UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

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Parasites, Poverty and Social commitment

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TUESDAY 21ST JUNE 2016

OPENING CEREMONY

PARASITES: THEORY AND PRACTICE

Luciano Canfora

University of Bari, Italy

Parasite is nowadays a disreputable term with several negative meanings in medical as well as in human Sciences. Among the ancients this term was used like something of sacred. Parasite is also a comic mask in Hellenistic and Roman theatre. The term Parasite, from meaning a "hanger-on" has been transferred to any living creature which lives on another one (university, industrial production, politics …). Therefore, the crucial question about parasites is where the theory bounds, in the real life, the practice.

PARASITES AND POVERTY

Maria Elena Bottazzi

Tropical Medicine, Baylor College of Medicine, Houston, Texas, USA

The neglected tropical diseases (NTDs) are the most common infections of the poorest people in the world and who live on less than US$2 per day. They include ancient scourges such as hookworm and other soil-transmitted helminth infections, Chagas disease, amoebiasis, schistosomiasis, leishmaniasis, and dengue. Together, these NTDs produce a burden of disease that in certain regions even exceeds HIV/AIDS, while simultaneously trapping "bottom billion" in poverty through their deleterious effects on child physical and intellectual development, pregnancy outcome, and worker productivity.
The high prevalence and incidence of the major NTDs afford an opportunity for joint cooperation and alliances to address the highest prevalence conditions and accelerate the development of alternative control tools such as vaccines for the major NTDs. One of the major hurdles in the critical path for the development and testing of novel and translational discoveries is overcoming the "valley of death", or product development gap for taking a bench discovery to the point where it shows a clear path to the clinic. A perspective of a sustainable model to accelerate translation of discoveries into new vaccines and applied by the Sabin Vaccine Institute Product Development Partnership (Sabin PDP) founded to develop recombinant protein vaccines targeting NTDs will be presented.

WEDNESDAY 22ND JUNE 2016

RESEARCH HIGHLIGHTS 1

Human and veterinary parasitology and social commitments

TROPICAL PARASITIC DISEASES IN THE 21ST CENTURY

Marco Albonico

University of Torino

In the last two decades remarkable changes have occurred in the epidemiology, research, and control of tropical parasitic diseases. These poverty-related (rather than tropical) diseases still thrive on socio-ecological conditions and unhealthy environments that sustain their persistence or re-emergence. Nevertheless, important successes have been achieved. Malaria's deaths have been halved due to effective interventions, including artemisinin combination treatment and impregnated bednets; neglected tropical diseases (NTD) like lymphatic filariasis, onchocerciasis, and human African trypanosomiasis are in the elimination trend targeted for 2020. Schistosomiasis has been eliminated from several endemic areas and preventive chemotherapy coverage for NTDs globally has reached 50% with about 5 billion anti-parasitic treatments delivered over the past ten10 years, mostly sustained by drug donations from the pharmaceutical industry. Research on diagnostic tools for mapping, clinical diagnosis, monitoring treatment outcome, and surveillance, is both improving affordable techniques in direct diagnostic parasitology, and moving towards DNA-based methods. Effective vector control, improved access to safe water supply and sanitation, integrated with drug development, have been key to reduce burden and transmission of several parasitic diseases. The one-health concept is a promising approach to tackle parasitic zoonosis. Yet, despite successes, challenges remain to be faced. Drug resistance is a world-wide threat. Global warming facilitates the development of vectors in previously non endemic areas with emergence of new pathogens. Migration of populations focuses the attention in Europe to parasitic diseases that were only a problem in tropical countries: Chagas disease, neurocysticercosis and even schistosomiasis and malaria are some examples and are re-emerging in some foci. The dynamic economies of low-income countries have changed, and despite several million people have emerged from the scourge of absolute poverty, inequalities in the distribution of wealth impact also on parasitic diseases; yet the shift from communicable to non-communicable diseases generates a double burden of diseases of transitioning populations that will modify the epidemiology of the "diseases of the tropics" in the years to come.

ZOONOTIC PARASITIC DISEASES IN EUROPE

Peter Deplazes

University of Zurich

Neglected helminthic zoonoses such as alveolar and cystic echinococcosis (AE, CE), fasciolosis, toxocarosis and *Taenia saginata* taeniosis are persisting or even re-emerging in parts of Europe. Many reasons may account for these trends such as changing eating habits (consumption of uncooked wild vegetables, meat and fish) or the close emotional tie between people and companion animals. A number of canine parasites have extended their distribution ranges in Europe. Among the reasons for this development are the displacement of dogs, the reduction of border controls within Europe but also political and socioeconomic transitions especially in the states of the former Soviet bloc. Furthermore, high populations of stray dogs and cats maintain a permanent infection pressure of many parasites towards pet populations. Ecological changes also contributed to parasite dispersal: Fox populations have grown in many areas of Europe. In fact, human AE emerged in Europe as a consequence of fox populations invading urban areas. Urban recreational environments that are increasingly designed close to natural ecological systems may lead to boosting populations of voles which represent urban reservoirs for zoonotic parasites as was shown for *Echinococcus*, *Toxoplasma* and *Toxocara* spp. Based on molecular analyses, some very rare zoonotic infections have been discovered recently such as *Dirofilaria* spp., *Thelazia* spp. and *Spirocerca* sp. infections or *T. martis* cysticercosis. Understanding of the parasites' epidemiology, close collaborations between veterinary and public health professionals in a 'One Health' concept as well as political decisions and funding are required for implementing effective prevention strategies.

NEW MOLECULES FOR PARASITES OF ANIMALS AND HUMANS

Ivan Scandale[1], Marc Hubner[2], Suzanne Gokool[3], Coralie Martin[4], Achim Hoerauf[2], Simon Townson[3]

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The World Health Organization approach for treatment of filarial infections primarily relays on mass drug administration of ivermectin and diethylcarbamazine. These two medicines have significantly reduced transmission and burden of these diseases by targeting the larval stage of the parasite (microfilariae) and by temporarily sterilizing adult nematodes. However there remains an urgent need to design and develop modern anti-filarial treatments that are cidal to adult worms (macrofilaricides). Indeed, the life span of adult worms being of 5 years for lymphatic filariasis and of 12 to 15 years for onchocerciasis, several consecutive annual treatments are currently required to clear an infection.
To address this need, the Drug for Neglected Diseases *initiative* (DND*i*) has recently constituted a small pipeline of macrofilaricde drug candidates entering into clinical or preclinical development. However, given the attrition rate in drug development, it is important to strengthen the pipeline by adding new chemical entities into the drug development pathway. Thus, DND*i* has initiated a drug discovery program aiming at constituting a research pipeline.
To identify compounds with such potential, DND*i* has accessed commercial libraries and worked with pharmaceutical companies to access focused libraries from their drug discovery programs. These libraries were evaluated through a screening cascade encompassing three steps: phenotypic screen against *Onchocerca* spp., determination of *in vivo* ADME, and proof of principle testing in rodent models using *Litomosoides sigmodontis.* Through this process, several molecules, existing drugs or advanced clinical candidates with good activity against filarial parasites were identified.
Despite this strategy has led to the identification of a large number of active compounds, a lead optimization process is required for designing a new drug candidate. In this context, promising chemical series, showing outstanding *in vitro* potency and *in vivo* activity, have been identified from the compounds collection of AbbVie and Celgene Global Health. These molecules are currently under development through a Lead Optimization program undertaken in collaboration with these companies.

ALLERGY PARASITES AND THE HYGIENE HYPOTHESIS

Rick M Maizels[1], Danielle J Smyth[1], Chris CJ Johnston[2], Henry J McSorley[2]

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Helminth parasites remain highly prevalent throughout the world today reflecting their ability to suppress host immunity, down-regulating both anti-parasite responses and inflammatory responses to bystander specificities such as autoantigens, alloantigens and allergens. To analyse immunological mechanisms underpinning these effects, we are studying a model intestinal nematode *Heligmosomoides polygyrus*, which is related to human hookworm parasites. Infection of susceptible mouse strains expands immunosuppressive host cell populations including both natural and adaptive Foxp3^{+} regulatory T cells (Tregs) and regulatory B cells, which inhibit allergic inflammation and autoimmune disease when transferred to uninfected animals. Promoting Tregs in resistant mice (with IL-2: anti-IL-2 complex) enhances infection, while Treg depletion in susceptible mice engenders worm expulsion. The immunoregulatory effects of *H. polygyrus* can be recapitulated *in vivo* with soluble products (termed HES) secreted by live parasites *in vitro*, which inhibit systemic autoimmunity, airway allergy and colitis *in vivo*. HES induces *de novo* Foxp3 expression and Treg function in peripheral T cells, acting through a parasite-encoded mimic of host TGF-β, termed TGM. HES also blocks the release of the alarmin IL-33 by epithelial cells, in a TGF-β-independent manner; through a novel molecule termed ARI (allergic response inhibitor). Hence, two parallel anti-inflammatory pathways have been identified which target the adapative and innate immune systems, and provide a first insight into a mechanistic explanation for the hygiene hypothesis.

THE IMPACT OF *TOXOPLASMA GONDII* ON HOST BEHAVIOUR: CAN THIS PARASITE PLAY A ROLE IN SOME CASES OF HUMAN SCHIZOPHRENIA?

Joanne P. Webster

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The ability of parasites to alter the behaviour of their hosts fascinates both scientists and non-scientists alike. One reason that this topic resonates with so many is that it touches on core philosophical issues such as the existence of free will. If the mind is merely a machine, then it can be controlled by any entity that understands the code and has access to the machinery. One key example is the potential epidemiological and neuropathological association between some cases of schizophrenia with exposure to the protozoan *Toxoplasma gondii. Toxoplasma gondii* establishes persistent infection within the CNS and can alter host behaviour. Altered dopamine levels have been reported for both *T. gondii* infection and schizophrenia. Moreover, several of the medications used to treat schizophrenia and other psychiatric disease demonstrate anti-*T. gondii,* properties *in vivo* and *vitro*. Furthermore, it appears that the parasite itself may actually be a source of this neurotransmitter. Using the epidemiologically and clinically applicable rat-*T. gondii* model system, I present a series of studies and discuss them in terms of their theoretical and applied implications for animal and human health.

HOOKWORMS, GUT MICROBIOTA AND COELIAC DISEASE

Cinzia Cantacessi

Department of Veterinary Medicine, University of Cambridge, Cambridge, UK

Multiple recent investigations have highlighted the promise of helminth-based therapies for the treatment of inflammatory disorders of the intestinal tract of humans, including inflammatory bowel disease and coeliac disease (CeD). However, the mechanisms by which helminths regulate immune responses, leading to the amelioration of symptoms of chronic inflammation are unknown. Given the pivotal roles that disturbances in the intestinal microbiota play in multiple immune disorders, and the fact that gastrointestinal parasites and the commensal flora share the same environmental niche, there is an increasing interest in understanding helminth–microbiota interactions and their relative contributions to health and disease. Indeed, based on the outcomes of recent studies in both humans and animal models of chronic inflammatory disorders, it has been hypothesized that helminth-induced modifications of the gut commensal flora may be responsible for the therapeutic properties of gastrointestinal parasites. In this presentation, recent progress in the elucidation of host–parasite–microbiota interactions in human chronic inflammatory disorders, with a particular emphasis on CeD, will be reviewed and discussed. A working hypothesis of the role of the gut microbiota in helminth-induced suppression of inflammation will also be provided

THURSDAY 23RD JUNE 2016

RESEARCH HIGHLIGHTS 3

The treatment of parasitoses of pets: science, economy and future perspectives

PROPHYLAXIS, VACCINES OR TREATMENTS FOR CANINE AND FELINE VECTOR BORNE DISEASES?

Guadalupe Miró

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Canine and feline vector borne diseases are caused by a diferent bacteria, parasites, and less frecuent, viruses which are transmitted by a variety of arthropod vectors mainly: ticks, fleas, mosquitoes and phlebotomine sand flies.
Clinical management of these diseases in dogs and cats are very complex and effective control requires a thorough knowledge of the infectious agents, their vectors and major hosts.
The different degrees and severity of each disease have implications for treatment and prognosis. Choosing the right treatment depends largely on the patient's clinical condition but must be based on the ethiological diagnosis in order to succeed. However sometimes it is not possible to prevent recurrences due to immunosuppression (stress, debilitating diseases ...).
Treatment and clinical follow-up protocols have changed considerably in recent years, due to improved veterinary care, owners collaboration, better diagnosis tools, a wide range of antiparasitic drugs and new available vacines for some of these diseases. By the way, expectations of a good clinical recovery, but not parasitological cure are very common.
Preventing these vector borne diseases involves both global intervention against the vector (environmental treatments, topical/systemic treatments and the pathogen (immunoprophylaxis) when might be available.
But each patient requires care tailored to its individual needs taken in consideration different factors such as age, health/immune status and life style.

HOW THERAPEUTIC PROTOCOLS HAVE CHANGED IN VETERINARY PARASITOLOGY OVER THE PAST 20 YEARS

Claudio Genchi

Università degli Studi di Milano

Over the past 20 years, the relationship between humans and pets animals has dramatically changed. The number of animals in households is increased, owners have acquired more awareness of the role of parasites on their animal health, possible risks of zoonotic transmission and to act responsibly not only for their own pet's health but for the health of other pet animals and people in their communities. Furthermore, pet movement has increased, both following owners'travels or for relocation, increasing the risk of new infections. At the same time, new antiparasitic drugs have been discovered and new medicinals have been made available on the veterinary market. The first new "revolutionary" compound used in pets was the ivermectin (IVM) for the prevention of patent heartworm (HWM) infections and then, milbemycin oxime (MBO), les toxic than IVM in dogs and cats. Such a characteristic, allowed to increase the safe dosage and expand the spectrum of efficacy further to HWM prevention, to intestinal worms and some ectoparasites. Selamectin was the first endoectocite for pets (HWM prevention, intestinal worms, fleas and other ectoarasites) and finally the discover of moxidectin (MOXI), very safe for pets, has allowed to further expand the broad spectrum of antiparasitic medicinales for pets. Nevertheless, the need to protect pets from Cestoda infections (such as *Echinococcus* sp. and *Dipylidium caninum*) and from arthropod-borne infections (such as *Leishmania* end tick-borne infections) has open the way for several combination of anthelmintics with compounds active against ectoparasites or compounds with repellent activity against arthropods. About the medicinals active against ectoparasites, in these last years instead of the topical formulations, new active molecules to be given orally have been made available on the market. This ensures a more accurate dosage, a better owner compliance and less dispersion of active compounds in the environment.

NEW PARASITES AND NEW CHALLENGES FOR THEIR TREATMENT

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[2]Dipartimento di Medicina Veterinaria, University of Sassari, Italy

The unveiling of new organisms as well of new strategies for fighting diseases has always been an important benchmark and a primary goal for scientists since the beginning of times. In the last decades, progresses in technology and in molecular engineering opened new ways for researchers in every fields of knowledge, and of course, in Parasitology.
At the beginning of last century, a young brilliant scientist wrote: “The more I learn, the more I realize how much I don't know” [Albert Einstein], and every person that focus on research can confirm this quote, as well another, less famous but equally true: “The more you look, the more you will find”.
Papers on new parasite descriptions, new approaches to control and new therapeutical protocols, are continuously published year by year making not easy to summarize the current state of art in this field. Nevertheless, we should clear to ourselves what is really “new” from what is probably not new but maybe “re-discovered”, “emerging” or, as unfortunately it happens, “neglected” in Parasitology. What truly is a new parasite? And, if they really exist, can we say that are “new” or “new for us”? Some times we need “new enemies” to fight for making our research more fashionable and, may be, funding attractive. However, the factual impact of “new parasites” is often not-well estimated and may distract the efforts from old-fashioned parasitic diseases having a significant burden in human and animal populations. Therefore, new challenges for treatment do not always means treatment of “new species” but
may be represented by new strategies to fight neglected parasitic diseases still affecting pets and humans.
In the lecture some parasite species affecting pets will be presented and used as key examples for new, emerging or neglected diseases. Treatment challenges for new and endemic parasitoses will be also discussed under the one-health perspective.

FRIDAY 24TH JUNE 2016

JOINT EVPC-SOIPA SESSION

Parasites, biodiversity and public health in a changing world

PARASITES AND RELIGION

Maria Elena Bottazzi

Tropical Medicine, Baylor College of Medicine, Houston, Texas, USA

The neglected tropical diseases (NTDs) are a group of 17 chronic diseases of high prevalence amongst the poorest populations of the world and spanning a vast global geographic distribution. An interesting aspect of the geographic distribution of these ancient scourges is their disproportionate impact on populations living in regions with a variety of mixed religious philosophies and affiliations. Several publications and reports by organizations such as the Pew Research Center have highlighted the global changing religious landscape. For example, in 2010 from a world population of 6.9 billion, approximately 2.2 billion are Christians (32% of the world's population), 1.6 billion are Muslims (23%), 1 billion are Hindus (15%), nearly 500 million are Buddhists (7%) and 14 million are Jews (0.2%). If you then superimpose the dimension of NTDs global distribution and regional religious affiliations, we can rapidly see a link between the global burden of NTDs and these populations. For example, a recent analysis of the world's intestinal helminth infections, schistosomiasis and significant numbers of trachoma, filariasis and onchocerciasis show that approximately 35-46% occur in the Organization of the Islamic Conference. Similarly, approximately one fourth of world's cases of helminth infections and schistosomiasis occur in Catholic-majority countries. Together, these data highlight the need to have in place advocacy programs that can help facilitate participation of many of the world's Islamic and Catholic universities and institutions in the ongoing global research & development strategies and tackle the control and elimination of NTDs.

CLIMATE CHANGE, BIODIVERSITY, TICKS AND TICK BORNE DISEASES

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Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães, Brazil

For generations, we have killed wild animals for obtaining food and decimated forests for many reasons. Nowadays, we are burning fossil fuels as never before and even exploring petroleum in deep waters. The impact of these activities on our planet is now visible to the naked eye and the debate on climate change is warming up in scientific meetings and becoming a priority on the agenda of both scientists and policy decision makers. It may be anticipated that warmer winters and extended autumn and spring seasons will continue to drive the expansion of the distribution of some tick species (e.g., *Ixodes ricinus*) to northern latitudes and to higher altitudes. Nonetheless, further studies are advocated to improve our understanding of the complex interactions between landscape, climate, host communities (biodiversity), tick demography, pathogen diversity, human demography, human behaviour, economics, and politics, also considering all ecological processes (e.g., trophic cascades) and other possible interacting effects (e.g., mutual effects of increased greenhouse gas emissions and increased deforestation rates).

EMERGING ISSUES IN PARASITE FOOD ZOONOSES: OPISTORCHIS FELINEUS IN EUROPE

Federica Berrilli[1], **Claudio De Liberato**[2]

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[2]*Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Roma, Italy*

Opisthorchiasis by *Opisthorchis felineus* is a foodborne zoonosis endemic in the former Soviet Union and Eastern Europe. Although focal outbreaks causing significant human morbidity have been reported in Western Europe, little information is available regarding the epidemiology of this trematode in EU countries. To date, *O. felineus* infection has been documented in humans and/or animals, either final or intermediate hosts, in 13 EU countries. In Italy, it was first described in 1884 in dogs and cats and for over 100 years only sporadic animal infections were reported. However, since 2003 an upsurge of human outbreaks occurred, with cases involving also other EU countries, all caused by the consumption of *Tinca tinca* from two lakes in Central Italy. The biology of the parasite and local social determinants could have contributed to the recent Italian human outbreaks: i) the high zoonotic potential of *O. felineus*; ii) a stable sylvatic life cycle, involving many species acting as definitive hosts; iii) changes in feeding habits of people, switched towards consumption of raw or undercooked fish; iv) an increased demand and export for tench fished from the two lakes; v) difficulty in parasite control in the area, also due to shortcomings in preventive measures (e.g. fish labeling, traceability, information campaigns, detection of alimentary frauds).
At present, many unresolved issues involving the life cycle, the epidemiology and the population genetic structure of *O. felineus* in the EU countries are pending, such as: 1) its actual distribution in Western Europe; 2) the involvement of just the tench as second intermediate host in Italy; 3) possible unexpected definitive hosts, such as rodents and birds; 4) from a molecular point of view, divergent haplotype of Italian specimens, evading the uniform genetic structure observed so far. All these intriguing questions are yet to be addressed and need thorough investigations and integrated efforts by scientists.

SPONSORED LECTURES

WEDNESDAY 22ND JUNE 2016

VIRBAC Sponsored Lecture

"*Fighting against vector-borne diseases, why a rapid onset of action matters?* "

K De Mari[1], N Rizzi[2], C Navarro[1]

[1]*Virbac Medical Department, France*
[2]*Virbac Italy*

Canine vector-borne diseases are caused by various pathogens transmitted by several arthropods. The transmission time varies between a few seconds (e.g. *Leishmania infantum* transmitted by *Phlebotomus perniciosus*), a few hours (e.g. *Ehrlichia canis* transmitted by *Rhipicephalus sanguineus*) and up to a few days (*Babesia canis canis* transmitted by *Dermacentor reticulatus*). To prevent the transmission of these pathogens, it's therefore crucial to use an ectoparasiticide solution having a rapid onset of action and/or repellent properties.
Effitix® spot-on solution is a new fixed combination of permethrin and fipronil. Permethrin is a pyrethroid with both acaricidal and insecticidal activities and well-known repellent properties. Its acaricidal efficacy is reinforced by the acaricidal spectrum of fipronil.
The rapid onset of action of Effitix® against tick infestations was evaluated in a recent study (1). When put in contact with the dog's treated skin for as little as 1 minute, ticks (*R. sanguineus*) had a strong reduction of their viability (59%). This reduction of viability increased with the duration of contact with the dog's treated skin (95% after a 10-minute contact time). In another study (2), Effitix® proved to have a faster speed of kill (as quickly as 30 minutes) against *R. sanguineus* than an oral parasiticide tablet (afoxolaner), and the repellent activity of permethrin against ticks on dogs was observed, whereas the afoxolaner-based treatment had no statistically significant repellent effect. The repellent activity was also confirmed in experimental studies using flying insects such as *P. perniciosus* (3) or C. pipiens (4). One of the practical benefits of the rapid onset of action / repellent activity is to prevent the transmission of vector borne-diseases. This point was confirmed in an experimental study (5) in which Effitix® successfully reduced the risk of transmission of canine babesiosis from infected *D. reticulatus*.
The optimal management of Canine vector-borne diseases requires a multimodal approach using preventive measures (e.g. avoiding development of the vector, avoiding contact with the vector), vaccines (when available), and topical products having repellent properties and/or rapid activity to prevent the transmission of the pathogen by the vector.

SPONSORED LECTURES
THURSDAY 23RD JUNE 2016

ZOETIS Sponsored Lecture

Simparica™ – a novel oral isoxazoline ecto-parasiticide

Thomas Geurden, Csilla Becskei, Tom McTier, Steven Maeder, Robert Six

The efficacy and safety of Simparica™ (sarolaner) has been demonstrated in a comprehensive research and development program involving more than 1,500 dogs worldwide. Laboratory studies confirmed that sarolaner protected against weekly re-infestations with ticks commonly found to infest dogs in Europe (*Ixodes ricinus*, *I. hexagonus*, *Dermacentor reticulatus* and *Rhipicephalus sanguineus*). The onset of acaricidal efficacy is within eight hours after oral administration. Further studies have also demonstrated the effectiveness against infestations with dog and cat fleas, including the KS-1 resistant cat flea strain. The pulicidal efficacy starts within three to four hours after treatment administration and effective control reached within eight hours. For both ticks and fleas, efficacy against weekly re-infestations was sustained for 35 days. The high efficacy and safety were further confirmed in veterinary patient studies conducted in several European countries. Sarolaner was also found to be highly effective in the treatment of three common mite species. Furthermore, sarolaner treatment was well tolerated and with no drug-related adverse events observed in any study, and can be used in puppies from the age of 8 weeks onwards.

ORAL-SHORT PRESENTATIONS

WEDNESDAY 22ND JUNE 2016

ARTHROPOD-BORNE PARASITOSES

PARATRANSGENESIS TO CONTROL MOSQUITO BORNE DISEASES: FROM BENCH TO FIELD

Maria Vittoria Mancini[1], Roberta Spaccapelo[2], Claudia Damiani[1], Alessia Cappelli[1], Aida Capone[1], Paolo Rossi[1], Matteo Valzano[1], Anastasia Accoti[2], Luca Facchinelli[2], Aurelio Serrao[1], Irene Ricci[1], Guido Favia[1]

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Malaria still represents a major public health concern health in developing countries, where it causes more than 1 million deaths annually. Given the lack of effective vaccines against its major etiological agent, *Plasmodium falciparum*, and the growing resistance of this parasite to the currently available drugs repertoire and of *Anopheles* mosquitoes to insecticides, the development of innovative control measures is strongly required to reduce malaria transmission.
Paratransgenesis, the modification of symbiotic organisms to deliver anti-pathogen effector molecules, represents an innovative strategy to contrast *Plasmodium* development in mosquito vectors. However, the field application of laboratory-based evidence of paratransgenesis requires the use of more realistic confined semi-field environments.
Large cages were employed to evaluate the ability of bacteria of the genus *Asaia* modified to express the green fluorescent protein (Asaiagfp), to diffuse in *Anopheles stephensi* and *Anopheles gambiae* receiving mosquito populations. Asaiagfp was introduced in large cages through the release of paratransgenic males or by sugar feeding-stations. Recombinant bacteria transmission was directly detected by fluorescent microscopy and bimolecular analysis.
We report the first known trial in semi-field condition on paratransgenic anophelines showing that modified bacteria were able to spread at high rate in different populations of *An. stephensi* and *An. gambiae*, two major malaria vectors, exploring horizontal ways and successfully colonising mosquito midguts. Moreover, in *An. gambiae*, vertical and trans-stadial diffusion mechanisms were also demonstrated.
Our results demonstrate the efficient ability of modified *Asaia* to colonise different populations of malaria vectors, including species where its association is not primary, in large environments. The data support the potential to employ transgenic *Asaia* for malaria control, disclosing promising perspective for field application with suitable effector molecules. The paratransgenic approach to control malaria and other mosquito borne diseases seems more feasible than in recent past.

IMMUNOLOGICAL EFFECTS INDUCED BY *ASAIA* SYMBIONTS ENGINEERED TO EXPRESS *WOLBACHIA* PROTEINS

Sara Epis[1], Elena Crotti[2], Nicoletta Basilico[3], Yolanda Corbett Rodriguez[4], Chiara Bazzocchi[1], Elena Martin[1], Claudia Damiani[5], Mauro Mandrioli[6], Luigi Gradoni[7], Guido Favia[5], Claudio Bandi[8]

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The symbiont *Wolbachia* is able to stimulate innate immune responses in mosquitoes; this immune stimulation is likely responsible for the reduction of vector capacity of mosquitoes, toward a variety of pathogens. However, the use of *Wolbachia* in vector-borne diseases control is impaired by the characteristics of this bacterium: it is an obligate intracellular symbiont, not culturable in cell-free media, not very resistant outside cells, and thus it is unsuitable for release in the environment. An alternative approach could be the identification of the *Wolbachia* effector molecules responsible for the reduction of the vector capacity of infected arthropods. Once *Wolbachia* effector molecules are identified, culturable and transformable insect symbionts could be modified for the expression of these molecules.
Following these assumptions, we realized a chimeric symbiotic bacterium of arthropod vectors (genus *Asaia*) to express the *Wolbachia* surface protein (WSP), the main antigenic protein of *Wolbachia,* shown to induce innate immune responses in mosquito cells and able to determine Th1 response in mammalian hosts.
This bacterium has been used to: i) evaluate the activation of the immune response in arthropods, induced by the WSP expression; ii) evaluate its capacity of activating mouse macrophage function and iii) to determine Th1 responses. Finally, we have tested this chimeric organism in *Leishmania*-infected macrophages.
We expect that the activation of the immune response in arthropods could provide a novel approach for the development of further tools for the integrated control of vector-borne diseases; furthermore, the activation of the immune response in *Leishmania*-infected macrophages could be suitable for the development of a prototype of vaccine against a variety of infectious agents.

VECTOR-BORNE PATHOGENS IN WILD CARNIVORES FROM ROMANIA

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Romania holds a significant number of wild carnivore populations, among the highest in Europe. Its geographic position and hematophagous arthropod and snail biodiversity are also remarkable, hence the epidemiological pathways of associated diseases are multiple. Our research from the last 10 years on the ecology and epidemiology of vector-borne and snail-borne diseases in wild carnivores revealed a large diversity of such pathogens. We included in this survey golden jackals (*Canis aureus*), grey wolves (*Canis lupus*), red foxes (*Vulpes vulpes*), wildcats (*Felis silvestris*), Eurasian lynxes (*Lynx lynx*) and badgers (*Meles meles*), collected from hunters or found as roadkill from various regions of Romania. All pathogens were found following complex necropsy procedures followed by identification using morphological and/or molecular methods. We report here data on the presence of the following pathogens: *Thelazia callipaeda*, *Dirofilaria repens*, *D. immitis*, *Acanthocheilonema reconditum*, *Angiostrongylus chabaudi, A. daskalovi*, *Anaplasma phagocytophilum, Borrelia burgdorferi* s.l., *Cytauxzoon* sp. and *Hepatozoon cf. canis*. Our results suggest that wild carnivores represent an important natural reservoir of infection with vector-borne diseases for domestic dogs and cats as well as for humans, highlighting the need of permanent surveillance of these epidemiologically dynamic pathogens.

AN EPIDEMIOLOGICAL AND ECOLOGICAL INVESTIGATION OF PHLEBOTOMINE SANDFLY: ABUNDANCE AND LEISHMANIA RISK MAPPING IN SOUTHEAST SPAIN

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Leishmaniosis caused by *Leishmania infantum* is an emergent, phlebotomine sandfly borne, zoonosis in Mediterranean countries. Incidence is spatially and temporally heterogeneous dependent on vector abundance and distribution. Understanding the latter is vital for developing evidence-based *L. infantum* control programs. The present study, carried out between May and October 2015, aimed at investigating sandfly distribution and its relationship with human and canine Leishmaniosis in Murcia Region, in southeast Spain. CDC light and castor oil sticky traps were placed for 24 hours on 8 separate weeks in 25 sheep farms and dog kennels in rural areas, in the main 5 ecoclimatic zones of the region. A total of 3620 sandflies were collected mostly on CDC traps. The species identified in 197 specimens (73% females) analyzed so far included, *Phlebotomus perniciosus* (78%), *P. papatasi* (13%), *P. longicuspis* (3%), *P. sergenti* (3%), *P. ariasi* (2%) and *Sergentomyia minuta* (2%). Sandfly abundance was markedly seasonal and differed between and within zones; it was spatially correlated with Leishmaniosis prevalence and was greatest in old, stone buildings with earth/straw floors that were not frequently disinfected. Sandfly abundance was not related to animal species or density or building ventilation. A hierarchical Poisson regression model indicated that sandfly counts increased with altitude, decreased with wind speed and were greatest when relative humidity was lowest (31-40%) and temperature was moderate (22-26ºC). Results from this and from previous canine and human *L. infantum* infection studies were used to generate a regional sandfly and Leishmaniosis risk map.

EXPOSURE TO VECTOR BORNE PATHOGENS IN OWNED AND FREE-ROAMING ASYMPTOMATIC DOGS OF NORTH-EASTERN ITALY

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The aim of this study was to understand the exposure to and circulation of pathogens transmitted to dogs by ticks, sand flies, fleas and mosquitoes in north-eastern Italy.
Asymptomatic owned (n=150) and free-roaming (n=338) dogs were screened in 2014-2015 by immuno-fluorescence for *Leishmania infantum*, *Ehrlichia canis, Anaplasma phagocythophilum*, *Babesia canis, Rickettsia conorii, R. rickettsii.* All the owned dogs and the free-roaming dogs seropositive to at least one vector-borne pathogen (VBP) (n=108) were tested by rt-PCR for *L. infantum*, *Ehrlichia*/*Anaplasma*, *Babesia*/*Theileria* and *Rickettsia* spp.
All the dogs were tested for filariae by blood filtration.
Overall, 40 owned (26.7%) and 108 free-roaming dogs (32%) were sero-reactive to at least one VBP. Seroprevalence in owned vs free-roaming dogs was: *L. infantum* (6.7% vs 7.1%), *A. phagocytophilum* (4.7% vs 3.3%), *B. canis* (1.3% vs 2.7%), *E. canis* (0.0% vs 0.9%), *R. conorii* (16% vs 21.3%) and *R. rickettsii* (11% vs 14.3%). There was no significant difference in seroprevalence between owned and free-roaming dogs, except for filariae found in 21 (6.4%) of free-roaming dogs only ($p<0.01$). Filariae were identified as *Dirofilaria immitis* (n=19) and *D. repens* (n=2). All the PCRs performed on blood were negative.
Although the dog owners declared to regularly use compound against fleas and ticks, their dogs have been exposed to VBPs similarly to free-roaming dogs. This call for the need to improve the owners education on the use of repellent compounds in order to prevent arthropod bites and therefore the contact with VBPs. However, none of the dogs had circulating pathogens at the time of sampling. The dog owners demonstrated instead to correctly use prophylactic measures against filariae. Finally, it's important to remind that *R. rickettsi* is not reported in Italy, thus the seroreactivity is likely a cross-reaction with other rickettsiae circulating in north-eastern Italy, such as *R. helvetica* and *R. monacensis*.

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FIRST DETECTION OF *CYTAUXZOON* SPP. INFECTION IN EUROPEAN WILDCATS (*FELIS SILVESTRIS SILVESTRIS*) OF ITALY

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Cytauxzoonosis is an emerging tick-transmitted protozoan disease, affecting domestic and wild felids and caused by *Cytauxzoon felis, C. manul* and *Cytauxzoon* spp. In Italy, *Cytauxzoon* spp. has recently been described in cats from a north-eastern and central areas. No further information on *Cytauxzoon* spp. infection in domestic or wild felids is currently available in Europe.
The present study aimed to determine the presence of *Cytauxzoon* spp. infection in *Felis silvestris silvestris* in Italy, in order to enhance the comprehension of its pattern distribution. The carcasses of 21 *F. s. silvestris* were collected from central and northern regions of Italy. All the animals were necropsied and samples of spleens were collected. *Cytauxzoon* infection was surveyed by a conventional PCR amplifying a portion of the SSU-rDNA of *Piroplasmidae*. The samples were also screened for *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp., *Babesia* spp., *Theileria* spp., and *Leishmania* spp. using SYBR Green Real-Time PCR (rtPCR) assays. Four animals were positive for *Piroplasmidae*-PCR assay and 3 sequenced amplicons were obtained (14.3%) and deposited in GenBank under accession numbers KR527491-KR527493. The sequences, identical to each other, clustered with Italian, Spanish, French and Romanian *Cytauxzoon* spp. isolates and with *C. manul* found in Mongolia. The samples were negative for the other pathogens screened. This work demonstrated that *F. s. silvestris* can be infected by *Cytauxzoon* spp. and may share the pathogen with domestic populations. Many aspects of the biology and epidemiology of the parasite remain uncertain. In this perspective, researchs should be focused on the pathogenicity of the European strains in domestic and wild felids and on the natural arthropod vectors and/or other routes of transmission.

TRANSMISSION BLOCKING EFFECTS OF *AZADIRACHTA INDICA* SEED KERNEL FRACTIONS AND ISOLATED MOLECULES ON *PLASMODIUM BERGHEI* EARLY SPOROGONIC DEVELOPMENT *IN VITRO*

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Antimalarial properties of *Azadirachta indica* are associated to the rich content in limonoids. Azadirone and gedunin have been demonstrated to inhibit the development of malaria parasite blood stages while azadirachtin A acts on the parasite transmission from human to the mosquito host.
This study aimed at characterizing molecules isolated from neem fruit kernel for *Plasmodium* transmission blocking activity directed against early sporogonic stages (ESS), the parasite's forms that develop in the mosquito mid gut after an infective blood meal.
Methanol extracts of powdered ripe and green seed kernels were subjected to solvent partitioning between water and ethyl acetate. Ethyl acetate phases were fractionated by MPLC and the major constituents of the obtained fractions were identified by NMR and MS. Extracts and 11 fractions (limonoid mixtures, nimbin, deacetylnimbin and salannin pure at 95%) were screened *in vitro* in the ookinete development assay, using the *Plasmodium berghei* CTRPgfp strain.
Nimbin at 50 µg/ml reduced the development of ESS by 40% (CI_{95}: 34,5 - 45.5) and affected specifically ookinete maturation. Indeed, only 30% (CI_{95}: 18,6 - 41.5) of round zygotes were able at this dose to develop into banana shaped ookinetes. Fractions containing mixtures of azadirachtins, inibited ESS development by 70% (predominantly azadirachtin A) to 40% (various azadirachtins), confirming previous results. Complete ESS inhibition was observed with the fraction containing almost pure deacetylnimbin and subsequent dose range experiments with HPLC-purified deacetylnimbin allowed to estimate an IC_{50} of 12 µM (7-21 µM). Deacetylnimbin appears to interfere with transmissible *Plasmodium* stages at a similar potency as azadirachtin A (IC_{50}: 17 µM (CI_{95}:15-19 µM). However, since it displays better thermal and chemical stability than azadirachtin A, deacetylnimbin could represent a valid alternative to azadirachtin A for the preparation of an anti-malarial transmission blocking combination drug or phytomedicine.

MOLECULAR MONITORING ON VECTOR-BORNE PATHOGENS IN CATTLE AND DROMEDARIES AT NOUAKCHOTT ABATTOIR, MAURITANIA

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The IZSA&M as has been worldwide engaged in projects on exotic animal diseases and zoonoses. In the framework of collaboration between CNERV Mauritania and IZSA&M as OIE ref Lab for Brucellosis and CBPP, a monitoring was performed at Nouakchott slaughterhouse for big ruminants aimed to assess the presence of selected vector borne pathogens and to evaluate the performance of molecular diagnostic tests. Blood samples from 119 cattle and 157 dromedaries have been collected.
All samples were tested for Trypanozoon DNA by Real Time PCR. Positive samples were confirmed to be *Trypanosoma evansi* by using the *VSG RoTat 1.2* specific PCR. DNA extracted from cattle blood samples were also analysed for *Theileria annulata* by Real Time PCR targeting the *18S RNA* gene. Furthermore, samples were tested by PCR targeting a portion of *pCS20* gene to detect *Ehrlichia ruminantium*. One hundred and thirteen ticks were collected from cattle, stored in ethanol 70% and identified by morphological observation. Eight out of 157 dromedaries resulted positive for *Trypanosoma evansi*. One hundred and four out of 119 cattle tested positive for *Theileria annulata*. All samples were negative for *Ehrlichia ruminantium*. The collected ticks were identified as *Hyalomma impeltatum* and *Hyalomma dromedari*.
Results confirmed the presence in Mauritania of two parasites (*T. annulata* and *T. evansi*). Molecular assays showed to be reliable for diagnosis also without clinical symptoms as for chronic forms of trypanosomosis in dromedaries and of theileriosis in cattle. Ticks species collected on cattle are well adapted to dry environment and both of them are known to be vectors of *T. annulata*. Considering negative results for *E. ruminantium,* it seems to be absent in the cattle or dromedaries as well as conceivable given the absence or scarcity of the tick vectors in the country and despite the report of clinical heartwater in bordering countries.

DIAGNOSIS OF PARASITIC DISEASES

IMMUNE STATUS AND RISK FOR NOSOCOMIAL INFECTION WITH *TRYPANOSOMA CRUZI, LEISHMANIA INFANTUM* AND *TOXOPLASMA GONDII*

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Opportunistic tissue protozoa may regulate their persistence/reactivation in peripheral blood depending on the immune status of the host. Aim of the study was to quantify this biological event during infections due to three parasites as *T. cruzi, L. infantum* and *T. gondii*, particularly important by the epidemiological point of view and, therefore, for the health safety of blood transfusions. The study was performed on health donors at risk of chronic Chagas disease (G1; n=42); asymptomatic/paucisymptomatic subjects with suspected leishmaniosis (G2; n=118) or toxoplasmosis (G3; n=65); subjects with ascertained immune deficit (G4; n=55). Screening was performed with serological (ELISA, ICT) and molecular (PCR) tests. The results are reported in the Table.

Group	**No.**	**Positive (%)**								
		T.cruzi		***L.infantum***		***T.gondii***			**TOT**	
		IgG	**PCR**	**IgG**	**PCR**	**IgM**	**IgG**	**PCR**	**Serology**	**PCR**
G1	42	2 (4.8)	1 (2.4)	11 (26.2)	0	2 (4.8)	14 (33.3)	*2 (4.8)	29 (69.0)	3 (7.1)
G2	118		0	14 (11.9)	4 (3.4)	0	0	0	14 (11.9)	4 (3.4)
G3	65	0	0	0	0	0	13 (20)	0	13 (20.0)	0
G4	55	0	0	5 (9.1)	23 (41.8)	0	2 (3.6)	9 (16.4)	7 (12.7)	32 (58.2)
TOT	280	2 (0.7)	1 (0.4)	30 (10.7)	27 (9.6)	2 (0.7)	29 (13.9)	11 (3.9)	63 (22.5)	39 (13.9)

*serologically negative

An overall 13.9% of blood samples were positive. Findings pointed out: i) unexpected *T.cruzi* and *T. gondii* in blood samples designed for transfusions (7.1%); ii) low presence of *L. infantum* in peripheral blood of subjects without evident clinical signs of disease (3.4%); iii) as expected, considerable presence of *L. infantum* and *T. gondii* in blood of patients with immune deficit (58.2%). Therefore, nosocomial transmission of parasitic species considered haematic only in the acute phase of the infection is always possible, mainly because in some cases preliminary serological tests failed in detecting the infection.

INNOVATIVE TOOLS FOR THE DIAGNOSIS OF *ECHINOCOCCUS GRANULOSUS* IN DEFINITIVE HOSTS

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Cystic echinococcosis (CE), caused by *Echinococcus granulosus*, is a widespread parasitic zoonosis. The aim of this study was to develop and standardize innovative tools for the diagnosis of *E. granulosus* in definitive hosts.
Fifty faecal samples collected from farm dogs infected by *E. granulosus* were used for the study. Each sample was examined by five different protocols using the FLOTAC apparatus and the following flotation solutions: satured sodium chloride (specific gravity, s.g.= 1,200), zinc sulphate (s.g.= 1,350), zinc chloride (s.g.= 1,450), Breza solution 1 (s.g.= 1,300) and Breza solution 2 (s.g.= 1,400). Moreover, four different protocols of DNA extraction were compared and standardized for the diagnosis of *E.granulosus*: (i) QIAamp Tissue Kit (Qiagen) from eggs, (ii) QIAamp Stool (Qiagen) from eggs, (iii) QIAamp Stool (Qiagen) from faeces, (iv) Wizard Magnetic Purification System for Food (Promega) from faeces. DNA extraction was followed by PCR amplification and sequencing of mitochondrial cytochrome C oxidase subunit 1.
The FLOTAC technique with zinc sulphate solution (s.g.1,350) resulted the best copromicroscopic method for faecal egg counts of Taeniidae eggs. Noteworthy, the FLOTAC device was also very useful recovering Taeniidae eggs to be used for DNA extraction. Indeed, the best kit for DNA extraction resulted the QIAamp Stool that provided the highest number of positive samples, i.e. 47/50 (94.0%; 95% Confidence Interval = 82.5-98.4%). The three negative samples had very low faecal egg counts (2 eggs per gram of faeces).
The best method for the diagnosis of *E. granulosus* in dogs resulted the combination of FLOTAC (with zinc sulphate) and QIAamp Stool (Qiagen) using the floated eggs.
These techniques could be very useful to control *E. granulosus* in endemic areas as the Campania region of southern Italy.

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WATERBORNE PATHOGENS CONTAMINATION IN DENTAL UNIT WATERLINE OF HOSPITALS AND DENTAL CLINICS

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Dental procedures generate aerosols containing microorganisms that proliferate within dental unit waterlines (DUWs). DUWs contamination is associated with environmental organisms such as *Legionella* spp., *Pseudomonas aeruginosa*, free living amoebae (FLA), which can increase the infection risk especially in immuno-compromised patients. We studied the microbial contamination and the potential health hazards for patients and dental staff in dental clinics where disinfection of DUWs was applied with different strategies. From October 2014 to November 2015, 11 dental clinics were investigated with 23 dental units in 4 teaching hospitals and 10 dental units in 7 dental premises. Tap water and DUWs systems (inlet, spittoon and handpiece) were sampled before and after the disinfection treatment, where applied, to determine Total Viable Counts (TVCs) (ISO6222,2001), *Legionella* spp. (ISO11731-2,2004), *P. aeruginosa* (ISO16266,2006) and coliform bacteria (ISO9308-1,2004). Water samples were subjected to FLA detection by cultural method and PCR amplification of 18SrDNA region.. To assign the FLA isolates to the species level, a comparison with available sequences in GenBank was performed by BLAST. Disinfection treatment was applied in 14/33 of dental units: 3% or 6% hydrogen peroxide (HP) (with and without surfactants) applied in DUWs for 1 hour and followed by water flushing was the prevalent shock disinfection practice followed by 0.2% peracetic acid or 2.5% quaternary ammonium compounds in continuous treatment. Microbiological quality of water varied between dental hospitals and smaller premises: *Legionella pneumophila* sg 2-14 was isolated in 30% (3/10) of DUWs housed in dental premises and 26% (6/23) of those in teaching hospitals. High concentrations (10^2-10^4 CFU/L) of *Legionella* spp. were detected in hospital tap water, handpieces (10^2-10^5 CFU/L) and spittoons (10^2-10^3 CFU/L). *P. aeruginosa* was frequently associated with high TVCs and it was detected in 58% (19/33) of all DUWs, mostly in handpiece devices. *Brevundimonas vesicularis* was detected in 6% (2/33) of dental units. FLA were recovered from 18% (6/33) of hospital DUWs. Among all cells microscopically positive to the culture examination, all PCR positive isolates belonged to *Vermoamoeba vermiformis* (identity of 99%). Shock disinfection with 3% HP resulted effective against *Legionella* spp. and it was never detected after 10 days from treatment. However high TVCs were still observed and *P. aeruginosa* was isolated from handpiece and spittoon in 79% (11/14) of hospital dental units. *Pseudomonas* was subsequently eradicated with 6% HP and surfactants shock treatment. Our data suggest the presence of a large contamination and biofilm persistence in DUWs, as a source of hazards specific for patients and dental staff. The presence of amoebae and biofilm justify the inefficacy of low-level disinfectants. This occurrence requires a water risk management and an effective choice of DUWs disinfectant to obtain an hazards control during dental practices.

MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF *DIROFILARIA REPENS* MICROFILARIAE IN AN ITALIAN PATIENT

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Dirofilarioses are zoonotic infections usually abortive in humans; otherwise, the parasites seldom reach the adult stage and only exceptionally yield microfilariae circulating in the bloodstream. Herein we present a peculiar clinical case observed in an Italian patient, in which at least two infectious larvae became mature adults that mated and produced active microfilariae.
In September 2014, a 30-year-old woman, resident in northern Italy, presented to the Umberto I Hospital (Rome). She reported a transient oedematous swelling on the left abdominal wall, with creeping eruption, followed by the occurrence of a subcutaneous nodular painless mass in the iliac region. One month later, analogue temporary swelling was appeared on the contralateral inguinal region, associated with intermittent joint discomfort in both knees. Biochemical values were within the normal range, whereas eosinophils were 15.7%. An ultrasound examination of the iliac swelling had evidenced a well-defined cyst with a big filamentous formation in continuous movement. A fine-needle aspiration of the lesion had been performed for parasitological, cytological and histological exams. The prompt microscopic examination of the aspired material had shown the presence of numerous microfilariae, which had been morphologically attributed to *Mansonella ozzardi.* Microscopic re-examination of Giemsa stained blood film and molecular analyses carried out in Rome identified *Dirofilaria repens* as etiological agent of the infection.
The described case is remarkable for at least three reasons: firstly, by the biological point of view, since in humans rarely more than one adult develops, and only exceptionally mature worms meet, mate and generate active microfilariae. Secondly, the new-borne larvae remained inside the nodule without reaching the peripheral blood, as only reported in three cases. Finally, in Italy this is the first diagnosis of human dirofilariosis carried out on microfilariae by microscopy and molecular diagnostics. Therefore laboratories must carefully consider this diagnostic possibility when dealing with microfilariae.

INFERRING *TRICHINELLA* SPECIES CAUSING INFECTION BY WESTERN BLOTTING

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Currently, the most used serological tests for the diagnosis of human trichinellosis are ELISA as primary screening test and western blotting (Wb) as confirmatory test. Both tests are based on excretory/secretory antigens (ESA) from *Trichinella spiralis*. *T. spiralis* ESA consist of a group of structurally related glycoproteins, which contain a predominant antigen epitope recognized by animals and humans infected with any species of *Trichinella*. However, tests based on ESA are unable to provide information regarding which species of *Trichinella* is the etiological agent of the infection. The aim of the present study was to define by Wb a distinctive pattern of reactivity of serum samples from mice infected with two species of the encapsulated clade (*T. spiralis*, *T. britovi*) and two species of the non-encapsulated clade (*T. pseudospiralis* and *T. papuae*) with the corresponding crude worm extract (CWE) in the same experimental conditions. By Wb, serum samples from mice infected with one species reacted with ESA and CWE proteins from all the four investigated species, displaying different band patterns recognizing a different number of proteins. Furthermore, serum samples from *T. spiralis* and *T. britovi* infected mice reacted stronger with CWE than serum samples from *T. pseudospiralis* and *T. papuae* infected mice. This procedure could represent an alternative way to infer if the etiological agent causing infection, belongs to the encapsulated clade or to the non-encapsulated clade, when is not possible to identify the *Trichinella* species by a direct method.

A SINGLE STEP-PCR ASSAY FOR THE DETECTION AND DIFFERENTIATION OF *BABESIA CANIS* AND *BABESIA VOGELI*

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Babesia species are tick-borne parasites of several mammalian species, including humans and domestic animals. *Babesia* infection in dogs is usually diagnosed by microscopic examination of blood smears for detecting merozoites within the erythrocytes. When their competent tick vectors occur in sympatry, *Babesia canis* and *Babesia vogeli* may affect dogs living in the same geographical area. However, their morphological differentiation of these species is unreliable, because of pleomorphic nature of these parasites. In the study, we describes a single-step polymerase chain reaction (PCR), targeting part of the cytochrome *c* oxidase subunit 1 (*cox*1) gene, for the simultaneous detection and differentiation of *B. canis* and *B. vogeli*. The PCR assay was standardized using genomic DNA of both species, extracted from individual and mixed infection samples that were microscopically positive for the parasite. Blood samples, negative upon cytological examination, were included as negative controls.
The PCR produced species-specific bands of the expected sizes for each pathogen (i.e., 750 bp, *B. canis*; 450 bp, *B. vogeli*), and two bands in the mixed infection samples. No non-specific bands, or amplification products from the negative control sample, were detected. The high analytical sensitivity and specificity of the PCR confirmed its reliability in detecting small amounts of genomic DNA, corresponding to $3x10^{-1}$ (*B. canis*) and $2.1x10^{-3}$ (*B. vogeli)* infected erythrocytes per reaction. The PCR assay developed represents a powerful tool for the epidemiological surveillance of *B. canis* and *B. vogeli* in areas where these species are endemic and/or occur in sympatry and provides a more sensitive/specific alternative to classical microscopic examination.

HEAT TREATMENT OF SERUM SAMPLES FROM STRAY DOGS NATURALLY EXPOSED TO *DIROFILARIA IMMITIS* AND *DIROFILARIA REPENS* IN ROMANIA

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Pre-heating of serum samples has been shown to reverse false negative antigen tests for *Dirofilaria immitis* infection in dogs. Here the authors report the results of modified Knott test for microfilariae, PCR and serum ELISA before and after heat treatment in a population of dogs naturally exposed to *D. immitis* and *Dirofilaria repens* infection. Of 194 dogs sampled from four cities in Romania, *D. immitis* circulating antigens were found in 16 (8.2%) non heated samples and in 52 (26.8%) heated samples. Of the 108 dogs examined by Knott test, 24 dogs (22.2%) were positive for circulating mf. Subsequent PCR identification showed six dogs had *D. immitis* mf only, 12 dogs had only *D. repens* mf, and 5 were positive for both. Fifty % of dogs with circulating *D. immitis* mf had positive antigen tests before and after heating, while the other 50% reverted to positive only after heat treatment. Sixty % of dogs with mixed *D. immitis/D. repens* infection were antigen positive before and after heating, while the other 40% converted to positive after heating. Antigen testing for *D. immitis* in the 12 dogs with only *D. repens* mf gave conflicting results. Only two dogs (16%) were antigen negative both before and after heat treatment. Six dogs (50%) became antigen positive after heating and four dogs (30%) were antigen positive both before and after heat treatment. Results would suggest that: false negative result for antigen testing can be reverted by heating of the serum sample; dogs infected with *D. repens* may have also an occult infection with *D. immitis*; heat treatment of serum from *D. repens*-infected dogs can reveal an occult infection with *D. immitis*.

MINI-FLOTAC, AN ACCURATE METHOD FOR THE DIAGNOSIS OF NEMATODE INFECTIONS IN HORSES AND SHEEP

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To improve the accuracy of nematode egg counting in equine faecal samples and establish whether the properties of equine faeces or the eggs affect the counts, the accuracy and precision of three faecal egg counting (FEC) techniques were compared: Mini-FLOTAC combined with Fill-FLOTAC, McMaster and Cornell-Wisconsin. Known numbers of eggs extracted from ovine or equine faeces were added to egg free ovine and equine faeces to give counts of 10, 50, 200 and 500 eggs per gram (EPG) of faeces. A series of cross-contaminations were performed: nematode extracted from horses' faeces were used to contaminate negative horse and sheep faeces and *vice versa*. For each level of egg density and for each series of cross-contamination twelve samples were taken and analysed for each technique. The study involving 768 counts showed that at all egg concentrations the Mini-FLOTAC had fewer counting errors than the other techniques which tended to underestimate FECs. The Mini-FLOTAC and Cornell-Wisconsin had 100% prevalence at all egg concentrations. The Cornell-Wisconsin technique significantly underestimated eggs count ($p< 0.05$) for all the measurements, both using sheep or horse faeces contaminated with nematode eggs. In contrast, Mini-FLOTAC counts did not differ significantly from expected values at any level of egg density ($p >0.05$). The McMaster techniques tended to underestimate the prevalence, revealing 100% only for concentrations greater than 200 epg (both using sheep or horse faeces contaminated with nematode eggs). The Mini-FLOTAC method is an easiest egg counting method for equine and ovine nematode eggs and it should therefore be considered to become the world standard. Combined with Fill-FLOTAC which provides an accurate method of weighing without need for a balance and filtering out debris, the two procedures together make the best method for egg counts on the farm as well as in the laboratory.

FIRST REPORT OF HUMAN CASE OF PSEUDOTERRANOVOSIS IN ITALY

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Members of the genera *Anisakis* and *Pseudoterranova* are responsible of human cases of anisakidosis worldwide. In Italy most human infestations have been associated to the species *A. pegreffii*, the most frequent in the Mediterranean area.
Here, a human case of pseudoterranoviasis from Italy is described for the first time: in 2015, a woman was found infected with an anisakid worm during a colonoscopy scheduled after aspecific clinical symptoms such as fever, epigastric pain and skin rash. Moreover, the patient declared a frequent consumption of marinated and raw fish.
The nematode was found penetrating the intestinal mucosa of ascending colon. Identification of species was performed by sequencing analyses of the mitochondrial region *cox*2 and a Taqman Real-Time PCR assay developed with two specific probes for *Anisakis* and *Pseudoterranova* genera. Sequence obtained was compared to GenBank retrieved material using tool.
BLAST search of the obtained sequence showed a 99% identity with *P. decipiens* s.s. The phylogenetic tree showed a very close relation with other *P. decipiens* s.s. isolates. Real-Time PCR gave positive amplification only with the specific *Pseudoterranova* probe.
Serum sample from the patient has given an IgE and IgG positive response at the Immunoblotting assay (WB) *versus* some aspecific antigenic bands obtained from crude extract (CE) of *Anisakis pegreffii* larvae, suggesting possible cross-reaction between *Anisakis* and *Pseudoterranova*.
This is the first case of human pseudoterranoviasis described in Italy due to the consumption of marinated fish and despite the information on fish consumed is missing, the recovery of *P. decipiens* ss could be explained in the light of the food global trade and consumption of imported fishes.

RECURRENT CRYPTOSPORIDIOSIS IN A CD40 LIGAND DEFICIENT CHILD AFFECTED BY SCLEROSING CHOLANGITIS

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Cryptosporidium spp. is a highly infectious protozoan parasite, responsible for cryptosporidiosis in humans, causing watery diarrhea, nausea, vomiting, and abdominal pain. Immunocompetent patients experience diarrhoea, while life-threatening infections can develop in immunocompromised patients with primary immunodeficiencies or HIV. *Cryptosporidium* spp. is a particularly pathogen in individuals with CD40L deficiency, that are at a major risk of developing severe liver disease with sclerosing cholangitis. Indeed, CD40–CD40L signalling plays an immunoregulatory role essential for the development of protective resistance to this parasite by enhancing IL-12 production, a crucial cytokine in resistance to *C. parvum*.A severe case of cryptosporidiosis in a six years old Ukrainian child with a hyperimmunoglobulin M syndrome due to CD40 ligand deficiency and affected by sclerosing cholangitis is herein described. During December 2015-January 2016, faecal samples, processed for microscopic examination by Ziehl Neelsen (ZN) and Merifluor® immunofluorescence staining, coupled to immunochromatografic assays, resulted positive for *Cryptosporidium* spp. Sanger sequencing-based identification characterized *Cryptosporidium parvum* species, belonging to the zoonotic lineage IIaA20G1 subgenotype. Biopsies were collected at ileum and colon and processed for ZN and Giemsa staining and GP60-based PCR analysis: *C. parvum* was diagnosed in high numbers at the only ileum site, as expected.
After azithromycin and paromomycin treatment for a month, the patient was further tested for *C. parvum*, revealing persistent cryptosporidiosis but with a significant decrease of parasite load. However, after three weeks new faecal samples were assayed and an increase of parasite load was again detected, with approximately 80-100 oocysts for slide. The patient is still hospitalized but parasite clearance has not been achieved.
This case highlights that CD40L deficient and cholangitis-affected patients, with either persistent diarrhoea or no symptoms, should be continuously monitored for cryptosporidiosis, as being an important co-morbidity and mortality factor often not treatable and with recurrence as the immune status worsens.

PARASITES IN AQUATIC FAUNA

SOUTH AFRICAN FUR SEAL *(ARCTOCEPHALUS PUSILLUS PUSILLUS)*: A NEW HOST FOR *DIROFILARIA IMMITIS*

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Dirofilariosis is a zoonotic mosquito-borne disease that is increasingly spreading worldwide, becoming a serious threat for animals and humans. Despite the importance of cardiopulmonary parasites, few studies are currently available in literature regarding *Dirofilaria* in pinnipeds (e.g. California sea lions *Zalophus californianus* and common seals *Phoca vitulina*). Recently, several nematodes were found in the right ventricle and pulmonary arteries, on the necropsy of two adult South African fur seals (*Arctocephalus pusillus pusillus*) from Algarve (Portugal). The nematodes were morphologically identified as *Dirofilaria immitis*. These first clinical cases prompted an epidemiological survey to assess the exposure to *Dirofilaria* spp. in pinnipeds kept in the Zoomarine park in Algarve.
Eleven blood samples were collected from three fur seal species (n=3, common seals *Phoca vitulina*; n=2, grey seals *Halichoerus grypus*; n=1, California sea lion *Zalophus californianus*; and n=5, South African fur seals *Arctocephalus pusillus pusillus*). Samples were tested by a commercial *D. immitis* antigen test (WITNESS® Dirofilaria), by modified Knott's technique and by real-time PCR assay based on SsoFast™ EvaGreen(®) coupled with melting-curve analysis. Overall, two common seals were antigen positive and one South African fur seal showed microfilariae consistent with *D. immitis*. Additionally, two of the tested samples from *A. p. pusillus* and *P. vitulina* were molecularly positive for *D. immitis*. To the authors' knowledge, this is the first worldwide report of *D. immitis* infection in *A. p. pusillus* and the first report of this nematode in a pinniped population from Portugal. Considering the emergence and the zoonotic potential of *D. immitis*, this report will increase the awareness on dirofilariosis in aquatic mammals in order to perform preventive therapy and future control strategies against *D. immitis*.

BREVIMULTICAECUM SP. (NEMATODA) LARVAE IN LIVER OF FISH *GYMNOTUS INAEQUILABIATUS* FROM PANTANAL REGION BRAZIL: LIVER PATHOBIOLOGY AND INFLAMMATORY RESPONSE

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Gymnotus inaequilabiatus is a fish of great importance in Pantanal region (Brazil). It is used as live-bait for collecting other fish species of commercial value and is one of the preferred prey for *Caiman yacare*. *G. inaequilabiatus* is paratenic host for the nematode *Brevimulticaecum* sp. and *C. yacari* is the definitive host where the adult parasites can be find in the intestine. In two occasions, one in the flood season and one in the dry period, 22 specimens of *G. inaequilabiatus* were sampled (mean total length ± standard deviation, SD: 31.88 ± 2.54 cm). Larvae of *Brevimulticaecum* sp. were encountered in likely all the visceral organs, but, this investigation was focused on liver. Twenty-one livers (95%) harboured *Brevimulticaecum* sp. larvae, with an intensity of infection ranging from 4 to 343 larvae (mean ± SD: 71.00±93.28 larvae for liver). In livers with high number of nematode larvae the vast majority of the hepatic tissue was occupied by the parasites. Most *Brevimulticaecum* sp. larvae were encapsulated on the surface of the liver, enclosed by a granulomatous response involving the peritoneal visceral serosa. The cellular immune response within liver was assessed by histological methods and transmission electron microscopy. The wall of the capsule was composed of two layers: the innermost, which was adjacent to the nematode, consisted of host connective tissue, mainly collagenous fibres, whilst the outer layer consisted mainly of mast cells (MCs) and macrophage aggregates (MAs). In infected livers, hepatocytes, notably those in close proximity to larvae, showed degenerative changes, i.e. swelling and hydropic degeneration. By comparison, hepatocytes in uninfected liver or in regions away from the larvae appeared normal. Emphasis will be placed on the role of MCs and MAs as important components of the host's inflammatory response.

GENETIC IDENTIFICATION AND PARASITIC INFECTION LEVELS OF *ANISAKIS* SPP. LARVAE IN COMMERCIALLY IMPORTANT FISH SPECIES OF THE MEDITERRANEAN SEA: A LARGE EPIDEMIOLOGICAL SURVEY

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The consumption of the European hake, *Merluccius merluccius*, European anchovy, *Engraulis encrasicolus*, sardine, *Sardina pilchardus*, mackerel species *Scomber scombrus* and *S. japonicus*, is widespread in Mediterranean European countries, where these fish species have a high commercial value. A total of 5894 specimens belonging to those fish species, fished between June 2013 - November 2015 from 13 different fishing areas of the Mediterranean Sea, were analysed to estimate infection rates by different *Anisakis* species in the framework of Parasite European Project. The parasitological analysis of viscera and fillets was carried out by traditional procedures and UV-press method. A large number (1941) of *Anisakis* spp. specimens were identified by allozymes, sequences analysis of the elongation factor 1 alpha1 nuclear gene (EF1 α-1 nDNA), mitochondrial cox2 (mtDNA *cox2)* loci and RT-PCR of mtDNA *cox2*. Based on those genetic markers, 1922 larvae corresponded to *A. pegreffii,* 17 to *A. physeteris,* while 2 *A. simplex* (s. s.) larvae were detected only from hakes of the Alboran Sea water, in sintopy with *A. pegreffii*. The infection levels resulted significantly different between the fishing areas, in accordance for the different fish species. Fish samples from southern Adriatic Sea showed the highest level for both prevalence and abundance of the infection by *A. pegreffii*, while fish from Balearic Sea, Ligurian Sea and Southern France showed low infection levels. The Alboran Sea resulted the only sympatric area for *A. pegreffii* and *A. simplex* (s. s.).
The great majority of *A. pegreffii* larvae were located in the body cavity (92.3%), only a small percentage were detected in the muscle of the fish (7.7%); while, *A. physeteris* was found only in the visceral cavity of the fish examined. A positive correlation between fish length and the infection levels by *Anisakis* larvae was observed in all fish species.

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THE EFFECT OF TEMPERATURE ON THE MIGRATION CAPACITY AND RELEASE OF EXCRETORY/SECRETORY PRODUCTS BY *ANISAKIS PEGREFFII* LARVAE CULTURED *IN VITRO* (NEMATODA: ANISAKIDAE): A MOLECULAR APPROACH

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The two zoonotic species, *Anisakis simplex* (s. s.) and *A. pegreffii*, have been found to show differential rates of infection in the flesh of their intermediate/paratenic fish hosts. In addition, the *post-mortem* migration by *A. pegreffii* larvae has been demonstrated to occur, *in vivo,* in some fish host, with a temperature dependent increase. However, the molecular characterization of the invasion mechanism by the two species of *Anisakis* has never been studied. On the other hand, it is also known that excretory/secretory proteins (ESPs), released by *Anisakis* spp. larvae, have an antigenic/allergenic role in IgE-*Anisakis* sensitization. Aim of this work was to study the effect of temperature on the migration capacity and release of ESPs by *A. pegreffii* larvae, the main zoonotic species infecting fish from the Mediterranean Sea.
The migration capacity of *A. pegreffii* larvae, maintained "*in vitro*" culture, was tested by agar penetration at different temperature and time intervals. qRT-PCR and western blot (WB) analyses were used to examine the expression levels of HSP90 and ESPs, under different temperature and time conditions. Primers used for mRNA expression of HSP90 in qRT-PCR were those previously described; whereas those used for mRNA of *Anis 1* and *Anis 7* were newly designed. A significant higher migration capacity by *A. pegreffii* larvae, correlated to the increase of temperature, was found. Increased trascript levels of HSP90 and of major antigens/allergens (i.e. *Ani s 1, Ani s 7)*, in response to the temperature, were progressively observed. In addition, the SDS-PAGE of the ESPs showed a differential production of those major antigens/allergens at different incubation temperatures and times. This result was also confirmed by WB-IgE response of positive human sera for Gastro-Allergic-Anisakiasis (GAA) due to *A. pegreffii.*

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SPREADING OF THE SWIMBLADDER PARASITE *CYSTIDICOLA FARIONIS* (NEMATODA: CYSTIDICOLIDAE) IN WILD AND FARMED SALMONIDS IN ITALY

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Cystidicola farionis is a nematode frequently reported in swimbladder of several species of wild salmonids from USA and Europe, and recently described in Italy. The life cycle is heteroxenous and involves as intermediate hosts several species of gammarid crustaceans widespread in the freshwater aquatic environment. After the previous report in salmonids (*Salmo trutta fario* and *Oncorhynchus mykiss*) from the wild in the northern part of Adige River (Bolzano province), the infestation seems to progressively widen its geographical area. During an ongoing parasitological survey on salmonids, high prevalence (P%) and mean intensity (MI) values of infestation have been recorded in wild salmonids (P% range 28.6-70%; MI range: 4.5-100) and in farmed rainbow trouts (P% range: 37.5-100%; MI range: 4.5-12.5) in northern Italy, expanding to different regions (Veneto and Friuli Venezia-Giulia). The pathogenicity and the impact of *C. farionis* on health of affected fish is still debated among the scientific community. Anyway in this study some gross lesions in heavily affected fish have been observed, such as a catarrhal-hemorrhagic inflammation of highly infected swimbladder, more severe in juvenile wild brown trout (*S. trutta fario*). Furthermore, although *C. farionis* has been generally detected at low intensity in farmed trout, it could affect productivity and quality of fish products and so has to be considered a potential health risk for national aquaculture. Although the authors in past surveys already alerted on the possible *C. farionis* switching from wild to farmed salmonids and vice versa, no preventive strategies to avoid the expanding of *C. farionis* geographic range have been adopted. Further studies are urgent to map the actual distribution of cystidicolosis in Italian salmonid populations and to apply appropriate containment measures useful to avoid its further spread.

FIRST REPORT OF GENUS *NEOSPIRORCHIS* IN SEA TURTLES FROM THE MEDITERRANEAN SEA

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Spirorchiid flukes (Digenea: Spirorchiidae) are parasites of circulatory system of freshwater and sea turtles. The presence of adults in heart and vessels and the spreading of eggs to various organs can lead to severe vasculitis, thrombosis and development of disseminated granulomas. *Neospirorchis* sp. in particular has been associated to meningitis and mortality mass event because of its tropism for Central Nervous System (CNS).
One hundred forty-four carcasses of loggerhead turtle (*Caretta caretta)*, stranded along North-Eastern coast of Adriatic sea in the period 2009-2015 were analyzed for spirorchidiasis. After necropsies, research of parasitic elements by stereomicroscopy in major vessels and organs was performed, followed by copromicroscopic analysis and histological examination of tissues. Internal transcribed spacer 2 region (ITS2) from parasitic elements was amplified and sequenced for comparison with data in literature.
Neospirorchis eggs (Type 3) were identified in faecal samples of six turtles (4.16%); four out of these had a mixed infection with *Hapalotrema*. No adults of *Neospirorchis* were found. Type 3 egg masses were grossly visible as black short stripes on the intestinal mucosa; big clusters of rounded eggs surrounded by granulomatous inflammation were histologically visible in mucosal and submucosal layers. Small granulomas surrounding isolated spirorchiid eggs were ubiquitous in several organs, but not in CNS. ITS2 sequences obtained from isolated eggs matched (100% identity) with those of *Neospirorchis* (Neogen11) described by Stacy (2008).
To identify the possible origin of the infected turtles, sequences of mtDNA encompassing the D-loop region were analyzed. All turtles were carriers of Mediterranean haplotypes.
This represents the first report of genus *Neospirorchis* in *C. caretta* living in the Mediterranean Sea. In this study spirorchidiasis seems not to have severely affected health status of the host, being lesions always mild in all districts.

CRASSICAUDOSIS IN FIN WHALES *(BALAENOPTERA PHYSALUS)* STRANDED ALONG ITALIAN COASTS

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Of several known crassicaudid infections, those caused by *Crassicauda boopis* (Nematoda, Spirurida) in whale are especially pathogenic. The giant adult nematode grows in the vascular and ureteral system of the kidney; it can cause complete vascular occlusion and kidney failure as described in Atlantic fin whales, *Balaenoptera physalus*; no data are still available in literature for this host species in Mediterranean basin.
Six fin whales, stranded dead along Italian coastline in the period 2006-2013, were analyzed for *Crassicauda* infection. The parasites were morphologically identified according to Lambertsen (1985); molecular analyses by amplification and sequencing of a portion of the 18S of the small subunit ribosomal and internal transcribed spacers 2 (ITS2) of the rRNA were carried out. Formalin-fixed tissues were routinely processed for histology.
Crassicaudosis was observed in four out six examined animals. Adult *C. boopis* were found in three fin whales, one of which had also nematode larvae in intestinal nodules and mesenteric vessels' wall. Another animal showed vascular lesions with ruined fragments of the nematode inside. The sequences obtained from the parasitic elements (adults, larvae and lesions) showed a high identity with each other for ITS2 region; the 18S sequences had high identity with the unique *Crassicauda* sequence (*C. magna*) registered in GenBank (Accession number: KM233410.1).
Chronic vasculitis and/or thrombosis were observed in renal vessels, vena cava and mesenteric arteries, leading to almost complete occlusion of vessels lumen in three cases. Histology showed renal fibrosis, perirenal granulomas and disseminated *Crassicauda* eggs in renal vessels, renal pelvis and adrenal glands.
This study provides data on the presence, pathology and biomolecular characterization of *C. boopis* in fin whale of the Mediterranean Sea.

ANISAKIS AND *HYSTEROTHYLACIUM* LARVAE IN ANCHOVIES *(ENGRAULIS ENCRASICOLUS)* AND CHUB MACKEREL *(SCOMBER JAPONICUS)* IN THE MEDITERRANEAN SEA: MOLECULAR IDENTIFICATION AND RISK FACTORS

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Nematodes belonging to marine ascaridoids comprise members of the families Anisakidae, including etiological agents of anisakidosis, and Raphidascarididae, considered not pathogenic to humans. Studies to improve knowledge for parasite control of fishery products for human consumption are needed (EFSA, 2010).
A molecular epidemiological survey was designed with the aim to evaluate the potential risk of human anisakidosis through the identification of nematodes found in anchovies and mackerel and the analysis of factors influencing the infection in fish.
Anchovies (n=179) and chub mackerels (n=84) were sampled at Milan Fish Market and coelomic cavity was visually examined. Statistics (epidemiological indices and predictors of infection) were evaluated (SPSS 20). Species identification was performed by PCR-RFLP of ITS (D'Amelio et al 2000; Abollo et al 2003; De Liberato et al 2013); sequencing of nuclear ITS and mitochondrial 12S were performed for *Hysterothylacium* and ITS dataset used to perform phylogeny (MEGAv.6).
An amount of 1264 larvae were collected. Both fish species resulted infected by third stage larvae of *Anisakis* Type I and *Hysterothylacium* (anchovies: P=6.7% and 54.18%; mackerels: P=55.95% and 13.09%, respectively). Predictors of *Anisakis* and *Hysterothylacium* infection were body length for anchovies and catching area for both anchovies and mackerels.
PCR-RFLP analysis identified *H. aduncum*, *A. pegreffii* and hybrid genotype (*A. pegreffii*/*A. simplex* s.s.). Preliminary results on NJ phylogenetic tree on ITS showed similarity among specimens analyzed, *H. aduncum* and *H. auctum*. However, species formerly recovered in the study area, i.e. *H. incurvum, H. corrugatum* and *H. petteri,* are missing in GenBank. Alignment of 252bp of 12S showed an average evolutionary divergence over all sequence pairs of 0.3%.
Epidemiological results are in agreement with previous reports (Cavallero et al 2015), suggesting a moderate risk of anisakidosis for anchovies. Molecular findings represent a first step for further investigations to clarify the "*aduncum* clade" systematics.

NEW AND NATURAL PARASITES OF *FISTULARIA COMMERSONII* REVEAL TRAITS OF THE BIOLOGY AND DYNAMIC OF THIS ALIEN FISH IN THE MEDITERRANEAN SEA

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The bluespotted cornetfish *Fistularia commersonii* is one of the most invasive marine fish in the Mediterranean Sea, as it has colonised almost all the Basin from 2000 to 2007. Sporadic findings of this species in shallow waters allowed to analyse the parasites of 34 specimens from Sardina and six from North Africa (Tunisia and Libya).
The parasite assemblage was composed by 22 species/taxa, and it was mainly characterised by two species, the larval circum-African cestode *Nybelinia africana* and the adult Indo-Pacific trematode *Allolepidapedon fistulariae*. Among the other parasites, most of them were larval stages, as the cestodes Phyllobothriidae and *Pseudogrillotia* sp., the nematodes *Hysterothylacium aduncum* and *H. fabri*, and gnathiid crustaceans. Adult stages were also found, among them the Indo-Pacific trematode *Neoallolepidapedon hawaiiense*, the hirudinean *Trachelobdella lubrica* and the acanthocephalan *Breizacanthus ligur*. Thus, the parasite fauna of this migrant in the invaded range is a combination of generalist species (acquired in the new habitat) and some of its natural parasites (co-introduced with migration).
The parasitological results offer elements to understand some traits of the biology and dynamic of this alien species in the Mediterranean Sea. For example, although first molecular studies suggested a colonisation of the Mediterranean Sea through larval dispersal, the presence of adult Indo-Pacific trematodes show that *F. commersonii* reached and colonised the Mediterranean Sea through adult individuals and not planktonic larvae. Besides, the infection of the deep-sea species *B. ligur* indicates that this fish spends part of its life in deep-water habitats. This could explain how this presumed littoral fish has been able to colonise the Mediterranean Sea in just seven years. Indeed, for its movements it could use the deep-sea/open-water shortcut, instead of the coast-to-coast circumnavigation of the Mediterranean Basin.

MOLECULAR DATA CONFIRM THAT *HETEROPHYES HETEROPHYES* (SIEBOLD, 1852) AND *HETEROPHYES NOCENS* ONJI & NISHIO, 1916 (TREMATODA: HETEROPHYIDAE) ARE TWO DISTINCT BUT CLOSELY RELATED SPECIES

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Heterophyes heterophyes and *Heterophyes nocens* (Trematoda: Heterophyidae) are among the most important zoonotic flukes found in brackish water fish. The first species mainly occurs in the Mediterranean and Middle-East regions, whereas the second one is widespread in several Far East areas. Due to the great morphological and biological similarities between these two species, in the past they were suggested as synonyms. One of the few characters useful to discriminate them is the number of rodlets of the genital sucker: 70-85 in *H. heterophyes* and 50-62 in *H. nocens*.
In the framework of a study on the zoonotic trematodes of Mugilidae from Sardinia (western Mediterranean Sea), 60 hosts (13 *Chelon labrosus*, 18 *Liza aurata*, 6 *Liza ramada*, 8 *Liza saliens*, 15 *Mugil cephalus*) were examined. A total of 17,899 metacercariae were isolated, and 14,113 were identified as *Heterophyes* sp. Afterwards, 300 of these metacercariae were used for an experimental infection of a hamster and eight adults were obtained. According to morphological characters (mainly the number of rodlets) six were identified as *H. heterophyes* and two as *H.* cf. *nocens*.
To corroborate the morphological identification, the sequence of a ITS2 rDNA fragment was analysed for 14 metacercariae of *Heterophyes* sp., 3 adults of *H. heterophyes*, 1 of *H.* cf. *nocens*, and 3 of *H. nocens* from South Korea.
PCR produced a fragment of about 450 bp, and the ML analysis revealed the occurrence of two well-defined clusters, one grouping the metacercariae of *Heterophyes* sp. and the adults of *H. heterophyes* and *H*. cf. *nocens*, and the other with the three Korean specimens of *H. nocens*. Results pointed out that *H. nocens* is clearly separated from *H. heterophyes*, thus suggesting caution in the exclusive use of the number of rodlets of the genital sucker to separate the two species.

THE TOXIC EFFECTS OF ESSENTIAL OILS ON MITES

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The use of synthetic pesticides and repellents to control veterinary ectoparasites is becoming increasingly problematic. Reports of resistance to conventional synthetic pesticides are increasing, and so are concerns in regards to environmental and human health risks. Thus, introducing novel acaricides is becoming more urgent than ever. Possible alternatives are the plant-derived essential oils. The objective of this study was to determine the toxic effects of essential oils on parasitic and economically important pest mites. *In vitro* experiments were performed using the mite *Tyrophagus longior*, which is a common pest of stored food, as a laboratory model. These experiments were used to identify the essential oils that gave the highest toxicity. Oils were used at a concentration of 5% (V/V) in ethanol. From the 37 essential oils tested, 13 caused over 90% mortality on these mites following a 24 hour exposure. From the most toxic essential oils, 8 were selected for further analysis (2-undecone, α-methyl-trans-cinnamaldehyde, methyl trans-cinnamate, ethyl cinnamate, benzyl alcohol, *Allium sativum*, *Melaleuca viridiflora* and *Mentha spicata*). The impact of lower concentrations (2.5%, 1.25%) was also examined after 24 hours, as was the residual activity of 5% (V/V) after 1, 2, 6, 24, 72 hours. Then, red poultry mites, *Dermanyssus gallinae*, were collected from a poultry farm in Gloucestershire. The toxicity of 5%, 2.5% and 1.25% concentrations of essential oils were investigated as described above as was the residual activity after 1, 24 and 72 hours. The results were comparable to those obtained with *Tyrophagus longior*, although with the red poultry mites the toxicity was less marked.

DEVELOPMENT OF *CRENOSOMA VULPIS* IN THE COMMON GARDEN SNAIL *CORNU ASPERSUM*: IMPLICATIONS FOR EPIDEMIOLOGICAL STUDIES

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Crenosoma vulpis is a metastrongyloid nematode, causing respiratory tract infections of wild (red foxes) and domestic (dogs) carnivores in Europe and North America. Larval stages develop in snails and slugs, with third stage larvae (L3) being infective to carnivore definitive hosts, which feed on these gastropods. The scant data available on the intermediate hosts of *C. vulpis* and the limited information about the morphology of larvae may impede epidemiological studies of this parasite. Here we provide i) data on the development of *C. vulpis* in *Cornu aspersum*, a common snail in regions of the Mediterranean and North-western Europe and ii) the morphological descriptions of L1 and L3. Snails were experimentally infected with single doses of 100 L1 of *C. vulpis* and then artificially digested at selected days post-infection (i.e., 3, 6, 10, 15, 20, and 180). The suspension obtained from gastropod digestion was microscopically examined and larvae were counted and morphologically identified. First- and third-stage larvae were preserved in 70% ethanol, cleared and examined as temporary mounts in glycerol for morphological descriptions. In all, 115 larvae were recovered from 12 infected snails (mean of 9.6 larvae per specimen). The 18S rDNA sequences obtained from the larvae collected from the dog and the snails' tissues displayed 100% identity to the nucleotide sequence of *C. vulpis*. *Cornu aspersum* is herein reported for the first time as a suitable intermediate host of *C. vulpis*. This snail species may play an important role for the infection of animals living in regions of the Mediterranean basin. In addition, this study provides more details on the morphological descriptions of L1 and L3 and supports future investigations on the epidemiology of this little known parasite.

CONTAMINATION BY CANINE GEO-HELMINTHS IN THE CITY OF PADOVA

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The soil contamination with canine geo-helminths has a relevant health-risk impact for animals and, for most of them, for humans.
The aim of this study was to improve knowledge on the pollution by canine geo-helminths in Padova municipality (Veneto Region, North-eastern Italy).
In order to homogenously evaluate the pollution in the six municipal districts, a grid representing rectangular sub-areas (0.8×1.1 km) was overlaid on the Padova map using the software Google Earth 7.1.2.2041. In each sub-area, a route was drawn involving sidewalks and public squares with high population densities. Sampling was performed in the early morning, from June to December 2013. The geographical location of each stool sample was recorded. Only fresh samples were collected and submitted to qualitative copromicroscopic analyses. A χ^2 test ($p<0.05$) was used to compare faecal pollution levels (n. of faeces/km) among the six Municipal districts.
A mean value of 10.1 faeces/km (1,861/184.2) was detected, with no statistical differences in faecal pollution ($\chi^2=4.267$; $p>0.05$) among the six Municipal districts. Of a total of 1,861 faecal samples observed on the city soils, 435 were collected and submitted to copromicroscopic analyses. The overall prevalence was 3.0% (13/435). The canine geo-helminths found in the examined samples were *Trichuris vulpis* (1.4%), *Toxocara canis*, Ancylostomatids, and *Eucoleus aerophilus* (0.7%).
The low prevalence values detected in this study confirm the data obtained in a survey performed in 2012 in green public areas of the same city. These results may be related to the common use of formulations for prevention of heartworm infection, that is endemic in Veneto Region. Anyway, the soil of Padova appears evenly contaminated by canine faeces as a consequence of the poor sense of civic duty of dog-owners. This finding highlights the need to enhance awareness of pet-owners in order to limit the threat of soil-transmitted geo-helminths.

OUTCOME OF CANINE STRONGYLOIDIASIS IN FIVE DOGS

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Canine strongyloidiasis caused by *Strongyloides stercoralis* is a zoonotic disease uncommon but potentially fatal. The clinical outcome of natural infection by *S. stercoralis* in five kennel dogs is herein reported along with the results of post-treatment fecal monitoring. In vivo diagnosis was achieved by detecting *S. stercoralis* first stage larvae (L1) in faecal samples. Dogs showed gastrointestinal signs from mild to severe degree, and laboratory alterations costing of mild anemia, severe hypoproteinemia, increase of alpha2 fraction at serum protein electrophoresis and increase of CRP. Mild eosinophilia was registered only in one case. Gastrointestinal tract was investigated in 4 dogs by ultrasound, endoscopy and biopsy and, in 3 animals, by scraping and histology at post-mortem. One dog died for the severity of clinical conditions at 48 hours from presentation, thus no specific treatment was started. In the remaining dogs a single course of treatment with fenbendazole 50 mg/kg/die OS for 5 consecutive days was started associated or not (two dogs each regime) to moxidectine plus imidacloprid spot-on once. A parasitological monitoring by using the Baermann technique on samples from ampulla was daily performed till the first negative result then twice a month on three days fecal pool for at least two times. Two dogs apparently eradicate the infection and recovered; one dog had reverse to positive fecal results in follow up and had euthanasia and one dog spontaneously died one month after treatment, despite the negative fecal results. Post-mortem scraping of intestinal wall revealed the presence of adult female nematodes. Canine strogyloidiasisis has to be included in the differential diagnosis of gastrointestinal disease in Europe. Treatment with fenbendazole alone or combined with moxidectine plus imidacloprid could be not effective against the infection. Therefore repeated fecal monitoring are advised to confirm the efficacy of treatments in curing this condition.

NOVEL MODES OF TRANSMISSION OF FELINE LUNGWORMS

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Snail-borne lungworms exert an enormous socio-economic impact on the health and welfare of animals and humans. Amongst these parasites, *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* are increasingly reported as parasites of the respiratory tract of felids. These lungworms share the same ecological niche and species of snail intermediate host (i.e. *Cornu aspersum*). Recently, we demonstrated the ability of dead or live *C. aspersum* to shed infective third-stage larvae (L3s) of these lungworms into the environment. These elimination pathways may represent alternative routes of infection for cats under field conditions and may affect the epidemiology of feline lungworms. However, little is known of the biology of these parasites in their snail intermediate hosts. Elucidating fundamental aspects of snail-parasite interactions will lead to a better understanding of the epidemiology of feline lungworms and other phylogenetically related species. In this study we assessed the potential of nematode transmission from infected to naïve, uninfected snails and evaluated the survival time of *A. abstrusus* and *T. brevior* L3s at different temperatures. Gastropods were experimentally infected with L3s of either *A. abstrusus* or *T. brevior*, or they were housed together with infected live (study 1) or dead snails (study 2). Previously uninfected gastropods scored positive for *A. abstrusus* (n=168) and *T. brevior* (n=5). In addition, L3s of both lungworm species were detected in naïve snails co-housed with live or dead infected snails. The maximum survival time of L3s at 4 and 26°C was 35 and 13 days for *A. abstrusus* and 27 and 7 days for *T. brevior*, respectively. Here, we describe the transmission of L3s from an infected to a naïve intermediate host and refer to this novel route of parasite transmission as *intermediesis*. The implications of snail-to-snail transmission in the epidemiology of feline lungworms and snail-borne diseases will also be discussed.

MEDICAL AND VETERINARY ENTOMOLOGY

BED BUG DETECTION DOGS: DIAGNOSTIC ACCURACY TO DETECT *CIMEX LECTULARIUS L.* (HEMIPTERA: CIMICIDAE) DURING OLFACTORY INSPECTIONS OF HOTELS IN ITALY

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The Bed bug, *Cimex lectularius* (*Hemiptera*: *Cimicidae*) is an obligate blood-feeding ectoparasite with a cosmopolitan spreading preferably fed on human blood. Its bites produce the onset of a highly itchy erythematous papular dermatitis with a consequent serious nuisance. A large number of bed bug infestations usually occurs in hotels. An early detection of infestations due to few bed bugs is essential for interventions to cut down their spreading, and consequently the pest control expenses and the social reputation damage. However, an early detection of bed bug infestations is difficult, because they are cryptic insects and mostly live in harborages made of cracks, crevices, etc. The purpose of this research is to evaluate the diagnostic accuracy of canine detection units trained for olfactory detection of bed bugs in hotels. The canine detection units were previously certificated by AICAC (www.aicac.it).
136 bedrooms of 11 hotels in Rome, Assisi and Perugia were inspected by two canine detection units, from October 2014 to January 2016. In those hotels the presence of bed bugs had been already reported three months before the inspections.
The Cohen's kappa test was calculated to assess the concordance between the canine inspections and the visual post-inspection, taken as gold standard. Analyses were performed using OpenEpi software. The overall diagnostic accuracy of the canine inspection was 96.32%, the Negative Predictive Value 99.11%, and the Positive Predictive Value 83.33%. Such results highlight the diagnostic accuracy of canine inspections as very high and reliable. In particular, the results show that the Negative Predictive Value is high, whereas some false positive results are possible (medium Positive Predictive Value). This means that negative results are reliable, whereas positive results obtained from canine inspections, should be always checked by a visual inspection of the handler.

ATTRACTION OF THE INVASIVE MOSQUITO *AEDES KOREICUS* (DIPTERA: CULICIDAE) TO HUMANS

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Aedes koreicus is an invasive mosquito discovered in Italy in 2011, quickly spreading in North Italy. Little information on its vector competence is available and its biology and ecology are poorly known and mostly related to the native place. There, it is reported to feed on humans and domestic animals during the daytime.
The behavior of *Ae. koreicus* out of its native range need to be urgently investigated, in particular the host feeding preference and the anthropophilic degree, that are a crucial aspect to evaluate the vector competence and human disease transmission. Herein, the anthropophilic degree was investigated by human landing catches (HLC), the best method for quantifying some entomological parameters. The present study was carried out in Belluno Province (three sites) and in Trento Province (two sites) in 2014-2015. Three collections of 30' each were set up to finish one hour before sunset.
At the same time a BG-Sentinel trap baited with lure and CO_2 was activated to evaluate the abundance of *Ae. koreicus*. Overall, 737 mosquitoes belonging to six species were collected by HLC. *Aedes koreicus* was collected in all the sampling sites (27 in 2014 and 33 in 2015). The human seeking activity was predominant at 18:00 and number of females collected ranged from 0 to 14 per hour. The total number of mosquitoes collected by HLC was significantly higher than those collected by the BG-CO_2 trap. Our results show that *Ae. koreicus* is attracted by human. HLC is confirmed as the best practice to determine host preference and biting rate, essential parameters to consider the species as a potential disease vector. Although this study is preliminary, our results represent the first data on feeding behavior of one of the most invasive mosquito species.
This work was funded by the Autonomous Province of Trento, Project LExEM.

UNEXPECTEDLY HIGH ZOOPHILY ASSOCIATED TO HIGH PLASMODIUM SPOROZOITE RATES IN *ANOPHELES COLUZZII* FROM A LINN-PROTECTED VILLAGE IN BURKINA FASO

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The global effectiveness of long-lasting insecticidal nets (LLINs) in reducing malaria transmission is indisputable. However, in areas where malaria transmission levels are extremely high, substantial reductions in transmission intensity only lead to a modest reduction in human parasitaemia. A paradigmatic case is represented by Burkina Faso where, after a few years of mass distribution of LLINs, the burden of malaria has not significantly changed as highlighted by WHO Country statistics and statistical bureau of Burkina Faso. We here report the results of a longitudinal survey on host choice and Plasmodium sporozoite rate (SR) in malaria vectors belonging to *Anopheles gambiae* complex in a rural village of Burkina Faso where LLINs were broadly distributed the year before the sampling (August - November 2011). The human blood index (HBI) - as determined by PCR-approaches - was 18.8% (N=112) and 8% (N=75), in *An. coluzzii* and *An. arabiensis*, the two most abundant malaria vectors in the area. These values are much lower than usually reported particularly for *An. coluzzii*, which is known as a highly anthropophilic species, but consistent with the hypothesis that LLINs reduced the availability of human hosts to mosquitoes. However, the *Plasmodium* sporozoite rates (SRs: 7.6%, N=449, and 5.2%, N=229, in *An. coluzzii* and *An. arabiensis*, respectively) were found to be in the range of those reported in the region before LLIN implementation. This suggests that, despite LLINs have significantly reduced human/vector contact, this has not apparently yielded to a substantial reduction of mosquito infection rates. Further investigations are needed to confirm these results; however, they are fully consistent with the lack of effectiveness of LLINs in stemming malaria transmission in the study area.

EPIDEMIOLOGY OF PARASITIC DISEASES 1

HERACLES COLLABORATIVE PROJECT ON CYSTIC ECHINOCOCCOSIS FUNDED BY THE EUROPEAN COMMISSION AND PRELIMINARY RESULTS FROM EXTENDED ULTRASOUND SURVEYS IN EASTERN EUROPE

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The global burden of human cystic echinococcosis (CE) is estimated in over 1 million cases and between 1-3.6 million DALYs, accounting for underreporting. However, diagnosed cases represent only a proportion of infected people and these figures are likely underestimated, contributing to the neglect of this zoonosis. Extended population surveys with ultrasound (US) are needed to estimate more reliably the prevalence of CE and therefore provide data to prioritize public health interventions in resource-limited settings.
HERACLES is a translational collaborative project funded by the European Commission aiming to break the vicious circle in which CE is maintained by identifying the affected population in Eastern Europe using extended US surveys and creating the European Register of CE.
US surveys were carried out in 2014 and 2015 in 3 partner countries, Bulgaria, Romania and Turkey, where 24,522 people were examined. Informed consent was obtained from participants, an epidemiological questionnaire was distributed, and blood samples were collected for serology and proteomic studies. Suspected cases were examined independently by 2 clinicians and patients were assigned to treatment according to WHO-IWGE Expert Consensus. Probable and confirmed cases were considered for prevalence calculations, resulting in the order of 1% of the population in rural areas. These results will support governments, European Commission, related European agencies (ECDC, EFSA), international organizations (WHO), and the Global Burden of Disease study to harmonize data collection, monitoring and reporting of CE.
HERACLES is funded by the European Community's FP7, grant agreement 602051.

THE EUROPEAN REGISTER OF CYSTIC ECHINOCOCCOSIS, ERCE

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Cystic Echinococcosis (CE) is a zoonotic parasitic disease endemic in many parts of the world, among which Southern and Eastern European countries, but its true burden is unknown due to lack of efficient and specific reporting systems. Neglect of CE hampers the collection of good quality data to support evidence-based diagnostic and therapeutic strategies, resulting in suboptimal case management and allocation of resources. In an attempt to improve this situation, the European Register of Cystic Echinococcosis (ERCE), was launched in October 2014 in the context of the HERACLES project. ERCE is a prospective, observational, multicentre register of patients with probable or confirmed CE enrolled in hospital or outpatient settings. The register is a single database where patient data, including demographic information, CE-related clinical data and biological samples collected from patients are recorded. Patients are assigned a unique ID code preventing data loss or duplication. ERCE is structured taking into account the peculiar features of CE and to address the evolution of cysts over time and as a response to treatment. As of March 2016, 28 centres in 13 countries have adhered to ERCE, with 835 patients recorded by 24 centres, while 6 centres in 6 countries expressed interest or are in the process of affiliation. ERCE responds to a long-standing need for a CE register with online data entry, and recorded data already largely outnumber the total of national cases reported by most European endemic countries. This confirms the need for a better reporting system of CE at the European level. ERCE will help collect data on a stage-specific approach to treatment, rate of adverse reactions, relapse rate, and costs of CE infection. We expect that ERCE will enable governments, the European Commission and related European agencies such as ECDC, to harmonize data collection, monitoring and reporting of CE.

TRICHINELLA SPIRALIS, A NEW ALIEN PARASITE IN ITALY, AND THE INCREASED RISK OF INFECTION FOR DOMESTIC AND WILD SWINE

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The circulation of nematodes of the genus *Trichinella* in Italy is known since 1887, when these parasites were discovered in the muscles of a woman at the autopsy in Camerino (Marche region). At that time, the parasite was identified as *Trichina spiralis* since only this species was known. On the basis of the present knowledge, we can suspect that the woman could be infected by *Trichinella britovi*. Between 1933 and 1946, trichinellosis outbreaks caused by the consumption of *Trichinella spiralis*-infected pigs occurred in Sicily. From 1968 to 2016, the identification at the species level of *Trichinella* spp. larvae collected from muscles of 351 wild and domestic animals of Italy, shows that 342 (97.4%) were *T. britovi*, 8 (2.3%) *T. pseudospiralis*, and 1 (0.3%) *T. spiralis*. The only one *T. spiralis* isolate originated from a fox (*Vulpes vulpes*), which had been shot at Jaffereau (Bardonecchia, Turin) at the border between Italy and France, in 1991. In January 2016, *Trichinella* sp. vital larvae (>100 larvae/g) were detected in muscles of a 2-year-old male fox shot in the Travo municipality (333 m asl), Val Trebbia, Piacenza province, Northern Italy. Larvae were identified as *T. spiralis*. The discovery of *T. spiralis* is a serious concern for the risk of introduction of this zoonotic pathogen among free-ranging and backyard pigs and in the wild boar population and, consequently, for humans. This species shows a higher larval burden and a longer survival time than that of the other *Trichinella* species in domestic and wild swine. This nematode species may have been introduced in the Emilia Romagna region from Eastern Europe by hunters, by a hunting dog, by imported animals (horses), or by immigrants, who illegally carried out infected meat in their personal baggage. However, other introduction ways cannot be excluded.

HUNTED WILD BOAR *(SUS SCROFA)* MAIN SOURCE OF TRICHINELLOSIS IN ITALY IN THE LAST 15 YEARS

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The history of human trichinellosis in Italy was characterized by old cases with two deaths, which were documented for the consumption of pig meat (#22) or unknown source (#2) from 1887 to 1930. Then, 209 infections with 22 deaths occurred for the consumption of pig meat infected by *Trichinella spiralis* in Sicily from 1933 to 1946. After the World War II to 2000, horse meat was the main source of trichinellosis (#1,031). In the last 15 years, there was a continuous sequence of trichinellosis outbreaks (#86 cases) caused by the consumption of raw sausages made with meat of hunted wild boar in Abruzzo, Basilicata, Liguria, Piedmont, and Tuscany regions. In December, 2015, a hunting team of the Mattinata municipality hunted two wild boars in the mountainous promontory of Gargano (Apulia region, southern Italy). The animal carcasses escaped any veterinary control. In January 2016, three household members with clinical signs and symptoms of trichinellosis, were hospitalized. The serological diagnosis confirmed the suspected diagnosis Two other person belonging to another family were positive to serology, one showing also clinical signs and symptoms of trichinellosis. *Trichinella* larvae (15-17 larvae/g) isolated by artificial digestion from two home-made salami, were identified as *Trichinella britovi*. Patients were successfully treated with mebendazole and promptly recovered. This is the second trichinellosis outbreak, which occurred in Mattinata. The first outbreak, which involved 8 persons from two households, occurred for the consumption of raw pork from a free-ranging pig clandestinely slaughtered because of furtive origin in 1968. The occurrence almost yearly of trichinellosis outbreaks caused by the consumption of uncontrolled meat from hunted wild boar is caused by the increased wild boar population. There is the need to educate hunters and their relatives on the risk to acquire this zoonosis if the game is not controlled by the veterinary services.

FACTORS ASSOCIATED WITH *TOXOPLASMA GONDII* INFECTION IN CONFINED FARROW-TO-FINISH PIG HERDS IN WESTERN FRANCE: AN EXPLANATORY STUDY IN 60 HERDS

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Pigs were among the first domesticated animals worldwide and pork production one of the main branches of meat industry. With different eating habits and cooking methods nowadays it is consumer's choice of how long and how intensively the meat will be treated before consumption, therefore increasing the risk of pathogen survival in the food. *Toxoplasma gondii* is an intracellular parasite from the phylum Apicomplexa, characterized by global distribution and a rather complex life cycle with Felids being the definitive hosts and practically all warm blooded animals including humans and pigs the intermediate ones. We assessed previously the prevalence of *T. gondii* in pig meat produced in France and determine the risk factors of pork contamination. The adjusted seroprevalence in pigs from intensive farms was 3.0%, highest in sows (13.4%), in 2.9% fattening pigs, and 2.6% in piglets. Outdoor farm seroprevalence in fattening pigs was 6.3%. The aim of the present study was to i) investigate the seropositivity of *T. gondii* in intensive pig farms from western-France; ii) identify the risk factors associated with infection. Stratified data and sera collection on 60 intensive farms from 3595 suckling-, weaned- piglets and fattening pigs. Questionnaire was used to obtain information about three classes of risk factors: i) breeding characteristics, ii) farm management, iii) biosecurity. The modified agglutination test (MAT) was used for detection of anti *T. gondii* antibodies in pig sera, starting from 1/6 dilution. The individual-level seropositivity was 6.9% confirmed previous data, but the proportion of herds with at least one positive pig was 100%. The average within farm prevalence was 7.0%. Multivariate mixed logistic model showed an increased seropositivity risk in weaned compared to suckling piglets, and a decreasing infection intensity in mid-sized and large farms. Facilities with a Danish entry system, that clearly separates buildings from farm environment, had a protective effect on *T. gondii* seropositivity as well. The observed *T. gondii* seropositivity provides further evidence that even in confined conditions of pig breeding, infection is widespread. The highest risk of acquiring *T. gondii* is at the end of weaning period. Smaller farms demonstrate higher *T. gondii* seroprevalence in confined conditions. This study also shows that Danish entry facilities provide effective protection against *T. gondii*.

DISEASE BURDEN AND CLINICAL IMPLICATIONS OF CHAGAS DISEASE IN TEXAS, USA: A CROSS-SECTIONAL ANALYSIS OF *TRYPANOSOMA CRUZI* SEROPREVALENCE AMONG VECTORS, MAMMALIAN RESERVOIRS, AND HUMAN RESIDENTS

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Chagas disease (*Trypanosoma cruzi* infection) is a considerable public health concern with over 7 million people currently infected. In fact, Chagas disease is the leading cause of non-ischemic dilated cardiomyopathy in Latin America, with 35% of patients developing sudden cardiac death as the first presenting symptom of infection. Disease acquisition is multifactorial with over 130 known Triatomine vector species, over 100 known mammalian reservoirs, and other known important non-vectorial transmission routes. Transmission cycles are established in either sylvatic or domestic settings, with peri-domestic vectors and mammalian reservoirs serving as bridge species. The southern United States has compounding factors that could contribute to *T. cruzi* transmission; however, epidemiologic studies are lacking. The aim of our current study was to ascertain the prevalence of *T. cruzi* in vectors and three different mammalian species (coyotes, stray domestic dogs, and humans) to understand the burden of Chagas disease among sylvatic, peridomestic, and domestic cycles in southern Texas. To further elucidate the origin and significance of disease among Texas residents, we performed a seroepidemiologic assessment of patients that presented for clinical management of known non-ischemic cardiomyopathy at one of three medical facilities.
To determine prevalence of infection, we performed PCR and ELISA based assays on vectors and mammals, respectively. Specifically, we extracted DNA from the posterior third of the vectors for detection of *T. cruzi* DNA and speciated the insects by mitochondrial 16S sequencing. Retrospective testing of banked sera samples was performed using two immunochromatographic assays (Chembio Stat-pak and DPP). A serum sample was considered positive if it reacted to both assays. Confirmation testing was performed by the US Centers for Disease Control for all human samples. Epidemiologic evaluations of transmission risk for positive patients included a validated questionnaire assessing residential, travel, occupational, and medical histories.
We identified a formidable disease burden attributable to Chagas disease in southern Texas. Over half of the vectors (56.5%) collected from peridomestic locations in the region tested positive for *T. cruzi* DNA, indicating likely infection. Similarly, mammalian seroprevalence was high, with rates ranging between 8% of coyotes, 3.8% of shelter-dogs, to 0.36% of human residents, further confirming risk of transmission in the region. From the large cohort of idiopathic cardiomyopathy patients, we identified a considerable proportion (up to 12%) of previously undetected *T. cruzi* infection. Transmission risks varied by patient population characteristics, with both imported and locally acquired cases identified in southeastern Texas.
Our findings have important clinical implications for veterinarians and cardiologists practicing throughout the United States, as well as clinicians in other non-endemic countries providing healthcare to Latin American immigrants. Furthermore, these results contribute to the growing body of evidence for autochthonous Chagas disease transmission in Texas. Considering a state population over 26 million, and up to 30% of *T. cruzi* infected individuals develop severe cardiac disease, it is imperative that we identify high-risk groups for surveillance and treatment purposes.

SURVEILLANCE OF MALARIA IMPORTED CASES IN ITALY, 2011-2014

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Imported malaria is a mandatory reportable disease in Italy, its surveillance being essential due to the presence and abundance of the former malaria vectors. This study provides epidemiologic profile and trends of recent confirmed malaria cases, identifying the main affected categories of travelers with the aim of implementing information targeted to at-risk people.
Cases diagnosed in 2011-2014 were analysed using a dedicated database. Demographic and epidemiological data were obtained from the surveillance reports sent by Local Health Authorities to the National Malaria Surveillance System (Ministero della Salute and Istituto Superiore di Sanità).
2,746 malaria cases were notified, with uneven distribution among Regions which strongly suggests underreporting from some of them. The majority of cases were males (78%) belonging to the age group 25-44 yrs (46%). The predominant species was *P. falciparum* (82%), followed by *P. vivax* (12%), *P. ovale* (4%), *P. malariae* (2%) and mixed infections (0.4%). All but four cases were imported: a probable autochthonous introduced case (*P. vivax*, 2011); a blood transfusion case (*P. malariae*, 2013); two cryptic cases (*P. falciparum* and *P. malariae*, 2014). Most of the infections were acquired in Africa (92%), followed by Asia (7%), South-Central America (0.6%) and Papua New Guinea (0.1%). Twenty% of cases occurred among Italians and the commonest reason for travel was work (38%), followed by tourism (17%) and voluntary activities (17%). Foreigners accounted for 80 % of cases, of which 80% were visiting friends and relatives (VFRs) and 12% refugees. Three deaths were reported. Only 16% of cases declared to have taken chemoprophylaxis. Preliminary analysis of the 2015 cases showed similar features as the study period.
Our study showed a stable trend of malaria cases in 2011-2015, confirming the trend of the previous five years. Underreporting remains challenging, as it may affect efforts for a reliable malaria surveillance. We recommend specific information campaign targeted to travelers, in particular VFRs, about the need for effective malaria prevention strategies.

OCCURRENCE AND MOLECULAR IDENTIFICATION OF FREE-LIVING AMOEBAE IN ITALIAN THERMAL WATERS

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Free-living amoebae (FLA) are ubiquitous protozoan commonly founded in natural and artificial aquatic environments. Some species, e.g. *Naegleria fowleri*, *N. australiensis, N. italica*, *Acanthamoeba* spp., *Balamuthia mandrillaris* and *Vermamoeba vermiformis* can be potentially pathogenic in human and animals. The purpose of this study was to isolate and identify FLA at molecular level in thermal waters, in order to increase their ecology and epidemiology knowledge.
Water samples were quarterly collected from April 2015 to January 2016, in two thermal hot springs (site A and B) in Latium Region. Eight sampling points were chosen for the whole area. Two-liter of water were collected for each point and processed within 48h. Water temperature and pH were recorded. Each sample was centrifuged and cultured onto NNA-media containing *Escherichia coli*. All agar plates were incubated at 37°C and 45°C and observed daily for amoebic growth. Molecular characterization was obtained through DNA extraction and amplification using three sets of primers. To determine species/genotype a phenetic analysis using MEGA 6 was executed.
Currently a total of 30 water samples (site A=20; site B=10) were collected and analyzed. Eighteen out of 30 samples were positive for growth of FLA, 14/20 for the site A, and 4/10 for the site B. Sequence analysis of positive isolates allowed to identify: *Vermamoeba vermiformis*, *Echinamoeba* sp. and *Platyamoeba* sp. (Amebozoa) and *Fumarolamoeba ceborucoi*, *Naegleria australiensis, N. italica* and *N. lovaniensis* (Excavata).
The present ongoing study is the first molecular based investigation providing an overview of FLA community composition in Italian thermal waters. Interestingly, among the species identified, *V. vermiformis* has been associated to human keratitis, *N. australiensis* and *N. italica* can cause disease in animals. The presence of potentially pathogenic amoebae in habitats related to human activities supports the relevance of FLA as potential public health concern.

FOOD AND PARASITES

ANISAKIS SPP. IN READY-TO-EAT FISH PRODUCTS

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Ready-to-eat fish products are more and more appreciated. Most of them are made of species frequently infected by *Anisakis* spp. (anchovies, sardines, mackerel and herrings). *Anisakis* spp. larvae are responsible of zoonotic infections and allergic symptoms: human cases have increased worldwide over the last 30 years. Fishery products intended to be consumed raw, marinated, salted or differently treated must undergo freezing to kill viable parasites (Reg. (EU) No 1276/2011). However, anisakids antigens are thermoresistant and may still be responsible for hypersensitivity reactions. This study aimed to assess the presence of *Anisakis* spp. larvae in ready-to-eat fish products. 44 products (average weight 190 gr, 80-650 gr) made of fillets of European anchovies (*Engraulis encralicolus*)(n=20), herrings (*Clupea harengus*)(n=10), mackerel (*Scomber scombrus*)(n=8) and sardines (*Sarda sarda*)(n=6), were purchased in supermarkets. Labeling information was recorded. Products were visually examined and, after counting the number of specimens, submitted to chloro-peptic digestion by Trichineasy® (CTSV srl, Brescia). Nematodes were identified to genus level and stored in 70% alcohol. Overall, 507 *Anisakis* spp. larvae were found in 22 products (50%). In particular, 466 larvae were found in 16 anchovies products (mean abundance, MA=1,01; mean intensity, MI=1,29; mean nr. parasites/g=0,1) and 41 larvae in 6 herrings products (MA=2,16; MI=5,86; nr. parasites/g=0,02). Products made of sardines and mackerel were negative. Molecular specific identification is in progress.
The high number of *Anisakis* spp. larvae in anchovies and herrings found in this preliminary study suggests the need to implement control during processing and to inform consumers about the sensitization risk associated with these products. In fact, although dose-response in allergic reactions to *Anisakis* spp. depends on individual sensitivity, it may even occur after ingesting a single larva.

LARVAE OF *ANISAKIS* SPP. IN FISHERY PRODUCT FROM APULIA REGION

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A survey was conducted over a one-year period with the aim of collecting systematic data on the presence of *Anisakidae* larvae in fish species caught or farmed off the coast of Apulia region. Overall 363 lots of teleosts and cephalopods were sampled: 1144 subjects belonging to 19 species of teleosts, 340 belonging to 6 species of cephalopods and 128 subjects belonging to farmed seabram and seabass. On arrival at laboratory each sample was identified to the species level, weighed and measured. Subsequently, coelomatic cavity and intestines of each item were inspected to evaluate the presence of visible *Anisakidae* larvae. 6150 larvae were collected and morphologically identified as belonging to the genera *Anisakis* (type I and II) (97.2%) and *Hysterothylacium* (2.8%). 171 larvae were sent to IZS of Sicily for species identification performed by RFLP-PCR molecular analysis of the nuclear ITS region (ITS1, ITS2 and 5.8 S subunit). *Anisakis* spp. larvae belonged mainly to the species *A. pegreffii* (98.8%) and to *A.physeteris* (1.2%).The data were processed to calculate Prevalence (P), mean Intensity (mI) and mean Abundance (mA) of each fish species and of overall species. Nematode larvae have never been detected in farmed fishes or in cephalopods examined, while 313 subjects of wild teleosts over 1144 were parasitised by *Anisakis* spp. (P=0.27 mI=19.1 mA=5.2). In line with literature *Scomber japonicus* was the species with the highest prevalence (0.67) and mean Intensity (0.55). As regards anchovies, most frequently involved in human anisakiasis in the countries of southern Europe, including Italy, the obtained data indicate a clear predominance of larvae of *Hysterothylacium* spp., alone or in co-infection with *Anisakis* spp., in conflict with those expected (overall P=0.31 mI=2.65 mA=0.84; *Anisakis* spp. P=0.20 mI=1.59 mA=0.32). Also in red mullet both *Hysterothylacium* spp. and *Anisakis* spp. infection was much lower than expected (P=0.03 and P=0.01 respectively).

A VALIDATION PLAN FOR A COMMERCIAL KIT AIMED TO THE DETECTION OF PATHOGENIC ANISAKIDS IN FISH PRODUCTS

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Anisakids are parasitic nematodes responsible for a zoonosis caused by the ingestion of fishes infected with larvae belonging to the genera *Anisakis* and *Pseudoterranova*. Rarely *Contracaecum* was found in association with gastric/intestinal illness while *Hysterothylacium* is considered to be not pathogenic. Although Real Time PCR assays have been recently used with the aim to detect and quantify parasites in food products, the methods applied did not undergo through extensive validation processes, a feature highly desirable or mandatory in the case of testing laboratories accredited for the ISO EN 17025:2005.
Here, a comprehensive study has been undertaken to validate a commercial kit based on multiplex real time PCR for the qualitative detection of *Anisakis* and *Pseudoterranova*.
Inclusivity/exclusivity trials were carried out on species of the genera *Anisakis, Pseudoterranova, Contracaecum, Hysterothylacium* and *Ascaris*. The assay gave positive amplification for several *Anisakis* and *Pseudoterranova* species, while providing no signal for the remaining genera. Each sample was correctly assigned either to *Anisakis* or *Pseudoterranova*, thus indicating that no cross-reaction occurred. The LOD was determined using two independent standard curves as the minimum amount of genomic units where the reactions gave positive amplification in 10 replications. Robustness was assayed by using two different thermocyclers in three distinct laboratories.
The establishment of a validation dossier will permit the use of the commercial kit for the detection of *Anisakis* and *Pseudoterranova* DNA in fish products intended for human consumption by public or private laboratories, following the requirements regarding the quality assurance processes described in the ISO EN 17025:2005.

IGE FROM SERA OF ITALIAN SUBJECTS PRESUMABLY SENSITIZED BY *ANISAKIS PEGREFFII* REACT WITH PROTEINS FROM *A. SIMPLEX*, *CONTRACOECUM OSCULATUM* AND *PSEUDOTERRANOVA* SP.

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Gastric anisakiasis caused by the zoonotic parasite *Anisakis pegreffii* is increasing in Italy. This parasite generates allergic reactions from urticaria to potentially life-threatening anaphylaxis. Epidemiological studies show that the induction of anti-*Anisakis* IgE requires infection by the parasite, since these antibodies occur in populations at risk, i.e. individuals regularly consuming raw or undercooked sea fish. The objective of the present study was to determine if parasite antigens from nematodes belonging to the Anisakidae family, different from those of *A. pegreffii*, are specifically recognized by IgE present in sera of Italian subjects. Twenty-one sera were collected from Italian raw sea fish consumers; of these, 18 (86%) originated from individuals who had shown allergic manifestations after fish consumption, and 3 (14%) from asymptomatic persons. Serum samples with detectable levels of IgE to *Ani s 3* but not to *Ani s 1*, were used as controls. All serum samples were further tested by ImmunoCap to determine the specific IgE levels. Each serum sample was tested for specific IgE by a western blot with recombinant proteins from *A. simplex* (*Ani s 4*, *Cystatin*, and *Ani s 5*, SXP/RAL-2), and with crude worm extract (CWE) of other anisakid species (*A. pegreffii*, *Contracoecum osculatum* and *Pseudoterranova* sp.). IgE present in sera of Italian individuals with allergic reactions and/or infection, as determined by ImmunoCAP, and thus presumably sensitized to *A. pegreffii*, strongly react with one (r- *Ani s* 1) or more recombinant proteins from *A. simplex* and with two or more CWE from other anisakid nematodes. Since it is believed that the allergic reactions observed in patients with anisakiasis are mediated by *A. simplex* allergen specific IgE, the presence of IgE reactive proteins shared among other anisakid species, e.g. *A. pegreffii*, *Pseudoterranova* sp., and *C. osculatum*, could increase the risk to develop allergic manifestations in patients sensitized or exposed to other species.

DETECTION OF PROTOZOAN PARASITES IN READY-TO-EAT PACKAGED SALADS

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Protozoan parasites commonly infect humans and animals worldwide, and *Giardia*, *Cryptosporidium Cyclospora* and *Toxoplasma* have been found in irrigation water, soil and vegetables. These products are covered by EU law only with regard to bacterial pathogens. The aim of this study was to investigate possible contamination by protozoans in 'ready to eat' (RTE) salads on sale in Italy. From March 2015 to February 2016, 486 packages of RTE salads produced by industrial brands and by local companies were purchased from supermarkets and grocery shops, respectively. Nine individual packages (100g each) were collected per sampling month (n=9), pooled and processed. After concentration, the pellets were subjected to microscopy, immunofluorescence, RealTime-PCR and sequencing. A total of 54 pooled samples were tested i.e., 243 industrial brand packages and 243 local brand packages. Four (7.4%, 95% CI 0.4-14.4) pools tested positive to *Blastocystis hominis* and 1 (1.8%, 95% C.I. 0-5.4) to *Dientamoeba fragilis*, by microscopy; whereas *Giardia duodenalis*, *Cyclospora cayetanensis* and *Toxoplasma gondii* were detected in 6 (11.5%, 95% CI 2.8-20.2), 7 (13.5%, 95% CI 4.1-22.7) 2 (3.8%, 95% CI 0-9.1) samples, respectively, but only molecularly. Mixed contamination was found in 4 (7.7%, 95% CI 0.5-14.9) samples. The mean number of protozoa contamination was 0.4 protozoa species/pool in industrial brands and 0.3 in local brands. This work represents the first large-scale study on packaged salads in Europe. These results show that 'ready to eat' salads are contaminated by one or more emerging protozoan pathogens and that the sanitation process (from harvesting to packaging) cannot guarantee a product free from protozoans of fecal origin. These results indicate the need for additional research on the sources of contamination of these foods and determination of parasite viability and infectivity, and for improvement of Hazard Analysis and Critical Control Points efficiency to reduce possible risks for human health.

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ZOONOTIC PROTOZOANS FROM FARMED AND MARKETED *MYTILUS* SPP. IN SARDINIA (ITALY)

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Bivalve mollusks are an important food resource worldwide, including Italy, and Sardinia Region is among the leading producers of *Mytilus* spp. EU legislation on shellfish products only regards bacterial pathogens, but emerging protozoan parasites are widespread and create health problems, mostly in young and immune-compromised persons. The aim of this study was to investigate the prevalence and seasonality of *Giardia duodenalis, Cryptosporidium* spp. and *Toxoplasma gondii* in two mussel species both farmed along the Sardinian coast, and available on the market.
From September 2013 to July 2014, 1620 shellfish specimens i.e. 1440 *Mytilus galloprovincialis* and 180 *Mytilus edulis,* were collected from 17 farms and 118 retail outlets in 8 areas of Sardinia and then pooled. Digestive glands and gills were removed from all specimens and a total of 135 pool samples were tested. After DNA extraction, the samples were tested by Real Time-PCR assay to detect *G. duodenalis (ssRNA), Cryptosporidium* spp. (*COWP*) and *T. gondii* (*B1*) and sequenced.
Sixty-two of the 135 (45.9%, 95% CI=37.5-54.3%) mussel pools tested positive for one or more investigated pathogens. Both *Mytilus* spp. and samples from all investigated areas harboured pathogens. Mussels were statistically more contaminated by *Cryptosporidium parvum* followed by *G. duodenalis* Assemblage A and *T. gondii*, and *M. galloprovincialis* was more contaminated than *M. edulis* ($p<0.01$). Contamination was more likely in farmed mussels ($p<0.05$) and those collected in spring ($p<0.01$). No statistically significant differences were registered between the anatomical sites. This study confirms that mussels farmed and marketed in Italy are contaminated by zoonotic protozoans. This is the first report of *T. gondii* in both Mediterranean mussel *M. galloprovincialis* and Blue mussel *M. edulis*. These results indicate a considerable threat to human health, particularly if mussels are eaten raw. Thus, modification/revision of the present EU law can no longer be delayed.

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EFFICIENCY OF THE Q3 LAB-ON-CHIP PLATFORM IN DETECTING PROTOZOAN PATHOGENS IN BIVALVE MOLLUSKS

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Cryptosporidium, *Giardia* and *Toxoplasma* have been recorded worldwide in economically important edible shellfish, and are thus likely to present a significant public health risk. The development of an innovative, user-friendly diagnostic tool is required to improve food safety control. The Q3 system, a miniaturized platform which integrates amplification and detection process for realtime-PCR on a Lab-on-chip, was developed by STMicroelectronics for the detection of zoonotic protozoans in three shellfish species according to realtime-PCR protocols previously set up in our lab. Q3's efficiency and applicability were investigated and compared with results obtained by standard realtime-PCR.
Tanks of saltwater containing acclimated pathogen-free *Mytilus galloprovincialis*, *Tapes semidecussatus* and *Ostrea edulis* specimens were spiked with purified *Cryptosporidium, Giardia* and *Toxoplasma* cysts/oocysts at different concentrations (i.e., 10^3, 10^4 and 10^5). Untreated control shellfish were included in each test. At 24h and 72h post-contamination (p.c.), thirty specimens of each shellfish species were collected from each group. Haemolymph, gills and gastric glands were removed and stored in pools at -20°C. After DNA extraction, all samples were tested by standard realtime-PCR and Q3, and we evaluated the sensitivity, specificity, predictive values, repeatability and the concordance between standard realtime-PCR and Q3-system. A significant concordance between standard realtime-PCR and Q3-system CT (Threshold Cycle) values was registered for all the shellfish species at the highest parasite load (i.e. 10^5) at 72h p.c. for *Toxoplasma* and *Giardia* in *M.galloprovincialis,* for *Toxoplasma* and *Giardia* in *O.edulis* and for *Cryptosporidium* in *T.semidecussatus* ($P<0.05$). No significant differences were registered between the anatomical sites. Q3 demonstrated an ability to detect all investigated pathogens, that was similar to standard realtime-PCR, and a very high level of ability to detect *Toxoplasma* in *M.galloprovincialis* and *Toxoplasma* and *Giardia* in *O.edulis*. Currently, the Q3-system can be used efficiently to detect *Toxoplasma* from whole mussels and oysters, and to a lesser extent also from clams.

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RISK ASSESSMENT OF *TOXOPLASMA GONDII* INFECTION THROUGH THE CONSUMPTION OF "PROSCIUTTO DI PARMA" DOP: PRELIMINARY DATA

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Toxoplasma gondii is considered one of the most common parasitic infections in the world due to its impressive range of hosts, widespread environmental contamination and the diverse means by which animals can be infected (EFSA, 2007). "Prosciutto di Parma" is a typical and popular Italian pork product known all over the world, highly valued for its flavor. *T. gondii* cysts in pork are persistent and they represent an important source of infection for human. However, little information is available concerning the effect of curing and salting on *T. gondii* cysts in ham. Furthermore, the scientific community and in particular the public opinion have diverse views on the possibility of transmission that could be observed through the consumption of cured ham.
The aim of this study was to evaluate the survival and viability of tissue cysts of *T. gondii* in cured hams according to the procedural guideline of the "Prosciutto di Parma" consortium. Twelve pigs were infected per *os* with 1000 sporulated *T. gondii* oocysts and slaughtered 4 months later. Twelve thighs were cured and 12 were immediately digested according to Dubey (Vet Parasitol. 15;74:75-7, 1998). To verify the infection, serology, meat juice serology, PCR from muscle tissue, cell cultures, and bioassay in mice were carried out. After 12 months of ageing, hams were digested and processed by cell culture, RT-PCR and bioassay in mice to evaluate the vitality and the presence of parasite DNA.
All pigs became infected. Preliminary data from the hams indicated the presence of *T. gondii* DNA, while the cell-cultures were negative. The bioassay in mice is still in progress. This study is the first in which the influence of processing of cured ham on the viability of *T. gondii* has been evaluated.

GENETIC CHARACTERIZATION OF *TOXOPLASMA GONDII* FROM INDUSTRIAL PIGS BRED IN THE "FOOD VALLEY" (ITALY)

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A significant association between human toxoplasmosis and the consumption of raw or undercooked meat or its products from pigs and other farm animals is registered worldwide. *Toxoplasma gondii* Type I, Type II, Type III, and atypical strains have been described. These lineages differ in their pathogenicity and prevalence in humans. Since there is little information on the genetic characterization of *T. gondii* in industrially-reared pigs, the aim of this study was to isolate and genotype *T. gondii* from pigs specifically bred for ham production.
Diaphragm and heart samples were collected from 103 pigs in 2 slaughterhouses in Abruzzo Region (Italy), giving a total of 206 samples. After DNA extraction, the samples were subjected to High Resolution Melting (HRM) assay coupled with next generation EvaGreen® Real Time PCR assay to detect *T. gondii* DNA using the B1 locus gene. Positive samples were sequenced for confirmation. Out of 103 animals, 14 (3.6%, 95% C.I.=7-20.2%) were positive to *T. gondii* with 12/206 (5.8%, 95% C.I.=2.6-9%) heart samples and 2/206 (1%, 95% C.I.=0-2.3%) diaphragm samples harboring the parasite DNA. According to HRM analysis coupled with melting curves and temperatures, Type I, Type II and Type III were detected in 14 samples: Type I was detected in two heart and two diaphragm samples, Type II in six and Type III in four heart samples. The heart was the most contaminated organ, with all three *T. gondii* types detected.
This is the biggest large-scale study in Italy on the genetic characterization of *T. gondii* in pigs. The use of a very innovative and specific molecular tool (HRM assay), has highlighted *T. gondii* lineages circulating in industrial pigs in Italy. Detection of the most pathogenic type (Type I) provides evidence that conditions in industrial breeding systems are still insufficient to guarantee '*Toxoplasma*-free pork', at least for pigs bred for ham production in the examined area.

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THERAPY AND DRUG RESISTANCE 1

EFFICACY OF SAROLANER AGAINST INFESTATIONS WITH *DEMODEX*, *OTODECTES* AND *SARCOPTES* MITES ON DOGS

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The efficacy of sarolaner (Simparica™) was investigated in dogs infested with *Demodex canis, Otodectes cynotis* or *Sarcoptes scabiei*. One laboratory study for each mite species as well as a European field study for *Sarcoptes scabiei* were conducted. In the laboratory studies, dogs were allocated to the control group (n=8) or treatment group (n=8; sarolaner at 2 mg/kg). In the sarolaner-treated dogs, *Demodex* mite counts were reduced by 97.1% and 99.8% at 14 and 29 days after the first dose, respectively, with no live mites detected thereafter. Weekly topical imidacloprid/moxidectin treatment (Advocate®; positive control group) resulted in 84.4% and 95.6% reduction at these two time points, respectively, with no *Demodex* mites detected from Day 74 onwards. Dogs in both the sarolaner and imidacloprid/moxidectin treatment groups showed improvement in the clinical signs of generalized demodicosis. In the *O. cynotis* study, sarolaner treatment resulted in 98.2% and 99.5% reduction in mite counts compared to placebo controls after a single dose and two monthly doses, respectively. In the laboratory study against *S. scabiei*, dogs received two monthly treatments with sarolaner or placebo (n=22/group) and skin scrapings were performed every 14 days. No mites were found on any sarolaner-treated dogs from 14 days after the first treatment onwards except for one dog that had a single mite on Day 44. In the *S. scabiei* field study, dogs were randomly allocated to receive two monthly treatments with oral sarolaner (2-4 mg/kg; n=53) or topical imidacloprid/moxidectin (n=26). No live mites were found in the skin scrapings in 88.7% and 100% of the dogs in the sarolaner-treated group and 84.6% and 96.0% in the imidacloprid/moxidectin group, on Days 30 and 60, respectively. The clinical signs of sarcoptic mange improved throughout the study. Sarolaner was found to be safe and efficacious in the treatment of mite infestations in dogs.

PROTEOMIC AND FUNCTIONAL ANALYSIS REVEAL MULTIPLE TARGETS OF THE ANTI-TUMORAL COMPOUND NBDHEX IN THE PROTOZOAN PARASITE *GIARDIA DUODENALIS*

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Giardiasis, caused by the protozoan parasite *Giardia duodenalis*, is an intestinal disease affecting almost one billion people worldwide. Infections may be asymptomatic or cause an acute and/or chronic diarrheal disease. Treatment of clinical giardiasis is highly recommended since prolonged/recurrent infections may cause cognitive and growth deficiencies in malnourished children or post-infectious long term consequences. Although the existence of effective antigiardial compounds, including nitroimidazoles and benzimidazoles, undesired side effects and cases of treatment failure occurred, posing concerns about future treatment of giardiasis. Recently, we demonstrated that NBDHEX, an anticancer agent, displays antigiardial activity, likely linked to its nitroreduction and ROS generation. Partially responsible for NBDHEX nitroreduction is the FAD-dependent glycerol-3-phosphate dehydrogenase that forms covalent adducts with the drug, thus inhibiting the enzymatic activity. To further clarify the mechanism of action of NDBHEX, a proteomic analysis was performed to define other binding partners of the drug in treated *G. duodenalis* trophozoites. Relying on the NBDHEX fluorescence, protein bands were isolated and, by ESI-LC/MS/MS mass spectrometry, a small, highly reproducible, subset of proteins forming covalent adducts with NBDHEX was identified. These include metabolic enzymes (e.g. thioredoxin reductase, gTrxR) and structural proteins (e.g. elongation factor 1γ, gEF1y, α-tubulin). NBDHEX-modified cysteines were univocally identified, being the catalytic cysteines of gTrxR irreversibly modified. NBDHEX effects on intracellular localization and protein profile of some targets were also studied, supporting a direct alteration of their role/function following drug exposure. In *E. coli*, recombinant His-tagged gTrxR and gEF1γ were covalently modified by NBDHEX in a similar manner. *In vitro*, both His-gTrxR and His-gEF1y bind to NBDHEX, but only TrxR was able to modify NBDHEX, thus resulting in adduct formation and impaired disulphide reductase activity. In conclusion, the characterization of NBDHEX targets will help the future design of more effective antigiardial compounds having NBDHEX itself as a leading compound.

RE-ORIENTATION OF THE HELMINTH CONTROL IN ADULT HORSES IN SWITZERLAND

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The epidemiological situation of strongyle infections in adult horses in Switzerland is characterized by a strong dominance of small strongyles (Cyathostominae) and an overall low level of egg shedding in the faeces. The prevailing strategy, usually comprising 3 to 4 annual treatments, considers neither husbandry conditions nor pasture management and hygiene measures. In the vast majority of the horse stables this approach is resulting in an over-use of anthelmintics. With respect to the increasing problem of anthelmintic resistance a re-orientation of the prevailing concept seemed to be mandatory. In 2011 a consensus has been agreed on between equine parasitologists and clinicians of the Vetsuisse Faculty in Zurich and Berne to focus on the concept of a selective control approach, based on individual faecal egg counts as the central element. Since then it is recommended that clinically healthy horses (≥ 4 y) are treated only when their strongyle egg count is equal to or higher than 200 eggs per gram of faeces (epg), or in the case of detection of *Parascaris* sp., *Strongylus* sp. or cestode infection. A yearly analysis of the strongyle population based on coprocultures, the regular control of drug efficacy with the faecal egg count reduction test and quarantine measures for newly incoming horses are mandatory components of the concept. A constantly increasing number of equine practitioners have adopted this strategy and a growing acceptance by the horse owners is clearly noticed. Data from a large monitoring program, including approx. 1000 horses indicate that only 7.4% of the McMaster analyses resulted in an anthelmintic treatment. From 5557 faecal samples, investigated during 2013-2015, a remarkably low mean strongyle egg count of 57 epg was calculated. For horses that did not receive any anthelmintic during the current season, a ‘safety’ treatment is recommended at the end of the grazing period.

MALARIA PHARMACOGENETICS IN BOTSWANA: INTERETHNIC DIFFERENCES AND PUBLIC HEALTH

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Human cytochrome P450 *2C8* and *2B6* are two highly polymorphic genes and show variation according to ethnicity. They are very important drug-metabolism genes in tropical medicine since the respective liver enzymes are involved in the metabolism of antimalarial drugs chloroquine, amodiaquine (CYP2C8) and artemisinin derivatives (CYP2B6). The *CYP2C8*2*, *CYP2B6*6*, *CYP2B6*16*, *CYP2B6*18* alleles are slow drug metabolism alleles and show high frequency in Black populations. The objective of this study was to assess their prevalence in Botswana among the San (or Bushmen) and the Bantu ethnic groups. For that purpose we recruited 544 children of the two ethnicities in three districts of Botswana from primary schools, collected blood samples, extracted DNA and genotyped them through PCR-based restriction fragment length polymorphism analysis. The results demonstrated that in the San the prevalence of the *CYP2C8*2* allele is significantly higher than among the Bantu-related ethnic groups, as well as is lower the prevalence of the *CYP2B6* alleles. These findings support the evidence of a different genetic background of the San with respect to Bantu-related populations, and highlight a possible higher risk of variable drug clearance and/or activation of pro-drugs CYP2C8 and CYP2B6-mediated among the San group to respect the Bantu of Botswana.

CHARACTERIZATION OF FUNGAL SYMBIOSIS IN *PHLEBOTOMUS PERNICIOSUS* AND EVALUATION OF ITS POSSIBLE IMPLICATIONS FOR THE CONTROL OF LEISHMANIASES

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The control of vector-borne diseases represents one of the greatest global public health challenges of the 21st century. In this context, biological control methods are an alternative to the use of chemicals, and the use of microorganisms is now well established in biocontrol. While arthropod-associated bacteria are the focus of several research programs aimed at developing strategies to control vector-borne diseases, such as malaria, dengue, and trypanosomiasis, arthropod-associated yeasts and their killer toxins have not yet been deeply investigated.
In this work, I studied the yeast community associated with the sand fly *Phlebotomus perniciosus*, the main vector of leishmaniasis in the western Mediterranean area, with the aim of investigating their potential to interfere with *Leishmania* development in insects. To reach the goal I associated culture-based methodology with culture independent methods: I performed yeast isolation and identification, 454 Pyrosequencing, PCR screening and whole mount FISH with specific probes to localize the yeast species. I focused my attention to the yeast *Wickerhamomyces anomalus,* isolated from both sexes of *P. perniciosus;* this yeast was phylogenetically characterized and tested against sensitive yeast strains, demonstrating its killer phenotype. Finally, in order to explore the possibility that this yeast could exert inhibitory/killing activity against pathogens, I tested the *in vitro* activity of *W. anomalus* strains against *Leishmania infantum* and *L. tropica.* This study offers the basis for the development of an environment-friendly and safe for human health method for vector-borne disease control that can be included in the integrated approach for the control of leishmaniases, a worldwide re-emerging public health problem.

MOLECULAR BIOLOGY AND PHYLOGENY IN PARASITOLOGY 1

THE PHYLOGENY OF ONCHOCERCIDAE (FILARIAL NEMATODE): A FOCUS ON THE GENUS *ONCHOCERCA*

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Onchocercidae is a family of filarial nematodes encompassing several species of medical or veterinary importance (e.g. onchocerciasis, lymphatic filariasis, loiasis, dirofilariasis). Because of their predilection for host tissue sites, filariae are difficult to collect and their diversity is far from being well-investigated. Given the lack of fossilized material, it is not possible to formulate a comprehensive evolutionary hypothesis for this group based exclusively on morphological studies. Molecular analyses on the other hand have so far been based on a limited number of filarial species only or have been based mainly on 12SrDNA and coxI gene sequences. While being suitable for species differentiation, these mitochondrial genes cannot be used to infer phylogenetic hypotheses at higher taxonomic levels. Thus a consistent evolutionary framework for this family is still not in place.
In the present study, a large number of species, representing seven of the eight onchocercid subfamilies, were sampled and sequences of seven gene loci (nuclear and mitochondrial) analyzed, resulting in the hitherto largest molecular phylogenetic investigation into this family.
Using this multi-gene dataset analysis, five major clades within the family are defined, including i) a group of ancestrally-derived subfamilies, ii) Setariinae as a sister group to all remaining subfamilies and iii) one large clade comprising genera of the Dirofilariinae, Onchocercinae and Splendidofilariinae. Finally an overview of the genus *Onchocerca* is also discussed.

ASSESSMENT OF PARAMYOSIN AS A DIAGNOSTIC MARKER FOR THE DETECTION OF *ONCHOCERCA LUPI*, A NEGLECTED ZOONOTIC NEMATODE

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Paramyosin, an invertebrate-specific protein, has been widely studied as a potential vaccine candidate against parasites. Antibodies against this molecule form the basis of a commercial kit for the serological detection of the human filarial nematode *Wuchereria bancrofti*. The anti-paramyosin antibodies also recognize antigens from *Dirofilaria immitis* and other filarial nematodes. The aim of this study was the isolation and characterisation of paramyosin from *Onchocerca lupi*, an increasingly important filarioid nematode that causes ocular disease in dogs, cats and also humans. Current methods to detect microfilariae of *O. lupi* include skin snips, whereas adults are removed surgically from ocular nodules. However, the procedures above are invasive, time consuming and laborious. Therefore, a more rapid, less invasive technique is required to detect the parasite. Development of a serological diagnostic test, based on antibodies to *O. lupi* paramyosin, would assist in the diagnosis of infestation and in the analysis of the prevalence and distribution of this zoonotic parasite. A full-length paramyosin was expressed as a glutathione S-transferase (GST) fusion protein in *Escherichia coli.* Bioinformatic analysis of the predicted protein was used to determine the properties of the protein, map potential antigenic epitopes and then design three smaller proteins. All expressed proteins were tested by both Western blot and ELISA for reactivity to serum from uninfested dogs and those infested with *O. lupi, D. immitis, Dirofilaria repens*, *Acanthocheilonema reconditum* and *Cercopithifilaria bainae*. The predicted full-length protein is 101 kDa, has a conserved nematode-specific proton donor site, no signal peptide and no transmembrane spanning domains. Epitope mapping suggested the presence of ~1800 potential antigenic sites. Data suggest that the paramyosin molecule is a potential candidate for the development and testing of a serological diagnostic assay to detect the presence of *O. lupi*.

THE *ANISAKIS* TRANSCRIPTOME – IDENTIFICATION OF NOVEL PUTATIVE ALLERGENS USING RNA-SEQ AND BIOINFORMATICS TECHNOLOGIES

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Food-borne nematodes of the genus *Anisakis* are responsible for a wide range of illnesses (= anisakiasis), from self-limiting gastrointestinal forms to severe systemic allergic reactions, which are often misdiagnosed and under-reported. In order to enhance and refine current diagnostic tools for anisakiasis, knowledge of the whole spectrum of parasite molecules acting as potential allergens is necessary. In this study, we employ high-throughput (Illumina) sequencing and bioinformatics technologies to characterise the transcriptomes of two *Anisakis* species, *A. simplex* and *A. pegreffii*, and mine these annotated datasets to compile lists of potential allergens from these parasites. A total of ~65,000,000 reads were generated from cDNA libraries for each species, and assembled into ~34,000 transcripts (= Unigenes); ~18,000 peptides were predicted from each cDNA library and classified based on homology searches, protein motifs and gene ontology and biological pathway mapping. Using comparative analyses with sequence data available in public databases, 36 (*A. simplex*) and 29 (*A. pegreffii*) putative allergens were identified, including sequences encoding 'novel' *Anisakis* allergenic proteins (i.e. cyclophilins and ABA-1 domain containing proteins). This study represents a first step towards providing the research community with a curated dataset to use as a molecular resource for future investigations of poorly known putative *Anisakis* allergens, using functional genomics, proteomics and immunological tools. Ultimately, an improved knowledge of the biological functions of these molecules in the parasite, as well as of their immunogenic properties, will assist the development of comprehensive, reliable and robust diagnostic tools.

COMPARATIVE GENOMICS OF *CRYPTOSPORIDIUM PARVUM* ISOLATES BY NEXT GENERATION SEQUENCING

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Next Generation Sequencing (NGS) has the potential to facilitate advances in pathogen typing and epidemiological investigations by providing information at the level of the whole genome, and many genomes have been delineated from parasites of human and animal health relevance.
The objective of our research is to generate whole genome sequences of *Cryptosporidium* isolates, and to exploit these data in the context of the complex epidemiology of human cryptosporidiosis. Infection is transmitted through both direct and indirect routes, could be of zoonotic origin, and large waterborne and foodborne outbreaks have occurred across Europe. High genetic variability characterizes isolates of *Cryptosporidium parvum* and *C. hominis*, the two main human pathogens, but the value of comparative studies at the genome level is largely unexplored.
Here, we established a protocol to generate whole genome sequences of *Cryptosporidium parvum* isolates of human and animal (calf, lamb, goat kid) origin. Briefly, oocysts present in fresh or frozen fecal samples were purified by cesium chloride density gradient and/or by immunomagnetic separation, and treated with bleach to reduce bacterial contamination. DNA was extracted by standard procedures and submitted to Whole Genome Amplification (WGA) technique. Whole genome shotgun sequencing of WGA products was performed on an Illumina platform. The resulting raw reads were processed using the CLC Genomics Workbench, assembled into contigs and mapped to reference *C. parvum* genomes.
Results indicate that the procedure generates highly purified genomic DNA of *Cryptosporidium* amenable to NGS. Indeed, 92-96% of the obtained reads could be mapped to the *C. parvum* reference genome, indicating minor contamination by non-target organisms. We will discuss how comparative genomics data can be used to identify genes under putative selection pressure and markers that can improve epidemiologic investigations.

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MOLECULAR DETECTION OF HAEMOTROPIC MYCOPLASMAS IN DOGS AND CATS OF NORTHERN ITALY

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Haemotropic mycoplasmas (hemoplasmas), the agents of infectious anemia, have been reported in several mammalian species. Few data are available on hemoplasma infections in dogs and cats of Italy. The aim of the present study was to evaluate the prevalence of haemoplasmas in dogs and cats of northern Italy. Blood samples of 211 owned dogs, 301 free-roaming dogs and 227 cattery cats were collected in 2014 and 2015. Among these, 17 owned dogs and 14 free-roaming dogs had symptoms referred to vector borne diseases.
Samples were screened using a newly developed Sybr green real time PCR based on the 16S rRNA gene amplifying 259 bp. RNaseP gene was also amplified to discriminate *Mycoplasma haemofelis* from *M. haemocanis.* All the amplified products were directly sequenced for species identification.
All the asymptomatic owned dogs were negative, except one positive for *M. haemocanis* (0.5%). The prevalence of infection in asymptomatic free-roaming dogs was 5.9% (17/287). Both *M. haemocanis* and *M. haematoparvum* were identified (4.2% and 1.7%, respectively). Among symptomatic dogs, 23.5% (4/17) owned dogs and 7.1% (1/14) free-roaming dogs were infected with *M. haematoparvum.* Symptomatic owned dogs showed higher prevalence compared to asymptomatic ones ($p<0.01$). The overall prevalence of infection in cats was 13.2% (30/227). All the three species affecting cats were found, i.e. *M. haemofelis* (4; 1.8%), *M. haemominutum* (25; 11%) and *M. turicensis* (1; 0.44%). The PCR assay here described is a suitable tool to screen for known and so-far-undiscovered hemoplasma species. The prevalence of hemoplasmas is negligible only in owned dogs, likely due to the regular use of compounds against arthropod vectors. The significance of hemoplasmas in symptomatic dogs should be evaluated.

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MOLECULAR DELINEATION OF *IXODES VENTALLOI* FROM CATS OF SOUTHERN ITALY

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Ixodes ventalloi has been implicated as a possible vector of pathogens of medical and veterinary relevance, including *Anaplasma phagocytophilum*, *Rickettsia helvetica*, *Rickettsia monacensis*, *Bartonella clarridgeiae*, and Eyach virus. Although *I. ventalloi* has occasionally been collected from cats, dogs and birds worldwide, no molecular data is available for this tick species. Therefore, the aim of this study was to provide genetic data on *I. ventalloi*, as an important support for its morphological identification. Adult tick specimens (65 females and 31 males) infesting owned and stray cats of the Aeolian Islands (Sicily, southern Italy) were morphologically identified and molecularly characterized, based on part of the mitochondrial 16S rDNA and cytochrome *c* oxidase subunit 1 (*cox*1) genes. All tick specimens were morphologically identified as *I. ventalloi*. Up to 4.7% nucleotide variation was detected amongst 8 and 16 haplotypes for 16S and *cox*1, respectively, suggesting the existence of two distinct genogroups. The mean genetic distance within each genogroup was low (i.e., 1.3% and 2% for 16S and *cox*1, respectively), in agreement with the mean intraspecific genetic distance of other *Ixodes* species examined (i.e., *Ixodes ricinus*, *Ixodes inopinatus, Ixodes spinipalpis, Ixodes nipponensis, Ixodes persulcatus*) (e.g., 16S: 1.3%, *cox*1: 0.6%). The presence of the two distinct genogroups was further supported by the separation of all haplotypes into two main clades, inferred from the phylogenetic analyses of both genes. Overall, molecular data herein generated suggests that a significant difference in the genetic structure occurs amongst *I. ventalloi* endemic population, advocating the existence of two distinct genogroups for this little-studied tick species.

SOIPA FOR HUMAN DEVELOPMENT.
CAPACITY STRENGTHENING OF YOUNG MALARIOLOGISTS IN BURKINA FASO

THE ROLE OF HUMAN GENETIC VARIATION IN MALARIA SUSCEPTIBILITY AND TRANSMISSION

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Malaria remains a public health problem worldwide. In 2015, about 214 million cases of malaria and about 438000 deaths were reported, with Sub-Saharan Africa alone bearing 90 % of the burden. In Burkina Faso, Fulani and Mossi/Rimaïbé sympatric ethnic groups have been previously shown to have distinct genetic background and marked differences in susceptibility to malaria (Modiano et al. *PNAS* 1996). Our research hypothesis is that genetic factors could also result in populations differences in the ability to transmit malaria to mosquitoes (Gouagna et al. *Nat Genet* 2010). Hence, the study objectives are: i) to assess differences in gametocyte reservoir, transmission blocking immunity and infectivity to mosquitoes between Fulani and Mossi; if any; ii) to investigate the genetic basis of the observed differences.
A total of 100 subjects per ethnic group will be recruited and venous blood samples will be collected during a cross-sectional survey that will take place in the 2016 transmission season (July-October) after approbation of the study protocol by the National Ethical Committee. The determination of the gametocyte reservoir and of the sex ratio between female and male gametocytes will be performed by microscopy and by Real Time qPCR methods. Transmission blocking immunity will be estimated by ELISA measurement of antibodies against gametocyte antigens. The infectious potential of individuals will be assessed by experimental mosquito infections (Direct Membrane Feeding Assay). Human DNA will be extracted from whole blood and genotyped for polymorphisms at candidate loci (e.g. *HBA*, *HBB*, *G6PD*) using standard protocols.
The generated data will provide original information about the impact of human genetic factors in malaria transmission at population level.

NATURAL IMMUNE RESPONSES TO *PLASMODIUM FALCIPARUM:* A WHOLE PROTEOME MICROARRAY APPROACH

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An effective antimalarial vaccine remains a highly desirable goal to control or to eliminate malaria in a context where the diffusion of malaria parasites resistant to currently used drugs and vectors to insecticides, is increasing.
The sequencing of *Plasmodium falciparum* provides an opportunity in the search for new drugs and vaccines to fight malaria. The genome sequence is stimulating vaccine development by the identification of hundreds of potential antigens that can be scanned for desired properties such as surface expression or limited antigenic diversity. For this purpose a protein micro-array, high-throughput immunological assay has been developed to identify novel candidate vaccine antigens that are potential targets of protective immune responses in humans.
This study will contribute to the identification of new *Plasmodium falciparum* antigens by using samples from populations naturally exposed to malaria and having different genetic background and different status of acquired immunity. Specifically, the study will assess the antibody responses profiles and link these profiles to clinical status, age, ethnic group and malaria transmission intensity.
The study will be conducted in two steps:
Existing databases will be used to select candidate antigens for the immuno-epidemiolgical screening. One logical approach is to focus on molecules with known biological functions such as invasion, sequestration and their association with the processes of pathogenesis in the host. The selected antigens will cover the sexual and asexual blood stages.
Antibody responses will be measured by Protein Micro-array screening of serum samples collected from populations with different genetic background, exposed to high malaria transmission and from adults (including pregnant women) and children with different susceptibility to malaria.
At the end of this immuno-epidemiological study, a panel of novel candidate vaccine antigens that are potential targets of protective humoral and cellular immune responses in humans will be identified.

CONTROL OF *PLASMODIUM FALCIPARUM* TRANSMISSION: ROLE OF GAMETOCYTES BIOLOGY

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The existence of submicroscopic gametocytemia and the low impact of artemisinin combination therapy (ACT) formulations on mature gametocytes [Peatey et al., J Infect Dis. 2009], constitute a major challenge to the current malaria control and elimination efforts undertaken in endemic countries.
The development of molecular tools such as transgenic *Plasmodium falciparum* (*Pf*)parasites expressing fluorescent tags and sensitive RNA based Real Time PCR assays offer opportunities for getting a better understanding on the biology of gametocytes. With those sensitive tools, sub-microscopic gametocytes were revealed and shown to contribute substantially to the human infectious reservoir [Ouédraogo AL et al., J Infect Dis. 2016].
As detection of gametocytes in health facilities is still mostly relying on microscopy, applying molecular tools for gametocyte detection will provide more accurate information to orient transmission blocking malaria control strategies.
Objectives: i) to set up continuous parasite cultures producing gametocytes; ii) to transfer RT-PCR assays enabling the detection of low level gametocytaemias and to validate assays with field samples; iii) to establish *in vitro* gametocyte production of *Pf* field isolates; iv) to investigate on the human infectious reservoir in populations living in Burkina Faso.
Set up of gametocyte cultures and molecular tools using the lab strains 3D7 and NF4 (Carter et al., Methods in Molecular Biology, 1993). Adaptation to *in vitro* culture and gametocyte production from field isolates of *Pf* parasites. Use of RT-PCR assays based on the gametocyte sex-specific transcripts from genes *pf*s25 and *pf*s230p RT-PCR (Schneider et al, Molecular & Biochemical Parasitology, 2015) to detect and quantify female and male gametocytes, respectively, in laboratory and field isolates. Determine gametoyte sex ratio from *in vitro* cultures and from blood samples. Blood samples from consent adult and children with uncomplicated *Pf* malaria will be used.
Data generated from this study will provide information on the levels and the sex ratio of gametocytes in wild parasite samples, relevant to study epidemiology and dynamics of *Pf* transmission in malaria endemic country in sub-Sahara.

IDENTIFICATION OF COMPOUNDS FROM MEDICINAL PLANTS WITH ANTIMALARIAL, TRANSMISSION BLOCKING PROPERTIES

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The renewed interest in malaria elimination has led to an increased investment in interventions that specifically aim to interrupt malaria transmission. Tools are needed that interfere with the transmissible *Plasmodium* stages, i.e. gametocytes or early sporogonic stages that develop in the human host and in the mosquito vector, respectively. Identifying compounds acting against transmissible stages and elucidating their targets on *Plasmodium* may provide new directions for the design of the next generation of transmission blocking drugs.
To screen extracts and fractions from known antimalarial plants to identify transmission blocking compounds.
The research will build upon previous studies conducted at the "Centre National de Recherche et Formation sur le Paludisme" (Ouagadougou, Burkina Faso) which showed prominent *in vitro* antiplasmodial activity against asexual *Plasmodium falciparum* parasites (*P.f.* K1 or W2 strains) of the following plant extracts: *Pavetta crassipes* Leaves **(**IC_{50}:1.23 µg/ml)*, Zanthoxylum zanthoxyloides* Bark (IC_{50}:1.2µg/ml)*, Terminalia avicenoides* Leaves (IC_{50}:1.2 µg/ml) and Bark (IC_{50}:2.9 µg/ml)*, Anogeissus leiocarpus* Leaves (IC_{50}:4.69µg/ml)*, Combretum collinum* Bark (IC_{50}:0.4µg/ml)*, Terminalia macroptera* Leaves (IC_{50}:1µg/ml) and Bark (8.57µg/ml). The plants have been collected in January 2016 and their extracts will be used for the bioguided fractionation studies. Active fractions will be further analyzed to identify the effective molecules. *In vitro* assays will be employed to screen for gametocytocidal (*P. falciparum* 3D7-CBG99luc strain) effects and activity against early sporogonic stages (*P. berghei* ANKA CTRP.gfp strain). Active fractions and compounds will be tested for *in vivo* transmission blocking effects using the *Plasmodium berghei* / *Anopheles stephensi* / BALB/c mouse model.
This study is expected to lead to the discovery of new molecules which display activity against gametocytes and/or early sporogonic stages *in vitro* and *in vivo.*

IMMUNOLOGY AND VACCINES

DNASEII ALLOW *TRICHINELLA* WORM TO ESCAPE NETOSIS INDUCED BY MICE MACROPHAGE

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A fascinating tool in the anti-parasite immunology based on the ability of the host organism to be able to block worm invasive forms. Nematode parasite *Trichinella* zoonotic, 1 mm long, is blocked at the intestinal mucosa in a few hours in the immunized animal or rats in a host restriction context. The analysis of differential transcriptome in *Trichinella spiralis* by different technologies (subtractive libraries, sequencing the transcriptome of different parasite stages, genome sequencing of *Trichinella*) allowed us to identify and describe a family of genes coding for type II DNase. More than 120 representatives of this family were identified and only one gene encoded a DNaseII not having the canonical catalytic triad (encoded protein called Plancitoxine3 for its strong identity with a starfish nuclease). We showed that the expression of different genes in this family, whatever the structure of the active enzymatic site, provides functional enzymes cleaving DNA. Secretion DNaseII was identified by electron microscopy and analyzed by confocal microscopy using antibodies specific for DNase II of *Trichinella spiralis*. The peripheral location of certain DNAseII secretion results from their co-localization in the pore level in the cuticle. Killed muscle larvae was put in contact with mouse macrophages and are the subject of direct attachment of cells during the phenomenon of "NETosis", originally described with neutrophils extracellular. The addition of DNaseII inhibitor in ex vivo model induced strong mortality of purified muscle larvae or new borne larvae. A major effector of parasite penetration to bypass the immune response is thus suggested for *Trichinella*.

MATRIX METALLOPROTEINASE (MMP-)9 AND CHEMOKINE PROFILE IN HUMAN TRICHINELLOSIS

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Matrix metalloproteinases (MMPs) are involved in many physiopathological processes. These proteins have been studied in some parasitic infections, but their role as well as their relation with other inflammatory markers such as chemokines in human trichinellosis are poorly known.
In the present study, the relationship between inflammation, clinical symptoms and the level of MMP-9 and two chemokines, namely CXCL10 and CCL2, respectively, were investigated in trichinellosis patients to assess their possible modifications and clinical significance.
Sera from 31 *Trichinella britovi*-infected individuals (20 males and 11 females), were analyzed for MMP-9, MMP-2, CXCL10, and CCL2 serum levels. Patients acquired infection after consuming raw or undercooked wild boar meat, during an outbreak occurred in Central Italy. Median age of patients was 49±0.33 years. Sera were collected before and 3 months after starting of anti-inflammatory and antihelmintic treatment, aliquoted and stored at -20°C until use. Sera from healthy subjects with normal erythrocyte sedimentation rate were considered as controls. The MMP-9 and the chemokine levels were determined with commercially available ELISA tests.
A significant (p<0.037) increase in MMP-9 serum level in patients compared to controls was observed. The MMP-9 levels showed significant correlation with those of CXCL10 (r2=0.48, p< 0.009), but not with CCL2. Furthermore, MMP-9 resulted higher in most of the patients suffering diarrhea, facial edema and myalgia. Serum levels of CXCL10 were increased (822.6±389 pg/ml) in 92.8% of patients, whereas the CCL2 levels were high (725.4±410 pg/ml) in 50% of patients, compared to values normally detected. After three months of infection, CXCL10 levels dropped to control values in all the evaluated patients, suggesting a decline of the inflammatory reaction, as expected from the low pathogenicity of the *Trichinella* species involved.
Our results suggest that MMP-9, CXCL10 and CCL2 are reliable markers of inflammation during human trichinellosis.

IMMUNE RESPONSES TO HELMINTH PARASITE ANTIGENS IN MALARIA ENDEMIC POPULATIONS

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Co-infection with *P. falciparum* and helminths in Sub-Saharan Africa could modulate the immune response towards the parasites as well as the individual susceptibility to clinical forms.
Our aim is to investigate the impact of helminth infections on immune responses and susceptibility to malaria in different ethnic groups from Burkina Faso (Modiano et al. *PNAS* 1996). The objectives of this work are: *i)* to measure immunoglobulins against helminth parasite antigens in plasma samples (N=288) collected among Mossi, Fulani and Rimaibe rural communities; *ii)* to assess differences in relation to age, sex, village, ethnic group, infection with *P. falciparum; iii)* to assess correlation with antibodies against malaria antigens (CSP, MSP1, MSP2, AMA1) and total IgE.
Measurements of IgG against *Strongyloides*, *Taenia spp.* and filarial infections are ongoing.
A custom ELISA protocol was used to measure IgG against *Schistosoma haematobium* Soluble Egg Antigen (SEA). The prevalence of anti-SEA IgG is 63%, in line with the prevalence of *S. haematobium* infection reported for the same area of Burkina Faso (55-85%, Traore et al. *Medecine d'Afrique Noire* 1990). The prevalence is zero in infants, increases during childhood to reach its peak in teens, and decreases from 20 years onwards. Females show a lower prevalence than males (P=0.003). Differences in prevalence are not observed among villages or ethnic groups, but the Fulani show lower levels of anti-SEA IgG (P=0.0001) suggesting that lighter *S. haematobium* infections occur in the ethnic group known for a marked lower susceptibility to *P. falciparum*. Individuals infected with *P. falciparum* show higher levels of anti-SEA IgG (P=0.0002). A positive correlation exist between anti-SEA IgG, total IgE (P<0.0001) and anti-CSP IgG (P=0.009).
These results suggest that common host factors may affect susceptibility to *P. falciparum* and *S. haematobium* (e.g. age, ethnicity) and warrant further investigation into the immunological cross-talk between the two parasites.

MONITORING AND CONTROL OF VECTORS

TRANSCRIPTOMIC APPROACH TO ANALYSE TEMPORAL PATTERNS OF INSECTICIDE RESPONSE IN *ANOPHELES STEPHENSI*

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Despite the encouraging results achieved in the last ten years, malaria epidemics are still taking a heavy toll on third world populations, especially in sub-Saharan Africa and South East Asia. Due to the unique and complicated life cycle of its pathological agent, *Plasmodium spp.*, vaccines are not in near sight. This, coupled with an increasingly widespread resistance to antimalarial drugs, has shifted the focus on its vectors, mosquitoes of the *Anopheles* genus. *Anopheles stephensi* is the main vector of *Plasmodium falciparum* in India, accounting for 12% of malaria cases in the area, where insecticides remain a staple in the war against the disease. As more and more mosquito populations display resistance to insecticides, it is relevant to understand molecular and regulatory mechanisms of insecticide action. Indian strain *Anopheles stephensi* larvae were exposed to permethrin, an insecticide of the pyrethroid class, and their expression profile compared by RNA-seq to control unchallenged larvae at six, 24 and 48 hours after exposure. Differentially expressed genes were investigated for enriched functional categories (Gene Ontology).
Gene families such as cytochromes P450, ABC-transporters, cuticular genes, glutathione S-transferases and carboxylesterases are known to be involved in toxic compounds responses and well studied in arthropods. We thus created a literature-based dataset of these relevant gene families. ABC-transporters play a major role in detoxification in insects but were found to be under represented in the dataset. We used an identity based approach to mine the transcriptome of *A. stephensi* for putative ABC-transporter genes, followed by a phylogenetic approach to classify them in families.
We extensively analyzed the expression profile of the above gene groups to reconstruct on-off temporal patterns of these relevant genes. Our temporal analysis helps to shed light on the changes of expression, after insecticide challenge, of relevant genes and pathways in a major malaria vector.

ECOCLIMATIC DRIVERS OF SPATIO-TEMPORAL HOT SPOTS OF *AEDES ALBOPICTUS* ABUNDANCE IN SOUTH EUROPEAN URBAN AREAS

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The stable colonization by *Aedes albopictus* of several south European urban areas represents an increasing public health threat due to the species competence in transmitting Dengue, Chikungunya and Zika arboviruses, whose expanding worldwide distribution is increasing the risk of an infected traveller to reach Europe. In fact, a Chikungunya outbreak has already occurred in northern Italy in 2007 and cases of autochthonous Dengue transmission have been recently reported from France and Croatia. Despite in the absence of vaccines the only way to prevent the risk of outbreaks of these diseases in Europe is mosquito control, this is rarely efficiently carried out by public administrations due to lack of appropriate resources to cover the large areas colonized by the species. It has been proposed that a more cost-effective method to prevent arbovirus outbreaks could be the focal treatment of hot-spot of highest mosquito densities. The aim of this work was to identify eco-climatic drivers of higher *Ae. albopictus* abundance on the basis of data from seasonal-round monitoring carried out over multiple years across and beyond the urban area of Rome and Bari. A fine scale (300 m radius) spatio-temporal dataset was built within each sampling site and exploited to analyse the effect of climatic (Land Surface Temperature, Daily Rainfall, Growing Degree Days), environmental (Land Cover as retrieved from digital multispectral aerial imagery) and demographic (human population density) variables on *Ae. albopictus* spatial abundance and temporal dynamics. Generalized additive mixed models highlighted a strong positive relationship between mosquito abundance and anthropic surfaces and population density and identified climatic drivers of the seasonal population dynamics. These results provide useful indications to prioritize public mosquito control measures in temperate urban areas in space and time for a more feasible and cost-efficient prevention of the risk arbovirus transmission in Europe.

N INTEGRATED CONTROL PROGRAM AGAINST *AEDES ALBOPICTUS* IN NORTHERN ITALY: A CASE STUDY

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Aedes albopictus is a major biting nuisance and a competent vector for many arboviruses. During the last decades, it has colonized almost all the Italian territory. Integrated mosquito management of *Ae. albopictus* is particularly difficult because many breeding sites are present in private areas. Therefore, public education campaign has become a basic tool to involve homeowners in mosquito control, and door-to-door active education has been recently carried out with success in Spain, the United-States and Thailand.
The present study, conducted in the municipality of San Michele all'Adige (Trento) in 2015, aimed to assess a community-based integrated mosquito control strategy including a public education campaign (public meetings and distribution of flyers), larvicide treatments of public catch basins, and door-to-door visits consisting in homeowners' education, garden inspection and/or delivery of larvicide tabs. All these control measures were implemented in one site (full intervention site), while only public education and public larvicide treatments were implemented in a second site (partial intervention site). A third site was used as control (no intervention site). Biweekly egg counts from 95 ovitraps were modelled by a zero-inflated negative binomial mixed model to evaluate the efficacy of the type of intervention against mosquito abundance.
In the full intervention site, 181/297 houses have been visited in June and in September, and 2210 larvicide tabs have been delivered. The total number of private catch basins with mosquito larvae decreased three times between June and September, showing a correct use of larvicide tabs by homeowners. The average egg density in the full intervention site was 2.2 lower as compared to the no intervention site, whereas the average egg densities in the partial and the no intervention sites were similar. Our results confirm that only an integrated mosquito control strategy targeting both public and private areas can be effective against *Ae. albopictus*.

INTRODUCTION OF THE ASIAN BUSH MOSQUITO *AEDES JAPONICUS JAPONICUS* (DIPTERA: CULICIDAE) IN ITALY

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The Asian bush mosquito *Aedes (Finlaya) japonicus japonicus* (Diptera: Culicidae) is one of the most invasive mosquito species worldwide and recently invaded several countries of Central Europe such as Austria, Croatia, Hungary, France, Switzerland and Slovenia. In 2011 the species was discovered in Slovenia and in southern Austria. Based on the results of the monitoring field studies performed in Austria from 2012 to 2014, an active spreading to northern Italy was supposed to occur.
Indeed several specimens of *Ae. j. japonicus* larvae were found in July 2015 during a survey carried out by Austrian scientists in three different sites in Udine province (Alps of Carnia). A second survey was conducted by Italian scientists in September 2015 confirming the records. A molecular analysis by PCR and subsequent sequencing confirmed the species identification.
Aedes j. japonicus was found in artificial containers, often with other species (*Culex pipiens, Cx. hortensis* and *Culiseta longiareolata*) and in one case also with *Ae. albopictus*.
The species colonizes a wide typology of natural and artificial containers and is adapted to tolerate the cold winter temperature. The species is known to be a pest problem and to have the vector competence for arboviruses such as West Nile, Dengue and Chikungunya viruses.
Other two invasive species, *Ae. albopictus* and *Ae. koreicus*, are already established in Friuli Venezia Giulia region. Thus, its establishment complicates the current surveillance system requiring well trained personnel for identification. From a Public Health perspective, a new competent vector of pathogens to animals and humans may represent a challenge for the Health System.
This work was funded by the Autonomous Province of Trento (Project LExEM), by the Friuli Venezia Giulia region and by the Austrian Climate Research Programme (ACRP7; KR14AC7K11954).

THERAPEUTIC AND RESIDUAL ACARICIDAL EFFICACY OF STRECTIS®, A FIPRONIL (17% W/V) (S)-METHOPRENE (8.5%) TOPICAL ADMINISTRATION, AGAINST TICK *(RHIPICEPHALUS TURANICUS)* INFESTING CATS

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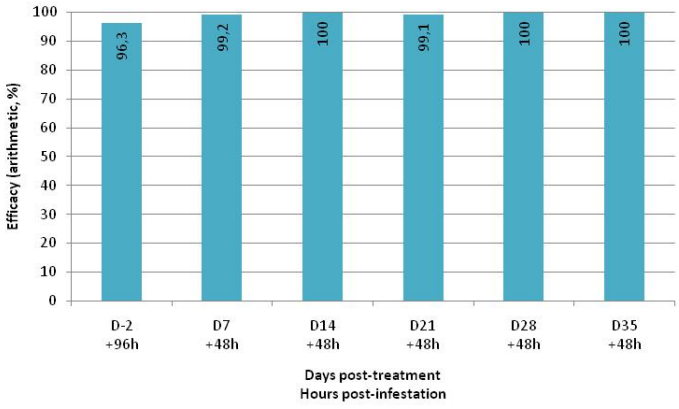
Despite their ability to groom themselves, cats can be infested by ticks. This study was conducted to assess the acaricidal efficacy of a fipronil (17 % w/v) (s)-methoprene (8.5%) topical ectoparasiticide (FM, Strectis®) against adult *Rhipicephalus turanicus* ticks in cats.
The protocol for this study was approved by an ethics committee. Sixteen mixed-bred cats (8 male and 8 female) were allocated based on their individual pre-treatment live attached tick counts (day -5) to 2 groups: an untreated control group (n=8, 2.69-5.19 kg BW) and a FM treated group (n=8, 2.52-4.11 kg BW). Cats in the treated group were administered the minimal recommended dose, 0.071 mL/kg BW of FM on day 0. Each cat was infested under sedation with 50 adult ticks on day -7, -2, 7, 14, 21, 28 and 35. The ticks were counted and removed from cats 48 hours after treatment or infestation. The ticks were categorized as live or dead, attached or free, engorged or unengorged. Arithmetic means of live and engorged ticks were calculated for each group at each time-point. Comparisons between groups were performed on the tick counts by ANOVA. Veterinary examinations, bodyweight, general health observations and clinical assessments were evaluated throughout the study.
The FM treatment provided a therapeutic efficacy of 96.3%. The preventive efficacy was ≥ 99% for 37 days after treatment. There was no tick found on the treated cats on days 30 and 37. The live and engorged tick counts differed significantly ($p<0.001$) between control and treated groups at each time-point. Topical application of FM was well tolerated. This study demonstrates that FM is a convenient ectoparasiticide for cats with a therapeutic and 5-week preventive efficacy against ticks.



COMPARATIVE ASSESSMENT OF THE RAPID ACTIONS AGAINST FLEAS OF A TOPICAL DINOTEFURAN-PYRIPROXYFEN-PERMETHRIN (VECTRA® 3D) AND AN ORAL SPINOSAD (COMFORTIS®) ECTOPARASITICIDE DESPITE SHAMPOO AND MOUSSE (DOUXO®) APPLICATIONS ON DOGS OVER 1 MONTH

Varloud Marie[1], Ollivier Elodie[1], Garelli-Paar Catherine[1], Liebenberg Julian[2], Crippa Alessia[3]

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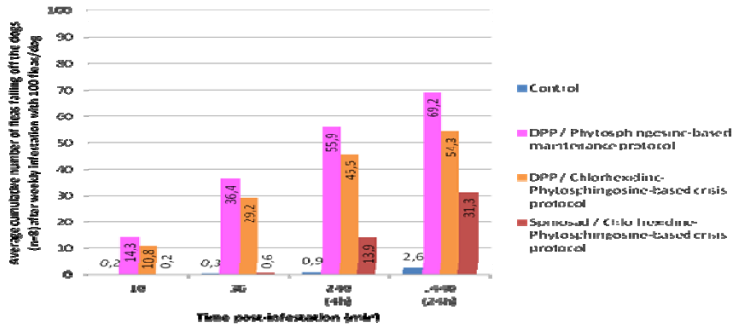
This study compares the insecticidal and knock-down actions of a dinotefuran-pyriproxyfen-permethrin topical ectoparasiticide (DPP, Vectra®3D) and a spinosad tablet (S, Comfortis®) against adult *Ctenocephalides felis* fleas in dogs despite Shampoo and Mousse applications (DOUXO®) over one month.
An ethics committee approved the protocol. Thirty-two mixed-bred dogs were allocated to 4 groups: an untreated control group (n=8), a S-treated group receiving Chlorhexidine-Phytosphingosine-based products (n=8, DOUXO®Pyo) according to crisis protocol and 2 DPP-treated groups receiving either Chlorhexidine-Phytosphingosine-based products (n=8, DOUXO®Pyo) according to crisis protocol or Phytosphingosine-based products (n=8, DOUXO®Calm) according to maintenance protocol. Dogs in the treated groups were administered the European label dose of ectoparasiticides on day 0. Chlorhexidine-Phytosphingosine-based crisis protocol consisted of applying Shampoo (20-30mL) on days 2, 9, 16, 23 and Mousse (1 pump/2kg) on days 5, 7, 12, 14, 19, 21. Phytosphingosine-based maintenance protocol consisted of applying Shampoo (20-30mL) on day 2 and Mousse (1 pump/2kg) on days 9, 16, 23. Each dog was infested with 100 adult fleas on days -6, -4, 3, 10, 17, 24, and 29. The fleas falling off the dogs were collected 10, 30, 240min and 24h after each infestation, for knock-down assessment; before being removed and counted from dogs 24h after infestation for insecticidal assessment. Fleas were categorized as live, moribund or dead. Comparisons between groups were performed on the flea counts by ANOVA. Veterinary examinations and clinical assessments were performed throughout the study.
All products were well tolerated. In average, control dogs retained 74 fleas, with less than 3 fleas falling off. Whatever the procedure, more fleas were dislodged in average from the DPP-treated than from the S-treated dogs. This study demonstrates that the flea knock-down performances of topical DPP are higher than systemic S despite DOUXO® protocols over 1 month.



ASSESSMENT OF THE RAPID ACTIONS AGAINST FLEAS OF A TOPICAL DINOTEFURAN-PYRIPROXYFEN-PERMETHRIN ECTOPARASITICIDE (VECTRA® 3D) DESPITE SHAMPOO AND MOUSSE (DOUXO®) APPLICATIONS ON DOGS OVER 1 WEEK

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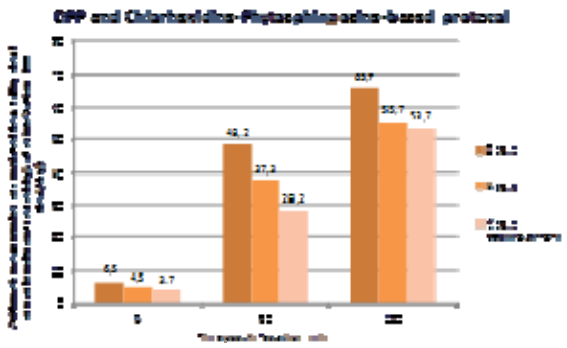
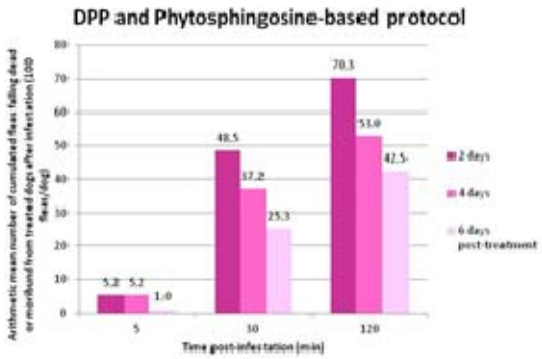
Washability of topical parasiticides by shampoos is often pointed as a weakness and it is generally recommended to wait for 48h between shampoo and treatment. This study investigates the action of a dinotefuran-pyriproxyfen-permethrin topical ectoparasiticide (DPP, Vectra®3D) against adult *Ctenocephalides felis* fleas in dogs despite Shampoo and Mousse applications (DOUXO®) as soon as 24h after administration.
The protocol was approved by an ethics committee. Eighteen mixed-breed dogs were allocated to 3 groups: an untreated control group (n=6), and 2 DPP-treated groups treated with either a Phytosphingosine-based (n=6, DOUXO®Calm) or a Chlorhexidine-Phytosphingosine-based (n=6, DOUXO®Pyo) topical products according to a crisis protocol. Dogs in the treated groups were administered the minimal recommended dose 0.12mL/kg of DPP on day 0. Shampoo (20-30mL) was applied on day 1 and Mousse (1 pump/2kg) on days 3 and 5. Each dog was infested with 100 adult fleas on days 2, 4, and 6. The fleas falling off the dogs were collected 5, 30 and 120min after each infestation before being removed and counted from dogs 2h after infestation. Fleas were categorized as live, moribund or dead. Comparisons between groups were performed on the flea counts by ANOVA. Veterinary examinations and clinical assessments were performed throughout the study.
All topical administrations were well tolerated. In average, control dogs retained 84 fleas, with less than 2 fleas falling off. In the treated dogs, there was in average >37 fleas in 30min and >55 fleas knock-down in 2h. The insecticidal efficacy in 2h of DPP was >90% only 2 days after administration and despite DOUXO® Shampoo application 24h after administration. This study confirmed the strong knock-down and speed of kill performances of DPP against fleas despite DOUXO® Shampoo and Mousse procedures.





SEASONAL ACTIVITY OF *DERMACENTOR RETICULATUS* IN A PERI-URBAN PARK IN NORTH-EASTERN ITALY

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Dermacentor reticulatus is a Palaearctic tick species widespread from western Europe to central Asia. In the recent years its distribution, as well as the number of pathogens transmitted, have been reported to increase. The presence of *D. reticulatus* was recently confirmed by molecular tools in a peri-urban park in north-eastern Italy, also in association with *Babesia canis* infection (Olivieri et al. 2016). The aim of this study was to investigate the distribution and seasonal activity of *D. reticulatus* in the park.
From April 2015 to March 2016, adult specimens of *D. reticulatus* were collected monthly from ground and bushes through dragging and flagging methods. Tick collection was performed in five transects compatible with ecological features of *D. reticulatus*. A GLM with negative binomial distribution was used to evaluate the effects of climatic variables on tick density (SPSS, Version 19.0).
A total of 106 *D. reticulatus* identified by taxonomical keys of Pomerantzev (1959) were collected. All transects tested positive for ticks; two were found to be more infested. The highest tick activity was recorded in March (60.3%, 95% CI = 51.16-69.44) and gradually decreased by the end of May (10.4%, 95% CI = 4.59-16.21) till the beginning of June (3.8%, 95% CI = 0.16-7.44); no ticks were found from July to December. The monthly means of both temperature and relative humidity were negatively correlated with the tick density.
The number of ticks collected is consistent with the findings of other surveys carried out in neighbouring countries with similar ecological and host features (Schaarschmidt et al. 2015) but is lower than in the other European countries.
In the study area, *D. reticulatus* shows a focal occurrence and the tick activity does not show the typical bimodal pattern, its activity being mainly restricted to the end of winter and spring when meteorological conditions are compatible with tick requirements.

FIELD EVALUATION OF A NEW LIGHT TRAP FOR SAND FLY SAMPLING

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Light traps are one of the most common techniques for sand fly collection. Although these traps are generally referred as "CDC light traps" different models, equipped with incandesced or UV lights or either with or without CO_2, have been developed in the years. Recently, a new light trap (Laika trap 3.0) equipped with led lights, for a lower battery consumption, and of handy design has been proposed. In this study, the sand fly capture performances of this new trap were evaluated and compared with those of a traditional light trap model in the field.
From May to November, a Laika trap and a CDC light trap were placed in a shelter located in an endemic area for canine leishmaniosis in Sicily. Traps were positioned at 50 cm and at 3 m of distance between them, and were activated biweekly from 06:00 pm to 06:00 am.
Captured sand flies were stored according to traps and date and, thereafter, sexed and identified at species level using morphological keys. Wilcoxon signed-rank test was used to assess any difference of sand fly abundance species and/or sex between the two trap models.
Overall, 256 sand flies, belonging to 3 species (*Sergentomyia minuta*, *Phlebotomus perniciosus, Phlebotomus neglectus*) were collected in the study. The Laika trap captured 126 sand flies: *P. perniciosus* (N=38); *S. minuta* (N=88). A similar number of sand flies (130) and species (3) were captured by the CDC light trap. No significant differences in the capture efficiency at each catch day, not either in the number of species or in the sex of specimens between the two traps were observed. Results of this study suggest that the Laika light trap may be a valid alternative to traditional light traps especially when handy trap use and long life battery represent key factors for field studies.

ESTIMATING MOSQUITO/HOST CONTACT FROM OVITRAP DATA: A CASE STUDY FOR *AEDES ALBOPICTUS* IN ROME

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Aedes albopictus is an invasive mosquito species now well established in Southern Europe, whose public health relevance is associated not only to the aggressive daytime biting behaviour, but also to the capacity to transmit arboviruses, such as Dengue, Chikungunya and Zika. In non-endemic European countries, the potential risk of autochthonous transmission of these exotic arboviruses is directly linked to the likelihood of importation of human cases (which itself is associated to their incidence worldwide) along with the potential vector abundance. The Zika epidemics occurring in 2016 in South America, in addition to the large number of yearly Dengue cases worldwide, increases the possibility of virus importation to Italy, where areas (mostly urban) with high density of *Ae. albopictus* are at risk for autochthonous transmissions. The actual potential risk should be addressed by an effective, and at the same time economically sustainable, surveillance system pertaining to stratify risk areas.
A common method to evaluate the likelihood of vector-borne disease transmission and spread is by assessing R_0, i.e. the number of secondary infections arising from a primary case. Although several models have been developed, the accuracy of estimates of relevant parameters for *Ae. albopictus* is often inadequate. We here present a linear regression model built to analyse the relationship between commonly used surveillance data obtained by ovitrap collections and the mean number of host-seeking *Ae. albopictus* estimated by Human Landing Collection (HLC), based on data collected every three days in Rome from July to October 2014. The model shows a positive relationship between ovitraps and HLC data and allows estimation of the number of expected daily mosquito bites per host based on eggs counts in ovitraps, opening the possibility of using ovitrap data to estimate actual entomological parameters of key epidemiological interest, such as human/vector contact, for *Ae. Albopictus*.

A PRELIMINARY ASSESSMENT OF THE INSECTICIDE RESISTANCE STATUS OF *AEDES ALBOPICTUS* AND *CULEX PIPIENS* POPULATIONS FROM ROME

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The indigenous *Culex pipiens* and the invasive *Aedes albopictus* are the most widely spread mosquito species in urban areas in Italy. Both species not only create considerable nuisance to citizens, but are also vectors of arboviruses such as Chikungunya, Dengue and Zika (*Ae. albopictus*) and West-Nile (*Cx pipiens*). Although insecticide-based interventions are carried out by citizens and public administrations in order to reduce the nuisance, little is known on the susceptibility of these species to insecticides. The aim of this study was to carry out a first assessment of levels of resistance of field populations of both species to permethrin, one of the most commonly insecticide used against adults.
Insecticide resistance tests were performed on adult mosquitoes obtained from larvae of *Cx. pipiens* and *Ae. albopictus* collected in 2015 in two sites in Rome (Rebibbia area and Verano Cemetery). The tests were performed using 0.75% permethrin impregnated filter papers in test tubes, following WHO protocols, and recording mortality at 24 hours after a 1-h-exposure. Three to 4 replicates were carried out for each population.
Mortality recorded was:

- 73.3% (CI 66.3 - 79.7) for *Cx pipiens* from Verano Cemetery.
- 96% (CI 90.0 – 99.0) for *Ae. albopictus* populations from Verano Cemetery.
- 100% for *Ae. albopictus* populations from Rebibbia.

Insecticide resistance tests showed that use of insecticides is starting to select resistance to permethrin, although differently between species and sites. Resistance is observed only in Verano Cemetery, which is heavily infested and subject to repeated insecticide-based treatments. The higher level of resistance recorded in *Cx. pipiens* than in *Ae. albopictus* populations suggests that insecticide space sprayings are mostly affecting nocturnal *Cx. pipiens* rather than diurnal *Ae. albopictus,* against which they are targeted. Overall, results highlight the need to carefully monitor insecticide resistance in Italian urban mosquito populations.

TOXOPLASMA GONDII IN BIRDS: ARE MAGPIES IMPORTANT?

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Wild birds may represent an important source of *Toxoplasma gondii* infection for domestic animals and for the spread of new, atypical recombinant genotypes (Wendte et al., Vet. Parasitol. 182:96–111, 2011). The aim of the present study was to evaluate *T. gondii* prevalence and genotype in magpies (*Pica pica*). Magpies have a strong bill with a sharp cutting edge, which is used for cutting the flesh of small mammals, digging up insects, and picking fruit. Magpies also frequently feed on carrion. These eating habits make them excellent indicators of *T. gondii* circulation in the environment and in natural, intermediate hosts.

In the present study, DNA was extracted from the hearts of 21 magpies, which had died from natural causes and were brought to the University of Parma's Pathology laboratory. PCR for the 529 bp marker was applied to identify the presence of *T. gondii* DNA. Positive samples showing good bands after amplification were genotyped using a nested-PCR protocol for several genetic markers (Su et al., Parasitol. 137:1-11, 2010), chosen based on their ability to discriminate among the three clonal types of *T. gondii*.

Of the twenty one birds studied, nine (42.8%) harboured *T. gondii* DNA in their myocardial tissue. Four samples were genotyped at varying loci: one at seven loci, showing a Type II/III profile; one at four loci, showing a Type–III pattern; two at two loci, with a predominant Type-III pattern.

The present study has shown that *T. gondii* infection prevalence is high in magpies. This was also reported by Darwich et al. (Vet. Parasitol. 183: 377-381, 2012) in Spain. The authors however, did not genotype the samples. In the present study, the genetic strains that are currently in the area of study are of the non-pathogenic type.

RED FOX *(VULPES VULPES)* AS A POTENTIAL RESERVOIR HOST OF CARDIORESPIRATORY PARASITES IN BOSNIA AND HERZEGOVINA

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Red fox (*Vulpes vulpes*) is considered as reservoir of different cardiorespiratory parasites of veterinary and medical importance. Since data on cardiorespiratory parasites in foxes in Bosnia and Herzegovina are still lacking, the aims of the present study were to (*i*) investigate the prevalence and geographical distribution of these parasites, (*ii*) determine genetic diversity of detected parasite species, and (*iii*) to estimate the role of foxes in the transmission cycle to companion animals and humans.
Four species, morphologically and molecularly identified as *Eucoleus boehmi* (64.6%; 51/79), *Eucoleus aerophilus* (69.7%; 154/221), *Crenosoma vulpis* (45.7%; 101/221) and *Linguatula serrata* (1.3%; 1/79) were retrieved from nasal cavity and lungs in 184 (83.3%) animals. The occurrence of heartworms, *Angiostrongylus vasorum* and *Dirofilaria immitis* was not detected by necropsy or PCR. Furthermore, three distinct haplotypes of *E. aerophilus* (I, III, XV) and two of *C. vulpis* (I, II) previously reported in pet animals and wild carnivores were confirmed in this study. A new haplotype of *C. vulpis* (designated as haplotype V) was also identified based on 12S rRNA gene for the first time.
The present study indicates a high prevalence and wide distribution of cardiorespiratory parasites in fox population in Bosnia and Herzegovina, and supports the existence of transmission patterns between wildlife and pet animals.

PREVALENCE OF LUNGWORMS IN FOXES IN SWITZERLAND, AND EVALUATION OF SEROLOGICAL METHODS FOR THE DETECTION OF *ANGIOSTRONGYLUS VASORUM*

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Angiostrongylus vasorum is a nematode occurring in the right heart and pulmonary arteries of dogs, foxes and other canids. Verminous pneumonia manifesting in respiratory impairment and several other health problems, including fatalities, are frequent in dogs. An important role as reservoir hosts is attributed to foxes, thus contributing to the increasing numbers of observed cases of canine angiostrongylosis. The aims of the study were to determine the prevalence, worm burdens and regional distribution of *A. vasorum* and other lung worms in foxes in Switzerland. Further, serological methods for the detection of circulating antigens and specific antibodies against *A. vasorum* which have previously been developed to be used for dogs were evaluated with foxes. Since 2012, 377 lungs, hearts and blood of foxes were examined. The mean prevalence of *A. vasorum* over the last four years was 45.1% (worm burden, WB: 1-44, mean 3.5), increasing from 20.5% in 2012 to 72.3% in 2016, while the prevalence for *Capillaria aerophila* (63.7%, WB: 1-99, mean 4.6) and *Crenosoma vulpis* (9.0%, WB: 1-48, mean 0.6) did not significantly increase. The ELISAs for the detection of circulating antigens and specific antibodies had a sensitivity and specificity of 91.2% and 89.4% and of 42.2% and 92.0%, respectively. Cross-reactions with other parasites were very limited. From our results we conclude that *A. vasorum* has increasingly established over the past five years within the fox population in Switzerland and that ELISAs are reliable, practical and fast methods for the detection of *A. vasorum* in foxes.

SURVEY ON PARASITIC INFECTIONS IN WILDCAT *(FELIS SILVESTRIS SILVESTRIS* SCHREBER, 1777) BY SCAT COLLECTION

Ettore Napoli[1], Stefano Anile[2], Carmelo Arrabito[2], Davide Scornavacca[3], Maria Vittoria Mazzamuto[4], Gabriella Gaglio[1], Domenico Otranto[5], Salvatore Giannetto[1], Emanuele Brianti[1]

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[3]*Dipartimento di Scienze della Vita, Università degli Studi di Siena*
[4]*Dipartimento di Scienze Teoriche e Applicate, Università degli Studi dell'Insubria*
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The study of the parasite spectrum of a threatened species, such as the European wildcat (*Felis silvestris silvestris*), is of importance to gain information on their health status and dietary habits as well as on it can provide ecological and geographical markers for monitoring distinct animal populations. In addition, wild animals have been suggested as reservoir and spreader hosts of uncommon parasitic species to sympatric domestic animals. Regrettably, scientific information on parasites of wildcats is particularly meager. In the present study, scat collection was used to assess the parasite spectrum of European wildcats living in the Etna Park (Sicily, Italy). Scat collection was performed, from May to September 2010, by weekly walking 4 transects. Faeces were identified using morphological characteristics and then analyzed by copro-microscopic techniques. In order to confirm donors of scats, genetic analyses were performed on 39 randomly chosen samples. A total of 121 scats were collected, and parasitic forms were retrieved in 110 (90.9 %) of the samples. Parasites found were: *Physaloptera* sp. (52.1 %), tapeworms (45.5 %), *Toxocara cati* (43.8 %), *Eucoleus aerophilus* (27.3 %), *Ancylostoma* sp. (22.3 %), *Troglostrongylus brevior* (15.7 %), trematodes (9.9 %), *Isospora felis* (4.1 %), *Cylicospirura* sp. (1.7 %), and Acanthocephala (0.8 %). The prevalence of endoparasitic infections herein recorded is similar to that described in other studies conducted using necropsy technique. The species richness of parasites found in the present survey, with a total of nine helminths and one protozoon, is the highest ever reported for wildcat in Europe. Genotyping of samples confirmed that all the tested scats were of European wildcats. Scat collection and examination are reliable and rapid non-invasive tools which can be used in systematic survey design to study the parasite spectrum of wildcat as well as that of other endangered wild species.

1995-2015: 20 YEARS OF PARASITES IN ITALIAN WILDLIFE. CREATION OF A WEBSITE AND DATA ANALYSIS

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The interest in wildlife has increased in the field of veterinary profession too. Parasitologists have made major contributions to the understanding of eco-pathological phenomena. It is therefore important to collect the national science activities over the years. This work was carried out back in the 1990s by a floppy disk. Another update appeared in the 2000s. It is along these lines that we have revisited the data concerning the last 20 years, thus creating a website, which can be consulted virtually and universally. The Cd in the conference kit provides the access to the website.
To create our database, we searched and classified the publications in annals of academic departments, journals, conference proceedings, university libraries, and data banks. Our database aims to be as up-to-date as possible and above all as updatable as possible. We analyzed this material on the basis of animal species, year, sites of interest, and parasitology research field. Then, works were also classified according to four interpretative levels from an eco-pathological viewpoint, from the isolation of an agent to the suggestion of management solutions.
More than 400 works produced mainly in northern Italy were collected. Most of these refer to cloven-hoofed animals (44%) followed by carnivores (25%), rodents (12%), lagomorphs (12%) and others (6%). The number of studies has proved to be homogeneous over the years with a peak on the occasion of the National Conference for Eco-pathology of Wildlife and a general tendency to concentrate when the SoIPa Conference was held. Among parasites, nematodes are in first place (39%), followed by: protozoans (19%), arthropods (16%), cestodes (15%), trematodes (5%), dermatophytes (4%), and acanthocephalans (2%). The analysis of eco-pathological levels revealed the predominance of the first (58%), followed by the second (29%), the third (11%), and the fourth (2%).
The authors believe they have provided an easy-to-use management tool for investigating parasitological topics in the wildlife field.

TREATMENT OF *SARCOPTES* MANGE IN LLAMAS AND ALPACAS WITH MOXIDECTIN

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An outbraek of sarcoptic mange was investigated in a herd of three female llamas (1, 2, and 4 years old) and four male alpacas (3-3,5 years old) in the Black Forrest (Baden-Wuerttemberg, Germany). The diagnosis was made by clinical picture and detection of mites in skin and ear scrapings. At the beginning numerous of *Sarcoptes* mites were found in the scraping samples. The llamas and alpacas were treated with 0,2 mg/kg bdw. moxidectin (Cydectin® 1% inj.: Zoetis Deutschland GmbH, Berlin, Germany) subcutaneously (2 ml per llama, 1,5 ml per alpaca) every 21 d on days 0, 21, 42, 63, 84, 105, 126, 147, and 168. No other treatment or environmental decontamination was performed during the trial. Because of the slow recovery of the South american camelids it was necessary to repeat the treatment eight times. On days 0, 42, 84, 126, and 168, all animals were examined clinically, and epidermal debris were collected from both auricular areas and other body regions for microscopic examination. The alpacas recovered rapidly and mite counts declined steadily. Llamas showed a slower remission of mite counts and clinical condition. Clinical signs had subsided by day 126 in 3/4 alpacas and on day 168 in 2/3 llamas. All epidermal samples were negative by day 168. No adverse reactions were observed. Under the conditions of our trial, injectable formulation of moxidectin was a practical and well-tolerated means of treatment for sarcoptic mange in South american camelids.

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EFFECT OF SEASONALITY ON PARASITE INTENSITIES IN ALPINE RUMINANTS

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Parasite infections are characterised by heterogeneities in host populations. In particular parasite-host-environment interaction may lead to temporal variability of infections as consequence of the effect of climatic seasonality on the abundance of parasite free living stages, host exposure and their susceptibility to the infections.
Trichostrongylidae nematodes undergo to these issues, since exogenous part of their life cycle. Moreover these parasites are represented by a large number of species with different response to climatic variability. Finally the strong seasonality of alpine environment determine variability of host exposure and susceptibility, related to the fluctuation of their body conditions.
The identification of the most influential mechanism among parasite abundance, host exposure and susceptibility, influencing temporal variability of parasite infection, is of crucial importance to predict and control parasite infections.
Here, we analysed the abomasal parasite community of 89 female alpine ibex (*Capra ibex*) sampled monthly over one year, in order to explore the effect of seasons on infection. Through multi-model selection approach we explored the effect of seasonality of vegetation (proxy of environmental climate), host reproductive status and host body conditions to identify which of these processes best describe the intensity of infection of these parasite species.
The parasite community was dominated by *Teladorsagia circumcincta*, *Marshallagia marshalli* and *Trichostrongylus axei*, which showed marked and contrasting temporal variability. While the first species shows low intensities in Jannuary-February followed by stable values, the seconds ones show peak intensities respectively in spring and summer months.
Models indicate that vegetation seasonality have the major role in the infection of the three parasites species, with a moderate effect of host reproduction status.
These results suggest that climatic seasonality principally influences free living parasite abundance with a lesser effect of host susceptibility. These findings highlight the importance of climatic monitoring as predictive tool to evaluate parasite spread.

CYSTICERCOSIS IN *LEPUS EUROPAEUS* HUNTED IN PLAIN AREAS OF BOLOGNA PROVINCE (EMILIA ROMAGNA REGION, ITALY)

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In the Bologna province, Emilia-Romagna region, a rapid hare population decline was noticeable from 2008: the captured hare for restocking in protected areas dropped from about 7,000 in 2007-'08 to 1,891 in 2014-'15. In the same period hunters (and mass-media) reported a sudden increase of hares infected by *Taenia sp*. larvae, whose appearance was consistent with *T.pisiformis* cysticerci. The aim of the survey was: i)to quantify the prevalence and abundance of cysticerci in hunted hares; ii)to identify the parasites through morphological features and molecular techniques; iii)to describe pathological aspects of parasite-induced lesions; iv)to evaluate possible genetic characters useful to assess the origin of the isolated hare parasites.
In 2013, from September 15th to October 5th, the viscera of 54 hares haunted in agro-ecosystems of the Po Plain (province of Bologna, ATC BO2) were collected.
Peritoneum, liver and lungs were examined for cysticercosis; abundance was estimated counting superficial parasites in liver; parasites were microscopically identified by shape and measure of both large and small hooks. One cysticercus from each hare was analized by a PCR targeting Taeniid species (Trachsel et al, 2007, Parasitology, 134:911-920) and then sequenced. Classical histologycal techniques were used. The sex and the weight of animals were recorded by hunters; age class was assigned observing foreleg Stroh's tubercle.
Generalized linear models were used for statistical analysis.
T. pisiformis was isolated in 8 hares (prevalence 14.8%; abundance range: 0-400; mean abundance 17.8). Identification was confirmed by both morphology and PCR. Infection was significantly related with female sex, adult age and low full-weight. Severe hepatitis was present in 1 infected hares only. The sequencing confirmed *T.pisiformis* in all samples. The sequences were all identical each-other and showed a 99% of similarity with a sequence from Japan, 97% with one from California and 94% with two from Germany and China, respectivey.

PRIN RESULTS "GENOMICS AND HOST-PATHOGEN INTERACTIONS IN CHAMOIS"

INTESTINAL PARASITES IN *RUPICAPRA* SPP. POPULATIONS: STUDY IN THE FRAMEWORK OF THE RELEVANT ITALIAN PROJECT (PRIN)

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Intestinal parasites can seriously threaten the performances and well-being of wild ungulates. In this study, we investigated the occurrence and parasitic burden of protozoans and gastro-intestinal helminths (GIH) in *Rupicapra* spp.
From September 2013 to January 2016, 352 fresh fecal samples were collected from *Rupicapra rupicapra rupicapra* in the Alps (N=262) and from *Rupicapra pyrenaica ornata* in the Apennines (N=90). Samples were examined using standard copro-parasitological methods for *Eimeria* and GIH and an immunofluorescence test for *Cryptosporidium* and *Giardia duodenalis.* Parts of *gp60* and *ssRNA/gdh*/β*giardin* genes were used to identify these protozoa species/genotypes.
In *R.r.rupicapra* and in *R.p.ornata*, 7 and 6 parasite taxa were identified, respectively, with a mean number of 1.7 species/host (min-max 0-5) and 2.05 (min-max: 0-4), respectively.
Overall, 85.3% (95%, C.I.=81.5-89.1) of the animals investigated scored microscopically positive to *Eimeria* spp. with a mean intensity of emission (m.i.e.) of up to 776 o.p.g.; 5.4% (95%, C.I.=3.0-7.7) were positive to *G. duodenalis* and 82% (95%, C.I. 77.91-86.15) to GIH with a m.i.e. of up to 147 e.p.g. Prevalence in *R.r.rupicapra* was 81.2% with a m.i.e. of 380 o.p.g. for *Eimeria*, 6.87% for *Giardia*, and 77.45% for GIH with a m.i.e. of 142 e.p.g. Prevalence in *R.p.ornata* was 94.4% with m.i.e. of 1,093 o.p.g. for *Eimeria*, 1.1% for *G.duodenalis*, and 94.4% for GIH with a m.i.e. of 151 e.p.g. Assemblages A/AI and E were identified in *R.r.rupicapra* and assemblage A/AIII in *R.p.ornata.* None of the animals tested positive for *Cryptosporidium*.
The results show that the prevalence of *Eimeria*, *G.duodenalis* and GIH in both host species is non-negligible, with a significantly higher parasitic burden in *R.p.ornata*. The detection of *G. duodenalis* in *Rupicapra* spp. is noteworthy.
This study updates the data on parasitic fauna of these wild bovids. The impact of these parasites on chamois population dynamics will be inferred from the results/variables obtained throughout the entire interdisciplinary project.

The study was funded by MIUR- Relevant Italian Project (PRIN n. 2010P7LFW4) - Genomics and host-pathogen interactions in chamois and by L.A.I.F.F. Project - Rete di laboratori per l'innovazione nel campo degli alimenti funzionali (codice n. 47);"PO Puglia FESR- 2007-2013, Asse I, Linea 1.2. Accordo di Programma Quadro in materia di Ricerca Scientifica. Intervento "Reti di Laboratori Pubblici di Ricerca"

EIMERIA SPP. IN ITALIAN CHAMOIS: MORPHOLOGICAL, STATISTICAL AND MOLECULAR ANALYSIS

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Coccidia of the genus *Eimeria* are common intestinal parasites affecting domestic and wild animals. In chamois, five species have been described: *Eimeria alpina, Eimeria suppereri, Eimeria yakimoffmatschoulskyi, Eimeria riedmuelleri, Eimeria rupicaprae* (the latter two highly prevalent in Italy). Accurate identification of these protozoa to the species level is crucial, particularly in host-pathogen interactions studies and when the parasitic pressure is high. However, this is often a challenging task. In this study, a survey was conducted to investigate *Eimeria* species and to explore their genetic features in the Alpine chamois, *Rupicapra rupicapra rupicapra,* and the Apennine chamois, *Rupicapra pyrenaica ornata.*
From September 2013 to November 2015, fresh fecal samples were collected from *R. r. rupicapra* in Alps (N=262) and from *R. p. ornata* in Apennines (N=90), and stored in 2.5% potassium dichromate until microscopy analysis. Oocysts were recovered by flotation, photographed, measured and identified. Based on oocysts morphometry (i.e. length, width) a hierarchical cluster analysis was performed. The morphological features of *Eimeria* oocysts were then correlated with their genetic variability after 18SrDNA fragment cloning.
Up to 94% of chamois harbored *Eimeria* oocysts and four species were morphologically identified: *E. yakimoffmatschoulskyi, E. riedmuelleri,* and *E. rupicaprae* in both *R. r. rupicapra* and *R. p ornata*, whereas *E. suppereri* was only found in Alpine chamois.
Cluster analysis based on 894 oocysts from *R. r. rupicapra* and 450 oocysts from *R. p. ornata* generated dendrograms mostly consistent with the results obtained by morphological identification. Phylogenetic preliminary analysis provided evidences of distinct clades: 27 *Eimeria* cloned sequences derived from *E. yakimoffmatschoulskyi* and *E. rupicaprae* formed two well-supported clades; the other clades included all isolates from other *Eimeria* species from ruminants available in the GenBank.
This study represents a first-step approach towards insights on the differentiating criteria and genetic variations of the coccidia in wild animals.

The study was funded by MIUR- Relevant Italian Project (PRIN n. 2010P7LFW4) - Genomics and host-pathogen interactions in chamois.

MULTIPLEX PCR ASSAY FOR THE IDENTIFICATION OF *HAEMONCHUS CONTORTUS* AND *TELADORSAGIA CIRCUMCINCTA* IN ALPINE CHAMOIS *(RUPICAPRA R. RUPICAPRA)*

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Trichostrongylidae nematodes are among the most important parasites of grazing ruminants affecting domestic animals with economic repercussion and wild species with impact on their population dynamics. *Haemonchus contortus*, known for its pathogenicity, and *Teladorsagia circumcincta* are among the most widespread species. Traditionally these helminths can be identified through examination of abomasal content, since trichostrongyle eggs are undistinguishable under copromicroscopy. In this regard, molecular approaches can provide useful tools based on the amplification of egg-derived DNA for a non-invasive identification of helminths from faeces.
We investigated the abomasal parasite community of alpine chamois (*Rupicapra r. rupicapra*) from Central Italian Alps in order to (i) recover adult male worms of the two target dominant species and (ii) develop a multiplex PCR assay for their simultaneous identification.
Overall 118 abomasa (83 and 35 from, respectively, Orobic and Lepontine Alps) of hunted chamois were gathered during two hunting seasons (2013 and 2014) and underwent standard parasitological examination together with the corresponding faecal sample when available. DNA was extracted from recovered adult male worms with the QIAamp DNA Mini Kit (Qiagen, Italy). Species-specific primers were designed annealing to ITS-2 gene and multiplex PCR assay was set-up.
DNA from 10 adult males of each *H. contortus* and *T. circumcincta*, confirmed as dominant species ($I \geq 1$) in both study areas, was detected separately and in mixed samples.
The designed primers were able to successfully detect and differentiate *H. contortus* and *T. circumcincta* and the corresponding mixed samples, thus supporting this method as potentially reliable to monitor the infections. The developed method is under validation on egg-derived DNA from chamois faecal samples. Once fully validated for the in-field use, this new assay will have potential applicability as a non-invasive and time-saving method suitable for specific parasitological analysis even for protected or endangered species.

The study was funded by MIUR- Relevant Italian Project (PRIN n. 2010P7LFW4) - Genomics and host-pathogen interactions in chamois.

EPIDEMIOLOGY OF PARASITIC DISEASES 2

THE SOCIETAL COST OF *TAENIA SOLIUM* CYSTICERCOSIS IN TANZANIA

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Taenia solium is a zoonotic parasite prevalent in many low income countries, including Tanzania. The parasite is recognised as a public health threat; however the burden it poses on populations of Tanzania is unknown. The aim of this study was to estimate the societal cost of *T. solium* cysticercosis in Tanzania, by assessing both the health and economic burden. The societal cost was assessed in humans and pigs based on data obtained by a systematic review. Experts' opinion was sought in cases where data were not retrievable. The health burden was assessed in terms of annual number of neurocysticercosis (NCC) associated epilepsy incident cases, deaths and Disability-Adjusted Life Years (DALYs), while the economic burden was assessed in terms of direct and indirect costs imposed by NCC-associated epilepsy and potential losses due to porcine cysticercosis. Based on data retrieved from the systematic review and burden assessments, *T. solium* cysticercosis contributed to a significant societal cost for the population. The annual number of NCC-associated epilepsy incident cases and deaths were 17,853 (95% UI, 5,666 - 36,227) and 212 (95% UI, 37 - 612), respectively. More than 11 percent (95% UI, 6.3 - 17) of the pig population was infected with the parasite when using tongue examination as diagnostic method. For the year 2012 the number of DALYs per thousand person-years for NCC-associated epilepsy was 0.7 (95% UI, 0.2 - 1.6). Around five million USD (95% UI, 797,535 - 16,933,477) were spent due to NCC-associated epilepsy and nearly three million USD (95% UI, 1,095,960 - 5,366,038) were potentially lost due to porcine cysticercosis. Our results show that *T. solium* imposes a serious public health, agricultural and economic threat for Tanzania. We urge that a One Health approach is taken to find sustainable solutions for prevention, control and elimination of *T. solium*.

INTEGRATED CONTROL OF *TAENIA SOLIUM*: EFFECT ON TAENIOSIS AND PORCINE CYSTICERCOSIS IN RURAL COMMUNITIES OF TANZANIA

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This study aimed to assess over a four year period the effect of school-based mass drug administration of praziquantel on the prevalence of taeniosis and porcine cysticercosis. *Taenia solium*, the cause of neurocysticercosis, is found throughout sub-Saharan Africa and co-endemic with schistosomiasis in many regions. Praziquantel, effective against a range of trematodes and cestodes, creates the potential for integrated control.

Five cross-sectional surveys, four including humans, were carried out in two endemic districts, Mbozi and Mbeya, in Tanzania from 2012 to 2015. Stool samples were collected from humans and prevalence of taeniosis estimated by copro-Ag-ELISA. Blood samples from pigs were collected to estimate cysticercosis prevalence by Ag-ELISA. School-based mass drug administration of praziquantel was delivered thrice in Mbozi and twice in Mbeya.

More than 12000 human stool samples and 4500 porcine serum samples were collected. Children (≤15) from Mbozi had significant decreased risk of being infected throughout the study compared to children from Mbeya that only showed a significant decrease after the first treatment. Adults in Mbozi had significant decreased risk of infection during the last survey ($p=0.031$, OR 0.40, CI: 0.17-0.89), where also the prevalence of porcine cysticercosis had dropped significantly ($p=0.002$, OR 0.49, CI: 0.32-0.76).

The study showed that a prolonged single approach intervention of repeated mass drug administration targeting a proportion of the definitive hosts not only had an effect on the target population, but spilled over into the intermediate host population and the remaining definitive host population.

EPIDEMIOLOGICAL ROLE OF FARM DOGS IN *ECHINOCOCCUS GRANULOSUS* LIFE CYCLE IN NORTHERN ITALY: PRELIMINARY RESULTS

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Cystic Echinococcosis (CE) is rarely reported in Northern Italy. In 2012, thanks to a project funded by the Veneto Region (DGRV 2221/2010), three clusters of cattle farms positive to CE were identified in Veneto by spatial scan statistic using a Bernoulli probability model.
The present research investigated dogs from cattle and sheep farms located in or nearby the cluster areas in order to identify autochthonous cycles of the parasite. Also shepherd dogs from traditional transhumant sheep farms were included in the survey.
Faecal samples from 157 dogs belonging to 84 farms (70 bovine and 14 sheep farms) were retrieved and subjected to taeniid egg isolation procedure (Davidson et al., 2009 Parasitol Res 104, 509-514) and then observed by inverted microscope. Positive samples were investigated by PCR (Trachsel et al., 2007 Parasitology 134, 911-920), and obtained amplicons were sequenced and blasted with sequences available in Genbank™. *Echinococcus* spp. positive samples were confirmed by the CeNRE, IZS Sardegna.
Totally, 11 samples (7%) were found positive at microscopy and confirmed as taenids by molecular analysis. Among shepherd dogs, the sequencing successfully identified *Echinococcus granulosus* in three dogs and *Taenia hydatigena* in seven, including two co-infested with both species. Among bovine-farm dogs, one was positive to *Taenia serialis* and one to *T. crassiceps*. One sample remained unclassified.
Notwithstanding the present results are still preliminary, the study suggests that also in Northern Italy traditional transhumant sheep breeding and shepherd dogs have an important role in maintaining and spreading *E. granulosus* and other parasite species characterized by a 'dog-sheep' life cycle. Moreover, bovine-farm dogs appear occasionally infested with tapeworm species characterized by 'dog-rodent' or 'dog-lagomorph' life cycles. Further surveys, increasing the number of sampled animals and including different canine populations (i.e. stray dogs), are necessary to confirm our preliminary results.

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BOVINE BESNOITIOSIS: FIRST OUTBREAK IN BEEF CATTLE IN SARDINIA ISLAND

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Besnoitia besnoiti, the causative agent of Bovine Besnoitiosis (BB), was firstly signaled in Italy in 1994 in cattle imported from France, while autochthonous cases were registered in North-Central Italy since 2009. Recently, a serosurvey detected cases of infection in dairy and beef cattle bred in North-eastern Italy, while any case was not found in Sardinia.
Here, an outbreak of BB in a sardinian beef cattle herd (Lula, Nuoro) is described. A Limousine bull 4.5 year-old, imported from France, showed since one year cutaneous lesions imputable to BB and pathognomonic cysts in the scleral conjunctiva. Any other animal in farm did not show clinical sign of BB. A serological screening was carried out to detect antibodies anti-*B. besnoiti* in all the herd using two sequential tests in order to increase sensitivity and specificity: a commercial ELISA (Id Screen® Besnoitia Indirect 2.0) followed by a confirmatory tachyzoite-based Western Blot (WB) performed under non-reducing conditions.
Seropositivity was detected in 22 out of 39 examined animals (56.4%) by ELISA but 17 were confirmed by WB (P=43.5%). Two positive individuals were males, including the bull with clinical signs. All other positive cattle were Limousine imported or born in farm besides a two year-old Sardo-Bruna cow, born and breed in Sardinia.
Our results confirmed the spread of the infection within the herd, probably as a consequence of the introduction of the infected bull from France. Animal trade among countries is thus confirmed as an important risk factor associated to BB. Sanitary authorities are therefore urged to update legislation (i.e. serological control of animals imported from endemic countries) and to set up adequate intervention on sanitary training.

FELINE LUNGWORMS IN EUROPE: RESULTS OF A MULTICENTRE SURVEY

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The occurrence of feline lungworms in Europe has been probably underestimated over the last few years due to diagnostic limitations and to their morphological discrimination at the species level. Along with the metastrongyloid *Aelurostrongylus abstrusus* and the trichuroid *Eucoleus aerophilus*, other nematodes have been identified as causative agents of verminous bronchopneumonia in cats. The reports of *Troglostrongylus* nematodes in 2010-2012 in cats from Spain and Italy has triggered studies leading to the "rediscovery" of other little-know nematode species, including *Oslerus rostratus*, *Troglostrongylus subcrenatus* and *Angiostrongylus chabaudi*, which were originally reported from some Mediterranean islands. However, subsequent reports of *T. brevior* in continental areas of Italy, Greece and, Bosnia and Herzegovina induced researchers to hypothesize a wider area of occurrence and their potential spreading. However, epidemiological data available for feline lungworm infections in Europe are mostly limited to those from Italy and Greece; therefore, information on the distribution of lungworms needs updating, including geographical areas where their occurrence has been poorly investigated. Thus, a multicentre survey on cardiopulmonary nematodes of cats was timely. From March 2015 to February 2016, about 10 cats with a history of regular outdoor access were monthly sampled at sixteen veterinary academic institutions in Europe, along with a complete anamnesis and freshly passed faeces. A quantitative modified Baermann-Wetzel technique and flotation with ZnCl was performed for the detection of metastrongyloid larvae and parasite eggs, respectively. Nematodes were morphologically and molecularly identified at species level. In the present communication, a detailed discussion on the risk factors associated with lungworm infection and parasite distribution in sampled sites will be provided.

SURVEY OF GASTROINTESTINAL AND LUNG PARASITES IN SHELTER CATS FROM NORTHWESTERN PORTUGAL

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Cats may harbour a large variety of parasite species, some of them responsible for important zoonosis, such as *Toxocara* spp. Other non-zoonotic parasites are increasingly reported throughout Europe and may cause illness of major clinical importance, like *Aelurostrongylus abstrusus*. Information regarding internal parasite occurrence in domestic cats from Portugal is scant or outdated. Thereby, an epidemiological survey was conducted in shelters from Braga and Viana do Castelo, two main urban centres in Northwestern Portugal, to study the current prevalence of gastrointestinal and lung parasites.

Between January and March 2016, 205 cat faecal samples were collected and analysed using qualitative coprological techniques: Willis Flotation, Natural Sedimentation, Faecal Smear and Baermann technique. Overall, 63.9% (131/205) were positive for at least one parasitic agent and 20.5% (42/205) showed co-infections. Nematodes were the most prevalent group followed by Protozoa, respectively detected in 50.7% and 13.2% of all the samples tested. The genera/species detected were *Toxocara* spp. with 45.9% (94/205), followed by *A. abstrusus* 22.4% (46/205), *Cystoisospora rivolta* 9.8% (20/205), Ancylostomatidae 5.9% (12/205) and *Cystoisospora felis* 5.4% (11/205).

This survey revealed a high prevalence level of parasitism among the cat population of Northwestern Portugal, namely by *Toxocara* spp., one of the most important zoonotic parasitic agents transmitted from companion animals to man. This high prevalence might be explained by the overcrowding conditions in shelters and lack of funding for regular prophylactic measures. In addition, the lungworm *A. abstrusus* was highly prevalent probably due to the Atlantic wet climate conditions (moderate temperatures and relative high humidity), that favours the development of its intermediate hosts. Cats adopted from shelters with asymptomatic or untreated parasitic infections pose ongoing risks for animal and human health. Data from this study highlight the urgent necessity to adopt strategies to prevent and control parasitic agents in domestic cats from this region.

HELMINTIC INFECTIONS IN ITALIAN DONKEYS: A NATIONWIDE SURVEY

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The donkey was traditionally used as working animal for transport and farm activities. Recently, in some European countries, including Italy, there is an increasing interest on donkeys due to their use as pet, for onotherapy and for milk production. Because there are few data regarding the donkey's parasitic diseases, a survey was conducted to determine the prevalence of the principal helmintic infections in 77 donkey farms in Italy. Between March 2012-2016, faecal samples were collected from 1,775 donkeys. Contextually to the faecal collection a questionnaire on management practices and parasite control was performed. Each sample was examined using a modified McMaster technique; in addition a centrifugation/flotation technique (Proudman test) and a sedimentation technique were used for the diagnosis of Anoplocephalidae and *Fasciola hepatica*, respectively. On each farm pooled coprocoltures were performed. The questionnaire results were correlated with results from the faecal examination to perform risk analysis via univariate regression analysis.
Strongyles were the most common parasites found, with a prevalence of 84.9%; 15.1% of donkeys were not infected and strongyle egg count was lower than the donkey cut-off selective therapy (300 eggs per gram) in 28.3% of animals. In all tested farms, coprocoltures revealed the presence of Cyathostomes (100%) followed by *Strongylus vulgaris* (31%), *Poteriostomum* spp. (25%), *Triodontophorus* spp. (9%), *S. edentatus* (7%) and *S. equinus* (5%). Other parasites were: *Dictyocaulus arnfieldi* (6.9%), *Oxyuris equi* (5.8%), *Parascaris* spp. (3.6%), *Anoplocephala* spp. (1.0%) and *Strongyloides westeri* (0.3%). Any positivity was detected for *F. hepatica*. The mean number of treatments/year was 1.4 (range 0-4). The majority of donkeys (63%) were dewormed using ivermectin. The rate of parasitic infection, particularly regarding *S. vulgaris,* in donkeys was higher than in horses. The risk factors analysis showed that grazing management significantly impact onto the presence of *Strongyles* infection as well as to the high patterns of excretion (FEC> 300 upg).

A LONGITUDINAL STUDY ON ENDOPARASITES IN GRAZING EQUIDS IN AN ALPINE ENVIRONMENT (NORTHERN ITALY)

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In recent years grazing of equids in alpine pastures became an increasing practice due to local legislation protecting equids in alpine environment (L.R. 31/2008 art. 24 bis). Aim of the study was to evaluate endoparasitic infection in grazing horses and donkeys on alpine pastures (Lombardy, northern Italy).
Fecal samples from horses (n=36) and donkeys (n=31) were collected at the beginning (May, BG) and at the end of the grazing season (end of October, EG) in 2014. In October also foals (n=11) born during grazing season were sampled. Faeces were analyzed by FLOTAC dual technique (FS2, s.g.= 1200; FS7, s.g.=1350). A data questionnaire was filled for each equids. Intrinsic and extrinsic factors predictors of parasitism were evaluated using Generalized Estimating Equations (GEE) (SPSS 20.0).
Equids were mostly infected by gastrointestinal strongyles (P=97% and 100% at BG and EG respectively); egg counts increase after grazing season (A=429 and 517 at BG and EG respectively). Equids had higher strongyles EPG excretion when were females (OR:1.471), less recently treated with anthelmintic drugs (OR:1.007) and grazing on southern slope (OR:2.508). Horses sharing pastures with donkeys showed lower EPG than horses reared alone (OR:0.649). The other parasites found *Parascaris equorum, Strongyloides westeri, Oxyuris equi, Dictyocauls arnfieldi* and *Anoplocephala* sp. showed an increase in prevalence at EG. Particularly, *Anoplocephala* eggs were absent before grazing and were exclusively detected in horses (P= 26.9% at EG). *O. equi* (P=7.5%) and *D. arnfieldi* (P=11.9%) were only detected in donkeys. Prevalence and EPG/LPG of *P. equorum, S. westeri* and *D. arnfieldi* were higher in foals when tested by chi-square and one-way ANOVA.
Endoparasites of grazing equids in the study area can be influenced by intrinsic and extrinsic risk factors; furthermore, donkeys didn't represent the main risk for horses. The results of this study may improve deworming strategies in grazing equids.

BROAD SCREENING ON PATHOGENS AND PESTICIDES OF HONEY BEE COLONIES *(APIS MELLIFERA)* IN GHANA (WEST AFRICA)

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Pollinators are essential contributors to global nutrition and food security.The productivity of many high value crops grown in the southern hemisphere is strongly tied to pollination services, being the honey bees one of the most important pollinators wordwide. The recent emergence of honey bee colony losses in many parts of the world have drawn much attention to elucidate the underlying causes, as their loss would seriously impact the world agriculture production for human consumption.

While very few surveys have been undertaken in West African countries, available data shows less vulnerability of African honey bee races to common diseases. However, prior to get deeper knowledge on their vulnerability, there is the need to know which pathogens are affecting them. With the aim to know about the local honey bee health status, samples from brood, adults and colony products from 45 colonies located in nineteen different locations all over Ghana were obtained during the summer of 2013.

Varroa detection was performed *in situ* and evidences of other pests noted on. Samples were taken to Spanish laboratories and analysed by molecular techniques to detect pathogens and determine the honey bee race and the Varroa haplotype using previously published methods. Wax samples were analysed by QuEChERS to detect pesticides.

Results show the presence and wide distribution of uniquely *Varroa destructor* korea haplotype (Genbank AF106899). The mean prevalence in brood was 15.33% and 0.92% in adult honey bees. *Aethina tumida,* the small hive beetle, was found in almost 50% of sampled colonies. Molecular diagnosis did not detect any *Nosema* species in samples of adult bees while demonstrated the presence of *Melissococcus plutonius* in four of them. No clinical signs of any disease were observed. Wax showed low levels of Amitraz, Chlorfenvinphos, Chlorpyrifos, Coumaphos, Fluvalinate and Thiabendazole.

CROSS-SECTIONAL SURVEY ON *TRITRICHOMONAS FOETUS* INFECTION IN ITALIAN CATS

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Tritrichomonas foetus (Trichomonadida, Tritrichomonadidae) is a flagellated protozoan parasite commonly regarded as a worldwide venereal pathogen of cattle. Also, it has recently been recognized as a cause of large-bowel diarrhoea in domestic cats in many countries (Gookin et al. 1999; Levy et al. 2003). In Italy, only few studies have investigated *T. foetus* infection in cat populations (Holliday et al. 2009; Mancianti et al. 2015) and no large-scale epidemiological studies have been conducted so far. Therefore, the aim of the present study was to carry a large-scale epidemiologic survey in cat populations across Italy.
Freshly individual faecal samples were collected from 267 cats kept in different environments (private household, breeding structures, catteries and colonies) from three different geographical districts of Italy, i.e North-eastern (Site 1, n=114 cats), Central (Site 2, n=90 cats) and Southern (Site 3, n=63 cats). Faecal samples were tested for the detection of *T. foetus* by a PCR protocol described by Gookin et al. (2002). The same samples were examined by copro-microscopic techniques for the detection of further enteric parasites and by a commercially available Direct Immunofluorescence Assay (DFA) (MeriFluor®) for the recovering of *Giardia duodenalis*.
The overall prevalence of *T. foetus* infection was 5.2%; all the infected cats showed diarrhoea at the sampling time, and 9 out of 14 positive cats resulted to be co-infected with *G. duodenalis*, 1 with *Toxocara cati* and 3 with *Dipylidium caninum*.
The risk factor analysis showed that breed, *G. duodenalis* and *D. caninum* infections were significantly associated with the presence of *T. foetus*. This study confirms the occurrence of *T. foetus* in cats living in Italy, suggesting that it should always be included in the differential diagnosis of patients referred with large-bowel disease symptoms especially if they are purebred animals and/or infected with other enteric protozoa such as *G. duodenalis.*

MOLECULAR DETECTION OF *GIARDIA DUODENALIS* AND *CRYPTOSPORIDIUM* SPP. IN CANINE FAECAL SAMPLES COLLECTED IN PUBLIC AREAS OF THE CITY OF PADOVA

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Giardia duodenalis and *Cryptosporidium* spp. are common intestinal pathogens in humans and animals; their occurrence in dogs is of potential significance from both veterinary and public health perspectives.

The aims were to evaluate the prevalence of *Giardia* and *Cryptosporidium* in canine faeces contaminating green (parks and playgrounds) and urban (streets and sidewalk) areas of Padua by molecular methods, characterizing the isolates, and to analyse potential risks for canine and human health.

A total of 705 canine faeces were collected in green (n=270) and urban (n=435) areas of Padua. Samples were processed by duplex Real Time PCR for detecting simultaneously both protozoa. Due to a possible effect of *Giardia* as inhibiting factor for *Cryptosporidium* detection, *Giardia* positive samples were tested by Real Time PCR SYBR® Green I to highlight *Cryptosporidium*. Positive samples were submitted to Nested PCR targeting β-giardin and/or SSU-rRNA genes for *Giardia* and SSU-rRNA gene for *Cryptosporidium*. Amplicons were sequenced and sequences were aligned with those available in Genbank™.

Giardia prevalence was 28.9% (n=204/705), with 16.3% (n=44/270) and 36.8% (n=160/435) of positive samples in green and urban areas, respectively. Among 44 Nested PCR positive samples, 21 were characterised as dog-specific assemblages C (n=11) and D (n=10), and 1 was identified as human-specific assemblage B. *Cryptosporidium* spp. was detected in 12/705 (1.7%) samples, 1/270 (0.4%) in green and 11/435 (2.5%) in urban areas. One isolate was identified as *C. canis*, the others were confirmed as *Cryptosporidium* spp..

The wide distribution of *Giardia* suggests a high risk of infection for dogs attending the urban areas. Conversely, *Cryptosporidium* prevalence was very low as already reported in the Italian context. Although data indicate a limited risk for human health, it is necessary to improve people's education to reduce canine faecal pollution towards a widespread awareness of health risks related to pet-animals.

GENE EXPRESSION PROFILING OF CHEMOSENSORY APPENDAGES IN THE TIGER MOSQUITO *AEDES ALBOPICTUS*

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The Asian tiger mosquito, *Aedes albopictus*, is a highly successful invasive species able to transmit several arboviruses (e.g. dengue, West Nile, chikungunya, Zika) as well as other parasites (e.g. *Dirofilaria*) of public health importance. Host seeking behaviour in mosquitoes relies on a combination of sensory functions (vision, chemosensation, thermosensation, mechanosensation, hygrosensation) that are carried out by specialized appendages mainly located on the head and comprising the compound eyes, antennae, mouthparts and maxillary palps. Compared to other mosquitoes, *Ae. albopictus* females exhibit several peculiarities, with very aggressive and generalist host seeking and severe biting behaviours that are also responsible for its high degree of nuisance. With the aim to characterize the *Ae. albopictus* olfactory repertoire, and possibly shed some light on the feeding-related behavioural peculiarity of this mosquito, we started a transcriptomic analysis of its main adult chemosensory appendages.
We used an RNA-seq approach for gene expression profiling of female antennae, female maxillary palps, male heads and female whole bodies. A comprehensive transcriptome of 33846 contigs was created by a combination of *de novo* assembly and comparison to genome-based peptide predictions (the first drafts of the tiger mosquito genome were recently released). The relative gene expression within each tissue (RPKM, Reads Per Kilobase per Million) and the pairwise differential expression in the different tissues (Fold Change values and False Discovery Rates) were evaluated. Comparative analysis allowed to identify and annotate around one hundred and fifty genes belonging to the four main sensory families: Odorant Binding Proteins (OBP), Odorant Receptors (OR), Ionotropic Receptors (IR) and Gustatory Receptors (GR). In addition, orthologues of genes expressed in the female/male maxillary palps and/or antennae and involved in thermosensation (e.g. *pyrexia* and *arrestin1*), mechanosensation (e.g. *piezo* and *painless*) and neuromodulation were identified.

RAPID IDENTIFICATION OF INVASIVE MOSQUITO SPECIES USING MALDI-TOF MS

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The identification of mosquitoes at species level by morphology requires an experienced entomologist and well conserved specimens, but can be difficult with sibling species. Genetic identification by PCR has been proved to be a reliable tool and was established for some species, but is expensive and time consuming. The protein profiling by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been recently reported as a cheap and quick method for the identification of different arthropods.
The aim of this study was to establish a reference spectra database of invasive *Aedes* mosquitoes using different body parts. Protein profiles were generated using legs and heads of three invasive species occurring in North-East Italy (*Ae. albopictus, Ae. japonicus* and *Ae. koreicus*) and of the exotic species *Ae. aegypti*. Female and male mosquitoes either field-collected or from laboratory colonies were used to create a database of reference spectra. Flex control, Flex analysis software 3.3, and MALDI-Biotyper v3.0 software (Bruker Daltonic) were used for acquisition, assessment of reproducibility, and analysis of protein mass spectra profiles.
Legs and heads of mosquitoes yielded different spectra, therefore, two libraries were created. MALDI-TOF MS analyses showed spectra with peaks of high intensities in the range of 2-20KDa. Using these libraries, all the four species were correctly identified with good score values >2.3.
MALDI-TOF MS is an alternative, low-cost, high throughput tool for rapid identification of mosquito species. The reference databases herein described can be complemented with other mosquito species and can also be transferred and directly used by any laboratory equipped with the same MALDI system.

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MOLECULAR QUANTIFICATION OF MALE AND FEMALE *PLASMODIUM FALCIPARUM* GAMETOCYTES IN HUMAN POPULATIONS

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The human malaria parasite *Plasmodium falciparum* is responsible for 200-300 million clinical cases and 400000 deaths annually (WHO, 2015). The presence of *P. falciparum* gametocytes in peripheral blood is essential for human to mosquito malaria transmission. The detection and quantification of gametocytes as well as the estimation of the sex-ratio are essential to assess the human host ability to infect mosquitoes, and comprehensively evaluate malaria transmission risk.
The sensitivity of microscopic examination of blood slides is below the minimum density required for mosquito infection.
The aim of our work is therefore to develop sensitive and cheap Real Time qPCR assays for large-scale epidemiological surveys, based on the quantification of gametocyte-specific transcripts.
The selected target genes are Pfs25 expressed by females, and Pfs230p expressed by males.
RT-qPCR methods based on SYBR Green were developed for all targets; for the Pfs25 target the efficiency and sensitivity was compared with a TaqMan assay (Schneider *et al*., 2015).
RNA was extracted from blood samples collected in a rural village of Burkina Faso. To overcome the inherent variability of RNA and different reverse transcription and PCR efficiencies, the quantification obtained for each target were normalized by an human housekeeping gene (18S).
Preliminary results obtained for 50 samples showed that, as expected, all these methods are more sensitive than microscopic examinations. The normalization by 18S quantity, improved the correlation between gametocytes density estimated by microscopy and qPCR (*correlation coefficient* 0.13 *vs* 0.42). Pfs25 SYBR Green Assay seems to be more sensitive than Pfs25 TaqMan Assay (*limit of quantification* 10 copies/µl *vs* 100copies/µl).
Ongoing work is aimed at the conversion of transcript copies/µl into gametocytes/µl and at sex-ratio determination.

THE SUBTILISIN-LIKE PROTEASE SUB1 HAS A ROLE IN *PLASMODIUM BERGHEI* MALE GAMETE EGRESS FROM THE HOST CELL

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SUB1 is a *Plasmodium* serine protease expressed in liver and blood schizonts and secreted into the parasitophorous vacuole from parasite secretory organelles just prior to egress. Proteolytic activity of Sub1 is thought essential for egress of the invasive stages, the merozoites, both from erythrocytes and infected hepatocytes.
We have now shown that SUB1 is expressed in *Plasmodium berghei* gametocytes and resides in specialized male-gametocyte-specific secretory organelles called Male Osmiophilic Bodies (MOBs), shown to be involved in gamete egress from the host cell. Upon gametocyte activation, SUB1 is discharged into the parasitophorous vacuole.
We also investigated SUB1 function in *P. berghei* sexual stages by generating a transgenic line in which the *sub1* endogenous promoter was substituted with the promoter of AMA1, a gene only expressed in asexual stages. In this transgenic line, SUB1 expression is specifically abolished in gametocytes. Analysis of this transgenic line showed that male gamete egress is significantly reduced.
SUB1 is synthesized as a pre-protein that undergoes maturation by autocatalytic processing. The released prodomain is a potent and selective inhibitor of SUB1 activity. To determine whether SUB1 proteolytic activity is essential for its function in gametocytes, we produced a second transgenic line expressing an extra copy of the SUB1 prodomain under the control of a gametocyte-specific promoter. Analysis of this transgenic line showed that SUB1 activity is strongly impaired in male gametocytes, leading to a milder phenotype compared to that caused by complete absence of the protease.
In summary, our results demonstrate that SUB1 is expressed in *P. berghei* male gametocytes, localizes to male osmiophilic bodies (MOBs) and plays an important role in male gamete egress from the host cell.

GENETIC HETEROGENEITY AND PHYLOGENY OF *TRICHURIS* SPP. FROM NON-HUMAN PRIMATES BASED ON MITOCHONDRIAL DNA

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Trichuriasis is a Neglected Tropical Disease widespread in tropical and sub-tropical regions and one of major groups of soil-transmitted helminthiases. Regarding species infecting primates, recent evidences based on molecular and phylogenetic investigations on nuclear and mitochondrial markers suggest the existence of cryptic species associated to human and non-human primates (NHP) (Callejon R *et al.,* 2013 Parasitol Res 112:3933; Liu GH *et al.,* 2013 PLoSONE 8, E66249; Cavallero S *et al*., 2015 Infect Genet Evol 34:450; Hawash MB *et al.,* 2015 PLoSNTD 9:e0004059; Hawash MB *et al*., 2016 Parasit Vectors 9:37). The present study was aimed to investigate the evolutionary relationship between *Trichuris* spp. from *Macaca fuscata* (18 isolates) and *Chlorocebus aethiops* (12 isolates) using the mitochondrial region *cox*1 as molecular marker, and to compare data with sequences from human, NHP and pig from GenBank. Phylogeny was inferred using distance based method of Neighbor Joining (MEGA6) and Bayesian method (MrBayes).
Preliminary results from the analysis of *cox*1 (420bp) confirm previous evidences from the nuclear ribosomal ITS (Cavallero S *et al*., 2015 Infect Genet Evol 34:450) parasites from Japanese macaques and grivets form two highly supported clades. Other three clades are strongly supported: one includes *T. suis* from pigs, one *Trichuris* spp. from *Colobus guereza* and one with isolates from human, macaque and baboons, including *Trichuris trichiura*.
No clear monophyly either of human-derived and of NHP derived *Trichuris* has been detected, in agreement with a recent study on the origins and demography of whipworms in humans and pigs (Hawash MB *et al.,* 2016 Parasit Vectors 9:37). *T. trichiura* could be rather represented by a complex of cryptic species with a certain degree of host preference. Further investigations on wider samples are needed to clarify species delimitation and host affiliation.

PHYLOGENETIC ANALYSIS OF *CULICOIDES* SPECIES VECTORS OF BLUETONGUE IN SICILY

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Culicoides species (Diptera: Ceratopogonidae) are vectors of pathogens with public health significance, including bluetongue virus (BTV) and Schmallenberg virus. The increase of Bluetongue incidence in the Mediterranean is related to the abundance of competent vector species. For this reason, studies of *Culicoides* populations are of great relevance.
This study was aimed to the phylogenetic analysis of Sicilian *Culicoides* species.
The analysis concerned 66 *Culicoides* specimens collected in all the Sicilian provinces in 2014 using Blacklight traps and belonging to *C. obsoletus* complex, *C. pulicaris pulicaris, C. newsteadi, C. imicola.* Morphological identification of the species was based on wing pattern. DNA was extracted from single midges, quantified and amplified by a PCR targeting the *Mitochondrial COI* gene. PCRs products were sequenced. Sequence analysis allowed to integrate data from morphological analysis and to carry out a phylogenetic study. References sequences were obtained from GenBank.
Members of *C. obsoletus* complex were identified at the molecular analysis as *C. obsoletus* s.s. and *C. scoticus*. In the phylogenetic tree, sequences of *C. obsoletus* complex, including reference sequences, formed a cluster that didn't include *C. dewulfi* reference sequences. All *C. imicola* sequences from Sicilian samples were close to each other and to the reference sequences.
C. newsteadi sequences were close together with the exception of samples from Ragusa, more closely related to *C. impunctatus* reference sequences. Even *C. newsteadi* sequences from Syracuse samples represented an exception appearing phylogenetically closer to *C. lupicaris* references sequences and to *C. pulicaris* s.s., whose sequences, obtained both from Sicilian samples and reference strains, were all close together.
This study describes for the first time a phylogenetic analysis on Sicilian *Culicoides* specimens and highlights the usefulness of molecular and phylogenetic analyses in studies of *Culicoides* populations.

COMPLETE GENOME SEQUENCING AND PHYLOGENETIC ANALYSIS OF WEST NILE VIRUS STRAINS FROM *CULEX PIPIENS,* NORTH- EASTERN ITALY, 2010-2015

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West Nile virus (WNV) is a mosquito-borne flavivirus responsible for an increasing number of epidemic outbreaks in Europe. In particular, North-eastern Italy experienced human and animal outbreaks caused by different genovariants of WNV. During the entomological surveillance in Veneto and Friuli Venezia Giulia regions in 2010-2015, we analyzed a total of 17,018 pools of *Culex pipiens* and found several strains within WNV lineage 1 (WN1), and WNV lineage 2 of the Hungarian (WN2-H) and Volgograd clades (WN2-V), thus highlighting the high complexity of WNV circulation in the area.

In this study, we sequenced the complete genome of WN2-H and WN2-V strains in order to determine their phylogenetic relationships with other strains previously isolated in vectors and hosts in Italy and Europe. The complete genomes were amplified using published and new designed primer. The WN2-H strain showed the highest similarity (99.9% nucleotide identity) to the Italian strain (KP789954), which was detected in a patient from Lombardy Region in 2014, and 0.5% of difference with the Hungary/04 strain (DQ116961), which was isolated in 2004 from goshawk in Hungary and is considered the ancestor of the WN2-H clade. WN2-V showed a 0.7% of dissimilarity to the Russian strain Volgograd (FJ425721) isolated in 2007. Our sequences differ from each other by 4.1%. Amino acid analysis of the NS3 protein revealed a $T_{334}S$ substitution in the WN2-V strain, while neither WN2-H nor WN2-V strains carried the $H_{249}P$ variant, which has been associated with increased pathogenicity in some bird species. In conclusion, complete genome sequencing is useful to understand the epidemiology and evolution of WNV and to monitor the emergence of highly pathogenic strains. Also, complete genome and phylogenetic analysis allow to discriminate between endemic strains and novel introductions, helping to unravel the epidemiology of WNV in a territory.

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EXPERIMENTAL *TOXOPLASMA GONDII* INFECTIONS IN PIGS AND VERTICAL TRANSMISSION

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In order to study the vertical transmission of *Toxoplasma gondii* in pigs, experimental inoculations of 5 sows at 79-85 days of pregnancy (end-pregnancy) and 3 sows at 54-57 days of pregnancy (mid-pregnancy) were performed with 10,000 *T. gondii* (CZ Tiger clone H3, Type II) oocysts orally. Three sows inoculated at late pregnancy were re-inoculated with 100,000 oocysts in a further pregnancy (75-78 days) together with 2 new seronegative sows. All 10 sows seroconverted (PrioCHECK Toxoplasma Ab porcine ELISA, Prionics, Switzerland) by 2-3 weeks post inoculation and remained seropositive for at least 38 weeks or until euthanasia. IgG levels increased significantly in all 3 re-inoculated sows within 1-2 weeks. All sows remained asymptomatic and delivered at term. The infection status of 2 out of 5 euthanized sows could be additionally confirmed by bioassay in Swiss Webster mice. Mice inoculated with heart tissues from both sows seroconverted and *T. gondii* DNA could be detected in their brains by real-time PCR. Piglets (*n*= 150) born from all 10 inoculated sows were serologically tested before colostrum ingestion and all were seronegative. Placentas and tissues (brain, liver, lung, heart, and masseter muscle) from 56 piglets euthanized or crushed by the dam during the first days of life were analyzed histopathologically and by real-time PCR for *T. gondii* with negative results. In addition, no seroconversion was observed in 14 piglets from seronegative dams that were transferred to infected dams one day after birth to detect a possible infection through colostrum or milk. Maternal antibodies were detected in some piglets until 2 months of birth but disappeared at 3 months of age. Next steps of this study would include the infection of sows with different *T. gondii* isolates, that could be more virulent, or with different parasite stages. Although vertical transmission of *T. gondii* was demonstrated in naturally infected pigs, many factors involved in the outcome of the infection are still unknown.

DIFFERENTIAL SUSCEPTIBILITY OF BOVINE CARUNCULAR AND TROPHOBLAST CELL LINES TO HIGH/LOW VIRULENCE ISOLATES OF *NEOSPORA CANINUM*

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Neospora caninum is considered to be one of the main causes of abortion in cattle. Tachyzoites are very effective at crossing the placental barrier and placental damage is key in the pathogenesis of abortion. Bovine trophoblast and caruncular cell layers constitute maternal-foetal interface in placentomes, playing a fundamental role in maintenance and immune regulation during pregnancy. Herein, we investigated tachyzoite invasion, growth kinetics and modulation of immune response elements (IFN-γ, TNF-α, TGF-β1, IL-12p40, IL-6 and IL-12 cytokines, TLR2, ICAM and VCAM) by high (Nc-Spain 7) and low (Nc-Spain 1H) virulence isolates, in primary cultures of bovine caruncular epithelial (BCEC-1) and trophoblast (F3) cells. The invasion and infection rates for the Nc-Spain7 isolate, determined at different time-points by a double inmunostaining plaque assay, were higher than for the low virulent isolate in both cell lines ($P < 0.05$). In addition, invasion was significantly disminished in BCEC-1 cells for both isolates. Tachyzoite growth kinetics along the lytic cycle determined by qPCR also showed tachyzoite exponential growth until egress at 58 hpi for both parasites in F3, with a higher doubling time (Td) for the Nc-Spain1H isolate. Interestingly, Nc-Spain1H showed a non-exponential growth pattern and earlier egress of tachyzoites in BCEC-1. Significant changes by RT-qPCR in the IL-6, IL-12p40 and TLR-2 RNA levels were observed in infected F3 and ICAM, TGF-β1 and IL-8 RNA levels in infected BCEC-1, regardless the isolate. These findings confirm the ability of *N. caninum* to proliferate in placental target cells and demonstrate the local immunomodulation that takes place. Different proliferation abilities were also confirmed in these target cells by the low and high virulence isolates. Remarkably, limited parasite growth in caruncular cells suggests a putative barrier function for this cell type in placenta, although parasite transmission should not be compromised due to the early egress of viable tachyzoites.

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♯Equal contribution

SEVERE SEIZURES IN PIGS NATURALLY INFECTED WITH *TAENIA SOLIUM* IN TANZANIA

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Neurocysticercosis (NCC) caused by *Taenia solium* is a serious neurological disease. In humans neurological symptoms have been thoroughly studied and documented, however, there is limited information on clinical signs in pigs infected with *T. solium* cysticerci. Among the scientific community, it is in fact believed that pigs with NCC rarely show neurological signs. The aim of this study was to describe clinical manifestations associated with NCC in pigs and correlate the manifestations to the number and distribution of cysticerci in brains of naturally infected pigs in Tanzania. Sixteen infected and 15 non-infected control pigs were observed for 14 days during daylight hours, and subsequently videotaped for another 14 consecutive days using close circuit television cameras. All occurrences of abnormal behaviour (trembling, twitching, mouth and ear paralysis, ataxia, dribbling, salivating, eye blinking, walking in circles) were recorded. At the end of the recording period, pigs were slaughtered and their brains dissected, cysticerci counted and locations noted. During the recording period, two infected pigs were observed having seizures. Some of the observed autonomic signs during a seizure were chewing motions with foamy salivation and ear stiffening. Motor signs included tonic muscle contractions followed by a sudden diminution in all muscle function leading to collapse of the animal. Stereotypic walking in circles was observed on several occasions. At dissection, both pigs had a high number of brain cysticerci (241 and 247 cysticerci). The two pigs with seizures were also older (36 months) compared to the others (18.3 months, ± 8.2 standard deviation). Results of this study have shown that pigs with NCC can develop clinical signs and suffer from seizures like humans with symptomatic NCC. Results of this study could potentially open up a new experimental pathway to explore the aetiology of neurological symptoms in humans with NCC associated epilepsy.

NEW FINDINGS ON DICROCOELIOSIS

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Dicrocoeliosis, caused by *Dicrocoelium dendriticum*, is one of the helminth infections most widely spread in small ruminants with high prevalence values (up to 67%) in Italy. To improve our knowledge about this disease and its pathogenesis we performed *in vivo* and *in vitro* studies. The relationship between the number of eggs per gram of feces (EPG) assessed by the FLOTAC technique, the burden of *D. dendriticum* and macroscopic changes of the liver was evaluated in 16 sheep naturally infected by dicrocoeliosis. The findings of this study showed a positive correlation between these three parameters. Interestingly, the significant linkage between EPG and macroscopic lesions highlights the importance of using high sensitive methods as FLOTAC for the estimation of intensity of this parasite and consequently as an indirect index of liver damage.
Furthermore, to clarify the complex mechanisms that regulate host-parasite interaction, studies *in vitro* were performed using somatic antigens of *D. dendriticum* isolated from the livers of the infected sheep reported above. In particular, the effects of *D. dendriticum* on cell proliferation, cell death mechanisms and oxidative stress induction were evaluated in human hepatocyte cell lines (HepG2 and HuH7). Results showed that short exposure time and low concentrations of the *D. dendriticum* somatic antigens induced a slight induction of cell proliferation while prolonged and high concentration caused a significant growth inhibition in both cell lines. This effect occurred with a 40% increase of the formation of autophagic vacuoles. A strong oxidative stress was also recorded with a 100% increase of the intracellular O2. These data are in agreement with previous results reported on the correlation between parasitic infections and activation of defence mechanisms by the host resulting in the generation of Radical Oxygen Species (ROS).

PRELIMINARY DATA ON CYSTIC ECHINOCOCCOSIS IN DAIRY CATTLE INTENSIVE BREEDINGS OF SARDINIA, ITALY

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A survey to determine the spreading of Cystic Echinococcosis (CE) was carried out after the incidental finding of this parasitosis in dairy milk cattle in the municipality of Arborea (OR) in Sardinia. The survey was carried out monitoring CE diffusion of cattle raised in Arborea (OR) between 2012 and 2015 through the analysis of 23,656 veterinary reporting forms (post mortem inspection visit) in 10 different Italian slaughterhouses. CE was found in 21.9% of monitored breedings (35/160), while 0.05% of adult slaughtered animals (>1 year) was infected by the parasite (13/23,656). Positive reports stratified per year showed the following rates: 0.09% in 2012 (5/5,673), 0.02% in 2013 (1/5,682), 0.08% in 2014 (5/6,261), 0.03% in 2015 (2/6,040).
In positive cattle, the metacestode was found in 71.1% (37/52) in the liver, in 55.8% (29/52) in the lungs and in 1.9% (1/52) in the kidneys. No significative difference was observed between the prevalence rates found in the liver and in the lungs (χ^2=2.65; P=0.103).
The prevalence rates for CE found in the Arborea region, even not high as in other Sardinian district, should be taken into serious account. The intensive farms monitored in Arborea region, a sort of enclave of 100 km^2, are intensive breedings with no dogs, no access to pasture, no direct contact with sheep and/or sheep breedings. However, all around this district, in some sheep breedings CE is unfortunately widespread as in almost 75% of Sardinian sheep. The diffusion of CE in about 20% of cattle farms of Arborea should be explained with the movement of shepard dogs or stray dogs coming from the surrounding endemic areas and even causing low prevalences in cattle, it's an alarming signal of high environment contamination that should taken into account not only for animals but also for human public health.

THE FOURTH STATE OF MATTER AND ITS BIOCIDAL EFFECT: PLASMA VS COCCIDIA

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Eimeria spp. are obligate intracellular parasites, the oocysts are their environmental resistance forms. The oocysts wall is extremely robust, it is resistant to mechanical and chemical damage. In this regard *Eimeria* spp. can serve as the prototype of pathogen elements with a very high resistance similar, in this aspect, to *Toxoplasma gondii* and *Cryptosporidium parvum* important zoonotic agents. Therefore, the occasion to test a non-chemical agent appear to be an interesting interdisciplinary opportunity. Dielectric Barrier Discharge (DBD) is a non-thermal plasma, that could be also used for sanitization purpose. The synergy of energetic electrons and ions, reactive species (such as atomic oxygen, ozone and nitric oxides and hydrogen peroxide), UV photons and intense electric fields is a key point in non-thermal plasma sanitization treatment. The efficacy of other kinds of plasma technology has already been demonstrated against bacteria, spores, virus, yeasts/moulds and parasites.
Petri dishes filled with 20 ml of water containing *Eimeria* spp. oocysts have been subjected to different DBD direct treatments and exposure times. After the treatment, the efficacy has been evaluated by the sporulation capacity of coccidia compared with untreated specimen. The number of sporulated and damaged oocysts has been counted considering a representative population of 100 oocysts both in control and in treated samples. Preliminary results have shown a statistically significant decrease of sporulated oocysts with long exposure times and high discharge voltages. Absence of possible resistance phenomena and pollution linked to the perspective of a good devitalization level is an incentive to go on in this way.

THERAPY AND DRUG RESISTANCE 2

RATIONAL DRUG USE AGAINST AFRICAN ANIMAL TRYPANOSOMOSIS

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Development of trypanocide resistance depends on a multi-factorial process driven by (i) trypanocidal drug use practices, (ii) quality of trypanocidal drugs, (iii) ability to detect resistance and (iv) availability of strategies minimizing and controlling resistance at the livestock keeper level, particularly smallholders. The TRYRAC[+] project addresses each of these factors through improving the capacity and capability of African laboratories and veterinary services to detect trypanocide resistance, to conduct quality control of trypanocidal drugs and to promote and monitor the use of best-bet control strategies aiming at a rational drug use approach.
Cross-sectional surveys were conducted to assess the trypanosome infection prevalence in randomly selected village cattle herds in the provinces of Kara and Savanes of northern Togo and in the Gurage zone of Ethiopia. Thereafter, drug sensitivity studies were carried out in hot spot areas (trypanosome prevalence > 10%). Based on these results, best-bet integrated strategies consisting of (i) rational use of quality tested trypanocidal drugs in symptomatic cattle, (ii) targeted insecticidal spraying of the lower body parts of cattle with a deltamethrin formulation and (iii) strategic use of anthelminthic drugs (albendazole) were implemented in risk group animals (calves < 2 years). Anthelmintic treatments took place at the beginning and end of the rain season.
Single and multiple drug resistance against the therapeutic drug diminazene aceturate and the prophylactic drug isometamidium chloride at the recommended dosages were detected with treatment failure rates varying between 0 to 50%. Up to 53.6% of the trypanocidal drugs from Togo and 34% from Ethiopia, respectively, did not conform to quality standards after analysis by HPLC. Rational use of trypanocidal drugs in symptomatic animals, combined with targeted insecticidal spraying and strategic deworming, resulted in much lower numbers of trypanocidal treatments, compared to control herds, whereby the animals remained in good health.
In the absence of new trypanocidal drugs, rational use of the available drugs will be an appropriate strategy minimizing the risk of drug resistance development.

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PREVALENCE OF INSECTICIDE RESISTANCE IN HOUSE FLIES (*MUSCA DOMESTICA*) ON PIG FARMS IN THE FEDERAL STATE OF SCHLESWIG-HOLSTEIN, GERMANY

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Disturbance by house flies (*Musca domestica*) is considered a major economic problem in animal husbandry. Therefore, fly control constitutes an integral part of livestock management. However, non-strategic use of insecticides might lead to rapid buildup of insecticide resistance (IR).
The susceptibility of *M. domestica* against deltamethrin (DTM) was assessed on 40 farms using the FlyBox®-test (280mg DTM/m^2). The field populations were further tested by a feeding assay against neonicotinoids (thiamethoxam, imidacloprid) and azamethiphos (phosphoric ester). Insect growth regulators (cyromazine, triflumuron) were additionally evaluated. Subsequently, 17 fly populations were lab-reared and their offspring exposed to topical applications of pyrethrum, DTM, azamethiphos, thiamethoxam and imidacloprid.
Resistance (mortality rates ≤ 90%) to DTM were observed in 85% of the fly populations. When exposed to thiamethoxam, 13 populations (33%) revealed moderate resistance (mortality rate 40-89%). Only 4 populations (10%) displayed 100% paralysis when imidacloprid was applied. Ten strains (25%) were sensitive against azamethiphos, the majority (75%) showing a moderate degree (≤ 90%) of resistance. Cyromazine proved highly effective (100% inhibition of larval development), whereas 30% of the fly populations emerged from the triflumuron treated culture medium at the recommended concentration of 5 mg/kg.
In the laboratory only 1 strain was susceptible to pyrethrum at the discriminating dose (DD) of 2.2 µg/fly. Six out of 17 strains (35%) showed moderate resistance to DTM. Resistance was confirmed against azamethiphos, 15 strains showed moderate to high resistance at 0.32µg/fly. The majority of the populations (59%) was also highly resistant against thiamethoxam (DD of 0.32µg/fly) and imidacloprid (41% at 2µg/fly).
Insecticide use has to be considered as last resort for managing house flies or other insect pests. Potential occurrence of IR should be evaluated before using insecticides. Rotation of active ingredients, frequent manure removal and biological means of control may delay IR development.

EVALUATION OF THE EFFICACY AND SAFETY OF MOXIDECTIN 2.5% W/V AND IMIDACLOPRID 10% W/V (ADVOCATE®) IN THE TREATMENT OF *THELAZIA CALLIPAEDA* IN NATURALLY INFECTED DOGS

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A GCPV negative/positive controlled, blinded and randomised study was conducted in a *Thelazia callipaeda* endemic area to evaluate the efficacy of Advocate® spot-on (moxidectin 2.5% w/v and imidacloprid 10% w/v) against eyeworm infection in dogs. At enrolment, 47 dogs (27 females and 20 males, from 7 months to 13 years old), infected by *T. callipaeda* were physically examined and infection was assessed by examination of both eyes for clinical score and live adult nematode count. Each dog was allocated to a treatment group (G1: Advocate® spot-on on Study Day (SD) 0 and 28; G2: Untreated control; and G3: Milbemax® tablets on SD 0 and 7). Clinical assessments and *T. callipaeda* adult counts were performed on SDs 7, 14, 28 and 35. The presence of *T. callipaeda* larvae was assessed on SD 35 by flushing of the conjunctival pouches. The primary efficacy variable was the percentage of animals showing a complete elimination of adult eyeworms, at each time point. Efficacy of Advocate® was 100% at each time point after first treatment on SD 0 (Fisher's exact test, $p<0.01$). Milbemax® efficacy was 57.4%, 92.8% and 100% (Fisher's exact test, $p\leq0.05$) on SDs 7, 14 and 28 and 35, respectively. The untreated dogs remained *T. callipaeda* positive, with a minor natural decline in the worm counts during the study. No significant differences were observed in ocular signs potentially related to the infection between groups and all dogs were negative for *T. callipaeda* larvae on SD 35. No adverse reactions were observed. Advocate® was safe and highly efficacious in treating *T. callipaeda* infections in dogs with a single treatment, eliminating the infection within 7 days. In contrast, Milbemax® had a slower onset of efficacy with less than 60% of dogs cured after a single treatment anda second treatment was required for 100% cure.

EFFECTS OF *AZADIRACHTA INDICA* EXTRACTS ON *PLASMODIUM BERGHEI* PARASITAEMIA AND PRO-INFLAMMATORY RESPONSE IN INBRED MICE

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Azadirachta indica is a medicinal plant popularly used for the management of various diseases including malaria. The plant's anti-plasmodial property is related to the presence of limonoids, such as gedunin, azadirone and azadirachtin. To explore whether *A. indica* harbors also inflammatory response modulatory components, we investigated the effects of neem extracts on *Plasmodium berghei* parasitemia in two inbred mouse strains (C57BL/6 and BALB/c) and compared plasma levels of metalloproteinase-9 (MMP-9) and tumor necrosis factor alpha (TNF-α) in infected treated and solvent control mice.

Experimental mice were infected with *P. berghei* ANKA strain ($1x10^6$ infected erythrocytes/mouse), divided into 4 groups and treated with: 1) artesunate; 2) solvent; 3) ripe, and 4) unripe fruit methanol extract. Fruit extracts were orally administered at a dosage of 150 mg/kg.

In plasma of infected and treated mice MMP-9 and TNFa levels were evaluated using commercially available ELISA kits.After 4 days of treatment a slight parasitemia decrease was found in C57BL/6 but not in BALB/c mice. Whereas infected solvent controls showed similar values in C57BL/6 (7,4%) and BALB/c (6,4%) mice, parasitemia of animals treated with the unripe neem fruit extract was slightly lower in C57BL/6 mice (5,5%) than in BALB/c mice (7,5%; $p< 0.05$). The same trend was observed in ripe fruit treated animals (C57BL/6: 5,1%); (BALB/c: 7,4% $p< 0.05$). Plasma levels of MMP-9 and TNF-α also differed between the mouse strains: in fact, if in BALB/c mice, independently on the treatment, MMP-9 levels were low, in C57BL/6 mice treated with the unripe fruit extract MMP-9 levels were twice ($p= 0.02$) and TNF-α levels three times ($p=0.052$) as high as in BALB/c mice.

These results suggest that the tested *A. indica* extracts do not contain anti-plasmodial limonoids at a concentration able to impact on parasite replication, but they can increase the pro-inflammatory response.

EFFICACY OF NATURAL ANTHELMINTIC PRODUCT IN SHEEP NATURALLY INFECTED BY NEMATODES AND TREMATODES: STUDIES IN CALABRIA REGION (SOUTHERN ITALY)

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Over the years, many anthelmintic drugs, have been suggested including the use of natural products without suspension times. To evaluate the effectiveness was conducted a test on a coplementary feed based on herbal extracts and essential oils of *Compositae, Cesalpinacae, Liliacae, Bromeliaceae, Labiatae* families, already on the market, registered for treatment of Nematodes and Trematodes infections in sheep.
The study was conducted in a farm located in the Calabria region where were identified 30 animals and divided, into three homogeneous groups of 10 animals for age, physiological state, and season of grazing: N10=treated with 10g and N20=treated with 20g respectively for normal or massive infestations as reported by the farmer; CG=untreated/control. The timing was: T_0: groups formation, sampling feces and treatment. T_7, T_{14} and T_{21} sampling feces and calculated the faecal egg count reduction (FECR) for evaluation anthelmintic effectiveness. All individual fecal samples were copromicroscopic examined using by FLOTAC *dual technique.*
The results of the copromicroscopic survey, in all the groups, show the presence of Gastrointestinal (GI) strongyles and *Dicrocoelium dendriticum.*
The results expressed in EPG (mean) and FECR (%) are summarized in table.

Groups		T_0	T_7		T_{14}		T_{21}	
		EPG	EPG	FECR	EPG	FECR	EPG	FECR
CG	GI-strongyles	348	349		377		284	
	D. dendriticum	129	264		142		143	
N10	GI-strongyles	413	555	-59	673	-78.5	505	-77.8
	D. dendriticum	151	257	2,6	81	42.9	77	46.1
N20	GI-strongyles	445	293	16	732	-94.2	286	-0.7
	D. dendriticum	181	55	79.2	89	37.3	293	-104

The present study reports that this formulation has proved ineffective against GI strongyles and *D. dendriticum* in the sheep, both at full dose or double dose. Indeed, the values of FECR were even negative. However, further research is required (i) to verify the validity of these products.

ITS BARCODING IN VETERINARY MYCOLOGY DIAGNOSTIC

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DNA barcoding is using short standardized sequences (DNA barcodes) for the identification of organisms at the species level by comparison to a reference sequence collection generated from well-identified species. In 2012 the internal transcribed spacer (ITS) region was selected as universal fungal DNA barcode, resulting from a community approach evaluating different loci. In addition, a curated reference ITS database (ISHAM-ITS) focusing on human and animal pathogenic fungi, with free access at http://its.mycologylab.org, was established in order to avoid possible misidentifications using e.g. GenBank, which contains many inaccurate sequence and obsolete taxonomy data. Veterinary mycology diagnostics faces a wide range of specimens coming from several environmental, clinical and food sources. Consequently, veterinary mycologists deal with a high heterogeneity of fungal species supported by limited epidemiological data. We applied DNA barcoding for the identification of all yeast species isolated routinely at IZSVE. A total of 731 strains were identified by sequencing of ITS1/ITS2 region, using the universal primers (ITS1/ITS4). Sequences were compared to the ITS-ISHAM database for identification and then aligned to generate unrooted trees (MEGA6 software). From the 731 strains, 459 were identified as Ascomycetes and 272 as Basidiomycetes yeasts representing 63 species from 20 genera. All strains are stored at the IZSVE fungal collection. The sequences will be deposited into the ITS-ISHAM database, increasing substantially the number of reference sequences for animal pathogenic fungi. In the last three years we successfully increased the quality and accuracy of fungal diagnostic at IZSVE, due to the introduction of DNA barcoding into the routine diagnostic workflow. This allowed us to strengthen our background in molecular epidemiology. Fungal isolates from animals or the environment represent an important “reservoir” that links humans with the environment they are exposed. This is another practical example of a One Health approach linking veterinary and medical scientific-health related disciplines.

THE ROLE OF DRUG EFFLUX PUMPS IN *MALASSEZIA PACHYDERMATIS* AND *MALASSEZIA FURFUR* DEFENCE AGAINST AZOLES

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Malassezia pachydermatis and *Malassezia furfur* are lipophilic yeasts, part of the normal animal and human skin microbiota, which can cause dermatitis as well as systemic infections in immunocompromised patients. Data on *in vitro* antifungal susceptibility to azoles showed that minimum inhibitory concentration (MIC) values of voriconazole (VOR) and fluconazole (FLZ) were higher in *M. furfur* and *M. pachydermatis* than those registered for other yeasts, thus resistance phenomena to VOR and FLZ were suspected in both species. The major cause of azole resistance in yeasts includes the overexpression of drug efflux pumps, namely ATP-binding cassette transporters (*i.e.,* CDR) and major facilitator superfamily (MFS - *i.e.,* MDR1) pumps.
This study aims to evaluate the effect of efflux pump modulators (EPMs), such as haloperidol (HAL), promethazine (PTZ) and cyclosporine A (CYS), on the FLZ and VOR MICs in *M. furfur* and *M. pachydermatis*.
The *in vitro* efficacy of azoles, in combination with HAL, PTZ and CYS against 21 *M. furfur* from human bloodstream infections and 14 *M. pachydermatis* from the skin of dogs with dermatitis, was assessed using a broth microdilution checkerboard analysis. Data were analysed using the model-fractional inhibitory concentration index (FICI) method. The FLZ and VOR MICs decreased in the presence of sub-inhibitory concentrations of HAL and/or PTZ. A synergistic effect was observed in strains with FLZ MIC≥128 µg/ml for *M. furfur*, FLZ MIC≥64 µg/ml for *M. pachydermatis* and VOR MIC≥4 µg/ml in both *Malassezia* spp.
Results suggest that the drug efflux pumps are involved as resistance mechanisms to azole drugs in *Malassezia* yeasts. The synergism might be related to an increased expression of efflux pump genes, resulting in azole resistance. Finally, the above FLZ and VOR MIC values might be considered as the cut-off to discriminate susceptible and resistant strains.

DETECTION AND GENETIC IDENTIFICATION OF *PNEUMOCYSTIS JIROVECII*

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Pneumocystis jirovecii (Pj) is a human specific uncultivable ascomycetous fungus for which the reservoir is represented by immunocompetent human individuals. Immunocompromised patients are at risk of developing *Pneumocystis* pneumonia (PcP) when exposed to *P.jirovecii* through their immediate environment. In the present study data on Pj occurrence and genetic identification at the *mtLSU rRNA* locus in an Italian hospital are reported. Epidemiological implications of genotypes distribution are also discussed.

From 2011 to 2015, DNA was extracted from 980 biological samples collected from patients admitted at the Tor Vergata University Hospital in Rome. For Pj detection a nested end-point PCR based on *mtLSU-rRNA* amplification was performed. Positive amplicons were sequenced for genotype identification. A Real-time PCR using a commercial kit based on TaqMan® technology was also applied on a subset (50) of positive/negative specimens. All the demographic and clinical data of patients were collected. Statistical analysis was performed using IBM SPSS.

One-hundred-seventy (17,3%) isolates of Pj were detected by PCR. Sixty-five isolates were sequenced and identified as genotype 1 (N=35, 0.53), genotype 3 (N=16, 0.25), and genotype 2 (N=11, 0.17). Three patients presented mixed infection. No genotype 4 was observed. The department of admission (p=.00383) and the underlying diseases (p=.02499) appear to be correlated with the likelihood of having a specific genotype. No correlation has been observed between specific genotypes and PcP prophylaxis, disease severity, need of second line treatments or a different outcome. The concordance between the two PCR assays was evaluated.

PcP remains an important disease associated with AIDS and in immunosupressed patients as well. The high rate of infections underlines the need of valuable tools for the diagnosis of pneumocystosis. Better understanding of the factors involved in the transmission and outcomes of PcP represents an essential requisite for establishing effective management and disease surveillance.

MORTALITY RATE OF *LUTZOMYIA LONGIPALPIS* SAND FLIES EXPOSED TO *BEAUVERIA BASSIANA* AND *EUCALYPTUS GLOBULUS* ESSENTIAL OIL

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Leishmaniases are diseases that affect several animal species including humans, being caused by protozoa of the genus *Leishmania* and transmitted through the bite of infected female phlebotomine sand flies. *Beauveria bassiana* is an entomopathogenic fungus, which can act as a biological control agent against several vectors of pathogens, such as ticks and mosquitoes. Few studies have demonstrated that *Phlebotomus papatasi*, the main vector of *Leishmania major* in the Old World, is susceptible to *B. bassiana*. Besides that, the essential oils of *Eucalyptus* trees have shown to be effective against *L. longipalpis*. Therefore, the aim of this study was to evaluate the effect of *B. bassiana*, *E. globulus* essential oil and their association for the control of *L. longipalpis.* Males and females of *L. longipalpis* were collected using CDC light traps in the municipality of Passira, Pernambuco State, Brazil. Treatments were carried out using *B. bassiana* (10^7 spores/mL), *E. globulus* (0.4 mg/mL), alone or in association. Distilled water and cypermethrin (0.1 mg/mL) were used as negative and positive control, respectively. The mortality rate was evaluated and dead sand flies examined by scanning electron microscopy (SEM). The mortality rate of treated groups was higher than that of negative control. The treatment with the essential oil in association with *B. bassiana* resulted in a decrease in the survival time of *L. longipalpis*. SEM observation revealed the presence of conidia, hyphae adhesion and degradation of the cuticle of *L. longipalpis* by *B. bassiana*, which may be related to the decrease in the survival time of sand flies treated with this fungus. Control groups and sand flies treated with *E. globulus* alone showed no changes in the structures of sand flies when analyzed by SEM. Thus, *B. bassiana* associated or not with *E. globulus* essential oil may play a role for the biological control of *L. longipalpis*.

ACARICIDAL EFFECTS OF *BEAUVERIA BASSIANA* IN COMBINATION WITH *EUCALYPTUS GLOBULUS* ESSENTIAL OIL AGAINST *DERMANYSSUS GALLINAE*

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The poultry red mite, *Dermanyssus gallinae*, is the most economically damaging ectoparasite of laying hens worldwide. The disadvantages of using chemicals against this mite (*i.e.*, environmental and food contamination and drug resistance) have spurred the interest of the scientific community in developing alternative methods for its control.
Eucalyptus globulus essential oil (EgEO) and *Beauveria bassiana* (*Bb*) have the potential to be used as alternative approaches for *D. gallinae* control. These compounds have some limitations in their use: the acaricidal effect of EgEO is rapid, but short-lived, whilst that of *Bb* is delayed, but long-lived. This study reports the effect of a native strain of *Bb* in combination with EgEO, against *D. gallinae* nymphs and adults. Batches of nymphs and adult mites (i.e., 360 individuals for each stage) for the treatment groups (TGs) were placed on paper soaked with a 0.1% tween 80 suspension of 10^9 conidia/ml of *Bb* (CIS) in combination with two different EgEO concentrations (*i.e.*, 0.2% and 0.1%), whilst 720 control mites for each stage (CGs) were exposed to 0.1% tween 80 (CG1), to *Bb* 10^9 CIS (CG2), to 0.2% EgEO (CG3) and to 0.1% EgEO (CG4).
A 100% mortality was recorded in adults at 9 days post infection (DPI) and at 10 DPI in nymphs, when using CIS in combination with 0.2% EgEO, but in CG2 at 12 DPI for adults and 14 DPI for nymphs. Used in combination with 0.2% EgEO, *Bb* displayed an earlier acaricidal effect towards both *D. gallinae* stages.
The combination of *B.bassiana* 10^9 CIS and *E. globulus* essential oil at 0.2% might be a promising natural control method for use in a pest management strategy against mite infestations in poultry houses.

IN VITRO TRIAZOLE SUSCEPTIBILITY OF CLINICAL AND ENVIRONMENTAL ISOLATES OF *ASPERGILLUS FUMIGATUS*

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Aspergillus fumigatus is the most common and life-threatening aerial fungal pathogen, especially amongst immunocompromised hosts. The mainstay of therapy is triazoles, but several studies have highlighted variable prevalence of triazole resistance amongst isolates from environmental and clinical settings. The presence of triazole resistance can hamper the management of aspergillosis. This study aims to assess the *in vitro* antifungal triazole susceptibility of *Aspergillus* section *Fumigati* isolates collected from different sources.

A total of 61 *Aspergillus* section *Fumigati* isolates from hospitalized human patients (Group I=20), animals (Group II=21) and laying hen farms (Group III=20) in Southern Italy, were analyzed. All isolates were identified by macroscopic and microscopic morphology on Czapek agar medium and molecularly by the amplification and sequencing of a portion of the β-tubulin and calmodulin genes to define the species present.

For azole susceptibility, the strains were screened on Sabouraud Dextrose Agar supplemented with itraconazole (ITZ, 4 μg/ml), voriconazole (4 μg/ml) and posaconazole (1 μg/ml). All isolates were molecularly identified as *A. fumigatus*. Azole resistance was recorded only to ITZ in 9 (45%) *A. fumigatus* strains from Group I.

Our results suggest that in southern Italy, ITZ resistance is only detectable in isolates from humans, in particular in those patients suffering from cystic fibrosis and haematological disorders. In these patients indeed ITZ resistance may develop during extended prophylaxis or therapy. ITR resistance needs to be further confirmed in future studies by the broth microdilution method according to the CLSI M38-A2 protocol.

ATYPICAL CASE OF SUBCUTANEOUS FILARIOSIS IN A CAT: DO WE EXPECT DIROFILARIA IMMITIS THERE?

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Subcutaneous dirofilariosis is a well-known disease caused mainly by *Dirofilaria repens* and described in several mammalian species including human, dog and cat [1]. Additionally, early developing stages of the heartworm, *Dirofilaria immitis*, are rarely reported in subcutaneous localization from humans and dogs. To our knowledge, evidence of this condition has not been described in the cat yet, even if the feline host can be affected either by the classic adult-related heartworm form or heartworm-associated respiratory disease (HARD) caused by immature stages.

A 2 year- old, spayed male cat was presented for three subcutaneous nodules on the head and trunk. The cat lived in Northern Italy and was regularly vaccinated and treated monthly with an antiparasitic spot on formulation containing selamectin (Stronghold®, Pfizer). One of the three nodules was surguically excided and examined. Histology showed in the subcutis the presence of a nodular lesion characterized by a severe inflammatory infiltrated composed by macrophages, small lymphocytes, with fewer eosinophils and mast cells, supported by a proliferation of mature fibroblasts (fibrosis). Inflammatory cells were multifocally surrounding sections of parasites identified as nematodes. The parasites were characterized by a thick cuticle with a smooth external surface, prominent and large lateral chords and a polymyarian-coelomyarian musculature. Microscopic features were compatible with *D. immitis* morphology. [2] After extraction from the paraffin block, DNA of the parasite was amplified with a PCR (ribosomal 5.8S-ITS2-28S region), the PCR product were purified, cloned and thereafter sequenced. A BLAST search revealed 97% identitiy to *D. immitis* isolate EU182331 and only 79% of identity the next related sequence of *Dirofilaria* genus (*D. repens*). The cat tested negative for *D. immitis* antigenemia and the two remaining nodules disappeared spontaneously in a few months.

Identification of a filaroid nematode with smooth cuticles in the subcutaneous tissues can be challenging. All species of the genus *Dirofilaria* are characterized by cuticular ridges, except from *D. immitis* and *D. lutrae* [2], with the latter described so far only in USA in the North American river otter. The parasite in the present case most likely represents an immature stage of *D. immitis* which developed in the subcutis (L3-L4) and was successively entrapped in this localization. The immunity of the cat, which is not a suitable definitive host for *D. immits*, likely played a role in preventing migration of the immature stage to the pulmonary arteries.

To author's knowledge this is the first reported case of subcutaneous localization of *D. immits* in a feline host.

ECOEPIDEMIOLOGY OF *DIROFILARIA* SPP. AND *ANGIOSTRONGYLUS VASORUM* IN PORTUGAL

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Dirofilariosis and angiostrongylosis are major and potentially fatal canine heartworms increasingly reported worldwide. Despite their importance, few studies have been performed, so far, in Portugal. For this reason, a large epidemiological survey was conducted in shelter dogs from Portugal between 2011-2015.

1. Blood samples were collected from 944 dogs. *Dirofilaria* antigen kit, Knott's technique and acid phosphatase staining were used. In total, 11.9% dogs were positive to *Dirofilaria immitis* and no case of *Dirofilaria repens* was detected. Furthermore, sandwich ELISAs were used for detecting antibodies and circulating antigens of *Angiostrongylus vasorum* in 906 dogs. In total, 0.66% were positive for both *A. vasorum* antigens and antibodies, and 1.32% for antibodies only. Regions with positive animals overlapped and were distributed over nearly all sampled areas, confirming the endemic occurrence of *A. vasorum* in dogs from Portugal.

2. Additionally, geospatial tools were used to assess the transmission risk of *Dirofilaria* spp. in Portugal, using a degree-days model based. Daily temperatures registered between 2003-2013 were recorded in five meteorological stations. Madeira was the area with highest number of days with suitable conditions for *Dirofilaria* transmission (209.9 days/year), followed by Faro, Lisbon, Azores and Porto. The risk period ranged from 5 months/year in Porto to 8 months/year in Madeira.

3. In order to characterize deworming practices currently implemented in companion animals, 312 owners were surveyed at the Small Animal Hospital, Faculty of Veterinary Medicine, University of Lisboa. The results showed that only 11.8% of dogs were internally dewormed at the recommended regimen and only 28.4% were uninterruptedly protected throughout the year against arthropods.

Given the severity of the conditions caused by *A. vasorum* and *D. immitis* and the zoonotic threat of *Dirofilaria* spp., these results sound an alarm bell to perform effective prophylaxis in pets and keep a One Health surveillance integrated approach.

Case report: Co-Infection of *Trypanosoma* (*Megatrypanum*) *pestanai* and *Anaplasma phagocytophilum* in a dog from Germany

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In Europe, Stercoraria trypanosomes are known to infect sporadically domestic ruminates and wild life. Canine trypasonomiasis was described as a rare, imported disease, which is caused by the species *T. evansi* or *T. congolense*. There is only few information about infections with stercoraria trypanosomes in dogs in Europe.

A dog was presented to a local veterinary clinic in Germany showing bad general condition, pale mucosa and stiffness of legs. Fever, slight leucopenia and thrombocytopenia were found during the clinical assessment. According to the owner declaration, the dog have had tick infestation. Therefore, the dogs blood was examined for *Anaplasma phagocytophilum*-, *Borrelia* spp.- and *Ehrlichia canis*-infections by real-time PCR. Only the PCR for *A. phagocytophilum* was found to be positive. After administration of doxycycline for three weeks, the dog´s condition improved rapidly and blood as well as clinical parameters were found to be in reference ranges.

A pre-therapeutic buffy coat was prepared and co-cultured with tick cells from the embryonal cell-line of *Ixodes scapularis* (ISE6) in L15B300 medium at 34 °C. Giemsa stained cytospins were prepared weekly. After 20 days of culturing, *A. phagocytophilum*-specific inclusions were visible in tick cells as well as extracellular Trypanosoma. The Trypanosoma had a pointed posterior end, length of 18.5 µm and a large kinetoplast. According the morphological criteria they were classified into stercoraria group. The sequencing of 870 bp fragment of 18S rRNA gene showed 99% identity to the badger trypanosoma - *T. pestanai*, which was described in European badger (*Meles meles*) in Great Britain, Ireland and France.

To our knowledge this is the first description of *T. pestanai* infection in a dog as well as first detection of *T. pestanai* in Germany.

THELAZIA CALLIPAEDA IN EASTERN EUROPEAN COUNTRIES

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Thelazia callipaeda (Spirurida, Thelaziidae) is a parasitic nematode of the eye of domestic and wild carnivores, lagomorphs and humans. This parasite is vectored, in Europe, by males of *Phortica variegata* (Diptera, Drosophilidae, Steganinae), a drosophilid displaying a zoophilic behaviour, which feed on ocular secretions. *Thelazia callipaeda* was for long time known as the "oriental eyeworm", for its original identification in Far Eastern Countries (e.g. China, Japan and Thailand). Since 1989, this nematode has been detected in many other European countries including Italy, France, Spain, Portugal, Switzerland, Germany and Greece, as agent of ocular infection of animals and humans. However, data on the occurrence of this parasite in the Eastern European countries were not available until 2014. Over the past two years, a number of autochthonous cases of ocular thelaziosis from animals living in Romania, Croatia, Serbia, Bosnia and Herzegovina, were published in the international scientific literature. In addition, the zoonotic potential of this parasite was further confirmed by the report of human cases of thelaziosis from a 36-year-old and an 82-year-old patients living in Serbia and Croatia, respectively.
Here we report 10 new cases of ocular infection by *T. callipaeda* from dogs living in Bulgaria (n=9) and Hungary (n=1). All animals had no history of travel outside their native countries, and were presented to the Department of Parasitology (Stara Zagora, Bulgaria) and to a veterinary practitioner (Pécs, Hungary) due to various degrees of ocular disorders (i.e. epiphora, conjunctivitis). Nematodes detected in the conjunctival sac were collected by flushing with saline solution, stored in 70% ethanol and, morphologically and molecularly identified.
The first confirmed autochthonous cases of thelaziosis in Hungary and Bulgaria have extended the geographical distribution of *T. callipaeda* from neighbouring countries (e.g. Bosnia and Herzegovina, Croatia, Romania and, Greece), where the occurrence of the parasite in humans and animals was already documented. Finally, since cases of human thelaziosis occurs in areas where the infection is prevalent in domestic animals and wildlife, a One Health approach is strongly advocated to tackle this vector-borne zoonosis.

POSTER
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ARTHROPOD-BORNE PARASITOSES

MOLECULAR AND SEROLOGICAL DETECTION OF *ANAPLASMA PHAGOCYTOPHILUM* IN GOATS: FOLLOW-UP OF A SEVERE OUTBREAK

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Anaplasma phagocytophilum (AP) is an emerging tick-borne pathogen transmitted by *Ixodes* ticks. Few data on clinical cases are available in goats. We describe the first evidence of a severe AP outbreak in naturally infected goats in Trento Province (North-Eastern Italian Alps) and the results of the molecular and serological follow-up in a subset of 22 goats.
In June 2015 a febrile syndrome was reported in five lactating goats, of which four resulted positive by PCR for AP and one showed antibodies by immunofluorescence (IFAT). Within three weeks other 90/200 (45%) showed similar symptoms, leading to a significant milk reduction and a total of four deaths. Animals were let to graze few hours per day from May to September. Afterwards 22 goats were selected and screened by PCR and IFAT in July, September and January. In general, 11/22 (50%) of the goats were PCR+ at least ones in the period and 18/22 (82%) have been exposed to AP. Particularly in July, 7/20 (35%) had circulating AP and 15/20 (75%) had antibodies; in September, 3/22 (13.6%) were PCR+ including two seronegative goats previously PCR- (likely new infections) and 9/22 (41%) had antibodies including one seroconversion. In January all goats were PCR- and 7/21 (33.3%) still had antibodies including two new seroconversions. In September, 73 questing nymphs and 9 larvae were also collected from the pasture by dragging. Two pools of nymphs resulted positive for AP, showing a minimum nymph infection rate of 2.7%.
Concluding, AP can have a significant impact on goat production. The high number of symptomatic animals in June imply a great exposure to infected ticks in a likely naïve population, followed by a decrease of exposure demonstrated by the fall of PCR+, seropositive and titles. However infected ticks were still questing at the end of the season.

PREVALENCE AND RISK FACTORS ASSOCIATED TO *EHRLICHIA CANIS*, *ANAPLASMA* SPP., *BORRELIA BURGDORFERI* SENSU LATO AND *DIROFILARIA IMMITIS* IN HUNTING DOGS FROM SOUTHERN ITALY

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Canine vector-borne diseases (CVBDs) are caused by a range of pathogens transmitted to dogs by arthropods including ticks and insects, and many of them are zoonotic, with dogs potentially serving as reservoirs for humans. The present study aimed to investigate the seroprevalence for *Ehrlichia canis*, *Anaplasma* spp., *Borrelia burgdorferi* sensu lato and *Dirofilaria immitis* in hunting dogs living in Campania region, southern Italy. Whole blood samples of hunting dogs (n=1,335) from Salerno and Avellino provinces were tested using a commercial in-clinic enzyme-linked immunosorbent assay kit (SNAP® 4Dx®-IDEXX Laboratories). Odds ratios (OR) were calculated by logistic regression analysis to identify independent risk factors of exposure. The seroprevalences for the four pathogens were: *E. canis* 7.56% (101/1335), *Anaplasma* spp. 4.34% (58/1135), *B. burgdorferi* s.l. 0.29% (4/1335) and *D. immitis* 0.22% (3/1335). Co-infection with *E. canis* and *Anaplasma* spp. was found in 29 dogs (2.17%), while co-infection with *Anaplasma* spp. and *B. burgdorferi* s. l. in only 2 animals (0.14%). Adult age was a risk factor for *E. canis* (OR 2.35), while fur animals (hares, foxes, boars) hunt for *E. canis* (OR 4.75), *Anaplasma* spp. (OR 1.87) and *B. burdorferi* s.l. (OR 10.51), respectively. The history, or presence, of tick infestation was identified as a risk factor for positivity to *E. canis* (OR 2.08) and *Anaplasma* spp. (OR 2.15). Finally, a large dog pack size was significantly associated with exposure to *E. canis* (OR 1.85) and *Anaplasma* spp. (OR 2.42). The results of present survey indicated that hunting dogs populations is at risk of CVBDs in southern Italy. Further studies are needed to evaluate the role of hunting dogs in the epidemiology of vector-borne agents due to sharing with the wild animals the same area as sympatric populations. Information on the prevalence and geographical distribution of CVBDs in dog populations is crucial for effective planning of surveillance and control measures.

PROTEOMIC APPROACHES TO INVESTIGATE THE BIOLOGY OF THE HARD TICK *IXODES RICINUS* AND ITS RELATION WITH THE SYMBIONT *MIDICHLORIA MITOCHONDRII*

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Hard ticks are considered the most important vectors of disease in Europe and North America. *Ixodes ricinus* is one of the important tick species in Europe, due to its widespread distribution and to its role of vector of pathogenic bacteria, viruses and protozoans. Furthermore, *Ixodes ricinus,* harbors a particular symbiotic bacterium named *Midichloria mitochondrii*. Most of these symbionts are localized in the tick ovaries (OT) and salivary glands (SG), and they are transmitted to the progeny and host. The relationship between the tick and its symbiont is not clear yet. Here we describe a proteomic approach aimed at expanding the knowledge of *I. ricinus* biology and its interaction with the symbiont.

We generated the protein profile of ovaries (OV) and salivary glands (SG) of adult female of this tick species. To compare the two different tissues, 2DE electrophoresis followed by LC-MS/MS protein identification was performed. Gel matching of SG and OV patterns revealed that, although the majority of spots were common to both tissues, some spots present in SG were absent in OV and conversely. Among the 21 spots showing significant difference in their relative abundance, ten showed 4-to 18- fold increase/decrease in density. This work allowed to detect proteins that exhibit a differential expression in OV and SG. However it was not able to detect symbiont proteins, possibly due to poor sensitivity.

To investigate the biological system at a finer detail, we are currently performing two additional approaches. The first is an immunoproteomic procedure using recombinant symbiont proteins and sera from tick-infested animals. This method allowed to detect the protein FliD from the symbiont. Finally we have performed shotgun proteomics experiments which, due to their high resolution, allowed us to detect thousands of tick proteins and tenths of symbiont proteins.

VECTOR COMPETENCE OF ITALIAN *AEDES ALBOPICTUS* FOR ZIKA VIRUS

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Zika virus (ZIKV), a single-stranded RNA virus (Family *Flaviridae*, genus *Flavivirus*), is transmitted to humans mainly through the bite of infected *Aedes* mosquito species, primarily *Aedes aegypti*.
The vector competence for ZIKV of a laboratory colony of Italian *Ae. albopictus* was assessed, and simultaneously compared to an *Ae. aegypti* colony, as positive control. Experimental infection was performed using an infectious blood meal with ZIKV at a final concentration of 10^6 PFU/ml.
Ten-day old females were fed on a membrane feeding apparatus and monitored for 24 days (T=26±1°C, 70% RH, light/dark of 14/10 h) to determine the length of viral extrinsic incubation period. ZIKV titre of infected mosquitoes was evaluated by quantitative Real Time PCR. Infection, Dissemination and Transmission Rates (IR, DR, TR) were assessed by virus detection in the abdomen, legs plus wings, and saliva, respectively, of mosquitoes collected at 3, 4, 7, 11, 14 18, 21 and 24 days post infection (p.i.).
Preliminary results indicated that *Ae. albopictus* was able to become infected and the virus to disseminate. Starting from day 11 p.i., ZIKV was present in legs plus wings and in saliva in 10% of mosquitoes tested, and an equal value of DR (10%) was observed up to day 18 p.i. However the mean viral titres in legs, wings and saliva of the infected *Ae. albopictus* were lower than those detected in infected *Ae. aegypti*. Furthermore, in *Ae. aegypti* the presence of ZIKV in legs, wings and saliva was detected starting from day 4 p.i., with a DR of 10% that increased progressively up to 70% by day 18 p.i.
Although the specimen analysis and the evaluation of possible vertical transmission are still in progress, our preliminary results have clearly shown lower vector competence for ZIKV of the Italian *Ae. albopictus* as compared to main vector *Ae. aegypti*.

APPLICATION OF A SOLUTION WITH SPORES OF THE FUNGUS *MUCOR CIRCINELLOIDES* AS A BIOCIDE TO PREVENT TICK EGGS DEVELOPMENT IN TROPICAL AREAS

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Ticks represent a very important problem among dairy farms due to blood sucking causes reduction in milk production, live weight and anemia. These parasites have the ability to transmit different pathogens as protozoa, rickettsia and viruses. The economically most important ixodid ticks of livestock from tropical regions belong to the genera of *Hyalomma*, *Boophilus*, *Rhipicephalus* and *Amblyomma*.
With the objective to reduce the risk of cattle infestation by ticks, the efficacy of the fungus *Mucor circinelloides* was assayed. An aqueous solution containing 10^7 spores of this fungus/mL was sprayed directly onto eggs of *Ripichephalus microplus*. A total of 40 Petri plates were placed 150 eggs, and then divided into 2 groups: G-Control was composed by 20 plates added 10.5 mL distilled water; G-Biocide consisted of 20 plates added 0.5 mL solution of spores + 10 mL distilled water. The development of the eggs was evaluated after 30 days at 28-30ºC.
By adding the spores of *Mucor circinelloides* to the eggs of *R. microplus*, the development of hyphae which attach to their surface and penetrate inside was detected. The percentage of viable eggs reduced to 40% after 15 days, and to 60% after 30 days. Significant differences between the G-Biocide and G-Control were demonstrated (F= 9.916, P= 0.001).
It is concluded that the distribution of spores of the fungus *Mucor circinelloides* by spraying provides a very helpful tool to decrease viability of eggs of the tick *Rhipicephalus microplus* in the soil.
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TICK BORNE PATHOGENS IN TICKS FROM DOMESTIC ANIMALS IN LEBANON

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Ticks (Acari: *Ixodidae*) are ectoparasites infesting livestock in every geographic zone in the world and they are vectors of several viral, bacterial and protozoal pathogens to animals and humans worldwide. A deep knowledge of pathogen prevalence in ticks, as well as the information about the geographical distribution of these arthopods, would have a key role in the control of tick-borne diseases. Few data are available about tick distribution and prevalence of tick-borne pathogens in Lebanon.
This study was aimed to the detection of pathogens in Lebanese ticks.
A total of 88 adult hard ticks was collected in 2014 from ruminants (cattle, sheep and goats) in four Lebanese provinces (Akkar, Bekaa, Nabatieh and South-Lebanon). All specimens were identified by morphological characters. DNA was extracted from each tick and analysed by PCRs to detect DNA of bacterial (*Coxiella burnetii*, *Anaplasma* spp., *A.ovis*, *A.marginale*, *A.phagocytophilum*) and protozoan (*Babesia bigemina*, *B.bovis*, *B.ovis*, *Theileria ovis*, *T.annulata)* tick borne pathogens.
Identified ticks belonged to the species *Rhipicephalus annulatus* (54; 61.4%) *R.turanicus* (21; 23.9%), *R.sanguineus* (6; 6.8%), *Hyalomma anatolicum* (6; 6.8%), *R.bursa* (1; 1.1%).
Anaplasma spp. DNA was found in 21 ticks (23.9%), *A.marginale* in 2 ticks (2.3%) and *A.ovis* in 5 ticks (5.7%) and 12 ticks (13.6%) were positive to *C.burnetii.* Only 2 ticks (2.3%) were found positive to *T.ovis,* while no positive samples were found for the other investigated pathogens.
This is the first survey of tick borne pathogens in ticks from Lebanon and it provides information on tick species distribution and the association with tick transmitted pathogens. An high prevalence of bacterial pathogens was reported in the analysed ticks.
This information is important for epidemiological studies of tick-borne pathogens in Lebanon and to evaluate the risk associated with pathogen transmission to humans and animals in this Country.
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THE AMERICAN COCKROACH (*PERIPLANETA AMERICANA*) AND ITS POTENTIAL ROLE IN THE EPIDEMIOLOGY OF THE CAT LUNGWORM

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The picture of the biology of the cat lungworm (*Aelurostrongylus abstrusus*) seems to be not yet completely delineated. Indeed, the life-cycle of this metastrongyloid may reveal one or more "actors" that have not been taken in account until now. Infections of cats based solely on the ingestion of snail intermediate hosts have been considered less likely to justify the widespread of this gastropod-borne disease. Therefore, alternative routes of infection have been investigated in the last years, and paratenic hosts (e.g. rodents and birds) have been suspected to play a key-role in the epidemiology of this parasite. The American cockroach (*Periplaneta americana*), a pest insect able to carry and/or transmit several pathogens including parasites, may share the same environment with the intermediate hosts of *A. abstrusus*. The aim of this study was to evaluate the potential role of *P. americana* in the epidemiology of *A. abstrusus*. Adult cockroaches (n=10) were forced to ingest 30 *A. abstrusus* third-stage larvae (L3s) each. Two cockroaches were dissected 24 hours after infection and every five days until day 20. Larvae found at the dissections were microscopically identified and their vitality assessed. A total of 5 *A. abstrusus* alive L3s were found at day 10 (n=1), day 15 (n=2) and day 20 (n=2). Although the low number of L3s recovered this finding suggests that *P. americana* could serve as transport host for *A. abstrusus*. Interesting, the detection of still alive L3s, 20 days after the infection suggests that cockroaches can harbor for many days infective larvae. According to these results, domestic cats, even those without outdoor access, may be exposed to *A. abstrusus* infection through the ingestion of cockroaches, which are pests commonly present in domestic environments.

DIAGNOSIS OF PARSITIC DISEASES

COMPARISON OF IFAT AND PCR ASSAYS TARGETING THE RE AND B1 REGIONS FOR DETECTION OF *TOXOPLASMA GONDII* IN MUSCLE TISSUES

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Toxoplasmosis is one of the most common food-borne zoonoses worldwide; among food animals, swine possess the highest presence of *T. gondii* cysts in meat, playing a major role as a source of human infection. Goal of the present study was to compare the detection of *T. gondii* infection in muscle tissues of swine by PCRs, based on amplification of the *T. gondii*-B1 regions and the repetitive 529 bp-elements (RE), and IFAT conducted on meat juice. Meat juice samples, obtained from the diaphragm pillars of 498 slaughtered pigs managed in intensive farms of Central Italy, were tested by a commercially available IFAT kit (MegaFLUO Toxoplasma g., Megacor Diagnostik). Genomic DNA was extracted from the same tissues and *T. gondii*-B1 and RE sequences were amplified by specific PCR protocols (Lin M.L. et al., 2000. J. Clin. Microbiol. 38(11):4121-5; Homan W.L. et al., 2000. Int. J. Parasitol. 30(2000): 69-75). Thirty-six meat juices (7.23%) tested positive for *T. gondii*-antibodies; among these 17 muscle tissues (47.22%) gave also *T. gondii*-DNA positive results. *Toxoplasma gondii* DNA was detected in 165 samples (33.13%). One hundred ninteen (23.89%) samples gave positive amplifications using both the genetic targets; however 31 (6.22%) and 15 (3.01%) were found to be positive only to RE and B1 respectively. The agreement beyond results from antibody detection and PCRs was assessed with Cohen's kappa coefficient and revealed a poor agreement (K=0.05); however a good correlation (K=0.77) between the results obtained targeting the two different genetic markers was detected. The results obtained allow to conclude that: i) standardized B1 and RE- PCRs applied on muscle tissues can detect higher rate of *T. gondii*- infection than IFAT on meat juice; ii) a multi-locus PCR approach is a recommended tool for accurate diagnosis of infection in swine with a wider application in food testing.

DETECTION OF *ELAEOPHORA BÖHMI* (FILARIOIDEA: ONCHOCERCIDAE) IN HORSE FROM NORTHERN ITALY

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Horses may be infected by different species of onchocercid nematodes, including *Onchocerca reticulata,* which reside in the connective tissue of the flexor tendons and the suspensory ligament of the fetlock, *O. cervicalis* and *O. gutturosa*, which parasitize the connective tissue of the nuchal ligament. The literature on equine onchocercosis is fragmentary, limited, and often dated. The present report aimed at describing a case of equine onchocercosis caused by *Elaeophora* (*Onchocerca*) *böhmi* in an 8-years old gelding Belgian show jumper from northern Italy (Liguria). The horse did not limp but presented a firm and not painful mass on the proximal third of the right metacarpal region. Ultrasound examination showed a peritendinous enlargement around the palmaro-lateral part of the tendons, characterized by an elongated hypoechoic and well-defined structure, with a coiled hyperechoic line within.The metacarpal nodule was resected and used to perform a histopathologic examination, which allowed to detect the presence of numerous nematode sections. Fragments of the nematode were isolated under a stereomicroscope and examined, with some of them molecularly processed. The total genomic DNA was extracted from individual specimen using a commercial kit. The nematode extract was morphologically identified as an immature female of *E. böhmi*. The BLAST analysis of *cox*1 sequence revealed the highest nucleotide identity (*i.e.,* 91%) with that of *O. lupi* available from GenBank™, since no other sequences were available. *Eleophora böhmi* has only been found in two isolated reports, and information about its biology are lacking. According to the original report by Supperer (1953), adults were found in the medial layer or within the artery wall. The present study provides new data on the unusual anatomical localization of this onchocercid, which most likely migrated from the circulatory system (*i.e.,* arteries and veins of limbs) to the subcutaneous tissues of the metacarpal region.

POST-SURGICAL FOLLOW UP OF HUMAN CYSTIC ECHINOCOCCOSIS (CE) IN SARDINIAN PATIENTS

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In human cystic echinococcosis (CE), caused by *Echinococcus granulosu*s, one of the major problems is the risk of post-surgical relapse or treatment failure. In this respect, post-treatment follow-up of patients for several years is mandatory to detect potential recurrences as soon as possible but no consensus actually exists as to which tests should be used, and how these tests should be applied and interpreted.
The current study reports the results of follow-ups up to 5 years, using ultrasound and serology, for a sample of 79 Sardinian CE patients clustered into 2 groups of either cured (CCE), or non-cured (NCCE) cases according to clinical course and imaging findings. Specific IgG, IgG1, IgG4, IgE against protoscolex soluble somatic antigens (PSC Ag) were assessed by ELISA test.
Out of a total of 71 CCE patients, followed for an interval of between 1 and 5 years after surgery, 17 relapse cases were detected, mostly long after surgery. As a whole, irrespective of length of follow up, at the end of the interval , 76% of relapsing patients were IgG positive vs 56% of non relapse cases, 53% vs 17% IgE positive, 65% vs 18% IgG1 positive and 47% vs 11% IgG4 positive. A decrease in positivity rate and antibody concentrations was detected particularly for IgE and IgG4 in non relapsing patients, while high titres of all subclasses persist in relapse cases and in NCCE patients, except in cases of calcified cysts.
In conclusion, the present results suggest that PSC Ag may represent a useful candidate for conducting serologic follow-ups of CE patients subsequent to treatment. CE-specific antibodies IgE, IgG4 and also IgG1, matching diagnostic images, may prove useful immunological markers for prognosis of CE patients.

PRELIMINARY RESULTS OF EXPOSURE TO *SARCOCYSTIS* SPP. IN HORSES FROM ITALY USING *SARCOCYSTIS NEURONA* AS ANTIGEN

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Sarcocystis spp. are coccidian parasites that can infect a wide range of animals and that need two hosts to complete their life cycle. Horses serve as intermediate hosts for several species of *Sarcocystis*, included *S.neurona,* that is responsible of the neurologic disease equine protozoal myeloencephalitis (EPM). To date, *S.neurona* is reported only in the Western Hemisphere, where it is restricted the definitive host, the opossum. In 2015, a horse located in the Forlì-Cesena district suddenly showed severe neurological signs, as pelvic limb and neck tremors and stiffness, decline of vigilance and lack of appetite and dead in few days. No presence of heavy metals and pesticides was found either in the beverage water, feed and the gastric content. Few weeks later, four horses from the same stable showed the same acute neurological signs; thus, they were tested for West Nile virus and botulinum toxin, resulting negative. Although none of the horses travelled abroad, but considering the protozoal myeloencephalitis-like symptoms evidenced, we serologically investigated the 4 symptomatic animals plus two asymptomatic ones from the same stable, using a *S. neurona*-specific rSnSAG2 ELISA. Considering a percent positivity (PP%) of 25%, all sera resulted positive with highest PP values found in symptomatic horses. However, when sera have been further evaluated by an rSnSAG4/3 ELISA and a Western blot analysis that used *S.neurona* SN3 whole-merozoite as antigen, they tested negative. The serological reactivity evidenced could be related to infection with other apicomplexan parasites, although it seems more likely that tested animals have been infected by other European *Sarcocystis* species closely related to *S.neurona*. Using the same serological methods, in Spain, a large proportion of horses resulted exposed to *Sarcocystis* spp. Although these serological findings cannot explain the neurological clinical symptoms of the animals, they can be considered the start for further studies on *Sarcocystis*.

IMPORTED *PLASMODIUM OVALE CURTISI* AND *PLASMODIUM OVALE WALLIKERI* INFECTIONS AMONG PATIENTS AT INMI L. SPALLANZANI HOSPITAL (ROME)

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Malaria caused by *Plasmodium ovale* infection has been considered a low-prevalence disease with limited geographic distribution, benign clinical course, and easy treatment. Diagnosis of *P. ovale* malaria can be difficult because of low parasitemia levels, mixed infections with other *Plasmodium* species, and false negatives from malaria rapid diagnostic tests (RDTs). The study of gene sequences demonstrated that *P. ovale* actually consists of 2 subspecies that cocirculate in Africa and Asia and that are unable to recombine genetically. The differences seem to be explained by real biological factors, rather than ecologic or geographic factors. *P. ovale curtisi* (*P.ov.c*.) and *P. ovale wallikeri* (*P.ov.w.)* were the names proposed for these species.
A second molecular test for *P.ov.c.* and *P.ov.w.* was applied to DNA samples with positive *P. ovale*-PCR, collected during August 2012–January 2016. DNA isolation from whole blood was performed by using the QIAamp DNA Mini Kit (QIAGEN). *P. ovale* molecular diagnosis was confirmed by using a nested malaria PCR. To differenziate *P.ov.c.* from *P.ov.w. potra* gene (*P. ovale* sp. tryptophan-rich antigen) was used in a nested procedure.
During August 2012–January 2016 a total of 12 samples positive by PCR for *P. ovale* were analyzed. Of these, we were able to amplify and genotype 10 samples. 3 patients had *P.ov.c.* infection and 7 had *P.ov.w.* infection. Parasitemia levels were not different between the 2 groups. Among the laboratory results, only platelet count was found decreased but at the same level in the patients with *P.ov.c*. and *P.ov.w.* infections.
In 2 groups we found only marked thrombocytopenia and no other significant differences. In many cases RDT was negative. RDTs still show a low sensitivity for detecting *P. ovale*, and this problem is explained by the genetic variability of the 2 subspecies and the low levels of parasitemia.

MOLECULAR AND IMMUNOLOGICAL DIAGNOSIS OF FOUR NEW CASES OF GASTRIC AND INTESTINAL *ANISAKIASIS* IN ITALY DUE TO *ANISAKIS PEGREFFII*, AND FIRST IDENTIFICATION BY RT-PCR HYDROLYSIS PROBE SYSTEM

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The larval parasites of the genus *Anisakis* are considered the most important biological hazards present in "seafood" products; they are indeed aetiological agents of human anisakiasis, a seafood-borne parasitic zoonosis. Among the nine species of *Anisakis* so far genetically characterized, only two – *A. pegreffii* and *A. simplex* (*s.s.*) – have been found to cause infections in humans. The species *A. pegreffii* is the most widespread zoonotic species affecting commercial fish from Italian waters; it has also been reported to cause in humans gastric, intestinal and gastro-allergic anisakiasis.

Here, four new cases of anisakiasis (three intestinal and one gastric) were described. Two *Anisakis* larvae were collected by endoscopy from the stomach of a patient; one larva was found at level of descendent colon, during a colonscopy of another patient. In addition, a granuloma formed at the intestinal level, surgically removed from a patient, and a biopsy of a lesion formed at the intestinal level of another subject, were examined. In the first and second case, the molecular identification of the larvae collected was performed by sequence analysis of the mtDNA *cox2* and the EF1 α-1 nDNA region. In the last two cases it was impossible to identify the parasite with those diagnostic methods, due to the very low quantity and concentration of the parasite DNA obtained (<0.01 ng/µl); while, the RT-PCR hydrolysis probe system - based on mtDNA *cox2* - was able to detect the parasite DNA quantity as low as 0.001 ng/µl. In all the four cases, the aetiological agent was: *A. pegreffii*.

In the sera samples from the same patients IgE/IgG response against the major ESP antigens of *A. pegreffii* was detected with the Immunoblot (WB-IgE/IgG).

This represents the first identification of human anisakiasis by RT-PCR hydrolysis probe system.

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PARASITES OF DOLPHINS STRANDED ALONG THE COAST OF TUSCANY (ITALY)

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In recent years, an increase in the number of stranded cetaceans has been observed. In order to give a contribution to the knowledge of the parasite fauna of stranded dolphins, in the period between February 2013 and July 2015, 10 *Tursiops truncatus*, 15 *Stenella coeruleoalba*, 1 *Grampus griseus* stranded along the coast of Tuscany (Pelagos Sanctuary) were screened for the search of parasites. All organs and faecal samples were examined with parasitological techniques. Immunological and molecular techniques were used for the search of *Toxoplasma gondii* on serum and brain tissue samples, respectively, and for *Giardia* and *Cryptosporidium* on faecal samples. The prevalence of parasites and the corresponding confidence intervals (95% CI) were calculated and data were statistically analysed (p <0.05). Twenty-one out of 26 examined animals (80%) tested positive for of at least one parasitic species. Specifically, 86% (13/15) *S. coeruleoalba*, 70% (7/10) *T. truncatus* and the single *G. griseus* were found positive. *Skrjabinalius guevarai* (7.7%, 2/26), *Halocercus lagenorhynchi* (3.8%, 1/26), *Halocercus delphini* (7.7%, 2/26), *Stenurus ovatus* (7.7%, 2/26), *Pholeter gastrophilus* (26.9%, 7/26), *Campula palliata* (3.8%, 1/26), *Bolbosoma vasculosum* (7.7%, 2/26), *Phyllobothrium delphini* (42.3, 11/26), *Monorygma grimaldii* (23.9%, 6/26), *Tetrabothrius forsteri* (7.7%, 2/26), *Strobilocephalus triangularis* (7.7%, 2/26) were the identified parasite species. Moreover, 6 out of 26 (23.1%) dolphins serologically examined for *T. gondii* were found positive, but PCR confirmed the presence of the parasite in the brain of a single animal. At statistical analysis, parasitological prevalence and seropositivity to *T. gondii* were not significantly different between *T. truncatus* and *S. coeruleoalba*. The high prevalence of endoparasitic infections in the subjects herein examined and the isolation of parasitic species considered as a cause of severe debilitation or death, highlight the importance of parasite monitoring in investigations aimed to evaluate the health status of dolphins in the Tyrrhenian Sea.

PARASITES OF THE HEAD OF THE ATLANTIC BONITO *SARDA SARDA* CAUGHT OFF THE SOUTHEASTERN IBERIAN PENINSULA (WESTERN MEDITERRANEAN SEA)

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The Atlantic bonito *Sarda sarda* (Bloch, 1793) is a pelagic fish widely distributed in tropical and temperate waters, including the Mediterranean Sea. Little is known about its biology, migrations and parasite fauna.
The aim of this study is to describe the metazoan parasites of gills and head of the Atlantic bonito from the western Mediterranean Sea.
Between 2011 and 2012, 40 specimens of Atlantic bonito were caught by tuna traps in La Azohia (Murcia, Spain). Fish were measured (fork length range 41-59 cm), the heads (including gills and heart) were excised, stored individually in plastic bags, and frozen at -20 °C. Samples were examined by naked eye and under a stereomicroscope for metazoan parasites.
Seven species were found: two monogeneans (*Capsala gregalis*, prevalence, P%=5%; *Hexostoma* sp., P%=5%), two didymozoid trematodes (*Unitubulotestis pelamidys*, P%=48%, and Nematobothriinae gen. sp., P%=50%), and three copepods (*Caligus bonito*, P%=53%, *Caligus pelamidys,* P%=60%, and *Unicolax anonymous,* P%=5%). The parasite assemblage of the head of the Atlantic bonito was dominated by the two species *Caligus*, which showed the lowest site-specificity, being distributed on gills, inner operculum and palate. The other parasites had high site-specificity. *U. pelamidys* and Nematobothriinae gen. sp. were found encapsulated in the inner margins of the gill filaments and under the epithelium of the gill chambers, respectively. *C. gregalis*, *Hexostoma* sp. and *U. anonymous* were located on the gill arches, between the gill filaments and in the nasal sinus, respectively. Platyhelminthes showed the highest host specificity, being didymozoids and monogeneans S*arda* genus specific; whereas copepods were more generalist, as *U. anonymous* and *C. pelamidys* parasitize several species within the Scombridae and *C. bonito* teleosts of several families.

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LARVAL ANISAKIDAE IN COMMERCIAL OMMASTREPHID SQUIDS FROM SARDINIAN WATERS (WESTERN MEDITERRANEAN SEA)

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Ommastrephidae (Cephalopoda) are common intermediate/paratenic hosts of marine parasites with life cycle based on prey-predator transmission, such as Anisakidae. Since the risk of human anisakidosis is associated to the consumption of raw or undercooked seafood, including cephalopods, this study focused on the epidemiology and taxonomy of these parasites.
Parasitological analyses were performed on 70 *Illex coindetii*, 69 *Todarodes sagittatus* and 26 *Todaropsis eblanae* sampled between 2013 and 2015, during experimental trawls (CampBiol and MedITS) around Sardinia (western Mediterranean Sea).
Anisakid larvae, visually detected, were morphologically identified under light microscope, and stored in 70% ethanol for molecular analysis. Among the 613 anisakid third-stage larvae found, 104 corresponded to *Anisakis* Type I (sensu Berland, 1961), 201 to *Anisakis* Type II, and 308 to the poorly known genus *Lappetascaris* (sensu Nagasawa and Moravec, 1995). For a subsample of 20 *Anisakis* sp. larvae, the ITS region of rDNA was amplified and PCR products digested with the restriction enzymes *Hinf*I and *Hha*I. Digestion of the PCR products showed that the five *Anisakis* Type I larvae had the RFLP pattern of *Anisakis pegreffii*, while all the 13 *Anisakis* Type II larvae showed the pattern of *Anisakis physeteris*.
The highest prevalence (60.9% of *A. physeteris*) was observed in *T. sagittatus*, and the highest mean intensity (5.6 of *Lappetascaris* sp.) occurred in *I. coindetii*. In the latter, *A. pegreffii* and *A. physeteris* had the highest (38.6%) and the lowest prevalence (7.1%) respectively.
The mapping of organ specificity showed that *A. pegreffii* was detected mainly in the stomach wall, *A. physeteris* in the gonads, and *Lappetascaris* sp. in the mantle of their hosts.
The results add information to the few available data on the presence of anisakid larvae in Mediterranean cephalopods, showing the need for a more accurate knowledge as a tool in the development of food-security strategies.

PREVALENCE OF *ECHINOPHALLUS WAGENERI* (BOTHRIOCEPHALIDEA) IN BLACKFISH (*CENTROLOPHUS NIGER)* OF WESTERN LIGURIAN SEA

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The blackfish *Centrolophus niger* (Perciformes: Centrolophidae) is a mesopelagic fish living offshore in depth range from 40 to 1000 m. Juveniles occur in surface waters while adults live deeper. Feeding of blackfish is not selective, including mainly small fish, squids and large pelagic crustaceans. Among the parasitofauna reported in blackfish, tapeworms of the order Bothriocephalidea are considered very common. One of them, *Echinophallus wageneri* (Cestoda: Echinophallidae) is a large worm, up to 50 cm long and 2 cm wide, with a flat strobila folded along longitudinal axis (convex dorsally and concave ventrally). The aim of this study is to define the prevalence of *E. wageneri* in a *C. niger* population of the Ligurian Sea. From March to April 2015, 42 specimens of blackfish were caught by local fisherman from the sea off the coast of Imperia (Italy). The visceral package of each blackfish was tied at its ends and placed in bags whit an identification code. In the laboratory, the samples were examined fresh or after freezing at -20°C. Parasites were fixed in ethanol (70%) and subjected to identification on the basis of morphological characters. The presence of *E. wageneri* was detected in all the samples examined with an intensity of infection ranging between 2 and 48 (MI=16,6). Several other species of not yet identified cestodes were found in coinfection with *E. wageneri*. These results provide preliminary data about the prevalence of *E. wageneri* in the blackfish, species with an increasing interest in local fish market.

NEW REPORT OF *ANISAKIS* SPP LARVAE FROM BLUNTHEAD PUFFER, *SPHOEROIDES PACHYGASTER* (OSTEICHTHYES: TETRAODONTIDAE) CAUGHT OFF STRAIT OF SICILY

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Aim of the present paper was to report and identify by morphological and molecular methods the presence of anisakid L3 larvae found in samples of *Sphoeroides pachygaster* (Müller & Troschel, 1848) caught off Strait of Sicily from 2012 and 2015 during trawl surveys: this host species has not been so far checked for anisakids. Seven specimens of *S. pachygaster* were identified, weighed and measured and the presence of metazoan parasites in the abdominal cavity was checked under stereomicroscope. Nematode larvae (n. 7), from three fish specimens, were collected, fixed in 70% ethanol and later examined for morphological identification at genus level, using light microscope after clarification with glycerol, according to morphological characters. The larvae identified as belonging to the genus *Anisakis*, Type I, and stored in 70% ethanol, were underwent molecular identification at species level by PCR- RFLP analysis of the rDNA (ITS1, 5.8S gene, and ITS2) region; sequencing of ITS regions and comparison with sequences in GenBank were also performed. The nematode larvae were molecular identified as belonging to *A. pegreffii* that is the predominant species in Mediterranean Sea while *A. simplex s.s*. is found in fish species of Atlantic Sea. The blunthead puffer *S. pachygaster* is a fish species of Atlantic origin, spread in all seas and oceans, warm and temperate. This alien species is known in the Mediterranean Sea where represent a definitively estabilished population. There are indeed numerous reports of *S. pachygaster* in the last years in the Mediterranean Sea included Italian Seas (Adriatic Sea, Tyrrhenian Sea, Ionian Sea, Sicilian Channel). The discovery of anisakid nematodes and identification of species such as *A. pegreffii*, may support the hypothesis of complete adaptation of *S. pachygaster* as well as of existence of a Mediterranean population.

HISTOLOGICAL DESCRIPTION OF ENTERIC INFECTION DUE TO THE MICROSPORIDIAN *ENTEROSPORA NUCLEOPHILA* IN GILTHEAD SEABREAM (*SPARUS AURATA*)

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Emaciative syndrome due to the enteric microsporidian *Enterospora nucleophila* is an emergent disease of gilthead sea bream (*Sparus aurata*) farmed in the Mediterranean. During 2015 and 2016 winters, two outbreaks of emaciative syndrome have been observed in caged gilthead sea bream from the Thyrrenian Sea. Sixty-two fish (3.5-110g) were necropsied and subjected to parasitological examination. Gastrointestinal tracts were fixed in 10% buffered formalin and processed for histology. Immunohistochemistry (IHC) was performed on some positive samples using polyclonal antibody anti-*Encephalitozoon cuniculi*. Intestinal tissue was also subjected to amplification of a fragment of 18S rDNA and sequenced. At necropsy, fish showed emaciation and melanosis; gills and internal organs appeared pale and intestine was often enlarged and filled with yellowish, mucoid exudate. Microscopical examination of intestinal scrapings and wall fresh mounts showed the presence of aggregates of minute spores of microsporidia identified as *E. nucleophila* by molecular analysis. Histology of stomach and intestine showed in massive infections a strong mucosal sloughing off and necrosis in association to inflammatory infiltration of lymphocytes and few mast cells. In mild infections, intestinal mucosa seemed to be preserved. Microsporidian spores have been detected both in gastric and intestinal mucosa, extending to the lamina propria and the sub-mucosal layer. In advanced phases of infection, the complete destruction of epithelial layers was observed, with thinning of the muscular layer, associated to the presence of conspicuous clusters of infected cells. Spores showed intracytoplasmic or intranuclear localization in enterocytes and only intracytoplasmic localization in macrophages. *E. nucleophila* spores were strongly positive by IHC. This study confirms the pathogenic role of *E. nucleophila* in gilthead seabream farmed in the Mediterranean and points out the need of studies aimed to clarify its biology and epidemiology.

NEOSPORA CANINUM OOCYST SHEDDING IN A NATURALLY INFECTED DOG

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Although the seroprevalence of *Neospora caninum* infection in dogs can be relatively high, there are few reports of dogs naturally shedding *N. caninum* oocysts and the prevalence of *Neospora* excretion in faeces is about 0.03% in Europe.
A mixed-breed male household dog of about 8 years in age living in the district of Pisa was referred for dysorexia, weakness and general lymph node enlargement. Clinical pathology evidenced mild normocytic and normochromic anaemia, thrombocytopenia and hypoproteinemia with hypoalbuminemia. Serology for *Leishmania, Ehrlichia canis* and *Anaplasma phagocytophilum* was negative. From lymph node and bone marrow analysis, T cell lymphoma, high grade, pleomorphic type, clinical stage V, was diagnosed. The dog was treated with a chemotherapy induction protocol with vincristine (0.75mg/m^2 IV once a week), cyclophosphamide (50mg/m^2 orally 3–4 days/week), and prednisone (40mg/m^2 orally daily for the first week, then tapered to 5 mg/m^2 orally daily in the further weeks) for 8 weeks. A faecal sample analysed by flotation test by using a low density solution 7 days after the beginning of the treatment, revealed the presence of *N. caninum*-like unsporulated oocysts of about 10-11 um in diameter. An aliquot of the same faecal sample analysed by PCR with species-specific primer pairs Np6+/Np21+ was positive for *N. caninum* DNA, while serology for *N. caninum* performed a month later by IFAT, was positive with a titer of 1:320.
Naturally occurring systemic illness or iatrogenic immunosuppression may predispose dogs to proliferation of the parasite. The dog described was receiving chemo-immunosuppressive treatment for T cell lymphoma. For these reasons it is not possible to suppose whether emission of *Neospora* oocysts in this dog was caused by reactivation of a latent infection or by a recently acquired infection.

CARDIORESPIRATORY AND GASTROINTESTINAL PARASITES IN DOGS IN LIGURIA

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Aim. This epidemiological study investigated the frequency of cardiorespiratory and gastrointestinal helminths in dogs in Liguria, Italy.
Materials and Methods. From 2009 to 2013 faecal samples were collected from 450 rural or semi-rural dogs (260 males, 190 females) in - Imperia (n=352) and Savona (n=98). Dogs had not received anthelmintic treatment for at least four months before the sampling. Their gender, age and lifestyle were recorded. Faecal samples were analyzed in centrifugal flotation (50% zinc sulfate solution, s.g. 1.350) and by Baermann's technique. Prevalences with 95% confidence intervals (CI) were calculated. Multiple parasitic infections were described. Pearson's chi square and Fisher's exact tests were used to compare prevalences among age classes (young dogs ≤1 year), genders, and lifestyles (hunting and non-hunting dogs).
Results. 197 dogs (43.8%, CI 38.7-48.9%) were infected, 3.3% by cardiorespiratory parasites, 32.4% by gastrointestinal species, and 8.0% with mixed infections. The most frequent gastrointestinal parasites were *Toxocara canis* (20.0%), *Trichuris vulpis* (17.8%), Ancylostomatidae (12.0%), Coccidia (2.7%), *Aonchotheca putorii* (1.8%) and *Toxascaris leonina* (1.8%). The cardiorespiratory species found were *Eucoleus aerophilus* (9.6%), *Eucoleus boehmi* (1.6%), *Angiostrongylus vasorum* (0.7%) and *Crenosoma vulpis* (0.2%). Larvae of the two latter species were detected in the same dogs also using Baermann's technique. 116 dogs (25.8%) were parasitized by a single species, and multiple infections (up to six species) were observed in 81 dogs (18.0%). Significant differences (P value <0.05) were found. Female dogs were more frequently parasitized by *E. aerophilus*. Hunting dogs (n=344) were more frequently infected than non-hunting dogs by *E. aerophilus*, *T. canis* and Ancylostomatidae, while the contrary was observed for *E. boehmi* and *T. vulpis*. *E. aerophilus* and *T. leonina* were more frequent in younger (n=57) than older dogs.
Conclusions. Cardiorespiratory parasites are more frequent than expected, suggesting a previous underestimation and expansion of their range.

ENDOPARASITES IN DOGS AND CATS OF SARDINIA, ITALY

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The present study aimed to update data on prevalence of intestinal and lung parasitic infections in owned dogs and cats and to identify potential risk factors in Sardinia, Italy.
From 2011 to 2016, 619 owned dogs and 343 owned cats, referred at the Veterinary Teaching Hospital, University of Sassari for examination, were also examined for parasites presence.
Individual faecal samples were analyzed using Wisconsin technique for copromicroscopical examination and Baermann technique for the presence of lungworms larvae.
Endoparasites were found in 34.9% and 43.4% of examined dogs and cats, respectively.
Helminthic infections (17% in dogs and 26% in cats) showed a slightly higher prevalence than protozoan infections (14% in dogs and 11% in cats). In both dogs and cats, the most common parasites were ascarids (12.1% and 15.7%, respectively), *Isospora* spp. (10.2% and 10.8%), *Giardia duodenalis* (9.4% and 8.5%), and hookworms (7.9% and 5.5%). Broncho-pulmonary nematodes were found in 0.8% of examined dogs and in 15.5% of examined cats (Yates corrected =80.97; $p<0.0001$). Data stratified by sex in dogs and cats showed similar prevalences in males and females ($p>0.05$). The statistical analysis of prevalence rates for each age group showed significant differences in both dogs (χ^2trend = 30.347; $p<0.00001$) and cats (χ^2trend = 16.066; $p=0.00006$).
The age was identified as risk factor, being animals younger than 6 months more frequently infected than older, while no significant association was observed for the sex. This study showed that endoparasites in owned dogs and cats of Sardinia have considerable high prevalences.
Veterinary practitioners should devote more attention to parasitic infections and should adopt more effective and standardized diagnostic and control practices as recommended by ESCCAP (http://www.esccap.org).

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DIROFILARIA IMMITIS AND *ANGIOSTRONGYLUS VASORUM* IN KENNELS IN CAMPANIA REGION, SOUTHERN ITALY

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The cardiopulmonary nematodes *Dirofilaria immitis* and *Angiostrongylus vasorum* are increasingly reported in dogs in Europe. The reasons for their apparent emergence have been discussed in several recent studies. In particular, global warming represents a fundamental factor for the seasonality and the spread of *D. immitis* and could be involved also in the development of infectious stages in the intermediate hosts of *A. vasorum*.
The aim of this study was to investigate the seroprevalence of *D. immitis* (by DiroCHECK® ELISA) and the fecal presence of first stage larvae (L1) of *A. vasorum* (by FLOTAC) in dogs from 68 kennels of the Campania region (southern Italy). The fecal samples were collected from pooled samples using the box as epidemiological unit. To the authors's knowledge, this is the first cross-sectional survey conducted at regional-scale in Italy and in Europe on the contemporaneous detection of *D. immitis* antigens and *A. vasorum* L1 in kennels.
Antigens of *D. immitis* were detected in 24/537 (4.4%; 95% Confidence Interval = 3.0-6.7) dogs in 6 out of the 68 kennels (8.8%; 95% CI= 3.6-18.9). The 24 positive samples for *D. immitis* antigen were tested also with AngioDetect® and only 1 sample was seropositive for *A. vasorum* with a prevalence of 4.2%. *A. vasorum* L1 were detected in dogs from 9 out of the 68 kennels (13.2%; 95% CI = 21.8-44.9). Pooled fecal samples from 25 boxes out of the 1,360 analyzed resulted positive to *A. vasorum* L1 (1.8%; 95% CI = 1.2-2.7).
The present study indicates that cardiopulmonary nematodes are present in Campania region in symptomatic dogs as well as in asymptomatic ones. Therefore, regular parasitological surveillance, appropriate treatment strategies and high quality standard of hygiene are required to guarantee the health and welfare of kennel dogs, as recommended by the European Scientific Counsel for Companion Animals Parasites.

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A SURVEY OF PORTUGUESE PET OWNERS' AWARENESS ABOUT PARASITIC ZOONOSES ASSOCIATED WITH DOGS AND CATS

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Parasitic diseases of companion animals comprise a group of globally distributed and rapidly spreading illnesses that are caused by a wide range of arthropods, helminths and protozoa. In addition to their veterinary importance, many of these parasites can also affect the human population, due to their zoonotic potential. The aim of the present work was to evaluate the knowledge of Portuguese pet owners from non-rural and rural parishes who attended veterinary medical centres from mainland Portugal by performing a multiple-choice questionnaire designed to obtain data knowledge about the meaning of zoonosis and parasitic diseases regarding their zoonotic potential.
Two hundred and ninety five (56.5%) of the 522 responders had heard of zoonosis/zoonoses, but only 35.2% (184/522) knew its meaning. Tick fever, mange, leishmaniosis and ascaridiosis/roundworms were the parasitic diseases most frequently identified.
The majority of pet owners that attended veterinarian clinics were not aware of the possible transmission of parasites from their dogs and cats to themselves, a fact which highlights the important role of veterinarians in the continuous implementation of effective control measures to reduce the risk of parasitic infections in both humans and companion animals.

EVALUATION OF THE INJECTION METHOD FOR THE EXPERIMENTAL INFECTION OF THE LAND SNAIL *CORNU ASPERSUM* WITH THE LUNGWORM *AELUROSTRONGYLUS ABSTRUSUS*

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The land snail *Cornu aspersum* (syn. *Helix aspersa*) is a widespread snail species, commonly used to produce infective third-stage larvae (L3) of feline lungworms (e.g. *Aelurostrongylus abstrusus* and *Troglostrongylus brevior*) under laboratory condition. Although several experimental methods of infection of snails with first-stage larvae (L1) of feline lungworms have been described, these are time-consuming and they often do not result in a satisfactory number of L3. The aim of this study was to evaluate the injection method for the infection of *C. aspersum* with L1 of *A. abstrusus*, instrumentally to reduce the infection time and to maximize the output of L3. In addition, the performance of this method was compared with others.
Three groups (i.e. A, B, C) of 15 *C. aspersum* snails each were infected with L1 of *A. abstrusus* (i.e. n=250 for each snail), whereas a fourth group (D) served as control. Snails were individually placed for 48 hours on a microfilm containing L1 (group A), on a potato slice previously irrigated with a suspension of L1 (group B), or they were inoculated by injection of L1 in the posterior-ventral portion of the foot (group C) and observed for 30 minutes after the injection. Eighteen days after the infection all the infected snails were sacrificed and tissues were digested to recover L3. No difference in mortality rate was recorded amongst groups and the mean number of retrieved L3 was significantly larger in group C (71.5 ±52.9) compared to group A (19 ±23.3; $p<0.0001$) and group B (38.2 ±44.9 $p=0.0161$). The injection of *A. abstrusus* L1 in the foot of *C. aspersum* proved to be a fast, easy to apply and effective method, resulting in the largest number of L3 obtained.

EXPERIMENTAL *ANCYLOSTOMA CEYLANICUM* INFECTION IN DOGS AND CATS: INFECTION RATE, WORM LENGTH AND FECUNDITY

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Ancylostoma ceylanicum (*AC*) is one of the most common species of hookworms parasitizing dogs and cats in southeastern Asia. *AC* readily establishes in humans in most of the regions where it is endemic in dogs and cats and thus control of this zoonotic hookworm is of specific importance. Deliberate infections in the course of anthelmintic efficacy studies allowed for the study of some biologic parameters of the *AC* infection in dogs and cats as there is only limited comparative data.
Eight (5M, 3F) dogs and eight (4M, 4F) cats, ~7-10 months old and nematode-naïve, were inoculated once orally with ~500 or ~300 infective larvae, respectively, of a canine-source *AC* isolate from Thailand which has been passaged in cats for three years before inoculation. Five weeks after inoculation, fecal egg per gram counts (EPG) were established, and hookworms were recovered from the small intestine of all animals, counted by sex and preserved for measurement of worm total length, width and spicule length.
Infection rate was significantly ($p<0.05$) higher in dogs than in cats (64.95% vs. 32.38%) and male and female *AC* recovered from dogs had a larger size (length, width, spicule length) than worms recovered from cats ($p<0.05$). Male-to-female *AC* ratio, fecundity (EPG divided by female worm burden) and number of eggs produced per female *AC* per day did not differ significantly between dogs and cats. Male hosts tended to have higher infection rate and higher female *AC* burden than female hosts (61.61% vs. 32.02%, $p=0.071$; 135.78 vs. 74.43, $p=0.080$). Significant host sex × species interactions were recorded for infection rate, total *AC* count, and *AC* size.
In conclusion, results indicated that both dogs and cats are highly susceptible to *AC* and that *AC* infection is potentially subject to regulation by host species (host size?), host sex and density-dependent processes.

INTESTINAL INFECTION WITH *ENCEPHALITOZOON POGONAE* IN A CAPTIVE BEARDED DRAGON (*POGONA VITTICEPS*)

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Encephalitozoon spp. are a genus of microsporidia that can be found in numerous vertebrate species. However, there is little information on their abundance and significance in reptiles. In bearded dragons a species first suspected to be a new strain of *E. cuniculi* recently has been classified as the new species, *E. pogonae*. Infection usually is associated with granulomatous inflammation in different organs whereby adrenal glands, gonads, and liver were observed to be the most affected sites.
A captive bearded dragon was presented in apathic and anorectic condition. It showed an inflated and very tense abdomen. As health condition worsened markedly it had to be euthanized. Subsequent histopathological findings after HE (haematoxylin and eosin) staining revealed a multifocal to coalescing granulomatous inflammation of the small intestine. In the intestinal lesions intrahistiocytic aggregations of numerous small (around 2 µm), oval organisms could be seen and an infection with microsporidia was suspected.
DNA was extracted from the intestine and amplified by PCR using the primers MSP3 and MSP2A which target the internal transcribed spacer region and parts of the small subunit and large subunit of the ribosomal DNA. After sequencing and BLAST analysis, a 100% similarity to the new species *E. pogonae* was revealed. Other infectious agents could not be detected.
This is the third description with molecular identification of *E. pogonae* in bearded dragons. In contrast to the previous cases, the main lesions were found in the small intestine. All these infections were found in bearded dragons only which suggests that this species might be host specific or at least specific for this group of reptiles.
Considering that it is very likely that *E. pogonae* was responsible for the disease described here further studies on the prevalence, transmission, diagnosis and treatment of this pathogen are necessary for improved disease control in these popular terrarium animals.

PREDATION EFFICACY OF CYCLOPOID COPEPODS AGAINST *AEDES* MOSQUITOES IN NORTHERN ITALY

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Aedes albopictus and *Aedes koreicus* are invasive mosquito species that have colonized northern Italy and are potentially zoonotic vectors. Cyclopoid copepods are natural predators of mosquito larvae and can be useful biological control agents in artificial containers used as breeding sites by *Aedes* mosquitoes. In this study, we evaluated the predation efficacy of two cyclopoid copepod species, *Macrocyclops albidus* and *Mesocyclops leuckarti*, common in natural conditions in northern Italy, against *Ae. albopictus* and *Ae. koreicus* larvae under laboratory conditions. In each predation test, one female adult copepod was placed with 50 first instar larvae of a single mosquito species in a small Petri dish filled with 10 mL of water. After 24 hours, the mean number (±standard error) of larvae killed by one *M. albidus* female was 18.6 ± 1.3 *Ae. koreicus* and 20.9 ± 1.3 *Ae. albopictus*, and the mean number killed by one *M. leuckarti* female was 25.8 ± 2.8 *Ae. koreicus* and 36.1 ± 4.2 *Ae. albopictus*. Predation tests were also conducted using larger Petri dishes filled with 30 mL of water, resulting in reduced predation rates. Our findings indicate that *M. albidus* and *M. leuckarti* are effective larval predators of *Ae. albopictus* and *Ae. koreicus*.

INSECTICIDAL REPELLENCY OF A TOPICAL ADMINISTRATION OF DINOTEFURAN-PYRIPROXYFEN-PERMETHRIN SPOT-ON (VECTRA® 3D) ON MICE AGAINST *AEDES ALBOPICTUS* MOSQUITOES

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Background: *Aedes albopictus* is an important vector for the transmission of numerous viral pathogens and filarial nematodes for human and animals. In the absence of licensed vaccines against numerous vector-borne diseases, the best protection mean is to avoid mosquito bites, by using for instance an insecticidal repellant. An ectoparasiticide combining three active ingredients: 4.95% dinotefuran, 0.44% pyriproxyfen and 36.08% permethrin (Vectra® 3D, DPP) has been used in mice in an experiment designed to evaluate its anti-feeding and insecticidal efficacy against *Aedes albopictus*.
Methods: Twenty two female adult mice (*Mus musculus*) were randomly grouped into two groups of eleven animals. A control untreated group and a DPP treated group. DPP was administered topically as a line-on on day 0. The dose administered (14 µL) was estimated by converting the doses expressed in terms of mg/kg in dogs to an equivalent surface area dose in mice, expressed as mg/m^2. Anesthetized mice from both groups were exposed individually during 1 hour to 27±2 starved female mosquitoes on days 1, 7, 14, 21 and 28 post-treatment. At the end of the exposure, mosquitoes were assessed for immediate survival and engorgement status. Live mosquitoes in both groups were incubated separately under controlled environmental conditions and observed for mortality counts 24 h after the end of the exposure.
Results: The anti-feeding efficacy of DPP after 1-h exposure period was 99.23%, 100%, 98.03%, 89.34% and 87.35% at 1, 7, 14, 21 and 28 days, respectively. Insecticidal efficacy evaluated at 1 h and 24 h after exposure at days 1, 7, 14, 21 and 28 was 36.65, 28.85, 30.84, 23.07 and 11.85% and 68.41, 44.96, 43.30, 37.89, and 19.9% respectively. At each time point, there was a significant difference ($p < 0.0001$) between the treated and control groups for both anti-feeding efficacy and insecticidal.
Conclusions: In this study, the laboratory mouse model allowed us to demonstrate that the DPP combination has a significant anti-feeding and insecticidal efficacy against *Aedes albopictus* for at least one month.

PHLEBOTOMINE SAND FLY SPECIES DISTRIBUTION IN CROATIA AND THEIR IMPLICATIONS IN *LEISHMANIA* TRANSMISSION

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Since early 2000s human and canine leishmaniasis foci were documented in Croatia from coastal and insular territories of central and southern Dalmatia.
We report on a 2005–2011 phlebotomine survey performed to confirm species composition and seasonality in three central-southern counties of Dalmatia previously inquired (Šibenik-Knin, Split-Dalmatia and Dubrovnik–Neretva) (Bosnić *et al*, Acta Trop 2006; 99:42-9); to investigate on the current species distribution in the westernmost Istria county, including the biggest part of the Istrian peninsula, for which available data date back 60 years (Simic and Zivkovic Arch Inst Pasteur Alger1956; 383-5); and to search for natural *Leishmania* infections.
Sand fly collections, carried out in the frame of bluetongue disease surveillance, used blacklight suction traps employed for *Culicoides* monitoring. Fifteen localities in four Croatian counties were investigated. Specimens were preserved in ethanol pending morphological identification and DNA extraction.
Sand flies were trapped from late May through early December. One thousand specimens were collected and seven species identified. Among *Phlebotomus* sand flies, *P. perfiliewi* was the most abundant species (54.6%), followed by *P. neglectus* (28.2%), *P. tobbi* (8.9%), *P. perniciosus* (5.4%), *P. papatasi* (0.6%) and *P. mascittii* (0,1%). *Sergentomyia minuta* accounted for 2.2%. A difference in prevalence distribution was detected, being *P. perniciosus* prevalent in Istrian peninsula (87.5%), *P. perfiliewi* in central counties (65.3%) and *P. neglectus* in the southernmost county (69.3%). A subset of 369 *Larroussious* females (*P.perfiliewi* 76.2%) organized in pools (1-27 specimens/pool) according to species, site and date of collection, was analysed for *Leishmania* DNA presence. All pools were found negative.
The long lasting sand fly season (May-December) and the high *P. perniciosus* prevalence, the main *L.infantum* vector, in the westernmost Istria county may represent a warning signal in re-emerging leishmaniasis.
This study was partially funded by the FP7-UE EDENext collaborative project, Contract Number: 261504.

CO-PARASITISM OF *PYEMOTES VENTRICOSUS* (ACARI: PYEMOTIDAE) AND *SCLERODERMA DOMESTICA* (HYMENOPTERA: BETHYLIDAE) ON *OLIGOMERUS PTILINOIDES* (COLEOPTERA: ANOBIIDAE): A CASE STUDY RELATED TO A PRIVATE APARTMENT IN SICILY

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The Pyemotes ventricosus and the *Scleroderma domestica* are two ectoparasitic arthropods affecting several species of insects. The *P. ventricosus* (Newport, 1850) (*Acari*: *Pyemotidae*) is a small mite around 0,2 mm long that parasitizes larvae of furniture woodworms. The *Scleroderma domestica* (Latreille 1809) (*Hymenoptera: Bethylidae*) is an aculeate bethylid wasp ranging from 2 to 4 mm in length that parasitizes larvae of xylophagous *Coleoptera* and some species of *Lepidoptera* larvae.
Both ectoparasites usually cause the onset of a highly itchy dermatitis in human beings. Our observations report a case of dermatitis appeared at the end of August 2014 affecting a man and a woman living in a private apartment sited in the city of Agrigento (Sicily). At the beginning of August 2015, four samples of wood-sawdust, related to the activity of xylophagous insects infesting the furniture in the apartment, were collected. The analyses performed on two of the four samples, using the stereomicroscope and the optical microscope, allowed us to identify numerous arthropods belonging to the *P. ventricosus* and *S. domestica* species. Among the environmental residues we recognised a significant amount of ligneous fragments together with faeces and several insects belonging to the species of the *Oligomerus ptilinoides* (Wollaston, 1854) *(Coleoptera: Anobiidae),* a xylophagous beetle that was infesting several pieces of furniture in the apartment.
Both the *P. ventricosus* and *S. domestica* are two ectoparasites that frequently affect xylophagous insects. Their coexistence in the analysed samples represents a clear sign of their contemporaneous parasitosis (co-parasitism) to the detriment of *O. ptilinoides.* The dermatitis affecting the two people disappeared after the treatment done on the worm-eaten furniture and that proved the relationship between the arthropods and the dermatitis. Unfortunately it was not possible to verify if both the arthropods were responsible for the skin lesions or only one of the two.

A CASE OF BIOLOGICAL ERADICATION OF AN INFESTATION OF BED BUGS *CIMEX LECTULARIUS* (*HEMIPTERA*: *CIMICIDAE*) BY THE SPIDER *STEATODA ALBOMACULATA* (ARANEAE: THERIDIIDAE)

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The *Steatoda albomaculata* (Degeer, 1778) (*Araneae: Theridiidae*) is an arachnid belonging to the *Theridiidae* family that includes approximately 2000 species of spiders all over the world. The *Cimex lectularius L.* (Latreille, 1802) (*Hemiptera*: *Cimicidae*) is a temporary obligate hematophagous ectoparasite on human being. In May 2015 a canine inspection was carried out in a private apartment sited in the Perugia Province, inhabited by a couple of young people, in order to find the main source of infestation. The canine olfactory inspection and the visual inspection did not reveal any presence of bed bugs or their eggs. The people living there had not been affected by bites since April 2015, although no treatment had been performed into the apartment. The infested room had a low sloping wood beam ceiling with several webs made by *Steatoda albomaculata* containing entrapped *C. lectularius*. The disappearance of the cutaneous lesions due to the bites of *C. lectularius,* together with the absence of their aggregation sources, led us to suppose that the eradication of the entire population of bed bugs was a consequence of the predatory activity of *S. albomaculata,* largely diffused on the wood beams of the infested room. Povolný (1957) reported an analogous case occurred in an Austerlitz Castle, in which a colony of *Cimex lectularius* was eradicated by the spider *Steatoda bipunctata.* Furthermore, the scientific literature describes similar cases involving other arthropods, such as: the ant *Monomorium pharoaonis* (Howard and Marlatt, 1986), the mite *Pyemotes ventricosus* (Kemper, 1936) and the spider *Thanatos favidus* (Lorando, 1929). In order to strengthen our hypothesis, we carried out a lab experiment that confirmed the capacity of the S. *albomaculata* to predate the *C. lectularius.*

ABC TRANSPORTERS INVOLVMENT IN *ANOPHELES STEPHENSI* DEFENSE AGAINST *AZADIRACHTA INDICA* EXTRACT

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Insecticides are a core component of malaria control programmes, but their massive use led to resistance insurgence in different vector populations that threaten the global malaria control efforts. Alternatives have been searched in botanical compounds used for centuries as traditional remedies. *Azadirachta indica*, also known as Neem tree, is particularly interesting for its wide action against vector arthropods, parasites and other agents of infection. Neem extracts have shown a strong larvicidal, anti-emergence, repellency, anti-oviposition effects on different mosquito species including *Anopheles stephensi*, the main malaria vector in Asia.
To understand the detoxifying mechanisms of *An. stephensi* against Neem extracts, we studied the implications of ABC-transporters at different time-points through a) bioassays in combination with the verapamil, an ABC-transporter inhibitor, and b) expression profile of 6 genes of 3 different ABC sub-families (ABC-B, ABC-C, ABC-G) through real-time PCR. Third instar larvae were fed with fish food alone or mixed with *A. indica* extract. Three pools of larvae were taken at 0.5, 24, 48 and 72 hours for expression analysis. A parallel bioassay with same treatments was performed to assess mortality due to different concentrations of Neem extract.
Our study shows an up-regulation of the ABCG4 gene for all time-points and an early activation followed by a down-regulation of ABCB2 and ABCBmember6 genes, when mosquito larvae were fed with food mixed to Neem. After 48h, we observed that mortality is dose-dependent for the insecticide but, surprisingly, verapamil seems to partly preserve larvae from dying when Neem concentration is higher. This can suggest an antagonistic effect of verapamil and Neem.
These results demonstrate the involvement of ABC-transporters on the detoxification of the *A. indica* extract and highlight the need of further investigations to understand the effect of this compound in combination with other toxic compounds against *An. stephensi* and other mosquitoes in general.

OCCURRENCE OF PHLEBOTOMINE SAND FLIES ALONG AN ALTITUDINAL CLINE IN CENTRAL ITALY: ANALYSIS OF PREDICTIVE CLIMATE PARAMETERS

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In Italy leishmaniosis is an important veterinary problem especially in rural areas where sand fly vectors are abundant. In recent years canine leishmaniosis had extended its distribution mainly because of the presence of competent vectors in regions and altitudes of the Country where sand flies were not previously reported. We present the preliminary data based on the first of a three-year survey aimed to obtain an update on phlebotomine distribution in the Province of Rome. From mid-July to mid-October 2015 a weekly longitudinal sampling has been performed using 5 CDC light traps located outdoors in 6 areas characterized by different altitudes (from 21m to 542m a.s.l.) and ecologies (urban-rural). The entomological collection is expected to be associated to *Leishmania* infection on sand flies and climatic data obtained from meteorological stations located within 2km from the sampling sites that will be instrumental to identify drivers associated to the spreading of sand flies in the study area according to an altitudinal cline and ultimately predict their local distribution and population density. In this first year of sampling a total of 475 sand flies has been collected. Preliminary results show that sand fly densities in the areas are very low, in particular for the presence of *Leishmania* vectors. In fact, in the whole sample the most represented species is by far *Sergentomyia minuta* (94%) followed by *Phlebotomous perniciosus* (3.4%), *P. perfiliewi* (2.6%) and *P. papatasi* (0.5%). Detection of *Leishmania* by molecular methods in collected specimens is ongoing. A neural network model, specifically developed for small data set analyses, has been applied for studying the relations between meteo-climatic drivers and local sand fly densities (in a fully nonlinear manner). We will present preliminary results of the model that will permit to identify the most influencing drivers and confirm its predicting ability.

INTESTINAL YEAST FLORA OF HONEY BEE IN PRESENCE/ABSENCE OF *NOSEMA* SPP.: PRELIMINARY RESULTS

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In the last years, several research are aimed at the study of the intestinal microbiota of honey bee and their role in bee health. The literature reports that the flora of healthy bees is dominated by bacteria while only 1% is represented by yeasts and other fungi. Some authors describe that various diseases or stress increase the number of yeasts. Borsuk et al (2013 Med. Weter. 2013, 69, 726-729) studying the yeast microflora in bees experimentally infected by *Nosema* spp. noted an increase of yeasts in presence of a weak degree of *Nosema* infection, while heavy infections reduced the number of yeasts. In the present work, we describe preliminary result about the research of yeasts in bee guts in presence/absence of natural infection by *Nosema* spp. A total of 200 bees were collected from June to October 2015 in apiaries of Bologna province. The bees were euthanasized by placing their 4-5 minutes in freezer, then each single bee was dipped in ethyl alcohol, the gut was aseptically removed and put in 500 microliter of sterile saline in eppendorf and ground with pestle. One hundred microliter of the suspension were used for counting the number of *Nosema* spp spores per gut in a Burker chamber; other 100 microliters were spread onto the surface of Petri plates containing Sabouraud Dextrose Agar with Chloramphenicol and incubated at 30°C for 7 days to count the number of CFU (Colony Forming Unit)/gut. *Nosema* spp was found in 25/200 (12.5%) bee, with number of spore/gut ranging from 5000 to 34 million. Yeasts were found in 55/200 (27.5%) bee, with an approximate number of CFUs/gut ranging from 5 to 14000. The yeast CFU was significatively correlated to the number of *Nosema* spores (Spearman's rho $p = 0.016$). The species composition of yeast will be further elucidated.

EVALUATION OF *VARROA* CONTROL TECHNIQUES IN *APIS MELLIFERA* BY A PROTEOMIC APPROACH

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One of the main biotic threat to honey bees all over the world is the ectoparasitic mite *Varroa destructor* (Acari: Mesostigmata). Without proper treatment, colonies are doomed and collapse within two years. Many treatments are used by beekeepers, with well-established acaricidal efficacy; however very little is known on the impact of the above-mentioned treatments on the well-being of the colony. Biochemical markers represent an interesting tool to assess the animal and human welfare. Particularly promising for our purposes seems to be the evaluation of vitellogenin in the hemolymph of a pool of bees. The potential of this protein can be foreseen considering his pivotal role in the homeostasis of social insect colonies. In fact, the function of vitellogenin in these species is not limited to egg yolk constitution: trophic, antioxidant and hormonal function are also described. The aim of this research was to test the impact of two different Varroa control techniques by analyzing the vitellogenin content in the hemolymph.
Brood interruption by queen caging (five colonies) and brood removal (five colonies) followed by trickling of Api-Bioxal (Oxalic acid based acaricide) were performed. The hemolymph of 30 bees per colony was sampled and pooled. Four samplings were considered: pre manipulation, after manipulation, autumn (wintering phase) and winter. All the samples underwent SDS-PAGE and quantification of vitellogenin, qualitative analysis of other relevant proteins was also performed.
Vitellogenin was successfully isolated and identified by mass spectrometry. Our data are confirmatory of the abundance of this protein in the hemolymph of worker bees and interesting differences were found depending on the season. Major differences were also found between the experimental groups, suggesting the expected different impact of the techniques and stressing the need of an objective tool to better evaluate and understand the techniques for *Varroa* control.

AEDES ALBOPICTUS (SKUSE, 1894) IN THE SOUTHERNMOST LIMIT OF EUROPE: FIRST RECORD IN LAMPEDUSA, LINOSA AND PANTELLERIA ISLANDS AND CURRENT DISTRIBUTION IN SICILY, ITALY

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Within the VectorNet Project, founded by Avia-GIS, the Istituto Superiore di Sanità (Rome) and the Istituto Zooprofilattico Sperimentale della Sicilia (Palermo) undertaken an entomological inquiry in order to verify the current southern limit of *Aedes albopictus* distribution in Europe.
The study was carried out in Agrigento, Siracusa, Ragusa, Trapani and Caltanissetta provinces from June to October 2015. Sporadic surveys were carried out in July and in October, in Lampedusa, Linosa and Pantelleria islands. Human bait collections were performed and CDC traps, BG-Sentinel® traps and ovitraps were used.
In Lampedusa, *Ae. albopictus* was found using 12 ovitraps placed in 9 selected sites that have been managed for nearly two weeks in July: all sites were found positive. In October 5 ovitraps in five sites were monitored for three days, and only one resulted positive. In Linosa during the same survey in October, the species was found in the village and in the nearby cemetery. In Pantelleria, *Ae. albopictus* was collected in 8 sites, by monitoring 20 sites with 22 ovitraps during two surveys in October. *Ae. albopictus* was also recorded in all the investigated Provinces of Sicily.
In conclusion the present study reports for the first time the presence of *Ae. albopictus* in Pantelleria, Linosa and Lampedusa, which represent the Southernmost European limit of this species and its occurrence in the whole Sicily. A monitoring activity during the whole year should be planned to study the phenology of the species, especially in such extreme environments. Finally, a constant mosquito surveillance system in ports and airports for the introduction risk of other invasive mosquito species, like *Aedes aegypti*, should be implemented. This aspect should be considered also in the light of the health problems potentially linked to the continuous landings of migrant people from Africa, Asia and Middle Est.

PREVALENCE OF *EIMERIA* SPP. IN DAIRY CATTLE IN NORTHERN AND CENTRAL ITALY AND CORRELATION BETWEEN SPECIES AND OOCYST EXCRETION

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Bovine coccidiosis is one of the most common parasitic diseases of cattle. Twelve different bovine species of *Eimeria* have been identified worldwide. Coccidiosis is mainly a subclinical disease and results in economical losses due to reduced growth, production loss and management issues. *E. bovis, E. alabamensis* and *E. zuernii* are highly pathogenic species causing mortality and morbidity specially in young animals. This study is part of the ElanCOX Program, developed in collaboration with Eli Lilly Italia, and carried out to determine the prevalence of coccidiosis in Intensive Dairy Production Systems in Northern and Central Italy. Despite the importance of Dairy cattle farming in Italy, little is known about the prevalence of bovine *Eimeria* spp. in this country. The aim of the program is also to define the correlation between pathogenic *Eimeria* species and oocyst excretion. From May 2013 we collected 403 pools of fecal samples from 1939 calves (one to six month of age) in 110 milk farms; we tested all the samples with Flotac® dual technique to evaluate the oocyst excretion per single pool; the fecal samples were also processed to identify the *Eimeria* species in every farm. Coccidiosis was present in all farms and in 341 of 403 fecal pools (84,6%). We observed ten *Eimeria* species; multiple infection with different species were found in 83,6% of samples. The main species detected were *E. ellipsoidalis, E. bovis* (both with a prevalence of 61,5%) and *E. zuernii* (45,9%). The ElanCOX Program is still ungoing, so the authors hope to be able to collect a greater amount of data to enlarge the knowledge about the prevalence and distribution of *Eimeria spp.* in Italy.

PREVALENCE OF EQUINE PIROPLASMOSIS IN CENTRAL SPAIN

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Equine piroplasmosis is a tickborn disease subject to international movement regulation caused by protozoans *Babesia caballi* and *Theileria equi* that affects equidae and is endemic in many European countries. Antibodies are long-lasting (4 years for *Babesia,* lifelong for *Theileria*). To date, information on the epidemiology of equine piroplasmosis in Central Spain is limited. The aim of this study is to determine seroprevalences of both parasites and to identify associated risk factors. Equidae sera (n=88) from three asymptomatic groups in different epidemiological situations (37 rural area donkeys, 31 city area Police horses and 20 breeding facility horses) were tested using *T. equi* and *B. caballi* Antibody test kit (VMRD®). Seroprevalences were determined with exact 95% confidence levels, and compared among and within groups to evaluate risk factors (individual characteristics and management) using Chi-Square or Fishers Exact Test, whenever appropriate. P value <0.05 was considered significant (3). Overall prevalence for *T. equi* was 23,9% (95% CI: 16-34%) 13,5% donkeys, 48,4% Police and 5% breeding horses. For *B. caballi* prevalence was 5,7% (95% CI: 2-13%); 5,4%, 6,5% and 5% in donkeys, Police and breeding horses respectively. Coinfection 1,1% (95% CI:0-6%). Prevalences except for the Police group are lower than those described by other authors (1,2). Differences related to the studied risk factors were not significant, except within the donkey group where age was significant. In conclusion, similar low prevalences of *Babesia* are observed in all groups while *Theileria* was higher detected in older animals, except in the breeding farm, where more studies are needed on animal characteristics, management practices and vectors presence.

PREVALENCE OF INTESTINAL PARASITES IN HUNTING DOGS IN CALABRIA REGION (SOUTHERN ITALY)

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Intestinal parasites are important pathogens in dogs, especially in groups where overcrowding and environmental contamination could favour transmission and maintenance of infestations. The distribution and intensity of parasitism in dogs are influenced by many factors including the attitude and among the different diagnostic techniques employed.
Therefore, the present study is intended to estimate the prevalence and to identify the different species of helminths in hunting dogs in Calabria Region.
The study was conducted between March/April 2015 on 60 hunting dogs, without any antiparasitic treatments by almost 6 mounths. The dogs were divided into two groups: HD1=wild boars hunting (34 dogs) and HD2=retrievers (26 dogs). All individual fecal samples were examined by FLOTAC *dual technique* which is based upon the use of two flotation solutions that have complementary specific gravity (s.g.), and are used in parallel on the same faecal sample.
The present study showed the presence of endoparasites in all dogs. The results are summarized in Table.

Parasitic elements	HD1 = 34 dogs		HD2 = 26 dogs	
	N° positive	Prevalence (95% CI)	N° positive	Prevalence (95% CI)
Toxocara canis	13	38.2 (22.7-56.3)	5	19.2 (7.3-40)
Toxoascaris leonina	-	-	1	3.8 (0.2-21.5)
Capillaria aerophila	16	47.1 (30.2-64.6)	3	11.5 (3.0-31.2)
Anchylostoma caninum	7	20.6 (9.3-38.4)	1	3.8 (0.2-21.5)
Uncinaria stenocephala	2	5.9 (1.0-21.1)	-	-
Trichuris vulpis	10	29.4 (15.7-47.7)	4	15.4 (5.0-35.7)
Angiostrongylus vasorum	2	5.9 (1.0-21.1)	-	-
Isospora canis	3	8.8 (2.3-24.8)	-	-
Dipylidium caninum	2	5.9 (1.0-21.1)	-	-

In conclusion, the intestinal parasitism is evident both in the wild boar hunting dogs and in the retriever, with higher prevalence in the group HD1 probably their hunting characteristics. The high prevalence in both groups emphasizes the need to adopt more effective control strategies in hunting dogs in Calabria.

INVESTIGATING THE ROLE OF ALPHA-THALASSEMIA AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN *PLASMODIUM FALCIPARUM* TRANSMISSION FROM HUMAN TO MOSQUITO

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Large evidence is available showing that human genetic variation affects susceptibility to infectious diseases, but it is unknown whether it also affects the host efficiency to transmit pathogens. Previous studies have shown that two variants of the *HBB* gene (HbS and HbC), known to protect from clinical *P. falciparum* malaria, increase the transmission of the parasite from the human host to the mosquito vector (Gouagna et al. 2010). In this study we evaluated the role of two further malaria resistance factors, 3.7 alpha deletional thalassemia (α-thal$^{3.7}$) and glucose-6-phosphate dehydrogenase deficiency (G6PDA-) in the ability to infect mosquitoes.

We conducted Standard Membrane Feeding Assays on blood samples from 69 children aged 3-15 years from the village of Soumousso, Burkina Faso (West-Africa) with known α-thal$^{3.7}$ and G6PDA genotypes. A total of 15515 *Anopheles* were dissected on day seven after membrane feeding and oocysts were detected by microscopy in mosquito guts. We found that α-thal increases both the prevalence (α-thal$^{3.7}$ vs wild-type OR=2.56; CI=1.7–3.9; P< 0.001) and density (P< 0.001) of *P. falciparum* infection in mosquitoes while G6PDA- increases the prevalence (G6PDA- vs wild-type OR=11.5; CI=5.4–25.4; P < 0.001) but not the density of infection (P > 0.05).

To evaluate the influence of α-thal$^{3.7}$ and G6PDA-mutations on the gametocyte reservoir we conducted a cross-sectional study in the village of Soumousso, and collected whole blood samples in RNAlater from 510 subjects aged 3-20 years. Real time qPCR assays will be performed to detect and quantify transcripts expressed specifically by *P. falciparum* gametocytes.

MOLECULAR IDENTIFICATION OF *BLASTOCYSTIS HOMINIS* ISOLATES IN SOUTH OF CÔTE D'IVOIRE

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Blastocystis hominis is one of the most common human intestinal protozoan in the developing countries. This protist, genetically and phenotypically heterogeneous, is characterized by a variable severity of the pathogenesis and in recent years is considered as emerging parasite. Several data suggest that some disorders *B. hominis*-associated are subtype-dependent and to date 10 subtypes (ST1-ST10) have been isolated in humans. In order to generate data on the subtype distribution of *Blastocystis* in Côte d'Ivoire, a survey was carried out on *Blastocystis* isolates from humans and domestic birds.

One hundred nine human fecal samples were randomly collected in four localities in the south of Côte d'Ivoire. Through a second sampling in a rural village, 31 fecal specimens (15 from humans, 14 from chickens, 2 from ducks) were further collected. After DNA extraction using the QIAamp DNA Stool Mini Kit (Qiagen), the specimens were tested by nested PCR for accurate subtyping. A 600 bp region of the small subunit (18S) rRNA *Blastocystis* gene region was obtained for subtype attribution by sequencing and phenetic analysis.

As regards humans, seventy one *Blastocystis* isolates were analyzed, 39 were from females, 32 from males, aged 1-74 years (mean 15.7). Only 9 patients presented symptoms. The phenetic tree revealed three clusters including 35 isolates for subtypes ST1, 16 for ST2, and 20 for ST3. For chicken, 4 samples resulted positive and were grouped in the ST7 cluster.

This study represents the first contribution on the molecular characterization of human and chicken *B. hominis* in Côte d'Ivoire. Although referred to only one village, *Blastocystis* transmission between human and domestic birds seems unlikely. A better understanding of the distribution of STs among human populations and domestic animals represents an essential requisite for establishing the epidemiology and the zoonotic potential of *B. hominis*.

UPDATES ON THE DISTRIBUTION OF *DIROFILARIA* SPP IN THE SOUTHERNMOST TIP OF APULIA REGION

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Dirofilaria immitis is the causative agent of canine heartworm disease. The increasing movement, of dog, climate modifications and the introduction of new vectors (such as Aedes albopictus), affected on the distribution of this filarioid in Italy in the last decades. In the southern regions, including Puglia, the occurrence of *D. immitis* was sporadic before the 90s', with few reports in the Daunian and Gargano areas. Starting from 2006, autochthonous cases of disease have been recorded in the southernmost tip of the region, including the Salento area, where the seroprevalence (ELISA) was 13.6 -24.6 %. Due to lack of data on the distribution of *D. immitis*, this study aimed to assess its presence in dogs living in the municipality of Lecce.
From April 2014 to October 2015, sheltered dogs living in Copertino and owned dogs living in the suburbs of Lecce were examined for filarial infection. Animals had never travelled in other areas. A modified Knott test was performed on blood samples to detect and identify microfilariae. Out of 132 examined dogs (69 from the kennel and 63 owned), 8 (6 %) tested positive for *D. immitis*, showing clinical signs, with 2 individuals coinfected by *D. repens*. Due to the distance, management and the ecological characteristics of the areas is possible to consider the two groups as different populations. Negative the dogs of Jonic coast, the prevalence in the Adriatic area for *D. immitis* and for *D. repens* raised to 13 % and 3 % respectively. In the same area, also a human case of ocular infection by *D. repens* was reported.
The results indicate that transmission cycle of filarioids is also active in Puglia. Further studies should investigate the dynamic of the infection and the mosquito species involved. Practitioners should be warned on the presence of *D. immitis* and undertake a chemoprophylactic regimen.

DIFFUSION OF *BABESIA CABALLI*, *THEILERIA EQUI* AND *ANAPLASMA PHAGOCYTOPHILUM* IN EQUIDS OF RAGUSA PROVINCE SICILY (ITALY) AND INCIDENCE OF DISEASE EPISODES REPORTED BY OWNERS

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As a consequence of climate changes related to global warming, vector populations and transmission intensity of piroplasmosis causing agents may increase and lead to a rise in the number of clinical episodes. This study aimed to assess the prevalence of *Babesia caballi*, *Theileria equi* and *Anaplasma phagocytophilum* in horses and donkeys living in Ragusa Province of Sicily (Italy) and estimate the incidence of disease episodes based on reporting of horse owners and donkey breeders.
The study was conducted during summer 2013 (April – September) on 115 farms corresponding to 10% of the officially registered horse/donkey/mule holders in Ragusa Province. Information on farm characteristics were collected by questionnaire and blood samples taken from totally 242 equids, analyzed by ELISA and by PCR for the presence of antibodies to and DNA of the 3 pathogens.
The majority of farms (80,0% (CI_{95} 71,5–86,9) and about two third of the tested animals (66.9% CI_{95} 60,6–72,8) resulted positive for one or more of the bacterial and protozoan agents by either PCR or ELISA. *T. equi* was revealed in more than one third of the samples. The PCR and ELISA positivity rates of this protozoan parasite (PCR: 39,3% CI_{95} 33,1–45,7; ELISA 35,7% CI_{95} 29,6–42,0) were higher compared to those of *B. caballi* (PCR: 7,1% CI_{95} 4,2–11,1; ELISA 14,2% CI_{95} 10,0–19,3) and *A. phagocytophilum* (PCR: 15,8% CI_{95} 11,4 –20,1; ELISA 7,0% CI_{95} 4,1–11,0). Horses and donkeys kept on pasture were more infected with the pathogenic agents (82,2%; CI_{95} 71,5–90,2) than those kept in boxes and paddocks (58,9%; CI_{95} 50,5–67,0; p=0,002). Only 3 horse owners and 1 donkey breeder of the totally 115 involved farms have reported a disease episode in the previous 12 months, indicating that piroplasmosis as a disease is not a major problem in the area.

COPROMICROSCOPIC MONITORING OF GASTRO-INTESTINAL HELMINTHS IN HORSES FROM ITALY

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During 2015 a parasitological monitoring of 3881 horses belonging to 292 stables from continental Italy was carried out in order to update data on most common gastro-intestinal helminths of these animals. Individual faecal samples were examined using McMaster slides with a NaCl flotation solution (Specific Gravity 1200). The 44.7% of the horses were positive for gastro-intestinal helminth eggs (1734/3881), while 42.1%, 6.9%, 1.1% and 0.13% respectively for eggs of Intestinal Strongyles (IS) (1632/3881), ascarids (267/3881), anoplocefalidae (44/3881) and oxyurids (5/3881). The higher prevalence rates for IS were found in May (50.3%; 153/304) ($\chi 2$ trend = 16:54; P <0.0001) and in horses between 1 and 5 years of age (55.2%; 423/766) ($\chi 2$ trend = 100.4; P <0.0001). The average value of EPG to IS was of 264.1 ± 761.3, while in positive animals was of 628 ± 1072.5 The 45.6% of positive horses to IS (745/1632) had averages EPG ≤ 200, that means in the threshold limit considered of contained contamination, while 20.9% (341/1632) had EPG averages ≥ 1000, indicative of severe infestation. EPG averages stratified per month of the year showed significant differences, with higher values in March (average of 374.6 EPG) (Kruskal-Wallis test - H = 54.34; P<0.0001), and in those aged ≤ to 1 year (436.9 EPG ± 833.3) (H = 149.46; P <0.0001).The stratification of prevalence rates for the year for ascarids showed statistically significant differences, recording a rise up of the values in February (11.2%) ($\chi 2$ trend = 12.10; (P = 0.0005). No significative difference was detected for the monthly prevalences for anoplocefalidae ($\chi 2$ trend 1.44; P = 0.231). Considering the high prevalence rates of gastro-intestinal helminths in horses and the 20.9% of animals with severe infestation, the responsible use of equine anthelmintics only after a proper diagnosis in parasite prevention programs should be mandatory.

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CROSS-SECTIONAL SURVEY ON THE OCCURRENCE OF *CAPILLARIA BOEHMI* INFECTION IN CANINE POPULATIONS OF CENTRAL ITALY

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Capillaria boehmi (syn. *Eucoleus boehmi*) is a trichuroid nematode that infects the mucosa of nasal cavities and paranasal sinuses of domestic and wild canids. In the past few years, nasal capillariosis has been reported in dogs from different extra-European and European countries, including Italy (Baan *et al.*, J Am Vet Med Ass, 2011; Veronesi *et al*., Vet Parasitol, 2013). Nonetheless, awareness on the distribution and clinical importance of this parasitosis is still poor. A copromicroscopic survey was conducted in dogs living in central Italy with the aim to provide new insights into epidemiology of nasal capillariosis.
Four hundred eighty individual faecal samples were collected between 2012 and 2015 from 225 asymptomatic dogs, mostly living in shelters, and 255 owned animals. Signaletic and anamnestic data were collected for each dog. Faecal samples were processed by flotation technique using a zinc sulphate solution; the identity of the barrel-shaped eggs was confirmed by a PCR based on amplification of *cox*1 gene of the Subfamily Capillarinae (Di Cesare *et al*., Vet Parasitol, 2015). A risk analysis was performed through a binary logistic multiple-regression models.
Overall 40 out of the 480 (8.33%) examined dogs showed to be positive for *C. boehmi*. Thirty-three of the positive dogs showed respiratory upper distress and the most commonly recorded symptoms were nasal discharge, sneezing and hyposmia/anosmia. Statistical analysis showed that the prevalence rate of *C. boehmi* infection was significantly influenced ($p < 0.05$) by type of housing (higher prevalence in kenneled dogs), attitude of dogs (mostly hunting animals), presence of copro/geophagia and co-infection with further trichuroid parasites (i.e. *Capillaria aerophila, Trichuris vulpis*); the presence of upper respiratory signs were also related to the occurrence of the nematode.
The present results confirm that nasal capillariosis is present and spread in canine populations of central Italy and underline its clinical importance.

HUMAN BABESIOSIS FROM DEMOCRATIC REPUBLIC OF CONGO

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Babesioses are worldwide emerging tick-borne diseases due to over 100 *Babesia* species that can infect a wide animal host range, humans occasionally included, in which induce malaria-like disorders. The infection in immunocompetent persons may run subclinically or cause mild symptoms, whereas it can be life-threatening in immunocompromised individuals, infants included. The most important zoonotic species is *B. microti,* responsible for most human infections described in US, and for rare cases in Europe and Asia. In Africa uncharacterized isolated babesiae have been found in humans. Aim of this work was to report two cases of human babesiosis detected in Tshimbulu (DRC) during a humanitarian survey carried out in 2014 to provide children with free preventive screening for malaria. Finger pricks, performed to 306 children, provided fresh blood for microscopic analysis and molecular diagnostics. DNAs were extracted from each dried spot and amplified using protocols previously described (Gabrielli et al., 2015. Vector Borne Zoonotic Dis. 15(9):535-8.). Amplicons were purified and sequenced. Sequences obtained were aligned and compared with those available in GenBank™ dataset. As expected, microscopy evidenced plasmodia in 80% of the smears, although the morphology of some parasites was suggestive also of *Babesia*. Indeed, intraerythrocytic ring forms, smaller than those of *P. falciparum,* were evidenced at least in 19 samples, always associated to *P. falciparum*. Sequencing confirmed the presence of *B. microti* (a.n. KT867773) in the blood of two asymptomatic children 2-years-old. Re-examination of all microscopically suspected samples is in progress. To the best of our knowledge, this is the first report of human infection with *B. microti* in Africa, where uncharacterized babesiae have been found in patients from South Africa and Egypt. Therefore, human babesioses could be an emerging sanitary problem also in this Country, where people are exposed to many other parasites that can induce severe anaemia.

AN ENDEMIC HOTSPOT OF *DIROFILARIA IMMITIS* INFECTION IN RURAL DOGS FROM ALBANIA

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Cardiopulmonary filariasis, caused by *Dirofilaria immitis*, is one of the most prominent canine parasitic disease in regions with a warm humid climate. Although documented as early as 1929 in the Balkans, epidemiology of canine heartworm infection has been studied in the countries of eastern and southeastern Europe after 1990 only. In Albania, serosurveys indicated 2.2% to 13.5% positivity for circulating *D. immitis* antigen in dogs, depending on their origin1-4, and heartworms were isolated from 1 of 30 dogs in a necropsy survey5. In the course of an anthelmintic efficacy study conducted in 2015, the cardiopulmonary organs of 24 mixed breed dogs deriving from the district of Durrës, Albania were examined for *D. immitis*. Plasma prepared from blood collected from all dogs was tested for *D. immitis* antigen using a commercial ELISA test kit (SNAP® HTWM, IDEXX Europe B.V.). In addition, skin biopsy samples (8 mm diameter) were collected from the face of six dogs. The skin samples were left overnight in saline and the sediment was then examined for microfilariae. Microfilariae were diagnosed to species by PCR[6]. Plasma samples of 8 of the 24 dogs tested positive for circulating *D. immitis* antigen. Only from these eight dogs, adult *D. immitis* (range, 5-44; mean 21.1) were recovered by dissection of the heart and lung arteries. Microfilariae which migrated from the skin samples of 4 of the 6 dogs were identified as microfilariae of *D. immitis* (four dogs) or *Cercopithifilaria bainae* (two dogs). This investigation demonstrates the existence of hotspots of *D. immitis* infection in Albania although serosurveys indicated low to moderate rates of infection and, for the first time, *Cercopithifilaria bainae*, a tick-borne filarioid parasite, was recorded in the country. In conclusion, veterinarians and dog owners should increase their awareness on canine vector-borne diseases comprising routine screening and preventive measures.

SEROLOGICAL EVALUATION OF *LEISHMANIA INFANTUM* INFECTIONS IN CATS FROM THE AEOLIAN ISLANDS, SOUTHERN ITALY

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Leishmania infantum infection in cats has been detected in endemic areas (e.g., southern Italy) and the role of this animal as reservoirs of *L. infantum* has been debated. Cases of feline leishmaniasis (FeL) have been reported, mainly in cats suspected of having an impaired immunocompetence. However, studies to ascertain the role of cats in the ecology of leishmaniasis are still scant.
The aim of the present study is to assess the antibody prevalence of FeL in a cat population from a selected area where the disease is endemic in dogs and clinical cases of feline leishmaniosis have been described. From January to February 2015, blood samples were collected from 250 cats living on the Aeolian islands (*i.e.*, Lipari and Vulcano). Sera were tested by an indirect immunofluorescent antibody test (IFAT) for *L. infantum*, with a cut-off value of 1:40. *Leishmania infantum* antibodies were detected in 66 animals, with an overall seroprevalence of 26.4% (titer range 40-640). The antibody titer was above 1:80 in 6 cats and the highest positivity rate was in in cats older 2 years of age (36.4%), followed by cats between 1 and 2 years (24.6%) old and <1 year old (15.6%).
The study confirmed that cats living in the Aeolian islands are exposed to *L. infantum* infection, highlighting the need for adopting preventive measures to reduce the risk of infection in these animals.

COPROLOGICAL SCREENING ON PREVALENCE AND INTENSITY OF INTESTINAL PARASITES IN HORSES, ROMANIA, WITH EMPHASIZES ON STRONGYLES

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Gastrointestinal helminth parasites are ubiquitous in grazing horses, and have been associated with poor growth, weight-loss, and clinical disease. Predominant research elsewhere has been and continues to be on strongyles and ascarids, the most potentially pathogenic endoparasites of horses. The present study describes the epidemiology of parasite infections in Romanian horses, excretion pattern, including accurate fecal eggs per gram (EPG) counting and identification of the high parasite egg shedders within different horse populations. A total number of 295 horses were enrolled in the study, including horses residing in two stud farms (n=138) and working horses (n=157) originated from 10 villages, in northeastern and southeastern Romania. Fresh fecal samples were analyzed qualitatively for presence of intestinal parasites using sodium chloride flotation technique and quantitatively, using a modified McMaster technique. For analysis of age- and gender- related differences in strongyle EPG profiles, animals were assigned in age and gender groups. Analysis of distribution of horses with positive strongyle EPG counts, stratified by classes of intensity (from <250 to >2000), was performed to comprise the excretion pattern. Fecal samples of 87.20 % and 73.2 % horses residing in studs and working horses, respectively, were positive for strongyle. Of them, 72.50 % and 46.10 %, respectively, exceeded the cut-off value of 250 EPG. Other parasites, such as *Parascaris equorum*, and *Strongyloides westeri*, were detected in both, horses from stud farms and working horses, but in lower prevalence, while *Anoplocephala* spp. infection was detected only in working horses. The results showed that strongyle infections are highly prevalent in Romanian horses. Moreover, the findings provide further evidence that the egg shedding levels are influenced by both age of horses and level of pasture hygiene, bur clearly demonstrated a higher infection pressure in stud farms than in working horses. These findings represent a base for further studies in designing sustainable control program of equine parasites.

FOOD AND PARASITES

UNDERSTANDING THE JIGSAW OF *ANISAKIS PEGREFFII* CAMPANA-ROUGET & BIOCCA, 1955 IN THE MEDITERRANEAN ANCHOVY AND PILCHARD: THE PIECE OF NORTHERN SARDINIA

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In Europe, human anisakidosis is usually associated to the consumption of exotic raw-fish dishes, whereas the cases described in Italy are related to traditional preparations of marinated anchovy *Engraulis encrasicolus* and pilchard *Sardina pilchardus*.
In order to add information on the presence of *Anisakis* spp. in these two commercially-relevant species, an epidemiological and molecular survey of 1,650 anchovies and 1,800 pilchards from northern Sardinia (western Mediterranean Sea) was carried out in 2014 and 2015.
Larvae, visually detected, were identified under light microscope and stored in 70% ethanol. For a subsample of worms, the ITS region of rDNA was amplified and PCR products digested with the restriction enzymes *Hinf*I and *Hha*I. All the 777 third-stage larvae of *Anisakis* sp. found corresponded to the Type I (sensu Berland, 1961). Digestion of the PCR products showed the RFLP pattern of *Anisakis pegreffii*. No *Hysterothylacium* sp. larva was observed. The total prevalence of infection in the two years was 21.3% and 23.0% in anchovy, and 12.1% and 2.7% in pilchard. The mean intensity was 1.9 and 1.7, and 1.4 and 1.5, respectively. In 2014, the highest prevalences were recorded in August (anchovy 38.0%, pilchard 21.3%), and the lowest in November (10.0%, 4.0%). In 2015, the highest values were in October (55.3%, 7.3%), and the lowest in April (2.0%, 0.0%). Such a pattern may be partially related to seasonal changes, but also to host size, as suggested by the highest intensity of infection (up to 11 larvae in anchovy) observed in largest fish. The results add a piece to the jigsaw on the presence of *Anisakis pegreffii* in Mediterranean small pelagic fish, showing the need for an accurate knowledge as a tool for the development of food-safety strategies.

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SEQUENCE VARIATION IN THE B1 GENE AMONG *TOXOPLASMA GONDII* ISOLATES FROM SWINE AND CATS IN CENTRAL ITALY

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Toxoplasma gondii is an obligate intracellular parasite that infects a wide range of warm-blooded vertebrates, including humans. Although usually benign in immunocompetent individuals, the infection presents a significant health risk in developing foetus and immunocompromised patients. Swine and cats are traditionally considered two primary routes of *T. gondii* transmission to humans through oral ingestion of infectious oocytes from the environment and tissue cysts from undercooked meats. The present study aimed to assess the sequence variation in the B1 gene among *T. gondii* isolates of swine and cats collected in central Italy in order to better define the epidemiological circuit of human infection. Genomic DNA was extracted from diaphragm pillar tissues of 36 pigs managed in intensive farms and 40 wild boars hunted in regional districts of central Italy, where domestic and wild swine populations were previously screened for the presence of *T. gondii* infection. DNA was also obtained from stool samples of 77 cats of the same areas. The genetic target B1 was amplified by specific PCR protocols (Lin et al., 2000. J. Clin. Microbiol. 38(11):4121-5). Amplicons were then purified and sequenced. Sequences were aligned and compared with those available in GenBank™ by BLAST analysis. A total of 36 specimens, including 10 pigs, 13 wild boars and 13 cats tested positive to the B1 gene. Sequences analysis showed the 99-100% identity with *T. gondii* sequences reported in GenBank™. PCR amplification of the B1 gene produced a single product of about 130 bp showing a single base pair polymorphism (C/T) at the position 42 in sequences from swine and cats, respectively. Preliminarily results of the present study evidenced an intra-specific variation of the B1 gene among isolates, suggesting to investigate and compared the sequences here detected with those recovered from human population of the same geographical district.

THERAPY AND DRUG RESISTANCE

CONTROL OF INFECTION BY *EIMERIA TENELLA, EIMERIA MAXIMA* ED *EIMERIA ACERVULINA* IN BROILERS WITH THE ASSOCIATION SULFADIMETHOXINE AND TRIMETHOPRIM

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Eimeria spp. infections in broiler still cause huge economic losses. An experiment was conducted to evaluate the efficacy of a combination of drugs in the control of multiple infections of these parasitic protozoans in chickens experimentally infected.
Five groups of 20 Ross308 broilers at 21st day of life were infected *per os* with 1 ml of a suspension containing 5000 oocists of *Eimeria tenella*, 5000 of *E. maxima* and 10.000 of *E. acervulina* per liter.
At 35th day, the therapy with the association of 200 mg di Sulfadimethoxine and 40 mg of Trimethoprim per ml was subdivided in 4 groups: "A" control; "B" with 0.5 ml/L in drinking water for 5 days; "C" with 1 ml/L for the first day and 0.5 ml/L for 4 days; "D" with 1 ml/L for 5 days, group "E" 2 ml/L for 1 day and 1 ml/L for 4 days. All dead specimens were necropsied and intestinal lesion score was evaluated using Johnson et al. (1970) method. Every day for each group oocyst number were counted on a pool of feces by McMaster method as well as direct observation and enumeration of oocyst were performed on the subjects who died.
Clinical signs attributable to Coccidiosis have appeared at 6th day after inoculation and first mortality was observed after 14 days. In the same day the treatments started and mortality go further for 2 days only in the control group.
The lesion scores show how the inoculation causes macroscopic lesions referable to *E. acervulina* and *E. tenella,* but less obvious for *E. maxima,* in the relative portions of the gut. The groups treated with different dosages had a highly significative reduction ($p<0.001$) of lesions compared to the control group. No significative differences ($p>0.05$) were found between constant dosage (B and D) or variable (C and E). The treatment has seen a good efficacy in reducing mortality, opening a good perspective for the control of these parasites.

MODULATION OF P-GLYCOPROTEIN (PGP) EXPRESSION IN AN *IXODES RICINUS* CELL LINE FOLLOWING AMITRAZ, FIPRONIL AND PERMETHRIN TREATMENT

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Recently, over-expression of ATP-binding cassette (ABC) transporter proteins (P-glycoproteins, PgPs) has been implicated in resistance to acaricides in the tick *Rhipicephalus microplus* (Pohl et al., Vet Parasitol 204:316-322, 2014). Tick cell lines could be useful for investigating resistance mechanisms (Mangia et al., Parasit Vectors., in press). The aim of the present study was to evaluate expression of several PgPs in the *Ixodes ricinus*-derived cell line IRE/CTVM19 and to determine modulation of expression following treatment with amitraz, permethrin and fipronil. Cells were treated with different drug concentrations (amitraz and permethrin: 25, 50, 100 µM; fipronil: 50, 100, 150 µM) and incubated for 10 days. Evaluation of viability and relative expression of ABCB1, ABCB6, ABCB8 and ABCB10 genes were carried out at day 10 post treatment. Cell viability following treatment with amitraz and permethrin (79-84%) was not significantly different from the control (83%); fipronil elicited a decrease in viability (63% at 150 µM condition). qRT-PCR showed that the drugs significantly affected ABC pump expression: fipronil treatment led to down-regulation of ABCB1 and up-regulation of ABCB6, ABCB8 and ABCB10; amitraz treatment resulted in down-regulation of ABCB1 (significant difference between 25 µM and 100 µM) and up-regulation of ABCB6 and ABCB10; permethrin led to up-regulation of ABCB6 and ABCB10 only at 25 µM. Development of an *in vitro* model for the study of acaricide resistance mechanisms would greatly facilitate screening for drug resistance in ticks. However, it is important firstly to determine if and when PgP expression is modulated and how (up-regulation, down-regulation, etc.) and by which classes of drugs. The present study shows that amitraz, permethrin and fipronil are able to significantly up- and down-regulate the expression of different PgPs in tick cells, thus indicating this as a potential model for the study of resistance to these acaricides in ticks.

EVALUATION OF THE EFFECTIVENESS OF A NOVEL ORAL FORMULATION OF SAROLANER FOR THE TREATMENT AND PREVENTION OF *CTENOCEPHALIDES CANIS* FLEA INFESTATIONS ON DOGS

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The efficacy of a single oral dose of sarolaner (Simparica™) for the treatment and prevention of *Ctenocephalides canis* (dog flea) infestations on dogs was evaluated in a laboratory study. The study was conducted using adult purpose-bred Beagle dogs. Eight dogs were enrolled per treatment group. All animals were individually housed and were randomly allocated to treatment with either placebo or sarolaner based on pre-treatment flea counts. Dogs were infested with 100 unfed, adult *C. canis* prior to treatment and at weekly intervals post-treatment for 35 days. Dogs were treated on Day 0 with a placebo or a sarolaner tablet providing a dose of 2 mg/kg. Comb counts were conducted to determine the numbers of viable fleas at 24 hours after treatment and after each subsequent infestation. The dogs were thoroughly combed to remove fleas for counting. Fleas able to stand upright and/or move in a coordinated manner were considered live. There were no adverse reactions to treatment with sarolaner. Dogs in the placebo group maintained *C. canis* infestations throughout the study with post-treatment arithmetic mean counts ranging from 72.0 to 95.6 *C. canis*/dog. No live fleas were recovered from any of the sarolaner-treated dogs at any post-treatment count. Thus efficacy against *C. canis* was 100% for 35 days after a single oral dose.

THE SPEED OF KILL OF EFFETIX® WHEN COMPARED TO NEXGARD™ AGAINST ARTIFICIAL INFESTATIONS OF TICKS (*RHIPICEPHALUS SANGUINEUS*) AND FLEAS *(CTENOCEPHALIDES FELIS*) ON DOGS

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This study compared the speed of kill of a pyrethroid spot-on (Effitix, Virbac, France) to an oral isoxazoline (Nexgard, Merial, France) against artificial infestations of ticks (*Rhipicephalus sanguineus*) and fleas (*Ctenocephalides felis*) on dogs.
On Day 0, 18 dogs were randomly allocated to one treatment group each: untreated, Effitix or Nexgard. Dogs were infested with 50 (± 5) *R. sanguineus* ticks on days 7, 14, 21 and 28, and with approximately 100 *C. felis* on days 8, 15, 22 and 29. Tick counts were performed 0.5, 2, 6 and 12 hours after infestation. Detached ticks were collected at 0.5 and 2 hours after infestation. Tick counts and removal were performed 24 hours after infestation. Flea counts were performed 30 minutes (with re-infestation) and 24 hours (with removal) after infestation.
Fewer ticks were recorded for the Effitix group compared to the Nexgard group ($p < 0.05$) for all days, up to 12 hours after infestation. More ticks were repelled in the Effitix group compared to the untreated group and the Nexgard group ($p < 0.05$) for all time points and days. No statistically significant differences were recorded for ticks repelled in the Nexgard group compared to the untreated group at any time point or day. The anti-attachment effect of Effitix against tick infestations on dogs was greater than that of Nexgard for up to 12 hours after infestation. Fewer fleas were recorded for the Effitix group compared to the Nexgard group ($p < 0.05$) at 30 minutes up to Day 22. Both products had a similar efficacy against *C. felis* infestations at 24 hours post-infestation.
The permethrin-based spot-on (Effitix) had a faster speed of kill effect (from as soon as 30 minutes against both ticks and fleas) and a better repellent activity against ticks on dogs than the afoxolaner tablet (Nexgard).

EVALUATION OF THE ACARICIDE ACTION OF SOME VEGETAL AND/OR SYNTHETIC SUBSTANCES IN COLONIES OF *APIS MELLIFERA LIGUSTICA* NATURALLY INFESTED BY *VARROA DESTRUCTOR*

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Goal of the present work* was to evaluate the action of some vegetal and/or synthetic substances, with low impact on the beehive system, on *Varroa destructor*.
The substances, tested at 2% concentration, were: Test1 = Propolis+Cinnamyl-alcohol; Test2 = Propolis+Orange Terpenes; Test3 = Propolis+Cytral; Test4 = Propolis+Cinnamyl-*alcohol*+Orange Terpenes; Test5 = Octanoic acid; Test6 = 1-decanol; Test7 = Octanoic+Nonanoic+Decanoic acid; Test8 = Cinnamyl-*alcohol*; Test9 = Ajowan essential oil; Test10 = Methyltrans-cinammate.
The efficacy trial was carried out during the year 2012-2013 on no. 66 beehives, showing a high different level of natural infestation, ranging from 407.5 to 4,266.5 total mites (average 1,736.08 mites, d.s. ±1,249.44). The trial was performed on 11 groups of 6 beehives constituted by 10 honeycombs full of bees and broods each one. Ten groups were tested with the substances applied using 2 wood strips (3 mm of poplar plywood) and one group was maintained as control.
In none of the beehives bees died or the brood was damaged. All the tested substances showed a low lethal action against *V. destructor*, increasing in the 4 weeks after the treatment. The highest rates of efficacy were obtained in Test2 (24.79%), followed by Test1 (19,88%) and Test7 (18.63%); the lower values were observed in Test10 (5.27%).
The low rates of efficacy might be explained by the low dosages used, applied for a preventive goal because these substances had never been employed before in the beehives.
Since the absence of toxic effects, the use of these substances at higher dosages may be suggested, especially during warm seasons, when the anti-*Varroa* treatments don't allow the use of essential oils, which rapidly volatilize, and may induce the colonies to abandon the beehive.
**Research supported by Mi.P.A.A.F.*

MOLECULAR BIOLOGY AND PHYLOGENY IN PARASITOLOGY

MOLECULAR IDENTIFICATION OF *HYSTEROTHYLACIUM* SPP. FROM FISHES OF SICILIAN COASTS (SOUTH MEDITERRANEAN SEA, ITALY)

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In this work a total of 3017 fish samples belonging to 11 species (*Lophius piscatorius, Zeus faber, Engraulis encrasicolus, Merluccius merluccius, Mullus barbatus, Trachurus trachurus, Trachinus draco, Trigla lyra, Conger comger, Phycis phycis. Trachuru trachurus* and *Pagellus erythrinus*) from Sicilian Mediterranean coasts (SE Mediterranean Sea; FAO zone 37.1.3, 37.2.2), were examined for the detection of *Hysterothylacium spp.* larvae and subsequent phylogenetic studies. The fish samples were examined for the research of nematodes by visual inspection and digestion method according to the EC Regulation 2075/2005. Detected larvae were subjected to morphological identification through the optical microscopy. By this method it was detected the gender and the morphotype for the genus *Hysterothylacium spp.* Subsequently, the DNA was extraction and polymerase chain reactions targeting the complete nuclear ribosomal internal transcribed spacer (ITS-1, 5.8S, ITS-2) and the cytochrome c oxidase subunit II (*cox2*) were performed and subjected to sequence reaction. Fifty-eight Raphidascarididae parasitic nematode were found in the examined fishes with prevalence values from 0.2% in to *Engraulis encrasicolus* to 60% in *Phycis phycis* samples. Parasites were characterized as *Hysterothylacium fabri*, *Hysterothylacium aduncum* and *Hysterothylacium bidentatum* by sequencing of ITS and *cox2* regions. Pairwise comparison between the ITS region of the *Hysterothylacium fabri* isolated in Sicilian coasts and *Hysterothylacium fabri* isolated from the Mediterranean Sea (Turkey, KC852206) showed differences ranged from 0.015 to 0.018. The *Hysterothylacium aduncum* samples isolated in our study revealed very low genetic differences with *Hysterothylacium aduncum* from Adriatic sea (KP979763; 0.00-0.003). Finally, among *Hysterothylacium bidentatum* and reference sequence (AY603539) a genetic distance of 0.053 was detected. The ITS and *cox2* Neighbor Joining tree indicated three distinct clusters among specimens. The results confirmed a correlation between *Hysterothylacium* spp. species infestation and fish hosts as a result of co-phylogenetic phenomena. Furthermore a genetic difference was verified in accordance with geographic distribution.

NUCLEAR GENETIC MARKERS FOR MULTILOCUS PHYLOGENY OF *RHIPICEPHALUS* TICKS

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The genus *Rhipicephalus* (Acari: Ixodidae) comprises tick species of great veterinary and medical interest, being vectors of several pathogens to animals and humans worldwide. The phylogeny and systematics of ticks belonging to this genus remains debated. Molecular identification and genetic relationship of *Rhipicephalus* spp. have been based mostly on the analysis of mitochondrial markers (e.g. ribosomal 16S and 12S subunits, cytochrome *c* oxidase subunit 1 or cytochrome b genes). Nuclear marker (i.e. ribosomal internal transcribed spacer-2 (ITS-2) region) showed to be unsuitable for the genetic delineation of some *Rhipicephalus* spp. Because the use of mitochondrial data alone might be potentially problematic at lower taxonomic levels due to potential pitfalls of introgression and incomplete lineage, the aim of this study was to discover new nuclear DNA markers useful for phylogenetic and population studies of *Rhipicephalus* spp. The EvolMarkers database was used for developing single-copy coding sequence (CDS) and exon-primed-intron-crossing (EPIC) markers. Twenty-four candidate markers were identified and oligonucleotide primers designed for eight of them. Three loci showed successful amplification on brown dog ticks from Europe, Asia, Africa and Americas, that have been previously analyzed by morphological and genetic analyses. These markers represent a step forward for more extensive characterization of *Rhipicephalus* spp.

VARIABILITY INSIDE *TAENIA MULTICEPS*: MORPHOLOGY AND HAPLOTYPES

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ABSTRACT

Coenurosis is a zoonotic parasitic disease caused by the metacestode stage of *Taenia multiceps* (Cestoda, Taeniidae) commonly known as *Coenurus cerebr*alis (Rostami et al., 2013). Information on the genetic variability of this parasite species is necessary for both epidemiological studies and for the implementation of control programs. The aim of this study was to investigate the genetic variation and population structure of *Taenia multiceps*, and to correlate morphological features of individual coenuri with haplotypes. Between May 2012 and July 2015, 92 animals (86 sheep; 4 goats; 1 cattle; 1 mouflon, *Ovis musimon*) showing clinical symptoms of cerebral coenurosis were included in this study. Putative *T. multiceps* coenuri (n = 118) were sampled from live animals and 52 metacestodes selected for a morphological and molecular characterization. Gene genealogies and population genetic indices were also determined. Morphological features of the selected coenuri (number and size of large and small hooks) were within the range reported in the literature. For the 379bp *cox1* dataset we identified 11 polymorphic sites of which 8 were parsimony informative. A high haplotype diversity (0.664±0.067) was recorded for the *cox1* sequences defining 10 haplotypes (TM01-TM10). The comparison of haplotypes generated in this study with published *T. multiceps* Tm1 variant pointed to the possible existence of a common lineage for *T. multiceps*. No correlation was detected between the size of the small and large hooks and the *cox 1* haplotypes. Polycystic infestation (2 to 9 coenuri) was recorded in 27.7% of animals (13/47). No statistical correlation between polycystic *T. multiceps* infection and haplotypes was detected. This is the first study that identifies haplotypes of *T. multiceps* in Italy and compares them with morphological features of coenuri. It is also the first report on the population structure of *T. multiceps* derived from various intermediate hosts from this island.

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MOLECULAR SCREENING FOR *MIDICHLORIA* BACTERIA IN HARD AND SOFT TICKS (ACARI: IXODIDA)

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Ticks can harbour microbial communities that play different roles in the biology of the host. Among these bacteria, *Midichloria mitochondrii* (Order Rickettsiales; family Midichloriaceae) was firstly described inside the intermembrane space of mitochondria of *Ixodes ricinus* females oocytes and subsequently observed in salivary glands. This bacterium is present in 100% of females and immatures, is vertically transmitted and is less prevalent in males (44%). In addition, the possibility of horizontal transmission through the blood meal is suggested by serological and molecular analyses showing positivity of mammalian bloods and sera to *M. mitochondrii* circulation. However, the role of this bacterium is still unknown. Several species belonging to the six most important genera of hard ticks (i.e. *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Rhipicephalus*) have been observed to harbour *Midichloria* bacteria, but only *I. holocyclus* presents bacterial loads similar to *I. ricinus*.
This work aims to expand the knowledge about the distribution of *Midichloria* bacteria in ticks, screening a total of 93 samples (16 Ixodidae and 1 Argasidae species) from different geographical areas by using qualitative (targeting the *16S-rRNA*) and quantitative (targeting the *gyrB* gene) PCR approaches. A total of 8 species out of 16 hard tick species were found positive for *Midichloria* at different inter-intraspecific prevalence levels. A 100% prevalence observed in *I. aulacodi* females and a high *Midichloria* load could indicate a mutualistic relationship. The obtained *16S-rRNA* sequences showed between 99% and 100% similarity with *M. mitochondrii*, and, basing on a phylogenetic analysis, we propose that these bacteria could be classified within the genus *Midichloria*. Furthermore, our results support the possibility of a horizontal transmission of these bacteria, as it often happens for tick-borne rickettsiae. Further studies focused on other genetic markers will allow to understand the genetic variability of *Midichloria* in ticks and to test the above hypotheses.

IMMUNOLOGY AND VACCINES

EXPRESSION ANALYSIS OF RELEVANT GENE TARGETS FOR HYGIENIC BEHAVIOUR IN HONEYBEES (*APIS MELLIFERA*)

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Honeybees (Apis mellifera) evolved social immunity mechanisms consisting in the cooperation of individuals to control disease levels in the hive, and in particular Hygienic Behavior (HB) is based on the uncapping and removal of dead, diseased or parasitized brood. Currently, the genetic and biochemical elements driving the manifestation of HB are largely unknown, and gene expression analysis can provide a valid tool to identify coding and non-coding HB marker genes.
This work was aimed to assess differential expression of selected gene targets between honeybee families with high and low HB, respectively.
The experimental activities were set as follows: a) in-field HB phenotypic characterization of 11 honeybee colonies during summer/spring 2015 through freeze-killed brood assay, with the cooperation of professional honeybee breeders (Elio Bonfanti, LC; Lorenzo Sesso, VA); b) a gene expression study on nurses from both high-HB and low-HB families, to assess differential gene expression between the two phenotypes. The following target genes were analyzed based on previous literature findings of potential link to HB and/or learning processes: ame-miR-219, ame-miR-276, ame-miR-932 and let-7a (non-coding microRNAs) and Act5C, Mblk-1 and Obp4 (coding genes). The expression analysis was performed through the following steps: 1) sampling of 10 nurse honeybees from each family, 2) dissection and pooling of brains, 3) total RNA extraction, quantification and retrotranscription, 4) RT-quantitative Real Time PCR, 5) selection of the normalizing gene through geNorm and NormFinder algorithms, 6) differential expression analysis of the candidate markers.
The preliminary results of the study were: a) identification of let-7a as the most stable normalizing gene; b) higher Mblk-1 expression in brains of nurses with higher HB.
During 2016, a larger number of families will be tested for HB and gene expression analysis in order to confirm the identification of candidate biomarkers to aid molecular evaluation of HB at the family level.

HOW *ANISAKIS PEGREFFI* (NEMATODA: ANISAKIDAE) MODULATES DENDRITIC CELLS DIFFERENTIATION

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Anisakis pegreffii is considered by the European Food Safety Authority (EFSA) as the most important Biological Hazard in the "seafood" (EFSA, 2010) since it has been identified as zoonotic agent of gastric, intestinal and allergic anisakiasis. So far, the mechanism of the interaction between the parasite and the human host immune system has not been fully elucidated. Aim of this study was to study and characterize the effects of the larval anisakid *Anisakis pegreffii* on dendritic cell (DCs) biological behaviour.
Monocyte derived DCs were differentiated in IL4 and GM-CSF, matured with inflammatory cytokine cocktail, in the presence/absence of *A. pegreffii* crude extract (CE), to mimic parasite-DCs interactions. When parasite CE was present during DC differentiation, iDCs displayed a more immature phenotype, and DC maturation was also impaired (low CD86, HLA-DR and no CD83). These phenotypic changes were accompanied by a more pronounced ERK1,2 signalling as compared to control DCs, while NF-Kb signalling was not affected. Furthermore, the presence of CE in the culture increased DC apoptosis (up to 25%) and when such parasite exposed DCs were used as Antigen Presenting Cells (APCs), a decreased of IFN-ϒ production by T cells was observed. Interestingly, when iDCs were exposed just before maturation to CE, DC maturation was not affected.
These results indicated that the exposure to *A. pegreffii* CE impaired DC viability and differentiation and maturation, modulating the ERK1,2 signalling. These events results in holding up DCs in an immature state, reducing their ability as APCs to trigger an efficacious Th1 response. The interaction between *A. pegreffii* and DC precursors seems to be relevant for inducing a "crippled" immune response, thus suggesting DC impairment induced by *A. pegreffii* as a possible mechanism of immune escape adopted by the parasite.
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PRELIMINARY RESULTS OF A PILOT FIELD STUDY: IMMUNOPARASITOLOGICAL EVALUATION AND LONG TERM FOLLOW-UP OF DOGS VACCINATED WITH THE LiESP/QA-21 VACCINE (CANILEISH®) IN ENDEMIC AREAS OF CANINE LEISHMANIOSIS

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This study aimed to follow the impact of the LiESP/QA-21 vaccine (Canileish®, Virbac, France) on selected cellular immune parameters considered as key markers of the appropriate Th1 response, in dogs living in leishmaniosis endemic areas. Seven healthy client owned dogs from Greece were vaccinated with Canileish®. They all received a primo-vaccination (one subcutaneous dose every 21 days; total of three doses), followed by annual booster injections (one dose per year). The dogs were followed for a duration of forty months after the primo-vaccination. The results were compared to control sentinel dogs (unvaccinated dogs coming from the same regions). The cell-mediated immunity response was assessed at 6, 12, 15, 22, 27, 33 and 40 months post-vaccination through the analysis of PBMC, lymph nodes (peripheral response), and skin biopsies (local response). At each time, the status (infected/non infected) was assessed with rapid-tests (Speed Leish K®, Virbac/BVT; Idexx Snap® Leishmania test), confirmed by the isolation of the parasite.
In the vaccinated dogs, a Th1-dominated cell-mediated immune response (leishmanicidal activity, induction and activation of effector cells and expression of various Th1-profil cytokines) was observed from 6 to 40 months post-vaccination, at a peripheral level, and also locally in the skin, with a stimulation of the response after each booster.
These preliminary results on a small number of vaccinated dogs with LiESP/QA-21 naturally exposed to infection with L. infantum are encouraging since they are in line with those previously observed in experimental studies where the vaccination was capable of stimulating an appropriate protective Th1-dominated cell-mediated immune response. The local immune response induced by the vaccination could play a key role in the protection against infection through phlebotomes. These results may allow, when validated on a larger number of dogs, to evaluate and follow the vaccination of dogs with Canileish® in endemic regions of canine leishmaniosis.

POSTER
FRIDAY 23ND JUNE 2016

MONITORING AND CONTROL OF VECTORS

PRELIMINARY RESULTS ON THE EFFICACY OF FOUR ALGAL EXTRACTS AGAINST LARVAE OF *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

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Aedes albopictus is one of the most aggressive and invasive mosquito species worldwide and it is also a competent vector of various pathogens.
The most used control methods include chemical treatments against adult mosquitoes and biological control of larvae. However, it is crucial to assess and develop efficient and sustainable vector control tools, in order to face the insecticide resistance and the current restriction to use of biocides in the EU. Few studies are available on the potential larvicidal activity of seaweeds and their compounds. The aim of this study was to investigate the potential activity of crude extracts obtained from different algae species collected in Mediterranean Sea against larvae of *Ae. albopictus*. We screened dried ethanol and acetone extracts of four algal species (*Ulva rigida*, *Asparagopsis taxiformis*, *Dictyota dichotoma*, *Cystoseira barbata*). Tests were carried out using ten specimens of third instar larvae per test, following the WHO standard protocol with little modifications. Since a substance is considered active as larvicide when it shows a LC_{50}< 100 mg/l (LC_{50}: concentration that causes 50% of mortality), a preliminary screening using high concentration of extracts (>100 mg/l) was carried out. In case of positive test (larval mortality) new tests were performed at lower concentrations. Among algal extracts, only one species was active at high concentration: *D. dichotoma*, which killed all larvae by ethanol extract and 7/10 by acetone extract. Then, an assay using *D. dichotoma* at lower concentrations was carried out. The values of LC_{90} was 100 mg/l and of LC_{50} was 50 mg/l for ethanol extract. A significant mortality was obtained with high concentration of acetone extract. In conclusion, *D. dichotoma* is a potential candidate as larvicide against *Ae. albopictus*. This preliminary test can be the starting point to evaluate a new approach of larval mosquito control using natural compounds.

INTRASPECIFIC COMPETITION AMONG LARVAE OF *AEDES ALBOPICTUS* IN CONDITIONS OF FOOD ABUNDANCE AND SHORTAGE

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Aim. Competition among larvae of *Ae. albopictus* under food abundance and shortage was examined in order to predict ecological, economic and health factors for humans and animals as well as for the biological control of mosquitoes.
Methods. 60 cups of water were used as breeding containers of *Ae. albopictus* larvae. Different levels of competition were set by varying larval density (cups with 20, 40, 80, 120, 180 larvae) and food regime with food shortage (0.06 g of food per cup) and food abundance, in proportion to the number of larvae (0.07g/20, 0.14g/40, 0.28g/80, 0.42g/120, 0.63g/180), with six replicates for each condition. Mortality of larvae, time of adult emergence, number and gender of emerging adults, and length of their bodies were measured. Statistical analysis was performed using Pearson's chi-square test, Wilcoxon sum of ranks test and Shapiro-Wilk normality test (significance: P value <0.05)
Results. The total number of emerging adults was 555 (144 females and 411 males) under food shortage, and 1,079 (467 females and 612 males) under food abundance (highly significant difference between adult numbers, chi-square test P<<0.01 and between proportions of emerging males and females, P<<0.01, fewer females emerged under food shortage). In both food conditions, adults emerged between the 8th and 13th day. For all larval densities, there were significant differences with Wilcoxon's test for body size between genders under food abundance, the bodies of males being smaller than females. Under food shortage, no significant differences in size between genders were found for low larval numbers, indicating that food competition tends to homogenize body size. Adult sizes of the same gender were significantly different under food abundance and shortage, both genders being bigger with abundant food.
Conclusions. Food competition determines high mortality, smaller adult size and smaller proportion of surviving females, but does not affect the time of adult emergence.

THE "FIRST LINE OF DEFENSE" AGAINST ACARICIDES IN THE BROWN DOG TICK *RHIPICEPHALUS SANGUINEUS* SENSU LATO

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Understanding the molecular mechanisms of cellular defense against insecticides is a critical research issue in medical and veterinary entomology, as insecticide/acaricide resistance is seriously affecting the effectiveness of control strategies on arthropods species. Recently, mounting data are showing a relevant role of ABC transporters in the defense/resistance to insecticides in vector species. However, at present this detoxification mechanism has been poorly investigated in ticks and no data are available for *Rhipicephalus sanguineus* sensu lato, the most widespread tick species worldwide. Here, we aimed to: *i*) investigate the possible role of ABC transporters against the acaricides fipronil (FIP) and ivermectin (IVM) in *this species* using toxicological assays; *ii*) isolate putative ABC transporter genes by transcriptomic analyses. Toxicological assays showed an increased toxicity of about 14- and 22-fold for FIP and IVM respectively, after ABC transporters inhibition by Cyclosporine A. Transcriptomic analyses identified 81 genes encoding for ABC transporters and assigned them to seven of the eight subfamilies identified in arthropods. The results presented here showed for the first time a strong association between ABC transporters and acaricide detoxification in *R. sanguineus* s.l. and add new genomic data for future studies on this important tick species. Funding for this work was provided by the Ministero dell'Istruzione, dell'Università e della Ricerca (PRIN 2010).

ANTI-FEEDING EFFICACY OF A HUMAN TOPICAL FORMULATION OF NEEM OIL AGAINST *AEDES ALBOPICTUS*

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Prominent botanical pesticides are those based on the neem tree extracts (*Azadirachta indica*, Meliaceae) whose seed kernels are rich in bioactive azadirachtin and other limonoids. Besides insecticidal effects, neem oil was shown to have anti-feeding activity against bloodsucking insects including anopheline and culicine mosquitoes, and phlebotomine sand flies. As with other natural products, neem oil has an excellent safety profile, however its persistence is affected by the rapid degradation of azadirachtin under natural conditions. Our objective was to determine the effective dose and efficacy duration of a commercial neem-oil product against the bites of the mosquito *Aedes albopictus*, a proven or potential vector of emerging Flaviviridae viruses in urban settings.
A topical formulation of neem oil containing 2400 ppm azadirachtin (RP03® Human Care, Farmaneem, Italy) was employed at different dilutions using laboratory-reared *Ae. albopictus* and human volunteers. Assays were performed following to the WHO Guidelines for efficacy testing of mosquito repellents. Each of 2 volunteers performed 6 replicate assays using 50-100 caged mosquito females, and consisting in 5 min exposure of the untreated hand (control) followed by 5 min exposure of the hand treated with 0.25 ml of serial (in dose-response assays) or fixed product dilutions (in duration assays). Insect landing and probing attempts were recorded.
In the dose-response assays, the anti-feeding efficacy increased significantly from 38.5% at 1% dilution to 99.5% at 15% dilution, with an ED_{90} in the range of 6-10%. In the efficacy duration assays, a fixed dose of 6% or 10% conferred an average protection of 92.4% at 5 min, which dropped to 38.1% (range 28-53%) at 3 hours from treatment and further decreased thereafter. We conclude that topical neem oil can be highly effective for short-term exposures to *Ae. albopictus*, whereas long-term efficacy would require frequent applications which, however, are not contraindicated because of the excellent product safety.

A COMPARISON BETWEEN TICK AND MOSQUITO POPULATIONS IN AGRIGENTO AND RAGUSA PROVINCES (SICILY, ITALY)

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Ticks and mosquitoes are important vectors of pathogens causing diseases to humans and animals.
Sicily is a typical Mediterranean ecosystem, suitable for entomological studies. Sicilian provinces facing the Mediterranean Sea act as cross border between Italy and Tunisia.
The study aimed to analyse composition and distribution of tick and Culicidae population in Sicilian cross-bordering provinces.
Sampling was carried out seasonally for one year in Agrigento and Ragusa provinces. Ticks were collected directly from animals (ovine, bovine, equine), while Culicidae were sampled in equine farms using light CDC traps, BG Sentinel traps with lurex, Universal Traps with UV light and lurex. Traps worked for 48 consecutive hours to capture mosquitoes active in the day and in the night. Egg-traps were placed in ovine, bovine and equine farms to monitor *Aedes albopictus* egg laid.
The two provinces showed a different tick distribution profile. *Rhipicephalus* spp. ticks (*R.turanicus*, *R.sanguineus* and *R.bursa*) were predominant in Agrigento, while in Ragusa *Hyalomma lusitanicum* was the most representative species. A remarkable variety of mosquitoes species was collected, including *Culex pipiens*, the most competent vector of West Nile virus in Europe, and *Aedes albopictus* (Asian tiger mosquito), responsible of arboviruses transmission. In Ragusa province, a higher number of mosquitoes was found with respect to Agrigento. *Aedes albopictus* eggs were found in the two provinces with differences related to the animal breeding.
This study provides information on distribution of ticks and Culicidae relevant for animal and human health and focuses on the importance of sampling methods and sites. These one have to be chosen basing on vector ecological behaviour, integrated by the environmental characteristics of the area in terms of macro and micro habitats.
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PARASITES IN WILDLIFE

THE ROLE OF RED FOX (*VULPES VULPES*) IN THE EPIDEMIOLOGY OF *TAENIA MULTICEPS* IN SARDINIA, ITALY

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ABSTRACT

In this study, the role of red fox as competent definitive host for *Taenia multiceps* was investigated towards a better understanding of its epidemiology.

Between 2012 and 2014, 63 carcasses of red foxes (*Vulpes vulpes*) were examined at necropsy. *Taenia multiceps* collected were identified using morphometrical keys and stored for molecular identification. Eggs were separated from gravid proglottids, and after performing egg vitality test as described by Deplazes (2005), the egg suspension was enclosed into gastro-resistant capsules and four sheep and one goat were orally inoculated with 3000 (Sheep1), 5000 (Sheep2, Sheep3 and the goat) and 7000 eggs (Sheep4).

Animals were daily examined for clinical signs and neurological alterations, and magnetic resonance imaging scan was executed every 30 days up to cysts removal. Size, localization and number of coenuri detected were documented. Surgical treatment of positive animals was performed at the end of the challenge. Collected coenuri were identified using morphological and molecular methods.

Four of 63 foxes (6.3%) were found infected by *T. multiceps*. Three of the small ruminants challenged developed coenuri at 30th days. Sheep2 developed one coenurus while sheep4 and the goat developed 4 and 3 coenuri, respectively.

All the infected animals fully recovered following surgical excision of the coenuri (days 180-240 p.i.). Worthy, all cysts recovered by surgery were fertile with viable protoscoleces inside. Morphological features of cysts were consistent with those of *T. multiceps* cysts and molecular analyses of both adult tapeworms and metacestodes displayed 100% nucleotide identity to *T. multiceps* Tm1 strain.

This is the first study providing unequivocal data showing that foxes excrete *T. multiceps* proglottids with viable and infective eggs, thereby confirming that red fox is a competent definitive host for tapeworm. This finding clearly indicates that coenurosis by *T. multiceps* can be maintained and spread also by this wild canid.

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INTESTINAL HISTOPATHOLOGY DUE TO AN ACANTHOCEPHALAN IN TWO CORVIDS SPECIES FROM NORTH ITALY

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In the control program of wild fauna the competent authority of Emilia Romagna region has identified as indicator animals wild boar (*Sus scrofa*), fox (*Vulpes vulpes*) and corvid species and regular monitoring should be done by Experimental *Zooprophylactic* Institute to assess changes in infectious and parasitic diseases. The Corvidae (order Passeriformes) belong to the most developed avian group. These birds live in very close contact with human residential areas as well as poultry farm and, being migratory species especially in search of the food, they can act as vector for a wide range of pathogens and parasites. There is no previous information on occurrence of endoparasitic helminths in corvids in Italy nor on their histopathological effects on hosts. During this investigation, we studied the histopathology due to an enteric helminth, namely *Sphaerirostris picae* (Acanthocephala) in 80 corvids belonging to two species, 29 hooded crow (*Corvus corone cornix*) and 51 magpie (*Pica pica*). The prevalence of *S. picae* was 10% in both bird species. The intensity of infection was 2-12 worms for hooded crow and 1-9 for magpie.
Intestinal helminths often induce changes in the morphology of the host tissues, which can lead to alterations in the digestive physiology of the host. The histopathology induced by *S. picae* in the intestines of birds was carried out by light and transmission electron microscopy. Both male and female acanthocephalans penetrated deeply through all the layer of the hosts intestine by means of their neck and proboscis, in some instances the proboscis emerged in the peritoneal (abdominal) cavity. At the site of attachment, *S. picae* provoked a complete loss of intestinal architecture and a catarrhal enteritis. The main cellular immune response was an intense eosinophil granulocytes infiltration.
S. picae is not a parasite species that may pose a public health risk.

MOLECULAR SURVEY OF *EHRLICHIA CANIS* INFECTION IN RED FOX (*VULPES VULPES*) FROM SOUTHERN ITALY

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Ehrlichiosis (canine monocytic ehrlichiosis, CME) is a tick-borne disease with a widespread geographical distribution caused by the obligate intracellular bacteria *Ehrlichia canis* that infects domestic dogs and wild canids and, on occasions, humans. In Italy, studies on the CME in dogs are numerous but surveys on red fox (*Vulpes vulpes*) include only two studies reporting the absence of the infection in Tuscany and the 31% prevalence of the examined animals from Sicily. In the present study we molecularly investigated the occurrence of *E. canis* in red fox carcasses (n=105) shot during the hunting season or killed on the road due to traffic accidents between October 2012 and February 2015 from Calabria and Campania regions, in southern Italy. Genomic DNA was isolated from samples (spleen, liver and kidneys) using the commercial kits QIAamp DNA Micro Kit (Qiagen, GmbH, Hilden, Germany). All samples were screened for the presence of *E. canis* DNA, using real-time PCR assay targeting 16S rDNA gene of pathogen (~123 bp). Real-time PCR-positive samples were then tested by nested-PCR. The amplicons were purified and sequenced, and sequences were aligned using ClustalW program and compared with those available in GenBank. Of the 105 red fox examined, 55 (52%) were positive for *E. canis* to the real-time PCR. Nucleotide sequences of *E. canis* from red foxes showed a high homology (from 99 to 100%) with that available in GenBank (KP844663). Results indicate that *E. canis* infections are common in red fox in southern Italy suggesting that a sylvatic life cycle of this pathogen occurs. To the best of our knowledge it represents the first study reporting *E. canis* infection in free-ranging mammals of the peninsular Italy.

MOLECULAR EVIDENCE OF *BABESIA VULPIS* AND *HEPATOZOON CANIS* INFECTIONS IN RED FOXES (*VULPES VULPES*) FROM THE PROVINCE OF PISA (CENTRAL ITALY)

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Piroplasmosis are among the most relevant tick-borne diseases of domestic and wild animals. Wild canids are closely related to dogs and represent a potential reservoir for haemoparasites by harbouring tick-transmitted haemoparasites that can infect dogs. We investigated the occurrence of *Babesia* spp and *Hepatozoon* spp in red foxes (*Vulpes vulpes*) from the province of Pisa to determine their prevalence of infection. For this purpose, conventional PCR assays were carried out on spleen samples from a cohort of 78 red foxes. These included 27 females and 32 males as well as 41 adults and 18 young subjects shot during a fox population control program in 2015. The remaining subjects were of unknown sex and age. *Babesia spp* were detected by using primer PIRO-A and antisense oligonucleotide primer PIRO-B that amplify an approximatively 400 bp portion of the small subunit ribosomal DNA of most *Babesia* species. A fragment of the 18S rRNA gene was amplified using the primers HepF and HepR to detect the presence of *Hepatozoon* spp. All PCR-positive samples were sequenced to determine the species of amplified *Babesia* and *Hepatozoon* DNA. Forty-two (53.8%, 95% CI: 42.8-64.9%) and 29 (37.2%, 95% CI: 26.4-47.9%) foxes tested positive for *Hepatozoon* and *Babesia* DNA, respectively. Sequencing results analysis identified *Babesia vulpes* (formerly *Theileria annae*) and *Hepatozoon canis* as the two piroplasmid species harboured by the red fox population that was examined. These results highlight the current prevalence of hemoprotozoa in red foxes in an area of Central Italy and support their role as sylvatic reservoir of *H. canis* infection for domestic dogs.

EVALUATION OF *TRICHINELLA PSEUDOSPIRALIS* AND *TOXOPLASMA GONDII* INFECTION IN FREE RANGING CORVIDS FROM THE PROVINCE OF PISA

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Magpies (*Pica pica*) and hooded crows (*Corvus corone cornix*) are scavenger birds feeding on carcasses, arthropods, vegetables, small preys and food waste. In Italy, they tend to remain in the same territory and establish large populations both in urban and rural environments. For these reasons, these birds have a potential role as sentinels for the spread of *Toxoplasma gondii* in a given area. *Trichinella pseudospiralis* has been previously reported in corvids. In the present study, 798 free ranging corvids, including 678 magpies and 120 hooded crows deceased following a program of population size reduction of the province of Pisa, were examined for *T. pseudospiralis* and *T. gondii* infections. More specifically, sera from 651 magpies and 120 hooded crows were examined by IFAT for antibodies specific to *T. gondii*. In seropositive birds, the heart was homogenized and DNA was extracted to perform a nested polymerase chain reaction (nPCR) detection method for B1 gene of *T. gondii* and genotyping for SAG. In the case of *Trichinella*, breast muscle samples (50 g each) from 678 magpies and 91 hooded crows were tested by an artificial digestion method. After digestion, eventually recovered larvae were processed for molecular typing. Data were statistically analysed (p <0.05). Forty-five, 41 magpies and 4 hooded crows, out of the 771 examined animals (5.8%) scored positive for *T. gondii* with antibody titers ranging from 1: 25 to 1:100. Seropositivity to *T. gondii* was not statistically different between magpies and hooded crows. *T. gondii* DNA was detected in 15 out of 45 heart samples and the occurrence of genotypes II and III of *T. gondii* was evidenced. No *Trichinella* larvae were detected in muscle samples. This is the first report of *T. gondii* infection in corvids in Italy.

RISK FACTORS OF GASTROINTESTINAL PARASITES AND LUNGWORMS IN DONKEYS IN THE ASINARA NATIONAL PARK (SARDINIA- ITALY)

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The study was aimed to investigate the prevalence, the abundance and risk factors associated to gastrointestinal and lungworms parasites of the autochthonous breed of albino and grey donkeys from the Asinara island.
From June to November 2015 a total of 91 fecal samples were collected from 36 albino and 55 grey donkeys. Sedimentation, Baermann technique and a modified McMaster method were performed; the EPG/OPG (eggs-oocysts/gram) were calculated. Larval cultures were performed from 15 pooled fecal samples and third-stage larvae (L_3) were recovered by a Baermann technique. The association between the endoparasites and the individual variables, the geographical distribution of donkey herd, and land cover types were analysed through a GLM with a ordinal logistic regression (SPSS 20.0, Chicago, IL).
Ninety out of ninety-one donkeys were infected by intestinal strongyles (98.9%), *Strongyloides* (6.6%), *Parascaris equorum* (15.4%), *Oxyuris equi* (2.2%) and *Eimeria leukarti* (2.2%). No eggs of cestodes and trematodes were found. *Dictyocaulus arnfieldi* larvae were found in 46.1% of samples. Fecal pools were positive for Cyathostominae (61%), large strongyles (30%) and *Trichostrongylus axei* (9%) L_3. Strongyles showed the highest egg excretion (mean abundance=1176.4 EPG; min-max=0- 4575 EPG).
Significant risk factors associated to strongyle infection (EPG) were: season; geographical distribution of herds and the land cover types. Egg shedding was 10.887 times higher in autumn than in summer and 2.865 times higher in donkeys from the North than those in the rest of the island. Donkeys from spare vegetation areas shed more eggs than other animals (OR=2.507)
Albino and young donkeys were more at risk for *P. equorum* than coloured and old donkeys (OR=4.289 and OR=0.978 respectively). *D. arnfieldi* larvae shedding was higher in autumn than in summer (OR=5.577).

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

TICKS AND LICE OF THE DONKEYS IN THE ASINARA NATIONAL PARK (SARDINIA - ITALY)

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The aim of this study was to investigate Asinara donkeys for the presence of ticks and lice and associated risk factors.
A total of 113 Asinara donkeys (41 albino, 72 coloured) were surveyed for ticks and lice within a health monitoring plan. Parasites collected were morphologically identified. The infection's ticks level were recorded defining three categories: no infestion, low (1-1 ticks and lice 0 ticks) and high infestion (>10 ticks). Three land cover types were defined to estimate the risk: sparse vegetation; mediterranean shrubland; grassland. The association between the tick level of infestion, the individual variables, the geographical distribution of donkey herd, and land cover types were analysed through a GLM with an ordinal logistic regression (SPSS 20.0, Chicago, IL). *Haemaphysalis punctata* (46,2%), *Hyalomma marginatum* (10.7%) and *Rhipicephalus bursa* (43.1%) were found. A total of 58.4% (66/113) of donkeys were infested by ticks (28.3% albino; 30,1% coloured). The prevalence was 78% (32/41) and 47% (34/72) respectively in albino and coloured donkeys. Albino donkeys group had the highest percentage with high infestion (39% vs 15%; OR= 2.865; P= 0.021). The highest percentage of donkeys with no ticks (57.77%) were from land with "sparse vegetation" and had a low number of ticks (OR=0.185; P=0,001) than donkeys from other areas. *Haematopinus asini* were found on nine donkeys (8%), 8 albino and 1 coloured (OR=17.212, 95% CI 2.067-143,321, P= 0.009).
Significant risks to tick infestion were associated to the colour of coat and the types of land cover. Albino donkeys show a 3.120 times higher risk than coloured donkeys to be infected by ticks. Donkeys from areas with sparse vegetation cover showed a lower risk to be infected by ticks (OR=0.227). This study confirms the presence of *Haemaphysalis* sp. and *R. bursa* but not the presence of other species founded in previous study.

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

FIRST REPORT OF AN INFESTATION BY *HYPODRMA ACTAEON* IN ROE DEER (*CAPREOLUS CAPREOLUS)*

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Two subcutaneous larvae of *Hypoderma* spp (Diptera: Oestridae) were detected in the dorsal region of one female roe deer hunted killer on January 23rd 2016 in the North of Guadalajara province (Central Spain). The larvae were observed in the inner side of the hide during the skinning of the animal; one of them was surrounded by an intense hemorrhagic reaction. The larvae were collected and stored into 10% formaldehyde. Identification was made on the basis of the morphological features indicated by Otranto *et al.* (2003).
Both larvae were identified as third instars of *Hypoderma actaeon* Brauer, being the first confirmation of this species in roe deer. The most common species of *Hypoderma* in cervids are *H. actaeon* and *H. diana,* characterized by the presence of subcutaneous warbles. *H. actaeon* is considered strongly specific of red deer (*Cervus elaphus*), being sporadically found in fallow deer (*Dama dama*); whereas *H. diana* is considered euryxenic, affecting different cervids. According to previous studies, *H. actaeon* are widely distributed in red deer from central Spain and its seasonal pattern of infestation show that the presence of warbles extends from January onward, so it is very probable the detection of more infested roe deer along this season. According to Price (1980), specificity in the Oestridae family results partially from limitations in the number of available hosts rather than from strong selection pressures for specialization on one or a few hosts. So that changes in the pattern of distribution of red deer in central Spain could be the cause of the expansion of this myiasis towards other hosts. Further studies to follow the evolution of this infection in roe deer are needed.

DEVELOPMENT OF *CEPHENEMYIA* STIMULATOR IN ROE DEER IN AN AREA OF OCEANIC CLIMATE (NW SPAIN)

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In order to determine the prevalence and intensity of infestation by 3 larval stages of *C. stimulator*, between 2012 and 2014, the necropsy of 98 roe deer from different localities of oceanic climate in Northwest Spain was carried out. In addition, to assess the prevalence of infection, 251 sera of animals from the study area were analyzed by indirect ELISA and L2 *C. stimulator* excretory-secretory antigens.
The antibody level was well connected with the active phase of the larvae, checking that, seroprevalence results coincide with the most active phase of the cycle of *C. stimulator*. By Spearman test a positive and significant correlation between seroprevalence and roe deer infested was found (ρ= 0.683, P= 0.029). The seroprevalence was also correlated with the mean intensity of infestation by larvae of the fly (ρ= 0.742, P= 0.014).
Considering the results of necropsy and immunodiagnostic, the chronobiology of *C. stimulator* was set to roe deer from Northwest Spain. Given that, L3 already observed in April and the period of pupation in oestrid flies is 20-30 days, the first adult flies would see in May and its activity would last all summer. The temperature registered between April and July determines the pace of development of the larvae, with the presence of 3 larval stages in roe deer. The larval diapause, in climatic conditions of the area, will take place between August and February, which coincides with a significant reduction in the seroprevalence and with a small number of L1 and L2, indicating that in this period the weather conditions are not favorable for the continuity of cycle of *C. stimulator*.

SETARIA TUNDRA, AN EMERGING FILARIOID NEMATODE IN ROE DEER (*CAPREOLUS CAPREOLUS*) IN NORTHEAST ITALY

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Coincidental with decades of warming (high temperature and humidity) in the sub-Arctic region of Europe, the mosquito-borne filarioid nematode *Setaria tundra* is now associated with emerging epidemic disease, resulting in morbidity and mortality for reindeer and moose. Similarly, roe deer is also target of this nematode, as documented in Denmark, Germany and Bulgary. In the early 2000s, *S. tundra* was first described in roe deer in Piedmont (north Italy). *Aedes* spp. mosquitoes seem to be the most competent vectors for *S. tundra* and little is known about its pathogenicity in roe deer. After a first report in 2013 of *S. tundra* in Friuli Venezia Giulia-FVG (northeast Italy) roe deers, during 2014-2016 an investigation was undertaken to evaluate the its presence in road-killed roe deer from the Gorizia province. Currently, parasitological dissections of 17 roe deer were performed. During the necropsy, nematode specimens (1 to 58 per host) were found in the abdominal cavity. Based on their morphology and biometrics, the nematodes recovered were identified as *Setaria tundra* and preserved in alcohol or cryopreserved for further analysis Moreover, pathological lesions were described, documented and damaged tissue samples were collected for histological evaluation. The prevalence of *S. tundra* in FVG roe deer population is 47.1%, mean intensity 11 (range 1-58) and mean abundance 5. The adult worms were located free in the abdominal cavity where tracks left by worm migration were also seen. Macroscopical lesions were polysierositis with thickening spleen and liver serosa with fibrinous deposition on surface of the abdominal organs. Whereas, histological lesions were diffuse serositis, granulomatous reactivity with Muller's giant cells and calcifications. In our study in FVG, this the first recognition of *S. tundra* and the alarming aspect has been that some road killed roe deers showed some significant pathological peritoneal changes.

MOLECULAR AND SEROLOGICAL EVIDENCES OF CIRCULATING *MIDICHLORIA MITOCHONDRII* IN ROE DEER (*CAPREOLUS CAPREOLUS*) AFTER *IXODES RICINUS* BITE

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Midichloria mitochondrii (order Rickettsiales; family Midichloriaceae) is an intracellular bacterium found in the reproductive apparatus and in the salivary glands of 100% of adult females of the hard tick *Ixodes ricinus*, vector of pathogens important for both human and animal health. Furthermore, direct (DNA amplification) and indirect (immunological analysis) evidences are published on the presence of *M. mitochondrii* in blood and serum of different mammalian hosts, including humans, suggesting a transmission during the tick bite.
This experimental work aims to investigate the circulation of *M. mitochondrii* in roe deer (*C. capreolus*), which is the host of choice for adult and nymph stages of *I. ricinus.*
Briefly, we evaluated: 1) the presence of circulating *M. mitochondrii* DNA in blood of seven roe deer from the INRA Gardouch experimental station (Haute Garonne; France); 2) the immunological response of the same roe deer individuals against a recombinant flagellar protein of *M. mitochondrii* (rFliD). Blood and sera were collected, from the same animals, during the spring of 2014 and 2015.
DNA was extracted from blood samples and molecular analysis were conducted using a specific qualitative PCR for 16S rDNA of *M. mitochondrii*. Instead, immunological analysis were conducted using the recombinant protein rFLID as antigen in ELISA assay.
After molecular analysis, positivity to *M. mitochondrii* DNA was found in some blood samples collected both in 2014 and 2015 springs. Furthermore, positivity in ELISA test was found in six out of seven roe deer sera collected in 2014, and in all samples collected in 2015. The specificity of these results was also confirmed by a Western blot analysis.
The obtained results demonstrate the circulation of *M. mitochondrii* in the blood of *C. capreolus* parasitized by *I. ricinus*, confirming once again that this mammalian host is a good subject to study the spread of tick-borne pathogens.

FIRST CASE OF SPIRORCHIIDIASIS (DIGENEA: SPIRORCHIIDAE) IN A MEDITERRANEAN LOGGERHEAD (*CARETTA CARETTA*) WITH MOLECULAR CHARACTERIZATION OF *HAPALOTREMA MISTROIDES*

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Cardiovascular flukes (Spirorchiidae) are considered as the most important parasitic cause of the sea turtle stranding and mortality worldwide. In the Mediterranean Sea the only spirorchiid known is *Hapalotrema mistroides* collected between the 1896 and 1902 in loggerhead (*Caretta caretta*) and green turtle (*Chelonia mydas*). Since, all following studies failed to reveal any cardiovascular fluke from the Mediterranean Sea. Here we describe the first case of spirorchidiasis from the Mediterranean Sea, along with the molecular characterization of *H. mistroides*. On June 2015 an adult loggerhead was found stranded on the coast of Latium (central Italy). The turtle, in poor condition and lethargic, showed an intestinal obstruction and died despite the emergency surgery to remove it. At post-mortem examination the serosa of the small intestine and pancreas was focally thickened with diffuse presence of black spots. Three adult *H. mistroides* were found from the spleen and massive presence of their eggs were recovered from feces. Histologically, *H. mistroides* egg granulomas were found in pancreas, liver, lungs, kidneys, urinary bladder, spleen, and intestine determining significant disorganization of the architecture of organs involved. Brain showed extensive areas of encephalomalacia with vasospasm, fibrosis and diffuse mineralization of meninges with microgliosis and astrogliosis. Secondary bacterial infection associated with or without spirorchiid egg embolisms were found from lungs, kidneys, pancreas, spleen, intestine, and brain. The initial BLASTn searches indicated that *H. mistroides* was most similar to *Learedius learedi* with 95% identity and *H. mehrai* with 94% identity already indicating the close relationship of *H. mistroides* to the *Hapalotrema*/*Learedius* "group". The *Hapalotrema*/*Learedius* group forms a distinct subclade within the marine spirorchiid and *L. learedi* phylogenetically bracketed by species of *Hapalotrema*. *H. mistroides* appears as a sister taxa to the *H. postorchis* and forms a paraphyltic sister group which is basal to the rest of the *Hapalotrema*/*Learedius* "group".

COMPARISON OF DIAGNOSTIC TESTS FOR *GIARDIA* DETECTION IN WILD UNGULATES: IS ELISA TEST A GOOD CHOICE?

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Giardiasis is one of the most common parasite intestinal infections in humans, wild and domestic animals worldwide, and therefore constitutes a potential zoonotic and zoo-economic risk. However, little information is currently available about its presence or its effect on wildlife populations. Immunofluorescence (IF) is the most widely used assay for *Giardia* detection, also in wild bovids. In the present study, IF was used as a comparative test in order to evaluate the performance of immunoenzymatic testing (ELISA) as a diagnostic option.
Fecal samples were collected from 166 alpine chamois (*Rupicapra rupicapra rupicapra*), culled during the 2013-2015 hunting season in the Central Italian Alps. Samples were divided into two portions; the first was stored in potassium dichromate (2.5%) and subjected to immunofluorescence analysis (MERIFLUOR® *Cryptosporidium/Giardia*), while the second was frozen at -20°C and subjected to immunoenzymatic testing. A commercial ELISA kit (RIDASCREEN® *Giardia*) was used, and the agreement between the analytical approaches was assessed by calculating the Kappa (K) value (EpiTools, Ausvet; CI 95%).
A *Giardia* prevalence of 7.8% (13/166) and 6.6% (11/166) was recorded by IF and ELISA respectively, thus showing a substantial agreement (k-value = 0.73) between the two tests.
The ELISA test could therefore represent a good choice and an alternative tool for direct giardiasis diagnosis in wild ungulates because it has several advantages: it is cheaper, can be used to carry out simultaneous screening of numerous samples, and also provides objective spectrophotometer reading and antigen detection. Molecular analyses will make it possible to obtain further data in order to evaluate the actual performances of the ELISA test for *Giardia* detection, and support its application in a context involving wildlife.

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DETECTION OF *GIARDIA DUODENALIS* IN FREE RANGING ALPINE CERVIDS

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Survival and diffusion strategy of *Giardia duodenalis* consists of its being a generalist pathogen infecting a wide range of animal hosts in different environments, including humans. Recently, this protozoan has been detected in alpine chamois (*Rupicapra rupicapra rupicapra*). In order to provide further knowledge on circulation of this protozoa in alpine environment, we investigate the occurrence and genetic identity of *G.duodenalis* in red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*). The study was carried in the Lepontine Alps in 2013-2014. Faecal samples were collected from soil in the protected area of the Alpe Veglia-Alpe Devero Natural Park and from culled animals in the contiguous VCO2 hunting district.
A total of 196 faecal samples were collected from red deer and 119 from roe deer. Faeces were frozen at -20° and a commercial ELISA kit (RIDASCREEN®*Giardia*) was used to detect protozoan copro-antigens. Positive samples were subjected to a nested PCR for molecular characterization.
G.duodenalis prevalence was 2.5% (5/196) (95%CI=0,3-4,7) in red deer and 8.4% (10/119) (95%CI=3.4-13.4) in roe deer. *G.duodenalis* was molecularly confirmed in 4 red deer and 3 roe deer. Zoonotic assemblage A was identified in red deer, while sequencing of PCR fragments from roe deer samples was not possible due to the poor DNA quality.
This study shows that wild alpine cervids harbour *G.duodenalis*, contributing to its spread in the alpine environment. Considering that *G.duodenalis* prevalence detected is not negligible and that zoonotic assemblage A was isolated, the increase of deer populations, outdoor and zootechnical activity suggest a potential zoonotic and economic risk. Moreover, assuming that the highest protozoan emission by deer occurs in the first weeks/months of life (at least for livestock), logistical difficulties due to late-spring field conditions in the protected area and sampling in autumn hunting season mean that real prevalence could actually be underestimated.

OCCURRENCE OF PHARYNGEAL BOT FLIES IN RED DEER FROM SOUTHERN SPAIN

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Larvae of the oestrids *Pharyngomyia* and *Cephenemyia* are obligate parasites of cervid hosts that deteriorate animal's welfare and cause mild to severe damage mainly due to their oral hooks and cuticular spines. This study was carried out to evaluate diptera infestation on red deer (*Cervus elaphus hispanicus*) in Sierras de Cazorla, Segura and Las Villas Natural Park (38º 5' N; 2º 45' W), a protected mountainous area in Southern Spain with Mediterranean climate.
From 2003 to 2005, a total of 64 red deer (36 female and 28 male; 41 adult, 14 subadult and 9 young) were randomly hunted during January-March for management purposes. After hunt, all animals were necropsied *in situ*, and naso-pharyngeal cavities were examined for larvae collection. Parasites were preserved in formaldehyde 10% for further identification.
The overall prevalence of infection was 37.5% (mean intensity of 10.58+/- 8.74 larvae), with much higher prevalence for *P. picta* (94.49%; 10+/- 8.87 larvae) than for *C. auribarbis* (5.51%; 1.75+/- 1.29 larvae); interestingly, both species coexist within the same host in 12.5% of the studied animals. Females and older animals showed higher prevalence and mean intensities of larvae, although statistical evaluation using a nonparametric analysis (Kruskal-Wallis) did not reveal significant differences between groups (p=0.67 and p=0.53, respectively). High prevalences have been previously described in Southern Spain, and differences in our results should be associated to the diptera species, the study area and the sampling season.

PARASITOSES OF LIVESTOCK

AWARENESS OF CYSTIC ECHINOCOCCOSIS AMONG SHEEP FARMERS IN SOUTHERN SARDINIA

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Due to widespread extensive or semi-extensive livestock farming and presence of large numbers of dogs, CE is on the decline but still an ongoing problem in Sardinia. Data from a questionnaire administered to sheep farmers, designed based on previous interviews and focus groups with stakeholders, was compared with an earlier survey conducted before the last tentative control programme carried out in Sardinia in the late 1980s. Survey investigates structural characterization of farms, management type, entrepreneurial conduct and farmers' attitudes to innovation, and specifically focuses on knowledge of CE, disease awareness, dogs' role, dogs' feeding practices, home slaughtering and offal disposal, together with direct and indirect actions suggested to be useful for preventive purposes.
All interviewees were aware of the relevance of dogs in the CE cycle and knew how they became infected, but many misunderstood transmission mechanisms and some actually believed sheep infect each other. All claimed they dewormed farm dogs at least once/year, though uncontrolled dogs were always present around farms.
Home-slaughtering of sheep is widely practised. All but one denied feeding dogs with raw offal, many reported boiling (probably inadequately) before feeding while the majority buried superficially or burned offal. But the majority of shepherds, when asked about how their counterparts behaved, stated they fed dogs with raw offal. Similar patterns of responses were observed for the disposal of carcasses.
Overall 58% showed a fair knowledge, while for 42% it was lacking and fragmentary, particularly in fully understanding the different role of definitive vs intermediate host, and on the role of direct contact in transmission. Knowledge of disease is concomitant with innovative attitudes towards management, while scanty information correlates more with the traditional approach. However a gap still remains between theoretical knowledge and practice, as the behaviour underlying diffusion persists, suggesting a need to rethink control information strategies.

UPDATING OF BOVINE ENDOPARASITES IN ITALY

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During the last decades, the impact of gastrointestinal parasites in adult cattle bred in Italy has been scarcely investigated. The objective of our study was to obtain up to date information on the presence and distribution of economically relevant helminthes in adult Italian cows by an abattoir survey. At this purpose from 2014 through 2015, 427 faecal samples were collected from culled cows in a slaughterhouse in the province of Bologna. From the same animals, 100 abomasa were randomly selected. Referring to the slaughter records it was possible to identify the tested animals in the National Livestock Identification System (NLIS) to obtain additional information about zootechnical parameters and geographical origin. Qualitative coprological examinations were performed on individual faecal samples, while abomasa were examined by necropsy techniques to evaluate worm burdens for gastric nematodes. The animals included in the study were 93% female (63% dairy cows and 37% brood cows) and aged between 1 and 26 years old. Adult cattle (> 2 years old) represent 90% of the total sample. The farms of origin were located in a wide geographical area within Italy, which included 11 regions and 33 different local districts. Prevalence rate for the pathogens under investigation was 31% by coprological examinations and 13% by necropsy. The genus detected were *Ostertagia sp*., *Trichosrongylus sp.* and *Cooperia sp*. The statistical processing of data and results pointed out a significant relationship ($\chi^2 \leq 0{,}05$) between the prevalence detected and the livestock category; at herd level the percentage of positive samples was dependent on a significant manner on the stocking density. The study results show that gastrointestinal parasitism by nematodes is a problem which must be considered ubiquitous in Italy in adult cattle, with relatively high prevalence rate; nevertheless it seems to be still underestimated by technical experts in the field.

UPDATES ON CLINICAL EVOLUTION OF COENUROSIS IN SMALL RUMINANTS

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Cerebral coenurosis is a common disease in small ruminants bred with extensive (traditional) methods. The present survey describes the evolution in vivo of *Coenurus cerebralis* infection in small ruminants (two Sarda sheep and one Maltese goat). Clinical signs, Magnetic Resonance Imaging (MRI) and cerebrospinal fluid (CSF) findings were used to describe the disease evolution from the acute to the chronic phase. The measuring of direct intracranial pressure (ICP) was also used. The surgical removal of cysts were scheduled when symptoms were detected. At the removal of the cysts, morphological and biomolecular identification of the parasites was performed. All the animals showed progressive clinical and neurological improvement, later neurological symptoms appeared again. Sheep 1 had 4 cysts: 1 on the left parietal lobe, 1 close to the left side of the falx cerebri, 1 on the right temporal lobe and 1 on the right frontal lobe; the cysts volume was 0.48 cm^3. Sheep 2 had 2 cysts: 1 on the left frontal lobe and 1 on the right temporal lobe; after 30 days, one cyst disappeared. The comprehensive cysts volume was 1.18 cm^3. The goat had 3 cysts: 1 in the right parietal lobe, 1 in the left side of the cerebellum and 1 in the 4^{th} ventricle; the comprehensive volume was 0.3 cm^3. Cytological examination was abnormal in sheep 1 and in the goat with a total nucleated cell count of 28/µl and 39/µl respectively, later CSF examination was normal in all the animals. The morphometrical features of the parasites were consistent with those of *T. multiceps* and also confirmed by the molecular sequencing of *cox*1 mitochondrial gene. The present survey unveils, for the first time, the clinical evolution of coenurosis in sheep and goat, thanks also to the use of diagnostic imaging and laboratory investigations like CSF and ICP.

MORPHOLOGICAL AND BIOMOLECULAR CHARACTERIZATION OF *GONGYLONEMA* SPP. FROM DOMESTIC AND WILD RUMINANTS OF SARDINIA, ITALY

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Nematodes of the genus *Gongylonema* (family Gongylonematidae) have a cosmopolitan distribution and are frequently encountered in domestic and wild animals, as well also in humans, being a minor zoonosis. Here, we examined the morphology and genetic structure of *Gongylonema* species isolated from domestic and wild ruminants in Sardinia, Italy. Twenty-five adult specimens were collected during post-mortem inspection from the oesophagus of naturally infected animals (cattle = 8; sheep = 7; mouflon = 6; goat = 4). All specimens were morphologically identified according with the morphological keys indicated by Sprehn (1932), collected in tubes with 70% ethanol until molecular analysis. After DNA extraction, PCR was performed on the ITS 1and ITS2 regions of the rDNA and *CoxI* region of the mtDNA. The long, transparent worms were identified as nematodes belonging to the genus *Gongylonema* through the presence of prominent bosses or scutes on the anterior end. The specimens were 16 adult females (cattle = 7; sheep = 4; mouflon = 3; goat = 2) and 9 adult males (cattle = 1; sheep = 3; mouflon =3; goat = 2). The mean length of females was 83.4 mm (±27.1) and the width was 326 µm (±54.2). The mean length of males was 76.1 mm and wide 304.5 µm (±73.9). Reported data for Sardinian parasites are consistent with those reported by Makouloutou et al. (2013) for *G. pulchrum* in domestic hosts and was also confirmed by biomolecular analysis.

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DETECTION OF *TOXOPLASMA GONDII* INFECTION IN SHEEP MEAT FOR HUMAN CONSUMPTION IN SARDINIA, ITALY

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Toxoplasma gondii is an Apicomplexan protozoa considered as one of the most important responsible of food-borne parasitic zoonoses worldwide. This study pointed out to investigate the presence of *T. gondii* in sheep meat for human consumption in Sardinia, through a serological and biomolecular survey. At slaughterhouses individual blood and tissues samples were collected from 112 sheep slaughtered in 8 municipalities of Sardinia (Italy). An ELISA kit was used for the detection of *T. gondii*-specific antibodies on sera and meat juice obtained from diaphragm and heart tissues. A Nested Polymerase Chain Reaction (PCR) was carried out for the detection of *T. gondii* genomic DNA on heart samples. Fifteen PCR-positive samples were typed at 5 genetic markers including 5 nuclear loci (SAG2, SAG3, BTUB, GRA6). Antibodies against *T. gondii* were found in the 87.5 % (96/112) and 84.8% (95/112) of the meat juice obtained from heart and diaphragm respectively and in the 42% (47/112) of sera samples. ELISA on meat juice allowed us to find higher prevalences in meat juce than in sera samples (85.7% and 84.8% *vs* 42%; $\chi 2 = 67.79$ with 2 degrees of freedom; $p < 0.001$). The presence of *T. gondii* DNA by PCR was detected in the 77.7% (87/112) and in the 82.1% (92/112) of the brain and heart samples respectively ($\chi 2 = 3.57$; $p = 0.058$). Genotyping showed *T. gondii* with clonal Type II and atypical genotypes in 9 samples. The high prevalence for *T. gondii* detected in this work highlights the strong pressure exerted by *T. gondii* in sheep in the island and poses the problem of the risk of transmission to humans through ingestion and handling of raw meat.

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OSTERTAGIA OSTERTAGI ANTIBODIES IN BULK TANK MILK FROM CATTLE HERDS IN ITALY

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Measurement of antibodies to *Ostertagia ostertagi* in bulk tank milk (BTM) is a diagnostic indicator for potential production losses and anthelmintic treatment responses in dairy herds. The purpose of this study was to assess *O. ostertagi* antibody levels in dairy cattle located in different northern, central and southern Italian regions. From March 2015 to December 2015, BTM samples were collected from a total of 588 dairy herds. Antibody titres to *O. ostertagi* were assessed by indirect ELISA (SVANOVIR *O. ostertagi*-Ab) and expressed as optical density ratio (ODR). A threshold of 0.5 (indicating production losses) was used for the analysis according to the manufacturer's instructions. Mean ODR, prevalence of samples with ODR ≥ 0.5 and standard deviation (SD) were calculated for each region. All the farms with ODR ≥ 0.5 were treated with anthelmintic drugs in order to maintain animal health, welfare and productivity. The ELISA results revealed a 53.2% (SD = 0.20) prevalence of samples with ODR ≥ 0.5. Specifically, mean ODR values ranged between 0.44 in Sardinia and 0.64 in Trentino Alto Adige, whilst the prevalence of samples with ODR ≥ 0.5 ranged between 24.4% (SD = 0.18) in Sardinia to 83.3% (SD = 0.16) in Trentino Alto Adige; variations between regions seems to reflect different husbandry practices, particularly those related to access to pasture. These results showed that *O. ostertagi* is a global problem in Italian herds of dairy cattle. This test will provide a quantitative assessment of the *O. ostertagi* status of dairy herd and the possible impact that may have on performance and potential responses to anthelmintic treatment. This represents a significant step forward in evidence-based medicine for dairy veterinarians, advisors and farmers.

PREVALENCE OF *CRYPTOSPORIDIUM* SPP. AND *GIARDIA DUODENALIS* IN SHEEP FARMS OF SARDINIA, ITALY

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Giardia duodenalis and *Cryptosporidium* spp. are enteric protozoa associated with diarrhea and illness that infect a wide variety of domestic animals and also humans.
The goal of this study was to determine their prevalence in lambs and dairy sheep of Sardinia.
A total of 915 individual faecal samples were collected in different class of animals: 305 from lambs > 5 days and <30 days, 305 from sheep in the last month of pregnancy and 305 from sheep 1 month post-partum in 61 farms between november 2015 and february 2016.
Samples, once in laboratory, were classified into three categories depending on the consistency of faeces: normal, soft or diarrhoeic. In each farm 15 samples (5 for each category) were individually analyzed using Ziehl-Neelsen modified staining technique for the diagnosis of *Cryptosporidium* spp. Individual samples from sheep and faecal pools from lambs were also analyzed with $ZnSO_4$ (Specific Gravity 1200) flotation, in order to verify the presence of *Giardia duodenalis*.
Oocysts of *Cryptosporidium* spp. were detected in 26.2% (16/61) of the examined farms and in 58.7% (47/80) of lambs belonging to positive flocks. *Cryptosporidium* spp. infection was significantly higher in diarrhoeic faeces ($\chi 2 = 8.03$, $P < 0.005$), with an Odds Ratio of 3.42. Oocysts of *Giardia duodenalis* were found in 6.6% (4/61) of the farms. The two examined categories of adult sheep, resulted negative to both parasites.
In 6.5% (4/61) of farms, the high number of oocysts of *Cryptosporidium* spp. found in diarrhoeic samples, could be considered as a possible cause of enteritis. The results of the present study indicate the presence of *Cryptosporidium* spp. and *Giardia duodenalis* in sheep farms of Sardinia. Infected sheep could be a possible source of infection for other animals but also, due to their zoonotic attitude, for people that could get in contact with these animals.

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INFECTIONS BY CRYPTOSPORIDIUM SPP IN PRE-WEANED HEALTHY CALVES (N.W. SPAIN)

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Cryptosporidium spp is a protozoan parasite associated with neonatal calf diarrhoea, leading to significant economic losses. Four species are mainly identified in cattle. *Cryptosporidium bovis* and *Cryptosporidium ryanae* usually infect weaned calves and yearlings, whereas *Cryptosporidium andersoni* is predominant in adults. In contrast, the zoonotic *Cryptosporidium parvum* is mostly observed in diarrhoeic pre-weaned calves, although it can also be found in healthy animals, meaning that its presence is not always causative.

In order to provide data on the prevalence of *Cryptosporidium* species in non-diarrhoeic pre-weaned calves, faecal samples from 88 healthy animals were collected in 47 farms from NW Spain. Oocysts were firstly concentrated using a diphasic sedimentation technique; *Cryptosporidium* species were identified by SSU rRNA PCR-RFLP analysis.

Our results revealed that *Cryptosporidium* is a prevalent parasite in pre-weaned healthy calves, since 29/88 (33.0%) shed oocysts of the protozoan. Approximately, half of the farms presented *Cryptosporidium*-positive animals (24/47; 51.1%). The prevalence increased from 10.0% to 66.7% in the three first weeks of life. Older animals presented lower percentages of infection ranging from 15.5% to 28.5%. Three *Cryptosporidium* species were identified: *C. parvum* (19/29), *C. bovis* (9/29) and *C. ryanae* (1/29). An age-related variation of *Cryptosporidium* species was also observed: *C. parvum* was especially prevalent (28.6-50.0%) in calves younger than 4 weeks. In contrast, *C. bovis* is the major *Cryptosporidium* species in animals older than 4 weeks.

Calves younger than four weeks, and especially those in their third week of life, are important carriers of *C. parvum* and therefore they should be considered as an important risk for the appearance of neonatal diarrhoea outbreaks and a threat to Public Health.

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PREVALENCE OF OSTERTAGIOSIS IN SLAUGHTERED DAIRY CATTLE FROM SÃO MIGUEL ISLAND, AZORES, PORTUGAL

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Ostertagiosis caused by *Ostertagia ostertagi* is considered the most important parasitosis in dairy cattle raised in tempered areas. The aim of this investigation was to determinate the prevalence of ostertagiosis in cattle slaughtered in São Miguel Island, Azores, the geographical distribution of positive cases and farmers' awareness regarding preventive and control measurements against this parasitosis. From the 2.000 animals sampled, 1.282 presented lesions compatible with ostertagiosis representing a prevalence of 48.99% in young animals and 75.73% in adults. Positive animals had worse carcass and fat ratings and less carcass weight when slaughtered. Our data also indicate that ostertagiosis has a widespread distribution throughout the island and that 85.7% of the farmers dewormed calves and 42.86% dewormed cows (only one administration was provided for each deworming). Data shows that ostertagiosis represents an important economical lost in dairy cattle from Azores and a significant lack of knowledge by farmers about this disease and its consequences. In an era where there is an increasing productive competitiveness it is crucial to improve farmers´ awareness regarding parasite life cycle, methods of prevention and control measurements.

INFLUENCE OF THE PRODUCTION SYSTEM IN THE OCURRENCE OF GASTROINTESTINAL AND RESPIRATORY PARASITES IN GOATS FROM NW SPAIN. PRELIMINARY RESULTS

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In Northwestern Spain goats are reared mainly in semi-extensive husbandry system. However, dairy farms apply intensive production practices that can reduce the possibility of infection with parasites acquired in the pasture. The objective of this study was to evaluate the effect of management system over some gastrointestinal and respiratory parasites, like gastrointestinal nematodes (GIN), coccidia and Protostrongylidae, in goats. Faecal samples from 197 goats in 23 commercial herds were collected, 103 were under extensive and 94 under intensive husbandry systems. Samples were processed by flotation technique to determine the presence of GIN and coccidia and by migration to detect lungworms. Associations between parasite presence and husbandry system were analyzed with a Chi-squared test. The overall prevalence for GIN was 62.7% (C.I.95% 55.3-39.6), 78.9% in goats reared under extensive and 45.5% under intensive system (OR = 0.223; C.I. 95% 0.117-0.425) and those differences were significant (χ^2 = 20.63; $p < 0.001$). *Eimeria* oocysts werepresent in 94.6% (C.I. 95% 90.6-97.2) of the samples; the prevalence was higher in intensive herds (100%) than in extensive ones (84.5%; OR = 2.228; C.I. 95% 1.283-385.195). Fisher test indicated that management practices influenced the prevalence of Coccidia ($p = 0.002$). Finally, protostrongylids were detected in 72.5% (C.I. 95% 65.6-78.6) of the samples; with a prevalence of 78.6% in extensive and 65.5% in intensive system. In this case, production system did not influence lungworm prevalence (χ^2 = 3.498; $p = 0.061$). These results indicated that intensive management practices favoured the infection by density-dependent parasites like coccidian, whereas pasture grazing offer more suitable conditions for the acquisition of GIN. Surprisingly, protostrongylid infection is not influenced by the management system; further studies analyzing the sources of infection in intensive system are needed.

Work was supported by a grant for Consolidating and Structuring Competitive Research Groups (R2014/005, Xunta Galicia, Spain).

FIRST EXPERIMENTAL INFECTION WITH *Besnoitia besnoiti* TACHYZOITES IN CALVES

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Bovine besnoitiosis, caused by the apicomplexan protozoa *Besnoitia besnoiti,* is a chronic and debilitating disease responsible for skin lesions and systemic manifestations. At present, the disease is re-emerging in Europe. Unfortunately, no treatments or vaccines are available for disease control. In this scenario, the development of animal models of infection is urgently needed. The aim of the present study was to develop an experimental model of *B. besnoiti* infection in cattle.
Twelve Holstein Friesian 3-month old calves were enrolled in the experiment. Prior to the inoculations cattle health scheme program included ectoparasite control, vaccination against bovine respiratory disease, serological analysis against *Neospora caninum*, *Toxoplasma gondii*, *B. besnoiti* and *Sarcocystis* spp. and coprological analyses to check for the absence of gastro intestinal parasites. Four groups of three animals each were inoculated intravenously with three different doses of tachyzoites (G1: 10^8; G2: 10^7; G3: 10^6) or with PBS (G4). Clinical monitoring included temperature, weight, and clinical signs compatible with acute and chronic besnoitiosis. Parasite presence was investigated in blood samples and skin biopsies from the femoral region that were weekly collected. The experimental infection was followed up to 70 days post-infection (dpi) when animals were euthanized and tissues were collected for lesions and parasite detection. A significant increase of temperature was observed in the infected groups when compared to control group and sporadic parasitaemia was detected in animals from G1 and G2 until 7 dpi. During the first month, all infected animals developed lymphadenopathy in at least two of the three lymph nodes observed (submandibular, prescapular and precrural). However, no clinical signs characteristic of the chronic stage of the disease were detected and no differences in weights between groups were found. Parasite DNA was detected in samples of conjunctiva, ocular sclera, epididymis, as well as, skin of scrotum and carpal zone. A future refinement of the model should be considered despite the acute stage of the disease was successfully reproduced with a mild-moderate severity.

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FUNGI AND FUNGAL INFECTIONS

PNEUMOCYSTIS IN LUNGS OF SQUIRREL, *CALLOSCIURUS FINLAYSONII* (RODENTIA, SCIURIDAE)

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Pneumocystis spp. are ubiquitous fungal commensals of the respiratory tract of many animals and represent a potential cause of life-threatening pneumonia in a wide range of mammals. All species show a restricted host-range, with a high degree of host-specificity. So far, only five *Pneumocystis* entities are formally described as species. The purpose of the present study was to investigate the occurrence of *Pneumocystis* in squirrels (*Callosciurus finlaysonii*) and the functionality of different markers for species recognition. Lung tissues of 54squirrels have been investigated for the presence of *Pneumocystis* DNA by amplification and sequencing of a portion of the mitochondrial large subunit (mtLSU), small subunit (mtSSU) and internal transcribed spacers 1 and 2 (ITS1/ITS2) of the rRNA genes according protocols previously reported (Danesi et al., 2016). Two gene datasets of *Pneumocystis* species, with mtSSU and ITS sequences, were analysed by Neighbor Joining with 1000 bootstrap replicates and conducted in MEGA6. *Pneumocystis* DNA was isolated from three squirrels. Amplification and sequences were obtained from mtSSU (n=3) and ITS (n=2) genetic targets, but not from mtLSU. In mtSSU and ITS trees, constructed without outgroup, all main clusters were well supported and consistent with mammal hosts. Mitocondrial SSU sequences compose a separate clade, which is polyphyletic with the only *Pneumocystis carinii* f. sp. squirrel (*Sciurus aestuans*) present in the database. The rDNA ITS tree confirmed Pneumocystis grouping according animal species. In general, squirrels were in apparently good condition, thus supposing animals were carrying Pneumocystis organisms without disease. The amplification of Pneumocystis mtLSU target did not succeed with these set of primers, suggesting this genetic target might be different in squirrels. Indeed, the detection of Pneumocystis spp. in a large variety of animals provides evidence of a highly diversified Pneumocystis genus. According to the Barcoding approach, ITS might be an excellent marker for species characterization.

INFLUENCE OF DIFFERENT STORAGE CONDITIONS ON THE VIABILITY AND VIRULENCE OF *BEAUVERIA BASSIANA* CONIDIA INFECTION SUSPENSION

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Beauveria bassiana myco-insecticides/acaricides are usually formulated as conidia infection suspensions (CIS) in sterile distilled water plus 0.1% tween 80 (v/v) for field and laboratory applications. However, stress factors i.e., temperature, UV radiation, oxygenation, can over time compromise the conidial viability and virulence. The native strain of *B. bassiana* has been shown to be highly virulent against the poultry red mite *Dermanyssus gallinae*. With the aim of investigating the best storage conditions for extending the CIS shelf-life, viability and virulence of native *B. bassiana* CIS stored at ambient and refrigerated temperatures (i.e., 20±1 °C and 4±1 °C, respectively), with and without light and agitation, were assessed over a one year period.
The viability of conidia was assessed monthly by quantitative plate counts of colony forming units (CFU)/ml on potato dextrose agar after incubation at 25°C for 4 days.
The virulence of *B. bassiana* was evaluated towards *D. gallinae* five times in one year (i.e., at T0 and every 3 months of storage).
Independently of light conditions and agitation, all CIS stored at 20±1 °C showed a reduction of about two logarithmic units of CFU/ml, starting from the 2nd month, which persisted until the 12th incubation month. CIS stored at 4±1°C showed a reduction of about one logarithmic unit of CFU/ml starting from the 7th month and remained unchanged until the end of the monitoring time.
The highest mortality rate of *D. gallinae* was registered for CIS stored at 4±1°C, in the dark and without agitation.
This study suggests that the viability of the spores during storage is only dependent on temperature, whereas the virulence of the fungus is influenced by temperature, light and agitation.
Refrigerating CIS (i.e., 4±1 °C), with storage in the dark and without agitation seems to guarantee the best viability and performance against *D. gallinae*, thus it can be considered the most appropriate storage method.

PREVALENCE OF THE MICROSPORIDIAN *NOSEMA CERANAE* IN HONEY BEE (*APIS MELLIFERA*) APIARIES IN SOME PROVINCES OF TUSCANY

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Nosema ceranae and *Nosema apis* are microsporidia which play an important role in the epidemiology of honey bee microsporidiosis worldwide. Nosemiasis reduces honey bee population size and causes significant losses in honey production. To determine the occurrence of *Nosema* spp infection in some provinces of Tuscany (Central Italy), 38 seemingly healthy apiaries (2 to 4 hives each) were randomly selected and screened from April to September 2014 (n=11) or from May to September 2015 (n=27). The apiaries were located in six Tuscan provinces, including Lucca (n=11), Massa Carrara (n=9), Pisa (n=9), Leghorn (n=7), Florence (n=1), and Prato (n=1). Light microscopy was used to screen the presence of microsporidiosis in adult worker honey bees and was carried out according to current OIE recommendations. As morphological characteristics of *N. ceranae* and *N. apis* spores are similar and can hardly be distinguished by optical microscopy, all samples were also screened by multiplex polymerase chain reaction (PCR) assay based on *16S rRNA-gene-targeted* species-specific *primers* to differentiate *N. ceranae* from *N. apis. Furthermore,* PCR-positive samples were also sequenced to confirm the species of amplified *Nosema* DNA. Notably, *Nosema* spores and *Nosema* DNA were detected in samples from 24 out of 38 (63.2%, 95% CI: 47.8-78.5%) apiaries, using both light microscopy and multiplex PCR. Positivity rates in single provinces were 10/11, 8/9, 3/9, 1/7, or 1/1 (n=2). A full agreement (*Cohen's Kappa=1)* was assessed between the two tests. Based both on multiplex PCR and DNA sequencing results, only *N. ceranae* was found. Overall, our results highlighted that *N. ceranae* infection occurs frequently in the cohort of the Tuscan honey bee population that was examined despite the lack of clinical signs, suggesting that colony disease outbreaks might result from environmental factors that lead to higher susceptibility of honey bees to this parasite.

USE OF *MUCOR CIRCINELLOIDES* AS A BIOCIDE TO PREVENT ZOONOTIC SOIL-TRANSMITTED HELMINTH INFECTIONS

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Soil Transmitted Helminth Infections (STHs) are parasitic diseases mainly caused by the accidental ingestion of ascarids, trichurids or ancylostomids. Zoonotic infection occurs after the ingestion of eggs from soils contaminated by faeces of domestic or wild animals, which frequently affects to the youngest people as well as poorest and most depressed communities.

In this study, the ovicidal fungi *Mucor circinelloides* has been tested to reduce the development of eggs of *Toxocara canis*, *Baylisascaris procyonis Trichuris* sp. and *Capillaria* sp. With this aim, spores of the fungus (5 mL containing 10^6 spores/mL) were poured directly onto plastic boxes with 5 grams of faeces of dogs and raccoons. The effect of this procedure was evaluated by estimating the percentage of viable eggs after being exposed to the spores.

Microscopical examination of the helminth eggs showed the ability of the fungus *M. circinelloides* to attach to the egg-shell, penetrate and destroy the inner embryo, thus its activity was classified as Type 3. After a period of 30 days, the percentage of viable eggs of *T. canis* reduced by 56-79%, and those of *B. procyonis* by 50-55%. The percentage of viable eggs of *Capillaria* sp. was reduced in the presence of *M. circinelloides* to 19%, and to 50% the eggs of *Trichuris* sp. Significant differences were obtained in all the cases in respect of the controls.

Our results led us to conclude that *Mucor circinelloides* exerts a notable and very useful biocidal effect on the eggs of soil-transmitted helminths responsible for zoonoses.

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MALASSEZIA SPP. IN RABBIT: AN UNSOLVED MYSTERY

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The recovery of *Malassezia* from rodents and lagomorphs has been rarely reported in literature. In 2011, Cabañes et al. (Medical Mycology, 49: 40–48) described, the skin of two rabbit from Spain, a novel yeast species, *Malassezia cunicoli*, able to grow only on Leeming & Notman agar (LNA). In a later research Galuppi et al. (2014, XXVIII Congresso Nazionale SoIPa, p.384), at microscopic examination of swabs from ear canals of 168 rabbits, observed the presence of yeasts *M. cunicoli* – like in 58.3% subject. Since all the samples showed no growth on LNA agar, it was hypothesize, the strains observed had different nutritional requirement. In the present study, isolation test from ear swabs canal of 23 rabbit microscopically positive for *Malassezia*-like yeasts, failed both on LNA and on 18 different modified medium. DNA extraction, PCR amplifying a ~300 bp fragment of 18s rDNA (Tampieri et al., 2004, Parassitologia, 46,205) and sequencing were carried out from 8 swabs. The sequences showed 95% identity with *M. cunicoli* (GU733708) and 99,9% identity with *Malassezia* Phylotype 131 (AB663497) described by Zhang *et al.* (2012) from human ear canal and foot sole skin. The latter was not able to grow on LNA medium similarly to our strains. Further research are needed to clarify if the strains present in rabbit in our country were different to the ones described by Cabanes et al (2011 l.c.) and would be considered another specie or simply a strains with different nutritional requirement. The results obtained in our research showed that there is still a lot to discover about the genus *Malassezia* as component of the skin microbiota.

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