. brevior* in the airways of cat A indicates an already patent infection at about one month of age. These data support the hypothesis of a vertical transmission route of *T. brevior* although it remains to be assessed whether this occurs *via* placenta or the milk. Further studies are warranted to evaluate the use of Profender® in the treatment of cat troglostrongylosis.

P33. Molecular epidemiology of *Blastocystis* in domestic and farmed animals in Italy: preliminary results

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Keywords: Blastocystis, Subtypes, animals

INTRODUCTION. *Blastocystis* sp. is a common intestinal protozoon distributed worldwide infecting humans and a wide range of domestic and wild animals, It exhibits an extensive genetic diversity and 17 divergent lineages (subtypes, STs) have been identified in mammalian and avian hosts, nine of them (ST1-ST9) reported in humans with varying prevalence (Afellani et al., 2013. Protist, 164:497-509). Since several STs are common to humans and animals (Cian et al., 2017. PLoS ONE 12(1): e0169659) it has been proposed that a proportion of human infections may result from zoonotic transmission. Aim of this study was to assess the prevalence of *Blastocystis* and to characterize the STs among a sample of domestic and farmed animals in southern Italy, in order to evaluate the potential risk of zoonotic transmission, considering the occurrence of zoonotic STs previously described in humans (Mattiucci et al., 2016. Epidemiol. Infect, 144(3):635-46).

MATERIALS AND METHODS. To date a total of 74 fresh faecal samples were collected from dogs, cats and farmed animals (horses, donkeys, ostriches, hens, ducks, pigs, calves, sheep and goats) in the provinces of Messina and Catania and submitted to direct microscopic examination. Genomic DNA was extracted from each faecal sample and submitted to PCR amplification using primers previously described (Mattiucci et al., 2016. Epidemiol Infect., 144(3):635-46), which target a fragment of about 600 bp from the SSU rDNA gene. The sequences obtained were compared to those of *Blastocystis* spp. deposited in GenBank by using the BLAST application. Subtypes were identified by determining the exact match (100%) or closest identity (99%), according to the classification given by Stensvold et al. (2007. Trends Parasitol, 23: 93–96).

RESULTS AND CONCLUSIONS. Overall 43% (32/74) of the animal samples scored positive to *Blastocystis* to the microscopical analysis. Molecular analyses are still in progress. However, preliminary results on the farm animal samples (20 PCR-positive out of the 37 examined) showed high prevalence of the zoonotic STs 5 and 7 in hens and pigs, respectively. Despite the present survey was performed on a few samples, preliminary results are interesting as they evidenced high circulation of *Blastocystis* sp. in both pets and farm animals, emphasising the potential risk of zoonotic transmission through their handling.

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P34. VTH (Veterinary Teaching Hospital), AVC (Atlantic Veterinary College), Prince Edward Island (PEI), Canada: an overview of eighteen years of parasitology traffic

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Keywords**:** Prince Edward Island, Veterinary Teaching Hospital, Endoparasite

INTRODUCTION. The Atlantic Veterinary College (AVC) is the only veterinary medicine faculty in Atlantic Canada. AVC was born in 1986 and also has a state-of-the-art teaching hospital. The parasitology diagnostic laboratory started a user-friendly data system that goes from January, 2000 to November, 2017. Really few surveys are present in literature like that (Raue et al., 2011, Parasitol. Res. 116:3315–3330).

MATERIALS AND METHODS. The objective of this study was to summarize the parasitology laboratory exam results. Descriptive analysis was carried out with the Stata statistical software package, version 15.1.

RESULTS AND CONCLUSIONS. A total of 35,513 samples were tested, belonging to exotic animals, wildlife, livestock and pets mostly from the eastern and Atlantic provinces of Canada. There was a mean of 1972.94 samples (525.05 SD) per year, and the range showed a minimum of 1365 and a maximum of 2954 per year. There were eight performed tests including: Baermann technique, Direct Smear, Egg counts, Sedimentation and Fecal flotation, Canine Heartworm Antigen Test, *Neospora caninum* Antibody Test, and Skin scraping. The kinds of specimens tested were feces, blood samples, sera and skin. The animal species represented in our study population were: dogs (19,570), bovine (4,797), cats (3,358), sheep (3,051), horses (2,312) and other (1,114), such as birds, foxes, goats, lynx, pigs and raccoons. Overall, out of 35,513 samples, 10,026 (28.2%) were positive for parasites. Protozoa were in 4068 (40.5%) of the 10,026 samples. Gastro-Intestinal Nematodes were found in 3658 samples (36%). Trichostrongyle eggs occupied approximately a third of the samples (40.9/). Cestoda were in 306 (3%) samples, of which *Moniezia* spp. played the greatest role (55.9%). Lungworms and *Capillaridae* were in 598 (6%) and 178 (2%) samples, respectively, where the most common species recovered was *Crenosoma vulpis*. *Ascarididae* were present in 803 (8%) samples, of which *Toxocara* spp. was the most frequent. Trematoda were found in 44 (0.43%) specimens. Mites were recovered in 28 of 97 (28.9%) skin scraping, and ten of those were identified as *Demodex* spp. Interesting results were 33 *Alaria* spp. cases, three in foxes and thirty in dogs. Also interesting was the detection of 21 cases of cardiopulmonary dirofilariosis in dog. Increasing surveillance of this vector-borne disease agent would be important as the climate, vector range, and habitat continues to change throughout Canada.

P35. Malaria surveillance in Italy: a public health topic of relevance

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Keywords: malaria, *Anopheles labranchiae*, autochthonous cases

INTRODUCTION. The re-emergence of malaria transmission chains in Greece has aroused concern in other Mediterranean Countries, such as Italy where the local vector *Anopheles labranchiae* is still recorded in Central-Southern regions and in the major islands. In those areas, the presence and abundance of the competent vector combined with the occurrence of gametocyte carriers, as possible reservoirs of infection, in a climatically favorable environment could induce the onset of introduced autochthonous cases. For this reason, in Italy imported malaria is a mandatory reportable disease, and a reliable National Surveillance System has been implemented by Istituto Superiore di Sanità (ISS) and Ministero della Salute to prevent malaria re-emergence by monitoring imported cases, promptly responding in autochthonous events, and providing guidance to travelers to endemic areas.
MATERIALS AND METHODS. At the ISS all notified cases were confirmed microscopically; demographic and epidemiological data were included in a dedicated database; molecular and genotypic analyses were also carried out for specific cases.
RESULTS AND CONCLUSIONS. The analysis of provisional 2013-2017 data showed 3,805 imported cases (677-888/year), 17% of which among Italians, while the most significant group was represented by settled immigrants. Twelve cases were autochthonous, 4 induced and 8 cryptic, with a peak of 7 cases occurred in summer 2017 that created a great concern for public health. Eight deceases were reported. *Plasmodium falciparum* was the most frequent diagnosis, mainly acquired in sub-Saharan Africa, although an increase in *P.vivax* cases was observed. Out of 517 *P. vivax* cases, 395 (76%) were potentially infecting and 304 (77%) occurred in a period favorable to malaria transmission (May-October). In Italy the potential occurrence of local malaria cases would be mainly associated to *P. vivax*, an early gametocyte-production species for which *An. labranchiae* is highly competent. Massive arrivals of migrants from endemic areas could introduce reserves of infection and local factors could place migrants and communities residing in the same way, in vulnerable situations, but migration is not a definitive risk for malaria. (Malaria and Migration, Southeast Asian J. Trop. Med. Public. Health, 2013, 44(Supp.1), chapter 4:166-200). Efforts should be paid to monitor migrants' health and to promptly respond in case of autochthonous malaria events.

P36. Parasites in cage and aviary birds: an update?

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Keyword Cage birds; Aviary birds, parasites

INTRODUCTION. In recent years, in Italy, there has been an increasing interest in cage and aviary birds and an increase of the owners of these animals. That pushes veterinarians to a deeper knowledge of this particular branch of avian medicine. In the present work a survey on intestinal parasites was carried out in particular during Ornithological Exhibitions, where breeders from different regions expose they specimens, because no research has ever been done on intestinal parasites in these locations in Italy. Some cage and aviary birds breedings were also sampled.

MATERIALS AND METHODS. 70 samples were collected from Ornithological exhibition of Faenza (RA) 2016, 101 from Ornithological exhibition of Faenza (RA) 2017 and 34 from Ornithological exhibition of Bologna 2017. During these events, each bird is housed in a single cage: fecal samples were obtained collecting the corncob litter or the blotting paper litter under each cage. Moreover 7 samples from the seat of F.O. I (Federation Italian bird breeders) and 23 samples from two parrot's breedings were also collected. Overall, 235 samples were examined: the papers were scrubbed to obtain fecal samples and analyzed by flotation technique; the corncobs were washed in one liter of water, that was filtered and submitted to sedimentation and flotation.

RESULTS AND CONCLUSIONS. *Ascaridia* sp. and *Capillaria* sp. are confirmed the most frequent helminthic species found in parrots, while in Passeriformes only Coccidia was observed. The low parasites prevalence seen in this survey suggests that breeders lead anti-parasitic therapies (which we have not been able to ascertain), and that they hold animals in compliance with good health and hygiene standards. The animals that are selected to participate in the exhibits are therefore in compliance with the characteristics of the breed standards, but also have a good state of nutrition and general well-being.

Place of sampling	Positive/total examined (percentage)	Parasites	Host
Ornithological exhibition Faenza (RA) 2017	5/101 (4.95%)	*Ascaridia* sp	2 *Psittacula krameri*, 1 *Nymphicus hollandicus*, 1 *Platycercus eximius*, 1 *Pirrhura molinae*
Ornithological exhibition Bologna 2017	1/34 (2.94%)	*Isospora* sp.	1 Estrilid shybrid
Ornithological exhibition Faenza (RA) 2016	0/70 (0%)	/	/
seat of the Italian Ornitocoltori Federation (F.O.I) + 2 breedings	5/30 (16.67%)	*Capillaria* sp. *Ascaridia* sp. *Capillaria* sp. Coccidia Coccidia + *Capillaria* sp.	1 *Aratinga solstitialis* 1 *Polytelis swainsonii* 1 *Nymphicus hollandicus* 1 *Agapornis* sp. 1 *Agapornis* sp
Total	11/235 (4.6%)		

P37. Canine faecal contamination and parasitic risk in the city of Bologna

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Keywords: Canine faecal contamination, dog's parasites, Bologna

INTRODUCTION. Canine faecal contamination of urban areas represents an important public-health problem. The aim of this study was to evaluate the degree of both canine faecal pollution and presence of canine parasitic elements in the city of Bologna, with particular attention to the Bolognina district, where a previous similar experience was done.

MATERIALS AND METHODS. The methods used in the previous study (Martini and Cassani,1984, Ann. Ist. Super. Sanità, 20:291-296) were employed to evaluate the canine faecal contamination of Bolognina district after more than 35 years, following a 6.2 km route structured in the middle of the quarter, for 12 times in a year. The centre of Bologna was also examined, dividing the overall area included in the circular avenue in 6 sub-areas. A sampling was performed just once in each sub-area, following a continuous route including the highest number of streets, for a total of 50.14 km. All the faeces found were collected and submitted to qualitative microscopic analysis by centrifugation-flotation technique. For each sample, a form was filled to specify date, time, address of the findings and some characteristics (weather in previous days, type of road, location, fresh state, aspect, presumed dog size, traces of trampling). All the data were reported in a Geographical Information System (QGIS 2.18) and on Excel datasheet (v. 2016).

RESULTS AND CONCLUSIONS. A total of 228 faecal samples was collected, of which 192 from Bolognina district (daily faecalization level: 2.6 faeces/km) and 36 from Bologna centre (total faecalization level: 0.7 faeces/km). A part of collected faeces was dried and traces of trampling were found in both areas. The prevalence of faeces attributed to large dogs resulted significantly higher in samples collected in Bolognina (17.19%), respect to the centre of city (2.78%) ($\chi^2_y = 3{,}89$; $p < 0.05$). In Martini and Cassani study (1984 l.c.), 2204 faeces were found in 10 months following the same route in Bolognina (daily faecalization level: 35.5 faeces/km). Comparing this value with our results, we can observe that the faecalization of the area decreased during past decades. Only two faecal samples (0.88%) were positive for parasitic elements, both from Bolognina district: eggs of *Trichuris vulpis* were found in both and Ancylostomatidae in one of them. The faecalization level and the presence of canine parasitic elements in the centre of Bologna and in Bolognina district, are lower compared to other studies (Poglayen et al., 2000, parassitologia, 42 (suppl1):220; Rinaldi *et al*., 2006, BMC Vet. Res. 2: 29; Zanzani *et al*., 2014, Sc. World J. http://dx.doi.org/10.1155/2014/132361, Beraldo et al., 2014, Atti XXVII SoIPa, 258). The low parasites prevalence in this area agree with the results of the diagnostic routine of our lab.

P38. Seroprevalence of the cat lungworm *Aelurostrongylus abstrusus*- in central Italy

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Keywords: diagnosis, aelurostrongylosis, ELISA

INTRODUCTION. *Aelurostrongylus abstrusus* (Metastrongyloidea, Angiostrongyliidae), i.e. the "cat lungworm" infects cats worldwide. The Baermann's method is considered the *gold standard* for the diagnosis of *A. abstrusus* infection, although its sensitivity and specificity can be impaired by different factors (e.g. prepatency, intermittent larval shedding and low parasite burdens) (Traversa et al., 2010, Parasit. Vectors, 3:62). To overcome these limitations an enzyme-linked immunosorbent assay (ELISA) has recently been developed (Zottler et al., 2017, Vet. Parasitol., 235:75-82) as an alternative diagnostic tool. This is the first field study relying on this ELISA for the serological detection of antibodies *vs* the cat lungworm *A. abstrusus* in endemic areas.
MATERIALS AND METHODS. Sera samples of 250 cats from two endemic regions of Italy were tested for the presence of antibodies *vs A. abstrusus* by the ELISA, *i.e*. 162 from Abruzzo (Site A) and 88 from Umbria (Site B). In particular, 20 serum samples of cats infected with *A. abstrusus* and 20 of cats negative for lungworms by Baermann's and PCR were used to select an OD cut off value (Subset A). Sera of 210 cats scoring negative for lungworms at Baermann's were also tested (Subset B).
RESULTS AND CONCLUSIONS. A cut off value of 0.347 OD (sensitivity 95% and specificity 100%) was determined. Antibodies *vs A. abstrusus* were present in 45 samples (21.4%) from Subset B, in particular 28/142 (19.7%) and 17/68 (25%) from sites A and B respectively. These results show a higher prevalence of *A. abstrusus* if compared with those obtained in other surveys carried out in the same areas (Traversa et al., 2008, Parasitol. Res., 103:1191-1196; Di Cesare et al., 2011, Parasitol. Res., 1S87:96; Di Cesare et al., 2015, Parasitol. Res., 114:4463-4469) that used the Baermann's test to diagnose the infection. This study confirms the endemicity of cat aelurostrongylosis in Italy, and shows that the newly developed ELISA represents a potential alternative for future large-scale epidemiological studies and for the clinical diagnosis of the infection, being able to detect even subclinical non patent infections and therefore overcoming the existing difficulties of copromicroscopic examinations.

P39. Parasitic contamination in off-leash fenced dog areas' soil: an underestimated zoonotic risk?

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Keywords: zoonoses, soil, dog parks, protozoa

INTRODUCTION. Off-leash fenced dog areas', created with the aim of controlling zoonotic risks posed by dog parasites, answer today to the problems of urban fecalization due to the increased numbers of owned pets. However, although faeces collection is mandatory, failure to comply with rules may pose a sanitary risk not only to dogs but also to owners of animals attending dog areas. With the aim of evaluating the zoonotic risk due to fecal contamination, a survey on parasitic contaminations in the dog areas' soil was planned in Milan, one of the first cities for the number of owned pets in Italy.

MATERIALS AND METHODS. In a period comprised between March 2016 and November 2017, 148 dog areas within Milan municipalities were sampled, collecting 200 g of soil from representative points of the area. For helminths detection, 20 gr of soil were processed according to Roepstorff & Nansen (FAO Animal Health, 1998) followed by FLOTAC® technique (NaNO3, s.g.=1200). Cysts/oocysts of *Giardia/Cryptosporidium* were detected by direct fluorescence assay (DFA) (MERIFLUOR® *Cryptosporidium/Giardia*, Meridian Bioscience) after concentration through flotation with sucrose solution (s.g.=1180); positive samples were subsequently submitted to molecular analysis (Pallant et al., 2015, Parasite Vector., 8:2; Traversa et al., 2008, Mol. Cell. Probes, 22:122–128).

RESULTS AND CONCLUSIONS. Considering FLOTAC® results, parasitic eggs belonging to the following taxa were found in 54 soil samples: *Trichuris vulpis* (Number of areas=32; Percentage=21.6%; Abundance per 20 gr of soil=0.74; Intensity per 20 gr of soil=3.43), *Toxocara* sp. (N=23; P=15.5%; A=1.06; I=6.87) and *Eucoleus aerophilus* (N=3; P=2%; A=0.02; I=1). Moreover, by DFA six samples resulted positive for *Giardia* sp. (4.1%) and four for *Cryptosporidium* sp. (2.7%). Molecular analysis confirmed the presence of *Giardia* sp. and *Cryptosporidium* sp. only in one sample each, probably due to possible PCR inhibitors in soil samples (Mayer and Palmer, 1996, Appl. Environ. Microbiol., 62(6):2081-2085). Data obtained in the present survey, in line with those previously reported on urban fecal contamination in the same study area (Zanzani et al., 2014, Scientific World Journal, Article ID 132361), confirmed off-leash fenced dog areas as crucial from an epidemiological viewpoint for the spread and maintenance of zoonotic parasitic infections, suggesting the risk not only for pets but also for public health.

P40. Keratinophylic fungi in off-leash areas soil of Bologna (Northern Italy)

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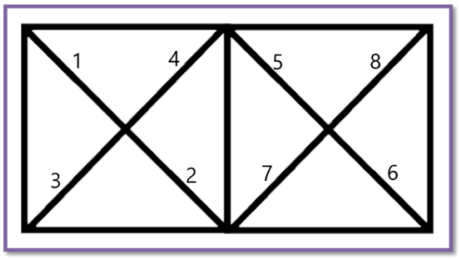
Keywords: Canine faecal contamination, parasites, Bologna

INTRODUCTION. Off-leash areas, created for dogs to exercise and play in a controlled environment, are increased in urban areas all around the world. Together the benefit, public health considerations can concern also zoonotic risk (Rahim et al., 2017 J. Comm. Health, 43:433–440). It is known that the presence and quantity of keratinophilic fungi in the soil is closely related to the animalization process: soils rich in keratin material of animal origin constitute a suitable pabulum for the survival and multiplication of potentially pathogenic agents for humans and animals (Mantovani,1978, Mycopathol., 65: 61-66). The aim of this study was to evaluate the presence of keratinophilic fungi in the soil of off-leash areas in Bologna.

MATERIALS AND METHODS. Six areas were selected; every ones was divided in two subareas in each of which a x sampling scheme was used (according to DM 11/5/1992), collecting a sample of superficial soil every 2 meters along the diagonals. From each area, 8 pools for mycological examination (see figure) were obtained, and handled according to Vanbreuseghem et al., (1978. Guide pratique de mycologie medicale et veterinaire, Masson, Paris) to isolate keratinophilic fungi, using human hair as baits. A total of 56 pools were examined.



RESULTS AND CONCLUSIONS. All the off-leash areas were positive for the geophilic *M. gypseum*, potentially pathogenic for humans and animals, found in 43/56 samples (76.8%); also the geophilics *Trichophyton ajelloi* (2/56-3.6%) and *Chrysosporium* sp (4/56-7.1%) were occasionally found. In this research, the assortment of genera of keratinophilic fungi was lower than other surveys carried out in soil from parks of urban areas or in soil from lairs of wild animals (Morganti and Tampieri, 1984, Nuovi Ann Ig. Microbiol, XXXV: 43-50; Gallo *et al*., 2005, Med Mycol. 43,:373-379; Galuppi *et al*., 2002, VI congresso FIMUA, 127-128), and no zoophilic dermatophytes were found. The lack of the latter should not be surprising, because they do not replicate in the ground and are rapidly destroyed by the environmental microflora, so their occasional finding in the soil is usually considered a consequence of a recent contamination due to infected animals (Lostia and Pinetti, 1970 , rassegna medica sarda, 73: 71). Vice versa, a higher percentage of positive samples for *M. gypseum* was found. Probably, the high attendance of a single animal species (dog) in these areas may promote the development of an ecological niche favourable to the development of this mycete. It could be of interest to verify, in collaboration with dermatologists, if an higher prevalence of dermatophytoses due to *M. gypseum* is observed in people attending off-leash areas.

P41. The fourth state of matter chapter II: biocidal effect of plasma against yeast

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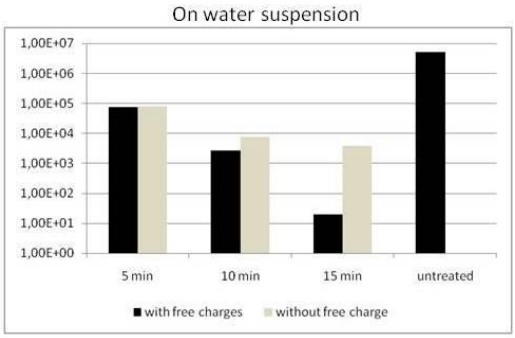
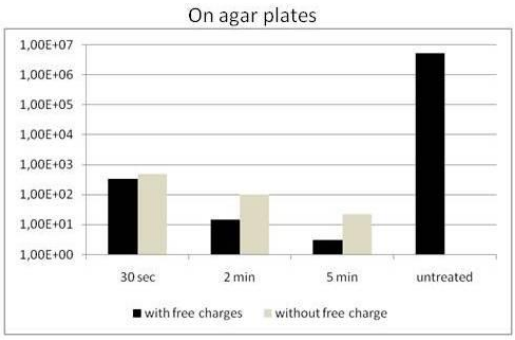
Keywords: annular plasma synthetic jet actuator (PSJAs), charged particles, *Candida guilliermondii*

INTRODUCTION. The use of plasma for sanitization purpose is of increasing interest. Annular plasma synthetic jet actuators (PSJAs) had demonstrated their ability to produce and induced tubular flow (Neretti et al., 2017, J. Phys. D: Appl. Phys. 50 015210, 9pp) normal to the surface where the dielectric barrier discharge is ignited. This typology of reactor enhances the transport of reactive species toward the treated samples (Taglioli et al., 2016, Plasma sources Sci. Technol., 25 06LT01-5pp.). Long life charged particles are generated within the plasma region and then can be advected together with the induced flow. In this work the effect of free charges into induced flow have been tested against *Candida guilliermondii* both in agar plates and in water suspension.

MATERIALS AND METHODS. Eighty ml suspension of *C. guiliiermondii* in saline solution (0.5 Mcfarland opacity) was prepared. The CFU of untreated suspension was determined performing multiple dilution that were plated on Sabouraud Dextrose Agar (SDA) petri dishes that were incubated at 26°C for 48 hours. Aliquots of 100 µl of suspension were spread on the surface of 6 SDA plates (9 cm diameter); aliquots of 20 ml of suspension were put into six 9 cm plates. Agar plates were subjected to induced flow for 30 sec, 2 and 5 minutes and suspension plates for 5, 10 and 15 minutes, both with and without blocking the charges by a grounded metallic mesh. After treating, from each suspension CFU was calculated as previous described, while in the agar plates the colonies were directly counted after incubation.

RESULTS AND CONCLUSIONS. The untreated yeast suspension contained 5.21 x 10^5 CFU/100 µl. In treated samples, the reduction of the CFU of the yeast was related to the treatment time and to presence/absence of free charges. The effect was greater on agar plate than on suspension: at the same time of treatment (5 minutes), only 1-log reduction of CFU were observed on suspension, while 4-log reduction was observed in agar plates. Additional 1-log reduction was observed when free charges have been allowed to reach the sample. On suspension, longer treatment time (15 min) was needed to obtain 2-log reduction without free charges and 4-log reduction with free charges.





P42. An epidemiological updating on the nematode *Sulcascaris sulcata* (Anisakidae, Nematoda) in Adriatic Sea

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Keywords: *Sulcascaris sulcata, Caretta caretta, Pecten jacobaeus*, Mediterranean Sea

INTRODUCTION. The nematode *Sulcascaris sulcata* is frequently recovered in stomach and esophagus of loggerhead sea turtles *Caretta caretta* in neritic feeding grounds worldwide. The existence of an intermediate host in its life cycle, represented by benthic gastropods and bivalves (Berry and Cannon, 1981, Int. J. Parasitol. 11: 43-54), accounts for its presence in shallow coastal waters, such as those of the Eastern Mediterranean and the Adriatic Sea (Gracan et al. 2012; DAO 99:227-236). The adult parasite, harboring in the stomach of marine turtles is associated to cases of ulcerative gastritis (Santoro et al. 2010, Parassitologia 52:364). The aim of this work is to provide an updating on the epidemiology of *S. sulcata* in Adriatic Sea.
MATERIALS AND METHODS. During five years (2013-2017), the whole digestive tract of 134 loggerheads stranded along Northern Adriatic Sea was opened and examined to search for *S. sulcata* parasites. The isolated parasites were fixed in 70% ethanol, clarified in Amman's lactophenol and identified following the dichotomous keys. Copromicroscopic exam by sedimentation and flotation method with high-density solution (s.g.1.450) was carried out in all animals (n=134). Besides, in 2017, during routine sampling, 37 fresh scallops (*Pecten jacobaeus*) caught in Northern Adriatic Sea were submitted to parasitological examination. All the collected parasites were morphologically and molecularly identified by a PCR-RFLP specific for the family Anisakidae and a PCR targeting the Cox-2 mtDNA gene.
RESULTS AND CONCLUSIONS. Adult and/or larval stages or of *S. sulcata* were found in the digestive tract of 40 loggerheads and eggs were observed in the feces of other 12 animals (overall prevalence: 38.8%). Gastric ulcers were observed in fifteen animals. In eight animals, parasites were detected, with copromicroscopic exam being negative; most of these turtles had only immature parasites or few specimens in the gastrointestinal tract. In all cases in which only eggs of *S. sulcata* were found, the bad conservation of the carcasses might have prevented the finding. Eighteen specimens (48.7%) of scallops were positive for larvae of *S. sulcata* in the muscle. The prevalence of *S. sulcata* in *C. caretta* in this study is higher than that previously reported by other Authors in Northern Adriatic Sea (Gracan et al. 2012; DAO 99:227-236). Although *S. sulcata* larvae have been found in several mollusc species in the world, *P. jacobaeus* is the only intermediate host detected in the Mediterranean basin. Further samplings will be required in order to define the real prevalence of *S. sulcata* in this intermediate host and to identify any other potential intermediate host of the parasite in this important foraging area for Mediterranean loggerhead population.

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P43. Comparison between two quantitative methods and two different matrixes for the evaluation of parasitic burden in *Hapalotrema mistroides* infections

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Keywords: Spirorchiids, egg burden, McMaster

INTRODUCTION. Infection by the blood fluke *Hapalotrema mistroides* (Digenea: Spirorchiidae) has been recently reported in sea turtles *Caretta caretta* in different Mediterranean regions (Santoro et al., 2016, Dis Aquat Org 124:101-8, Marchiori et al., 2017, Parasites&Vectors 10: 467). Eggs of *H*. *mistroides* are commonly found in all host's organs, but release of eggs probably happens by fecal route (Stacy et al., 2010, Dis Aquat Org 89:237-259). Observation of post mortem gross and microscopic lesions is generally used to assess severity of the disease, but few attempts have been done to standardize the evaluation of the parasitic burden by tissue egg counts (Work et al., 2005, J Parasitol 91(4):871-876).

MATERIALS AND METHODS. Feces and spleen homogenates of 105 loggerheads, stranded dead along North-western Adriatic Sea in the period 2013-2017, were submitted to a sedimentation-flotation technique for the research of spirorchiids eggs; quantification of eggs was then achieved in positive feces and spleen by a modified McMaster method (2 gr of feces/spleen homogenate in 6 ml of high density solution, s.g. 1450). Spleen homogenates were also submitted to a second quantification by the method previously proposed by Work et al. 2005 (J. Parasitol., 91(4):871–876), which includes a preventive chemical digestion of tissues with a 2% pepsin - 0,03% HCl solution. Concordance between research of eggs in feces and spleen was calculated and evaluated using the kappa-type statistics (Landis and Koch, 1977, Biometrics 33:159-174). Correlation between fecal and splenic egg counts and between splenic counts obtained with the 2 different methods were calculated using the test Rho of Spearman.

RESULTS AND CONCLUSIONS. High concordance was obtained between qualitative examination of feces and spleen (95%, k=0,874), revealing that copromicroscopic exam can be an appropriate method for the diagnosis of the infection *in vivo*. Low correlation (Rho=0,418) was found between fecal egg counts and splenic egg burden, thus fecal burden cannot be regarded as indicative of disease severity. High correlation was instead found between splenic egg burden calculated with the two methods (Rho=0,904). Thus modified McMaster method can represent a good alternative for counting tissue egg burden compared to that of Work et al. (2005), in consideration of the fact that it is faster and cheaper. However, counts result underestimated with modified McMaster method, therefore appropriate ranges must be calculated to compare results between the two methods and to correlate them with severity of the infection in the investigated geographic area.

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P44. First molecular detection of *Pennella* sp. (Siphonostomatoidea: Pennellidae) in cetaceans from the Mediterranean Sea

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Keywords: *Pennella*, *Balaenoptera physalus*, fin whale, Mediterranean Sea

INTRODUCTION. Copepods of the genus *Pennella* (Oken, 1816) are large mesoparasites infecting teleost fishes and marine mammals. Two species are mainly described from fish (*P. filosa* and *P. instructa*); while, one (*P. balaenopterae*) is described in cetaceans. The reports in cetaceans concern specimens of adult females embedded into the skin of various host species and the description of first naupliar stage of parasite; the male is free-living (Arroyo et al. 2002, Sarsia, 87: 333-337). The aim of this report is to describe immature stages of *Pennella* sp. collected in a fin whale (*Balaenoptera physalus*) stranded along Italian coast.

MATERIALS AND METHODS. Specimens of immature stage copepods were collected from the skin-blubber of two fin whales (*B. physalus*) stranded along the Sardinian and Tuscan coast on 2011 and 2013, respectively. Six entire parasites were studied by light microscope (Nis Elements D software, Nikon) and were referred to *Pennella filosa*, according to the morphological features reported by Thompson (1905, Biol Bull 8 (5): 296-307). A portion of one specimen was submitted to molecular analyses by a PCR, amplifying the mtDNA *cox*1 gene (using the primers LCO1490 and HCO2198 (Folmer et al., 1994; Mol Mar Biol Biotechnol. 3(5): 294–299)). The obtained sequence was compared with those previously obtained, at the same gene locus, from specimens of *P. balaenopterae* (from fin whale and sperm whale); *P. filosa* (from bluefin tuna) and *P. instructa (*from swordfish) Alignment of the sequences was performed using BioEdit and genetic analysis (MP, NJ) by MEGA6.0 was performed.

RESULTS AND CONCLUSIONS. The morphological features of the samples (size, characteristics of the cephalothorax and the abdomen) allowed to ascribe the specimens to the genus *Pennella*. The molecular analysis showed that sequence obtained clustered in the same clade with the specimens of *P. balaenopterae P. filosa* and *P. instructa*. The genetic similarity among all the different *Pennella* spp. suggests that other genetic/molecular markers should be used to clarify whether they are separated species, or morphotypes adapted to different hosts.

This work was financially supported by a Research Project of Padova University (Prot. CPDA149521/14).

P45. Seasonal variability of the parasite fauna of the Atlantic chub mackerel *Scomber colias* (Osteichthyes: Scombridae) in the western Mediterranean Sea

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Keywords**:** parasite fauna, fish parasite, parasite distribution

INTRODUCTION. The Atlantic chub mackerel *Scomber colias* (Osteichthyes: Scombridae) is a pelagic species with a relevant interest for human consumption in the Mediterranean area.
MATERIALS AND METHODS. Thirty specimens of *S. colias* were monthly caught in the Gulf of Asinara (North Sardinia, western Mediterranean Sea) from June to September 2014 and 2015 (only 21 in September 2015, total number = 231) for parasitological examination. Fish were measured, weighed, dissected and all the organs observed separately by naked eye and under dissecting microscope for metazoan parasites. Prevalence (P%) and mean abundance (mA) of parasite species were calculated. Yearly and monthly abundance of parasites were compared with the Welsh bootstrap t-test to estimate the possible association of infection with the temporal variables.
RESULTS AND CONCLUSION. Fifteen parasite species were found in *S. colias*: *Grubea cochlear* (P%= 5%, mA= 0.05), *Kuhnia scombri* (16%, 0.2), *Pseudokuhnia minor* (28%, 0.4), *Cephalolepidapedon saba* (1%, 0.01), *Lecithocladium excisum* (45%, 3.8), *Neonematobothrioides faciale* (17%, 0.4), *Nematobothrioides filiforme* (32%, 1.2), *Allonematobothrioides scombri* (4%, 0.1), *Allonematobothrioides* sp*.* (15%, 0.3), *Opechona bacillaris* (23%, 4.3), *Prodistomum orientalis* (23%, 7.4), *Hysterothylacium* sp. (80%, 2.9), *Anisakis* sp. (61%, 3.2), *Radinorhynchus lintoni* (46%, 0.9), *Clavellisa scombri* (3%, 0.03) The total mean abundance of *K. scombri*, *L. excisum*, *N. faciale*, *N. filiforme*, *Allonematobothrioides* sp*.*, *O. bacillaris* and *P. orientalis* differed between the two years (p-value<0.05). The comparison of the same months in the two years showed that the mA of none of the species differed between August 2014 and 2015; whereas, that of several species differed only in one month (*P. minor*, *Allonematobothrioides* sp., *Hysterothylacium* sp. in June, *P. orientalis* in July, *Anisakis* sp. in September); *N. filiforme* in two months (June and July) and *L. excisum* in three months. This fact suggests that the occurrence of parasites in *S. colias* from the Gulf of Asinara is not constant during the year and it is probably linked to the temporal variability in the marine ecosystem and host size/age. The study of these associations can elucidate the epidemiological patterns of parasites, specially those considered as zoonotic species and those eligible as biological tags to investigate the migratory pathway of *S. colias* in the area.

P46. A 9000 years long history of trematodes parasitism in brackish settings of Italy

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Keywords: *Abra segmentum*, Gymnophallidae, Italy, Paleoparasitology

INTRODUCTION. The rising global temperature and sea-level have led to concern about the increase of parasites at our latitudes. Ecological studies on parasitism is forcibly restricted to short-time intervals (months/years). The Holocene fossil record can offer a quantitative archive of ecological responses to geological short (10^2-10^3 yr) but societally relevant past climate transitions. The Gymnophallid digenetic trematodes encyst as metacercaria in the brackish bivalve *Abra segmentum* and induce the active growth of oval pits on the interior of the shell. These pits are preserved in the fossil record, thus providing a proxy for past parasitic dynamics. This study investigates prevalence of pits related to Gymnophallids through the last 9 ky in modern and Holocene brackish settings.

MATERIALS AND METHODS. From winter 2016 to summer 2017 three samplings were performed: 200 *Abra segmentum* were collected in the lagoons of Piallassa Baiona and Saline di Cervia (Emilia Romagna) while 300 *A. segmentum* from Lesina (Apulia). Each specimen was examined for the presence of larval stages of digenean trematodes. The soft tissues of infected clams were preserved in 70% ethanol for further parasitological analyses. The shells of all bivalves (modern and fossil) were observed with a stereomicroscope to detect the presence of parasite traces.

RESULTS AND CONCLUSIONS. In modern brackish setting parasite prevalence, when restricted to samples with only metacercariae, shows a low spatial variability. The mean prevalence is comparable among Lesina and Saline di Cervia (21%, and 20% respectively) and lower in Piallassa Baiona (7%). Within the Holocene specimens, significantly, elevated infections are recovered in samples proximal to previously documented flooding surfaces. In addition, sizes of the pits on the living bivalve shells are smaller and morphologically less pronounced (shallow) than the fossil counterparts. Finally, the prevalence of pits in the brackish deposits during phases of rapid sea level rise (flooding surfaces), is significantly higher (~57%) than the highest prevalence recorded in modern settings (~32%). This result hints a possible association between significantly elevated prevalence and centennial scale flooding events and support the link between sea-level rise and increasing parasite activity. The recognition of the link between parasite prevalence and past sea-level rises provides us with an important reference framework for assessing near-future parasite related threats ignited by global warming.

P47. Morphological and molecular studies on larval and adult stages of *Eustrongylides* sp. (Nematoda, Dioctophymatidae)

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Keywords: *Eustrongylides*, fish, cormorants

INTRODUCTION. *Eustrongylides* is a genus of Dioctophymatid nematodes characterized by a complex cycle linked to freshwater habitats and involving two intermediate hosts (oligochaetes and benthivorous fish respectively), transport hosts (predatory fish, amphibians and reptiles) and piscivorous birds as definitive hosts. Few cases of human infection by consumption of raw or undercooked infected fish have been reported worldwide, pointing out the zoonotic potential of this nematode. In fish, the fourth larval stage is encapsulated in muscles, visceral organs and on the internal surface of body cavities. In birds, adults live in the wall of proventriculus, with the middle of the body embedded in a capsule and the cephalic and caudal ends free in the lumen. This nematode is reported to be responsible of large outbreaks of mortality as it can affects nestlings of seven orders of birds worldwide. The presence of *Eustrongylides* sp. has been recently reported in fish from Trasimeno lake (Dezfuli, 2015, Parasit. Vectors, 8: 227) and surveys are currently in progress to identify the parasite species and define its epidemiology in Italian freshwater ecosystems. In this study a combined morphological/molecular approach has been applied to identify larval and adult stages of *Eustrongylides* sp. detected in fish and birds respectively.

MATERIALS AND METHODS. Twelve fourth-stage larvae were isolated from muscle and body cavity of freshwater fish from Trasimeno lake (*Perca fluviatilis* and *Atherina boyeri*), and 50 portions of adult worms were collected from proventriculus of two great cormorants (*Phalacrocorax carbo*) found dead along the lake shores. The nematodes were preserved in 70% ethanol for morphological and molecular analyses. A little portion of the central part of the body were cut for molecular analyses, whereas cephalic and caudal extremities were clarified in Amman lactophenol for morphological study. The DNA was extracted with a commercial kit, and the ITS rDNA amplified and sequenced.

RESULTS AND CONCLUSIONS. Morphological study of fourth-stage larvae allowed to identify them only at genus level as *Eustrongylides* sp., while morphological features of adults, with main reference to caudal end and genital system of males, were consistent with the species *E. excisus*. The ITS rDNA sequences obtained from larvae and adults were identical to each other and, when compared by BLAST search, gave 96-99 % of similarity with the sequences of *Eustrongylides* sp. available in GenBank.

P48. Nodular gill disease, an emerging pathology in Italian farmed rainbow trout (*Oncorhynchus mykiss*)

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Keywords: Nodular gill disease, Amoeba, Rainbow trout, *Oncorhynchus mykiss*

INTRODUCTION. Nodular gill disease (NGD) represents one of the most serious pathology affecting freshwater farmed rainbow trout (*Oncorhynchus mykiss*). This disease is caused by different species of amoeba, both testate amoebae (*Roghostoma minus*) and naked amoebae (*Acanthamoeba, Vermamoeba, Naegleria, Hartmannella, Protacanthamoeba* and *Vannella*) as described by Dykova *et al.* (2010; 2015). NGD has been reported in rainbow trout farms of North America and Europe (Denmark, Germany, Poland, Czech Republic and, recently, Italy).
MATERIALS AND METHODS. In the last few years, NGD has spread in rainbow trout farms of Northern and Central Italy. Affected fish show dyspnea and alterated swimming behavior, staying close to water surface and walls of the tanks. Farmers report higher mortality rates during winter, mainly at temperatures ≤10 °C, with losses up to 60% of the tank population. To investigate about outbreaks, several affected fish were collected for necropsy. Gills were submitted to microscopical and histological examinations with Giemsa stain.
RESULTS AND CONCLUSIONS. The macroscopic examination of gills revealed swelling, excessive production of mucus and multiple whitish nodular lesions (≥1mm diameter) localized mainly in the distal parts of filaments. Microscopically, the affected gill filaments showed a swollen and clubbed profile, especially in the apical part. In the most severe cases, several filaments coalesced into a single mass of proliferative tissue. Along the surface of gill filaments and lamellae, particularly in those interested by cell proliferation, a number of amoebic organisms (around 15 x 20 µm) was observed. The histology showed multi-focal and diffused in heavy cases epithelial hyperplasia of the gills causing lamellar fusion, with infiltration of inflammatory chronic cells, mucous cells hypertrophy, cellular sloughing and necrosis in presence of organisms referable to amoebae above the surface of the affected filaments. Studies are in progress to clarify pathogenesis, biological and environmental determinants of NGD in rainbow trout farms.

P49. Epidemiological studies to identify risk factors for *Saprolegnia* infections in Italian trout farms

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Keywords: Saprolegnia, trout farms, Italy, epidemiology

INTRODUCTION. *Saprolegnia* spp. are widespread oomycetes in freshwater environment and are among the main sources of economic losses due to disease in salmonid aquaculture. Our study was aimed at investigating risk factors influencing the emergence of Saprolegniosis in Italian trout farms.

MATERIALS AND METHODS. Five trout farms in northern Italy were selected on the basis of the documented presence of different management risk factors, besides temperature, for Saprolegniosis (e.g. vaccination, stripping, grading, frequent tank change). The study included rainbow trout *Onchorhynchus mykiss*, marble trout *Salmo trutta marmoratus* and brown trout *Salmo trutta fario*. In each farm, periodic visits were carried out every month during the cold season, at higher risk for Saprolegniosis, and once during summer, for a total of 8 visits from February 2017 to January 2018. A selected tank from each farm was equipped with a data logger for continuous monitoring of water temperature during the study period. Furthermore during each visit pH, temperature and dissolved oxygen were collected and clinical inspection of fish to assess prevalence of lesions referable to Saprolegniosis was performed. During each visit detailed information about management practices/fish handling, concurrent infections and mortality were collected. Only during the first visit, five fish with Saprolegniosis were sampled from each farm in order to characterize the circulating species of *Saprolegnia*.

RESULTS AND CONCLUSIONS. As expected, the prevalence of *Saprolegnia* was on average higher during the cold months (< 10 °C) and in strict correlation with different handling practices. Only in one farm Saprolegniosis prevalence increased at relatively higher water temperature (14 °C) after intraperitoneal vaccination. All isolates from fish with clinical signs during the first visit belonged to the species *Saprolegnia parasitica*. Preliminary results of this survey highlight the combination of low temperature and handling practices as relevant risk factors for Saprolegniosis. The complete statistical and epidemiological analyses of the collected data will be processed together with the results obtained from parallel surveys in Spain and Scotland in order to identify the main risk factors and design biosecurity and management strategies to control Saprolegniosis in salmonid farms.

The study was conducted within the framework of ParaFishControl, a EU H2020-funded project (634429) aimed at increasing sustainability and competitiveness of the aquaculture industry by controlling and mitigating parasitic species affecting the main European farmed fish species.

P50. SARCOPTIC MANGE AND CLIMATIC FACTORS AFFECT CHAMOIS POPULATION DYNAMICS IN THE PANEVEGGIO-PALE DI SAN MARTINO NATURAL PARK (ITALY)

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Keywords: surveillance, mortality, mange, chamois

INTRODUCTION. Sarcoptic mange is known to be the most severe disease affecting mountain ungulates. Notwithstanding, it is difficult to define its "standard impact", even in naïve populations, since other factors can contribute to mortality. The aim of the present work is to describe the trend of the mange epidemic occurring since 2007 in the Park "Paneveggio Pale di S. Martino" (PPPSM – TN, Italy), comparing two different approaches to passive surveillance and trying to disentangle the impact of mange from other causes of mortality in the alpine chamois population.

MATERIALS AND METHODS. Data analyzed came from routinary passive sanitary surveillance in chamois from 2006 to 2016 and population censuses of the same period on the whole study area. Besides, data from an intensive census and surveillance protocol, conducted from 2008 to 2013 in a specific and more restricted area, were also used to compare the effectiveness of the two approaches.

RESULTS AND CONCLUSIONS. 426 chamois were found in the field. The peak of mortality, including mange cases, occurred in 2009. An analysis of mortality causes showed an evident contribution of other factors, namely starvation and winter mortality, to this epidemic peak. In particular, high winter mortality appeared related to higher snowfall occurred in winter 2008-2009 when compared to previous winter seasons. Even though mountain ungulates are adapted to harsh winters, stochastic events can affect survival, population size and dynamics. Harsher winter and the peak of mange epidemic may have amplified each other's impact on chamois, leading its density to values close to the estimated threshold for the transmission of *Sarcoptes scabiei*. Since climatic events appear as more and more variable and unpredictable, association between pathogens and climatic conditions may require a greater consideration for conservation and management. Considering the surveillance sensitivity, the intensive protocol increased the probability of a proper diagnosis and confirmed the seasonal and gender mange patterns, whereas no effect was observed in detecting younger age cases in the field. This is probably due to the small size of this kind of individuals that are rapidly removed from the field by scavengers or remain as hidden in the alpine environment.

P51. Parasitological survey in red foxes (*Vulpes vulpes*) from central Italy

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Keywords: Endoparasite, Ectoparasite, Fox, Central Italy

INTRODUCTION. The parasitofauna of the red fox (*Vulpes vulpes*), one of the most common wild carnivores in Italy, is influenced by prey availability and geographical settings. Foxes may act as reservoirs of zoonotic endoparasites, e.g. *Toxocara canis*, *Trichinella* spp., *Echinococcus* spp., and ectoparasites, e.g. *Ixodes ricinus*, *Sarcoptes scabiei* and *Pulex irritans* (Sréter et al., 2003, Vet. Parasitol, 115:349-354; Magi et al., 2009, J. Wildl. Dis, 45:881-885). Information on parasitofauna of red foxes in Italy is limited (Magi et al., 2015, J. Helminthol, 89: 506-511; Perrucci et al., 2016, Parasite Epidemiol. Control, 1: 66-71), thus the present study has investigated the parasite occurrence in a fox population of a selected region (Marche) to increase current knowledge on the epidemiology of fox endo- and ecto-parasites.

MATERIAL AND METHODS. From February to April 2016, 22 hunted red foxes were investigated for helminths and ectoparasites by examination of hair (visual inspection and combing), fecal (flotation and Baermann's tests) and blood (Knott's test) samples. Also, lungs and heart were examined at necropsy. All parasites retrieved were identified in accordance to bibliographic keys.

RESULTS AND CONCLUSIONS. Overall 19 (86.4%) fecal samples were positive for endoparasites: *Angiostrongylus vasorum* (n.13), *Eucoleus aerophilus* (n.11), ancylostomatids (n.8), coccidia (n.5), *Trichuris vulpis* (n.3), taeniids (n.3), *Crenosoma vulpis* (n.2). Eleven multiple infections were recorded, being the most frequent association *E. aerophilus* + ancylostomatids (n.5). All blood samples were negative for parasites. Thirty adult nematodes collected from 11 foxes were identified as *A. vasorum*. All foxes were positive for ectoparasites and, overall, 36 ticks and 63 fleas were collected, being the most common species *Rhipicephalus sanguineus* and *Pulex irritans*. Necropsy findings consistent with angiostrongylosis included emaciation and multifocal to coalescing, bilateral, red to brown, firm foci throughout the lungs and myxoid degeneration of the right heart valve. Microscopic examination revealed granulomatous bronchopneumonia throughout the lungs, with aggregates of parasite larvae and obliterative vasculitis. The present study indicates that red foxes of central Italy harbor many parasites that were previously reported in other areas, thus suggesting that foxes could be potential sources of parasitosis to pets and, occasionally, humans in broad geographical areas.

P52. Myiasis by *Philornis* sp. (Diptera: Muscidae) in a young Mexican Harris hawk (*Parabuteo unicinctus)*

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Keywords: Myasis, *Philornis*, Harris hawk, *Parabuteo unicinctus,* México

INTRODUCTION. The genus *Philornis* (Diptera, Muscidae) comprises approximately 50 species of fly occurring throughout Central, South America, and extending north to southern North America. Larvae of the genus *Philornis* reside in bird nests and may feed on blood, tissues or fluids. In this abstract, we report the presence of *Philornis sp* fly larvae found on young Harris hawks (*Parabuteo unicinctus*).

MATERIALS AND METHODS. A total of sixty-eight fly larvae were removed from furuncle type lesions on the head, breast, thighs, legs and toes of two one-month-old Harris hawk from Morelos, Mexico. Larvae were identified at the Department of Parasitology of the Faculty of Veterinary Medicine and Zootechnics (FMVZ) of the National Autonomous University of Mexico (UNAM). Fly larvae were mounted by transferring them into clean glass slides with Hoyer solution; morphology of spiracles was observed by stereoscopy microscope.

RESULTS AND CONCLUSIONS. After structural examination, we determined that fly larvae belonged to the genus *Philornis sp.* The importance of this finding is that injuries caused by *Philornis sp,* larvae in muscular and cartilaginous tissues can produce irreversible deformations potentially affecting the movement of birds, decreasing their ability to hunt and, therefore, compromising free-ranging populations of Harris hawks.

P53. Haemoparasites affect fitness related traits in barn swallows (*Hirundo rustica*)

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Keywords: Avian malaria, *Hirundo rustica*, haemosporidian, fitness

INTRODUCTION. Parasitism is recognized as an important evolutionary factor. It influences many aspects of the host life, also compromising the health and survival of the host, and its mating success. In birds, haemosporidians, including species from the *Plasmodium*, *Leucocytozoon* and *Haemoproteus* genera, are very common blood parasites that cause avian malaria. In this study we investigated infection of long-distance migratory small passerine barn swallow (*Hirundo rustica)* by these parasites, and the consequences for host life-history traits.

MATERIALS AND METHODS. In 2013 we captured, sexed and marked 199 adult barn swallows breeding at 11 farms located in the Northern Italy, and a blood sample from each individual was collected. Nests were weekly inspected to record all breeding events during the breeding season. DNAs were extracted from blood and a nested PCR protocol was performed on the extracted DNA samples as reported in Hellgren et al. (2004, J. Parasitol*.,* 90(4):797–802). The PCR process permits the amplification of a conserved region of the cytochrome b gene allowing the discrimination of the haemosporidians. Then, the positive samples were sequenced to determine genera and species of the infecting parasites. We also analyzed blood samples from 88 offsprings before their first migration to Africa to determine if the most abundant detected haemosporidian could be transmitted also in Europe, and not only in Africa.

RESULTS AND CONCLUSIONS. We assessed infection by haemosporidians in all adult barn swallows; the prevalence was relatively high for *Plasmodium* (males: 14.3%; females: 16.2%), intermediate for *Leucocytozoon* (males: 6.1%; females: 4.5%) and very low for *Haemoproteus* (males: 1.0%; females: 1.8%). In addition, our results provided new evidences for the negative effects of haemosporidian infection on life history traits in birds, including sexually selected ones, and that these effects vary depending on age and sex of the host.

P54. Cystic echinococcosis in wild boars hunted from southern Italy

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Keywords: Cystic echinococcosis, Wild boars, Southern Italy, Hunting

INTRODUCTION. Cystic Echinococcosis (CE) is one of the most important parasitic zoonotic disease in the world and it represents an important public health and socio-economic concern (Dakkak, 2010, Vet. Parasitol, 174:2-11). In the Mediterranean basin, CE is widespread and it is endemic in Italy, with major prevalence in southern regions (Garippa and Manfredi, 2009, Vet. Res. Commun, 33:S35-S39). Several studies investigated CE in domestic pigs, however, data in wild boars are scant. In the last years the wild boar population in Italy is increased (Massei et al., 2015, Pest. Manag. Sci, 71:492-500) and this ungulate could play an important role in the spreading of CE in wildlife. This survey was carried out in order to determine prevalence and fertility rate of hydatid cysts in the wild boar population in Campania Region.

MATERIALS AND METHODS. The carcasses of wild boars, shot down in different hunting areas, examined during two hunting seasons (2016-2017) by 15 veterinary practitioners involved in the regional project "Piano Emergenza Cinghiali Campania". A detailed form was collected for each examined animals, including wild boar origin, gender and age. For each animal, liver and lungs were examined and when cysts were found, their number, morphology and fertility were determined by visual and microscopic examination (Varcasia et al., 2006, Parasitol. Res, 98(3):273-277).

RESULTS AND CONCLUSIONS. Out of a total of 2,107 wild boars examined for CE, 92 (4.4%) were found positive. Positive animals were 45 males and 47 females, aged between 1 to 8 years. The average number of cysts per wild boar was 1.3 (min 1-max 13). The number of cysts per organ examined, typology and viability of hydatids recovered are reported in Table 1.

Table 1			Typology of hydatid cysts		Viability of hydatid cysts		
Organs	N° positive animals	N° hydatid cysts	Unilocular	Pseudo multilocar	Fertile	Sterile	Acephalocyst
Liver	89	118	104	14	25	42	51
Lungs	3	4	4	0	2	1	1
Total	**92**	**122**	**108**	**14**	**27**	**43**	**52**

The total number of cysts collected was 122, of which 118 (96.7%) in the liver and 4 (3.3%) in the lungs. Cysts were 27 (22.1%) fertile, 43 (35.3%) sterile and 52 (42.6%) acephalocyst. The presence of fertile cysts in 19,6% of positive animals is noteworthy. Additional molecular studies are needed to investigate the species of *E. granulosus* sensu lato circulating in wild boar and to evaluate the epidemiological role of this ungulate in the transmission of CE in Italy.

P55. First detection of *Cucullanus carettae* Baylis, 1923 (Nematoda: Rhabditida) in loggerhead turtle (*Caretta caretta*) from the Adriatic sea

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Keywords: loggerhead turtle, *Cucullanus carettae*, Adriatic Sea

INTRODUCTION. *Cucullanus carettae* (Baylis 1923), is a nematode belonging to the subfamily Cucullaninae that has been described worldwide in loggerhead turtles (*Caretta caretta*). Regarding the Mediterranean, *C. carettae* has been just identified in Tyrrhenian and Ionian Sea (Santoro et al., 2010, Parasitol. Int., 59, 367-375). Conversely, until now a description of a unique specimen of *Cucullanus* sp. in loggerhead from the Adriatic sea is reported in literature (Piccolo and Manfredi, Proceedings, of First Mediterranean Conference on Marine Turtles. Rome, 2001, 207-2011).

MATERIALS AND METHODS. In a framework of a bio monitoring project of Abruzzo and Molise coasts, a parasitological survey was performed on stranded and accidentally caught sea turtles, at Istituto Zooprofilattico of Abruzzo and Molise "G.Caporale". During necropsy, the gastrointestinal system of 73 stranded sea turtles (72 *C. caretta* and 1 *Chelonia mydas*) was inspected for the isolation and the collection of the parasites. At the same time intestine samples were collected for histology. Furthermore, 60 and 62 samples of feces were also collected from dead and alive animals respectively and were submitted to sedimentation and flotation technique, using a high density solution (s.g. 1300) for the detection of parasites eggs. Adult parasites of the genus *Cucullanus* were identified according to the specific literature.

RESULTS AND CONCLUSIONS. A massive infestation by *C. carettae* were found in the intestine of one loggerhead, (1.4% positive) associated with chronic lympho-plasmocytic enteritis, while the remaining 72 were negative for the presence of the parasite. To our knowledge this is the first identification of *C. carettae* in loggerhead turtles from Adriatic sea. In addition, three fecal samples from alive turtles and five stools (8/122; 6.6%), collected during necropsy, were positive for *C. carettae* eggs. Additional studies are needed to gain knowledge on the real prevalence and distribution of *C. carettae* in the Adriatic sea compared to other Mediterranean areas. Its presence among the helmintofauna of the animal could indeed contribute to track turtles migratory routes and also to assess the possible impact on this endangered sea turtle species.

P56. Murine populations in the Ponziane islands: biomolecular evaluation of zoonotic pathogens

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Keywords: rodents, Ponziane archipelago, zoonosis

INTRODUCTION. Islands are privileged sites to investigate mechanisms of infection transmission due to their natural isolation and to a detailed knowledge of their biogeography (Zanet et al., 2014. Vet. Par., 199 (3–4): 247-249). Among the pathogens present in the Mediterranean basin, vector-borne protozoa and bacteria together with *Toxoplasma gondii* and *Neospora caninum* are especially relevant because of their burden on human and/or animal health. Within the Life Project PonDerat (LIFE NAT/IT/000544) we investigated presence and prevalence of selected pathogens in the population of Black rats *Rattus rattus* from the Ponziane Islands (Italy).

MATERIALS AND METHODS. Black rats were trapped on the islands of Ponza, Ventotene and Palmarola. Specific PCR protocols were carried out to detect *Leishmania infantum*, *Babesia/Theileria* spp., *Anaplasma/Ehrlichia* spp., *Borrelia burgdorferi* s.l., *T. gondii* and *N. caninum (*Zanet et al., 2014 Parasites & Vectors 7(1): 70; Zanet et al., 2014. Vet. Par., 199 (3–4): 247-249; Rijpkema et al., 1995, J. Clin. Microbiol. 33 (12):3091-3095; Munderloh et al., 1994. J. Clin. Microbiol. 34, 664–670).

RESULTS AND CONCLUSIONS. A total of 38 rats were trapped and analyzed. *Babesia/Theileria* spp. were detected with a prevalence of 36.84% (CI 95% 23.38% - 52.72%). Significantly higher prevalence was recorded in the rats from Palmarola and Ventotene. *L. infantum* was detected in 2 animals from Ventotene with a prevalence of 5.26% (CI 95% 1.46% - 17.29%) while *B. burgdorferi* s.l. was detected in one animal. *T. gondii* was detected in 42.11% of the sampled rats (CI 95% 27.85% - 57.81%). Animals from Ponza and Palmarola were more infected than those from Ventotene. None of the animals tested positive for *N. caninum* nor for *Ehrlichia/Anaplasma* spp. Understanding the role of rodents in the epidemiology of vector-borne diseases and of *T. gondii* and *N. caninum* is a relevant issue for improving disease management and pathogen control. Comparative studies carried out in ecological contexts where the main reservoir/definitive hosts are not present can give useful insights on the epidemiology of these parasites.

P57. Host partitioning of taeniid species in wild and domestic canids at the southern edge of the Italian Western Alps

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Keywords: Taenia spp, Wolf, Domestic dogs, Natural Regional Park of the Ligurian Alps

INTRODUCTION. Taeniids are relevant pathogens in humans and animal, circulating between carnivorous definitive hosts and a variety of wild and domestic intermediate hosts (Craig and Pawlowski, 2002, Vol.341, IOS Press). Their life cycle is based on predator-prey interactions and, especially in the rural areas where wolves, shepherd dogs and livestock are in close contact, transmission of pathogens from domestic to wild animals and humans might occur (Thompson, R. A., 2013, Int. J. Parasitol. 43, 1079-1088). Our study aimed to determine the distribution of taeniid species in canid definitive hosts in a mountainous area, at the interface between wildlife and domestic animals.

MATERIALS AND METHODS. From June to November 2017, we conducted an epidemiological survey in wild and domestic canids in a protected area of the Western Italian Alps in the Imperia province (Liguria region). We collected 152 wolf scats and 37 shepherd dog fecal samples covering 10 standard paths on a monthly basis. So far, 37 fecal samples of wolf and 28 of shepherd dogs were analyzed and taeniid eggs were isolated through a sieving-flotation technique (Mathis et al., 1996, J. Helminthol,70:219-222) from 3 dogs (10.7%) and 4 wolves (10.8%). Species identification was performed as described by (Hüttner at al., 2009, Int.J.Parasitol, 39(11):1269-1276) using the *nad1* and *cytb* mtDNA gene as marker, followed by sequencing of the amplicons.

RESULTS AND CONCLUSIONS. *Taenia hydatigena* was detected in all samples and its wide distribution is probably due to a synanthropic life cycle involving wolves and domestic animals, but also a wild one between wolves and wild ungulates. *Taenia krabbei* was identified in 2 wolves and in 1 dog, indicating that the parasite may circulate mostly in wild hosts but dogs could have a role in its transmission dynamics. *Taenia ovis* was identified in 2 wolves and 1 dog, confirming that domestic animals are part of the wolf diet and that dogs have an easy access to raw offal. *Echinococcus multilocularis* and *E. ortleppi* (G5) were also reported in both wolves and dogs but further molecular confirmations are needed. These results will be correlated with hosts' feeding ecology.

P58. *Trichuris* spp. infecting animals of the Bioparco Zoological Garden of Rome

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Keywords**:** *Trichuris*, zoological garden, conservation biology, zoonotic potential

INTRODUCTION. Animals in zoological gardens may be threatened by parasites, which are facilitated in their transmission especially in case of direct life cycles, high population density and stress conditions. Nematodes of the genus *Trichuris* infect several mammalian species, including humans, being one of three major groups of soil-transmitted helminths (STHs). The genus includes around one hundred recognized species, but only three, *Trichuris trichiura, T. suis* and *T. vulpis*, are considered zoonotic so far. With the aim to characterize whipworms infecting captive animals of the Bioparco of Rome, an integrative taxonomical approach was applied, for conservation purposes and for evaluation of potential zoonosic risk.

MATERIALS AND METHODS. Adults of *Trichuris* were found in intestinal caecum of the following host species: the Patagonian mara (*Dolichotis patagonum*), the Bactrian camel (*Camelus bactrianus*), the Desert antelope Addax (Addax nasomaculatus), the Ring-tailed Lemur (*Lemur catta*), the Japanese macaque (*Macaca fuscata*) and the grivets (*Chlorocebus aethiops*). Nematodes collected during necropsies were washed and measured for traits with high discriminatory value, such as presence of the spicular tube, shape and distribution of spines in the spicular sheath, length of spicule and vulvar morphology, along with classic morphometric characteristics (Spakulova 1994 Syst Parasitol. 29, 113; Robles et al 2014, PLoS ONE 9, 1). Molecular analyses were carried out by sequencing nuclear (ITS) and mitochondrial (*cox1* and *cob*) markers, in comparison to retrievable sequences for related species.

RESULTS AND CONCLUSIONS. A large number of *Trichuris* species have been described from the host species analyzed in the present study. Unfortunately, proper morphological descriptions and deposited DNA sequences are lacking for most of them. Concerning specimens from the Patagonian mara, Blast search selected two species occurring in rodents from South America, i.e. *T. pardinasi* and *T. navonae*, but with a very low (76-78%) percentage of identity, while molecular comparison with the species described in this host, *T. dolichotis*, was not possible. Many species have been so far described in addax and camels and the sequences obtained showed a high but not complete identity with *T. ovis*. Finally the molecular typing of specimens from macaques, grivets and lemurs suggested that *T. trichiura* is a complex of cryptic species.

Interestingly, the distribution of *Trichuris* species seems to be more related to host preference rather than to cross-species transmission in close or shared confined habitats.

P59. Sarcocystis gigantea in muscle fibres of a horse affected by granulomatous eosinophilic myositis

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Keywords: *Sarcocystis gigantea,* horse, granulomatous eosinophilic myositis

INTRODUCTION. Sarcocystosis is widespread in horses with a wide range of prevalence, from 0.5% to 93% (Aleman et al., 2016, Neuromuscul. Disord. 26: 85–93), however the presence of sarcocysts in equine skeletal muscle has been considered as an incidental finding. In fact there are just sporadic outcomes of clinical sarcocystosis in horses associated with *S. fayeri*, including few cases of severe granulomatous and eosinophilic myositis (Tinling et al., 1980, J. Parasitol., 66: 458-465; Fayer et al., 1983, Vet. Rec., 113: 216-217; Cawthorn et al., 1990, J. Vet. Diagn. Invest., 2: 342-345). Recently supported with a relevant casistic list a possible association between neuromuscular disorders and presence of sarcocysts in skeletal muscles of horses was recorded (Aleman et al., 2016, Neuromuscul. Disord. 26: 85–93).

MATERIALS AND METHODS. A Hunter gelding was referred to a private veterinary surgeon of the Hampshire (UK) for progressive swelling on right forelimb, extending to right forelimb and chest and the presence of 2 lumps in the pectoral region. Histopathological examination of biopsies from the lumps showed a multifocal eosinophilic granulomatous myositis associated with intact and degenerated encysted parasites, consistent with *Sarcocystis* spp.. For species classification, DNA extraction was carried out from formalin-fixed paraffin embedded (FFPE) muscle blocks and complete 18S ribosomal RNA (18S rRNA) gene was amplified by PCR (Herwaldt et al., 2003, Emerg. Infect. Dis. 9:942-948). Amplified product was purified and submitted to sequencing. The sequence generated was compared with those available in the GenBank using BLAST and deposited in the NCBI database under the accession no. KY594259. The sequence was phylogenetically analysed using Molecular Evolutionary Genetic Analysis version 7.0.20 software (MEGA7).

RESULTS AND CONCLUSIONS. In light of muscle histological and molecular findings a diagnosis of multifocal eosinophilic granulomatous myositis caused by *Sarcocystis gigantea* was formulated and daily oral administration of toltrazuril sulfone at 10 mg/kg for 28 days was added to the therapeutic plan with recovery of the clinical signs. The present report represents the first documented incidence of clinical disease related to *S. gigantea,* a non-pathogenic sheep-associated species, in equids, and is the first histologic description of *S. gigantea* sarcocysts in the tissues of a horse. The present finding can suggest that some *Sarcocystis* species have a wider intermediate host choice than previously thought and that *Sarcocystis* of further species than those considered horse-associated may invade muscle fibres of the equids causing myositis.

P60. Endoparasites of sheep in Majorca (Balearic Islands, Spain)

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Keywords: endoparasites; Majorcan sheep; diffusion

INTRODUCTION. Gastrointestinal and lungworm parasites is one of the main factors limiting sheep production. Nevertheless, their presence an intensity apart from the factors related to the host, is conditioned by farm management, climate and different rural ecosystem.

MATERIALS AND METHODS. From October to December 2017, 209 individual faecal samples were collected in 14 sheep flocks (n=67, White Majorcan sheep; n=17, Red Majorcan sheep, n=125, mixed breed) from Majorca (Balearic Islands, Spain) in order to examine the diffusion of bronchopulmonary and gastrointestinal parasites. The samples were processes using the McMaster and Baermann techniques, for gastrointestinal parasites and bronchopulmonary nematodes, respectively. The possible influence of breed and land cover types and climate (n=91, southern Marina, a semi-arid scrubland; n=73, Plà, a semi-humid flat plain; n=45, Serra de Tramuntana, a hyper-humid mountain range) on the prevalence values was also evaluated using unconditional exact test.

RESULTS AND CONCLUSIONS. Sheep were positive to Strongylidae (56.9%), *Nematodirus* sp. (5.7%), *Trichuris* sp. (1.4%); *Eimeria* sp. were found in 40.2% of sheep; 0.5% of animals were infected with *Moniezia* sp. and Paramphistomidae. The prevalence of lungworms is shown below: *Dictyocaulus filaria* (2%), *Protostrongylus rufescens* (2.9%), *Neostrongylus linearis* (2.4%), *Muellerius capillaris* (2%), *Cystocaulus ocreatus* (0.5%). Gastrointestinal nematodes and lungworms were broadly distributed since they were detected in all studied regions. In contrast, the prevalence of *Nematodirus* sp. was higher in the Marina than in the Tramuntana region (9.9% vs 0%; $p= 0.024$) and the prevalence of *Eimeria* sp. was higher in the Plà (56.2%) than in the Marina (29.7%, $p< 0.001$) and Tramuntana areas (35.6%, $p= 0.031$). *Moniezia* sp. was only found in Tramuntana flocks. Moreover, the prevalence of GINs was higher in the White Majorcan than in the Red Majorcan breed (68.7% vs 35.3%, $p = 0.013$). This is the first report of *Moniezia* sp. and Paramphistomidae in Majorcan sheep. These results suggest that the breed, management, land cover types and climate influence the presence of endoparasites. Further research is needed to detect other risk factors affecting the diffusion of endoparasite infections of Majorcan sheep, allowing the implementation of the most suitable control measures.

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P61. The influence of Geographical Indication disciplinaries on parasitic burden and epidemiology: the case-study of Italian dairy production system

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Keywords**:** GI parasites, breeding systems, dairy cows

INTRODUCTION. Gastrointestinal (GI) parasites are some of the most important pathogens affecting dairy cows (Charlier et al., 2009, Vet Parasitol, 164(1):70-79). Parasite load is mainly influenced by the breeding system, and cattle from intensive breeding are usually supposed to be GI parasites-free. The aim of the study was to evaluate the burden of parasitic infections on dairy cows and heifers from intensive breeding from three different areas of Northern Italy: Parmigiano-Reggiano area, Grana Padano area and Piedmont region. The analysed areas have three different breeding systems: for Parmigiano, grass and hay must represent the 50% of ratio, corn silage is not allowed and cows are kept indoor; in Grana Padano area, cows are kept indoor but corn silage is allowed; in Piedmont region, cattle can feed on grass and are allowed to graze on pasture.

MATERIALS AND METHODS. Overall, faecal samples of 1304 animals from 45 intensive stables in Northern Italy were analysed for this study. From each stable, a mean number of 15 dairy cows and 14 heifers was sampled. Farms were distributed equally among the three areas: Parmigiano (n=15), Grana Padano (n=15) and Piedmont (n=15). On each sample, Faecal Egg Count (FEC) was performed by using MINI-FLOTAC to detect Coccidia oocysts, GI nematode eggs and Cestoda oncospheres. Parasite load on pasture were monitored monthly during an entire vegetative season from April to November 2016. Briefly, larvae quantification was performed on 1 m^2 of freshly collected grass, and results were normalized on grass dry weight and expressed as larvae/kg of dry weight. Statistical analysis on risk factors for parasitic infections was performed by using generalized mixed linear models.

RESULTS AND CONCLUSIONS. GI nematodes were recorded with an overall prevalence of 10.35% (CI 95% 8.81-12.12%) in 18 out of 45 sampled farms. The mean parasite load was 2.52 epg (eggs per gram) (median= 0; standard deviation= 15.27). These parasites were more prevalent in Piedmont region than in Parmigiano area, while in the latter a higher infestation with *Moniezia spp*. was recorded ($p<0.01$), with a prevalence of 1.99% (CI 95% 1.36-2.91). Larvae seasonal trend on pastures showed two picks, one in the middle of June (with 93.05 larvae/kg of dry weight) and the second in October (73.04 larvae/kg of dry weight). This study clearly shows that GI parasites can be present also in intensive breeding cattle, even with low prevalence and burden. Different breeding systems show specific parasite risks, especially linked to the use of fresh grass and hay, and give helpful indications to farmers and veterinarians on how to maximize efficiency and efficacy of parasite management.

P62. Two cases of abomasal coccidiosis in goats by *Eimeria gilruthi,* a shirked enemy

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Keywords: *E. gilruthi*, abomasal coccidiosis, goat

INTRODUCTION. *Eimeria* (*Globidium) gilruthi* is a protozoan parasite belonging to the Phylum *Apicomplexa*, Class *Coccidia*, Family *Eimeridae*, and is responsible of Abomasal Coccidiosis (AC). The AC is reported in sheep and goat in many different regions worldwide, as a cause of anorexia, diarrhoea, and proliferative abomasitis (Maratea and Miller, 2007, J Vet Diagn Invest, 19(1) 118-121). Unlike intestinal coccidiosis, which is a problem mostly in young animals, AC can be seen in juvenile as in adult ones. Despite reported for over two centuries, little is known on biologic cycle, pathogenesis and immunology of *E. gilruthi*. As described by others, diagnosis of AC by faeces examination cannot be helpful in the absence of classifiable oocysts (Hermosilla *et al*., 2016, J Vet Med Res, 3(4): 1055). The present study reports on fatal cases of AC from two different farms of central Italy diagnosed with histopathological evaluation of tissue samples from two goats.

MATERIALS AND METHODS. A 3-months Saanen kid and a 2-years cross-breed goat were submitted for a complete post mortem examination at the Laboratory of General Diagnostic and Animal Welfare of the Istituto Zooprofilattico dell'Umbria e delle Marche "Togo Rosati", in Perugia. A fully necropsy examination of both animals was performed. Standard sampling protocols were applied for parassitological, bacteriological and virological diseases of small ruminants. Representative tissue samples were removed and fixed in 10% neutral buffered formalin for routine histological examination. Five 5 µm thick sections were obtained and stained with hematoxylin and eosin; Periodic Acid Schiff (PAS) staining on abomasal samples was also performed.

RESULTS AND CONCLUSIONS. The post mortem examination revealed in both cases a poor body condition with dehydratation and absence of perivisceral fat. Both animals showed a distended abomasum with low reddish brown fluid and multifocal areas with patchy mucosal haemorrhages. The adult goat also showed signs of diarrhea, a severe *Haemonchus contortus* infestation and enlarged mesenteric lymph nodes.

Microscopic examination of the abomasum of both animals revealed similar lesions. The mucosae were characterized by attenuation of epithelial cells, microhaemorrhages and slight diffuse inflammatory cellular infiltrate consisting mainly of lymphocytes and histiocytes. Necrotic cell debris in the fundic glands and in the lumen of the organ was detected. Multifocally abomasal mucosa was expanded by intact or degenerate giant protozoal schizonts consistent with *E. gilruthi* measuring 250-400 µm in diameter and characterized by homogeneous palely eosinophilic wall and thousands of merozoites inside. The *Eimeria* cysts were surrounded by numerous lymphocytes and macrophages. The periodic acid Schiff (PAS) staining showed marked positivity of the thick wall and merozoites of the detected schizonts. Morevoer, histological examination of the abomasum of the adult animal showed scattered nematodes in the lumen morphologically consistent with *Haemoncus* spp.

The adult goat showed important gastrointestinal lesions, mainly associated with abomasum parasites like the *E. gilruthi* and the *H. contortus.* Instead, the only lesions the kid showed were due to *E. gilruthi* that brought the animal to anorexia and subsequent death.

Clinical diagnosis of AC by *E. gilruthi* is difficult to confirm; the only diagnosis remains the post-mortem necropsy with histopathological examination of abomasal tissue. The Abomasal Coccidiosis should be considered as differential diagnosis in any clinical case of small ruminant

diarrhoea or weight loss, and tissue sampling for histopathology should be included in diagnostic protocols of sheep and goat diseases.

P63. Antiprotozoal activity of Neem seed oil

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Keywords: Neem seed oil, *Leishmania* spp., *P. falciparum*

INTRODUCTION. The evergreen tree, *Azadirachta indica* A. Juss (Meliaceae), commonly known as Neem, possesses a range of activities, including anti-microbial, anti-inflammatory, anti-cancer and neuroprotective activity (Gupta et al. 2017). In the tropics, Neem is used as a traditional antimalarial. The leaf and seed extracts, containing metabolites such as nimbolide, gedunin and epoxyazadiradione, are active against sexual and asexual stages of *Plasmodium falciparum* (Udeinya JI et al. 2008; Lucantoni L et al., 2010). Less documented is the activity of Neem against *Leishmania* spp. In the present work, the antiprotozoal activity of a seed oil of Neem was evaluated against *P. falciparum, L. infantum* and *L. tropica.*

MATERIALS AND METHODS. The antimalarial activity of an Italian certified Neem seed oil, was tested against the asexual stage of *P. falciparum* using the pLDH assay, as described (D'Alessandro et al. 2010). The anti-leishmanial activity was tested against promastigote stage of *L. infantum* and *L. tropica* using MTT method (Sereno D et al. 1997). Intracellular amastigotes were obtained infecting PMA differentiated THP-1 cells with metacyclic promastigotes of *L. infantum* and *L. tropica*. The amastigote susceptibility was determined counting the percentage of infected macrophages in control and in Neem treated cells using Lab-Tek culture slides stained with Giemsa. Cytotoxicity against human THP-1 cells was evaluated by MTT assay.

RESULTS AND CONCLUSIONS. The activity of the seed oil against W2, chloroquine-resistant and D10, chloroquine-sensitive strains of *P. falciparum* was comparable against both strains ($IC_{50} \simeq 200$ µg/mL), but lower than that observed using other extracts or isolated seed or leaf components.

The IC_{50s} of seed oil against promastigote stage of *L. infantum* and *L. tropica* were more than 200 µg/mL for both species while the IC_{50s} against intracellular amastigotes of *L. infantum* and *L. tropica* were 13.07 and 16.74 µg/mL, respectively. Low toxicity was observed against human macrophages- differentiated THP-1 cells ($IC_{50} > 700$ µg/mL) leading to a high selectivity index (IC_{50} against THP-1/IC_{50} against amastigotes of *Leishmania*).

The Neem seed oil demonstrated good activity and selectivity toward *Leishmania* amastigotes, but not promastigotes. Further studies are needed in order to investigate the mechanism of action.

P64. Ethnobotanical survey of antimalarial medicinal plants used by pregnant women to prevent and cure Malaria in West Cameroon

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Keywords: antimalarials, pregnancy, ethnobotany, medicinal plants

INTRODUCTION. The use of medicinal plants to meet health-care needs has greatly increased worldwide in recent times. The identification of new plant-derived bioactive agents that can be exploited for the development of new drugs to prevent and treat multi-drug resistant malaria cases is urgently needed. Particularly requested are also new drugs for women in pregnancy, both for prophylaxis and for cure. Thus, the aim of this study was to identify and collect antimalarial plants used to prevent and treat malaria during pregnancy in Cameroon and to document ethnobotanical information available on those plants.

MATERIALS AND METHODS. The investigation was conduct in Menoua division in the Western Region of Cameroon. Using a semi-structured questionnaire, twelve traditional healers (two women and 10 men) were contacted and interviewed; likewise, 38 women, members of four different women's associations and 100 pregnant women attending antenatal care at the district hospital were included in the interview assessments. The study was conducted in November 2017 and plants were collected between December 2017 and January 2018. Photographs and a specimen of the plants were deposited at the National Herbarium of Cameroon for scientific classification and voucher number registration.

RESULTS AND CONCLUSIONS. In total, 12 plants have been reported to be used for the treatment and prevention of malaria in pregnancy (*Aloe barbadensis*, *Cymbopongon citratus*, *Solanecio mannii*, *Senna alata*, *Mangifera indica*, *Picralima nitida*, *Ocimum gratissimum*, *Dacryodes edulis*, *Eucalyptus globulus*, *Persea americana*, *Bidens pilosa*, *Voacanga africana*). From the interviews emerged that leaves, bark and fruit were the main parts used for the phyto-preparations and decoction was found the principal method of extract preparation. Ten plants species could be collected at community level, the plant material dried and powders transported to the University of Camerino. Currently, methanol and water extracts are being prepared. *In vitro* studies will be performed during the next months to identify plants with activity against asexual blood stages and transmissible stages (gametocytes and early sporogonic stages). Results will be presented at the SOIPA congress in Milan.

P65. Ivo de Carneri Foundation - Italy-Zanzibar

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Keywords: de Carneri, parasitic diseases, health, Pemba

INTRODUCTION. Ivo de Carneri Foundation was established in 1994 to remember Ivo de Carneri, researcher and professor of parasitology at the University of Pavia. In Africa the Public Health Laboratory "Ivo de Carneri" is aimed to improve public health and knowledge of the community. In Italy the IdCF supports the updating of Ivo de Carneri' s "Parassitologia generale e umana" and "Medical parasitology and parasitological diagnostics"; furthermore the IdCF created the book series *fronte**retro***, focused on the catastrophic effects that transmissible diseases brought in Europe in the centuries, and "I Quaderni" to document research activities, training etc.

MATERIALS AND METHODS. A partnership with the MoH of Zanzibar, the PHL-IdC has been operational since June 2000. It is a WHO Collaborating Center since 2005 and National Center for TB Control since 2008. Fifty-one people work at the PHL-IdC and many more are involved in field campaigns.

Public Health Laboratory Ivo de Carneri

The PHL-IdC includes three well equipped units for more than 1000 m^2surface. In support of the MoH program IdCF and PHL-IdC have carried out a four years project to strengthen the Health Management Information System to improve health interventions. "Safe Water" is a six years project aimed at monitoring the island's hydric resources through microbiological and chemical-physical analyses. The PHL-IdC furthermore has a crucial role in surveillance and control of endemic and epidemic diseases: malaria, schistosomiasis, intestinal parasites infections, tuberculosis, etc. and in training for local/ international professionals.

RESULTS AND CONCLUSIONS. The PHL-IdC is a reference infrastructure in sub-Saharan Africa for the control of endemic diseases in partnership with international institution.

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