XXXII

Congresso Nazionale della Società Italiana di Parassitologia

Napoli, 27-30 giugno 2022

TRANSIZIONI PARASSITOLOGICHE

SOIPA

NAPOLI 2022 PADOVA

SOIPA
Società Italiana di Parassitologia

XXXII

Congresso Nazionale
della Società Italiana di Parassitologia

TRANSIZIONI PARASSITOLOGICHE

Napoli, 27-30 giugno 2022
Centro Congressi Federico II

XXXII Congresso della Società Italiana di Parassitologia

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PRESENTAZIONE

Il **XXXII Congresso della Società Italiana di Parassitologia** (SoIPa) si terrà finalmente **IN PRESENZA** dopo anni di attesa dovuta alla pandemia di Covid-19. Sarà un piacere rivederci tutti a **Napoli** dal **27** al **30 giugno 2022**, presso il **Centro Congressi** dell'**Università degli Studi di Napoli Federico II**, suggestiva e prestigiosa sede sul lungomare di fronte a Castel dell'Ovo.

Il Comitato Organizzatore ha voluto scegliere la tematica ***"Transizioni parassitologiche"*** che sottolinea l'**evoluzione della Parassitologia** tra passato, presente e futuro in un momento di accelerazione delle conoscenze, evidenziando anche la forte connotazione interdisciplinare che caratterizza la nostra Società nel panorama scientifico, non solo nazionale, in termini di competenze biologiche, naturalistiche, mediche e veterinarie.

Il Congresso è co-organizzato da un comitato composto da Colleghe/i di due sedi Universitarie – **Napoli e Padova** – geograficamente lontane ma intimamente connesse sia da un punto di vista umano e di amicizia che da un punto di vista scientifico.

Il programma è molto ricco perché oltre alle **Comunicazioni in sessioni parallele** ci saranno numerosi **Simposi** di cui alcuni sponsorizzati da Aziende, su vari argomenti di interesse parassitologico e svolti in modalità duale (contemporaneità di pubblico in presenza e in remoto).

Numerosi saranno gli **invited speaker** provenienti da diverse parti del mondo; anche per questa XXXII edizione sono previsti **premi per i soci giovani** messi a disposizione dal Consiglio Direttivo della SoIPa. All'insegna dello spirito che ha sempre contraddistinto i Congressi della SoIPa non mancheranno gli appuntamenti sociali per rinsaldare il **rapporto tra i vari Soci**.

A nome del Comitato Organizzatore, fiduciosi che questo periodo estremamente difficile possa finalmente terminare, vi aspettiamo tutti a Napoli.

Sarà un piacere incontrarci.

A presto,

Giuseppe Cringoli e *Mario Pietrobelli*

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LUNEDÌ 27 GIUGNO 2022

16:00 REGISTRAZIONE DEI PARTECIPANTI

18:00 CERIMONIA INAUGURALE

SALUTI ISTITUZIONALI

PLENARY LECTURES

PAST, PRESENT AND FUTURE OF PARASITE VACCINE DEVELOPMENT: HOW THE COVID-19 EXPERIENCE WILL ACCELERATE THEIR DEVELOPMENT

Maria Elena Bottazzi
Associate Dean of the National School of Tropical Medicine, Co-director of Texas Children's Hospital Center for Vaccine Development, Baylor College of Medicine, Houston, TX, USA

PARASITOLOGICAL TRANSITIONS IN OUR WORMY WORLD

Jürg Utzinger
Director, Swiss Tropical and Public Health Institute and Professor of Epidemiology, University of Basel, Switzeland

20:30 COCKTAIL DI BENVENUTO

Martedì 28 giugno 2022		
Sala A1	**Sala A2**	**Aula Magna**
9:00 - 11:00		
SESSIONE COMUNICAZIONI Companion animal parasitic diseases Antiparasitic drugs: efficacy and resistance	**SIMPOSIO** Genomics and the other -omics in parasitology: from epidemiology to functional studies	**SIMPOSIO MSD ANIMAL HEALTH** Uomo-Animale-Ambiente: tre facce di un'unica Salute da tutelare
Coffee Break		
11:30 - 13:00		
SESSIONE COMUNICAZIONI Vectors and vector-borne diseases	**SIMPOSIO** Endoparassiti dei ruminanti: una via italiana alla prevenzione dell'antielmintico-resistenza	**SESSIONE COMUNICAZIONI** SoIPa Awards
Lunch		
14:30 - 16:30		
SESSIONE COMUNICAZIONI Vectors and vector-borne diseases	**SIMPOSIO** Foods of non-animal origin and parasites: from a neglected to an emergent issue	**SIMPOSIO BOEHRINGER INGELHEIM ANIMAL HEALTH** New Italian Recommendations for parasite control of dogs and cats: the Italian perspective
Coffee Break		
17:00 - 19:00		
SIMPOSIO Malattie parassitarie in specie animali affini: differenze e analogie per una corretta diagnosi e gestione	**SIMPOSIO** Invasive arthropod vectors and emerging vector-borne diseases	**SESSIONE COMUNICAZIONI** SoIPa Awards
19:00 Assemblea dei Soci		

Mercoledì 29 giugno 2022		
Sala A1	**Sala A2**	**Aula Magna**
9:00 - 11:00		
SESSIONE COMUNICAZIONI Mycotic diseases Medical Tropical parasitic diseases	**SIMPOSIO** Progetti di ricerca in ambito nazionale ed europeo riguardanti le infezioni causate da *Echinococcus granulosus s.l.* ed *Echinococcus multilocularis*	**SIMPOSIO VETOQUINOL** A breakthrough in parasite protection for cats
Coffee Break		
11:30 - 13:00		
KEY NOTE *John Russell Stothard* **SESSIONE COMUNICAZIONI** Medical Tropical parasitic diseases	**SIMPOSIO** Aquatic animal parasites in the Anthropocene era	**SESSIONE COMUNICAZIONI** SoIPa Awards
Lunch		
14:30 - 16:30		
SESSIONE COMUNICAZIONI Farm animal parasitic diseases Parasitic diseases in wild and exotic animals	**SIMPOSIO** IN-NTD: il network italiano per le NTD	**SIMPOSIO** Nuove frontiere e prospettive della Leishmaniosi nel Bacino del Mediterraneo
Coffee Break		
17:00 - 19:00		
SIMPOSIO L'ecografia come ausilio diagnostico nelle malattie parassitarie	**SIMPOSIO** Le infezioni fungine dell'uomo e degli animali: "Pensa al fungo e salva la vita"	**SESSIONE COMUNICAZIONI** SoIPa Awards
20:30 Cena Sociale		

Giovedì 30 giugno 2022		
Sala A1	**Sala A2**	**Aula Magna**
9:00 - 11:00		
SESSIONE COMUNICAZIONI Innovative diagnosis of parasitic diseases Zoonoses and One Health	**SIMPOSIO** New frontiers in vector control	**SIMPOSIO** Gestione sanitaria della fauna: quali spazi per parassiti e malattie parassitarie
Coffee Break		
11:30 - 13:00		
SESSIONE COMUNICAZIONI Parasitic diseases in aquatic animals		**SIMPOSIO** Effetti di calamità ed eventi climatici eccezionali sulla trasmissione di parassiti
13:30 Cerimonia di chiusura		

Simposio in lingua inglese

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PAST, PRESENT AND FUTURE OF PARASITE VACCINE DEVELOPMENT: HOW THE COVID-19 EXPERIENCE WILL ACCELERATE THEIR DEVELOPMENT

Bottazzi M.E.

Baylor College of Medicine, Houston, United States of America

For the last two decades, the National School of Tropical Medicine and its Center for Vaccine Development in Houston, Texas has operated with the mission to develop and test new low-cost and effective vaccines against emerging, parasitic and neglected tropical diseases, build capacity for vaccine development locally and with foreign nations and guide and influence vaccine policy and advocacy. This approach relies on the need for international diplomacy, solidarity, and cooperation. This presentation will provide an overview of the past, present and future of Parasite Vaccine Development. Specifically, a behind the scenes vignette of the case study of Corbevax, a COVID-19 vaccine, suitable for global access will be used to highlight how the COVID-19 experience will accelerate the development of parasitic vaccines.

PARASITOLOGICAL TRANSITIONS IN OUR WORMY WORLD

Utzinger J.[1,2]

[1] Swiss Tropical and Public Health Institute, Allschwil, Switzerland; [2] University of Basel, Basel, Switzerland

In a seminal paper published in 1947, Dr. Norman Stoll, the then-president of the American Society of Parasitology, asked the seemingly straightforward question: “How much human helminthiases is there in the world?” Stoll’s scientific inquiry revealed that there was a lot! Indeed, he estimated that there were more than 2 billion helminth infections among just over 2 billion people living on planet Earth. Where do we stand today? In my talk, I will highlight some of the most important developments that occurred over the past 75 years, placing particular emphasis on diagnostics, spatially explicit risk profiling, treatment and integrated control and elimination of helminth infection. As an outlook, I dare looking into the crystal ball. What are key social, economic, environmental and geopolitical transformations that will govern global health? My take-home messages are that we must recognize and address super wicked problems and thus embracing sustainability sciences.

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KEY NOTE

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SURPRISINGLY TROPICAL PARASITIC DISEASES RIGHT ON YOUR DOORSTEP: A COPROLOGICAL STUDY OF A TROOP OF SEMI-CAPTIVE BABOONS AT KNOWSLEY SAFARI PARK, UK

Stothard R.*

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

With the impact of COVID-19, international travel opportunities to study neglected tropical diseases were severely curtailed at the LSTM during the 2020-2021 period. In response, we turned our attention to fieldwork opportunities within the UK, particularly where One Health studies could be performed. Upon the request of the head veterinarian at Knowsley Safari Park, a short drive from the LSTM, we conducted a parasitological investigation of their troop of semi-captive olive baboons during the summer of 2021. Each day, these animals typically interact with visitors' cars during a drive-through safari experience and often leave behind various faecal material on vehicle surfaces. Using a combination of standard parasitological and coproculture methods, we ascertained that both trichuriasis and strongyloidiasis were hyper-endemic within the troop of 240 animals. By contrast, even though many animals were positive for giardiasis by rapid diagnostic tests, standard coproscopy failed to detect *Giardia* cysts. Overall prevalence of trichuriasis was 48.0% (95% CI: 41.8-54.2%), giardiasis was 37.4% (95% CI: 33.7-41.1%) and strongyloidiasis was 13.7% (95% CI: 12.0-15.4%). A sub-set of parasite material was subjected to DNA characterisation, confirming the presence of *Trichuris trichiura* and rather surprisingly *Strongyloides fuelleborni* rather than *S. stercoralis*. From video analysis of cars and baboons, vehicles that spent more than 15 minutes inside the baboon enclosure were 1.5 times more likely to be defecated upon than those spending less than 15 minutes inside. Our parasitological findings will be discussed in relation to future park management plans for this baboon enclosure. The implications of these findings are discussed in terms of animal, keeper and public health, noting how One Health approaches to control of neglected parasitic diseases here, and in sub-Saharan Africa more generally, are needed.

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GENOMICS AND THE OTHER -OMICS IN PARASITOLOGY: FROM EPIDEMIOLOGY TO FUNCTIONAL STUDIES

THE INTRIGUING GENOMIC BIOLOGY OF THE EARLY-BRANCHING, BINUCLEATE TETRAPLOID, *GIARDIA*

Jex A.*

The Walter and Eliza Hall Institute of Medica, Parkville, Australia

Giardia duodenalis is an important and highly prevalent food and waterborne parasitic protist that causes more than 200 million symptomatic cases of diarrhoea every year and is a major cause of chronic, post-infectious gastrointestinal disorders, including irritable bowel syndrome. *Giardia* are also members of one of the earliest-branching lineages of eukaryotes, the Diplomonadida. Intriguingly, *Giardia*, like all diplomonads, are binucleated cells with each nuclei containing a diploid set of chromosomes. This creates intriguing questions but various challenges with its population genetic study. Understanding *Giardia*'s genomics and population genetics is highly relevant to new drug development, improved diagnostics and a better understanding of its transmission dynamics and epidemiology. Such studies are also important in addressing a range of fundamental questions around *Giardia*'s regulatory biology, genomic evolution, modes of genetic recombination, host-specificity, disease mechanisms and host-interactions. Here, I'll discuss the current state of research of *Giardia* genomics, new advances in understanding of its population genetics and how these studies are helping to better shape our understanding of the biology of this important parasite and intriguing eukaryote.

INTEGRATION OF SYSTEMS BIOLOGY AND COMPARATIVE GENOMICS TO UNDERSTAND GENOME EVOLUTION IN *LEISHMANIA*

Brilli M.*

University of Milano, Milano, Italy

Leishmania are arthropod vector-born etiological agents for several human and animal diseases such as visceral and cutaneous leishmaniasis. Like the other Trypanosomatids, they have peculiar genomic features: almost complete absence of specific transcriptional regulation, highly dynamic copy numbers (CN), long polycistronic transcripts of functionally unrelated genes, trans-splicing of the coding sequences therein, to name a few. Consequently, the abundance of proteins in the cell can be modulated through changing the CN of loci across generations, regulation of mRNA half-life and translatability, regulation of the export to the cytoplasm/splicing, regulation of protein half-life. The evolutionary optimization of these features is further complicated by the life cycle of these organisms in different hosts since optimal solutions in the vector might well be sub-optimal in the vertebrate host. After a short summary on the genomics of these species, we introduce concepts from systems biology and metabolic control theory that can help in understanding some of the atypical genome evolutionary patterns in these organisms, in particular the observed epistasis of the CN of functionally related genes located on different chromosomes.

LARGE-SCALE GENOMIC EPIDEMIOLOGY INVESTIGATIONS OF *CRYPTOSPORIDIUM PARVUM* IN EUROPE

Castelli M.*[1], Bellinzona G.[1], Nardi T.[1], Sannella A.R.[2], Blanchard Y.[3], Klotz C.[4], Troell K.[5], Chalmers R.[6], Robinson G.[6], Stensvold R.[7], Jokelainen P.[7], Autio T.[8], Embom T.[8], Sassera D.[1], Cacciò S.[2]

[1]University of Pavia, Pavia, Italy; [2]Istituto Superiore di Sanità, Rome, Italy; [3]ANSES, France; [4]Koch Institute, Germany; [5]Swedish Veterinary Agency, Sweden; [6]Cryptosporidium Reference Unit, United Kingdom; [7]Staten Serum Institute, Denmark; [8]Ruokavirasto, Finland

The apicomplexan genus *Cryptosporidium* includes globally distributed parasites that cause severe gastrointestinal disease in humans and animals. Two species account for the vast majority of human infections, namely the anthroponotic *C. hominis* and the zoonotic *C. parvum*, with the latter being highly prevalent in high income areas of the world, including Europe. Very young children and neonatal ruminants are particularly at risk of severe infections, and no effective drugs or vaccines are currently available.
Previous studies, essentially based on sequence analysis of the glycoprotein 60 (gp60) gene, revealed high genetic diversity in the *C. parvum* population, yet a few subtypes predominate and cause outbreaks in Europe. The reasons for this high differential prevalence of subtypes are still unknown.
To investigate the genetic diversity of *C. parvum* at the genomic level and understand its epidemiological significance, we generated over 100 whole genome sequencing (WGS) data of isolates collected from different hosts (humans, calves, goat kids and lambs) and geographic origins (13 European countries) and representing sporadic and outbreak cases. We also retrieved WGS data from public databases.
After removing WGS data showing high contamination or representing mixed *C. parvum* infections, we focused our analyses on the WGS data of 116 samples, 95 of which were newly sequenced while 21 were from public databases. Sequencing reads were mapped to a recently established and essentially complete reference genome (Iowa II-ATCC), allowing the identification of over 30,000 SNPs. We observed a non-random distribution of the SNPs across the chromosomes, with enrichment in subtelomeric regions.
Phylogenetic analyses based on genomic SNPs led to the identification of three strongly supported lineages of *C. parvum*, with no apparent correlation with host species, country of origin, or gp60 subtype. Additionally, pairwise SNP distances disclosed the presence of 11 distinct clusters, containing highly similar (<100 SNPs) and phylogenetically closely related isolates. Each cluster may represent a distinct outbreak, as supported in some cases by epidemiological links. Interestingly, all those “outbreak clusters” are affiliated to the same large lineage.
Further analyses are in progress to investigate the genomic differences between the three different *C. parvum* lineages in terms of gene content, allelic variations, and recombination patterns, in order to identify genetic determinants linked to the observed differential capability to cause outbreaks.

This work has been supported by funding from the European Union’s Horizon 2020 Research and Innovation Programme, under grant agreement No 773830: One Health European Joint Programme (PARADISE project).

TRANSCRIPTS AND MIRNA FROM *ANISAKIS PEGREFFII* INFECTIVE LARVAE AND THEIR RELEASED EXOSOMES: FROM THE PATHOGENIC REPERTOIRE TO HOST-CELLULAR RESPONSE

Cavallero S.*, D'Amelio S.

Department of public health and infectious diseases, Rome, Italy

Fish-borne parasitic helminthiasis caused by trematodes, cestodes and nematodes has recently emerged as a major food safety concern. *Anisakis* spp. (Nematoda: Anisakidae) is the etiological agent of the zoonotic disease anisakiasis. In the last decade, investigations on ecological and epidemiological aspects allowing to understand relevant features as biodiversity, geographic distribution and host preference, while pathogenic mechanisms of the disease are barely known. Despite humans are accidental hosts, and infective larvae cannot reach adult stage, they still elicit immunological and inflammatory response causing a gastrointestinal disease with mild to severe clinical signs, also of allergic nature. Moreover, reports of larvae co-localization with gastrointestinal tumors and larval behavior mimicking metastatic lesions are increasing, highlighting the need to deepen aspects related to fine mechanisms of infection. In this scenario, advances in omics studies and in cellular models may assist important discoveries in the biology of parasitic nematodes, including *Anisakis*, and in the host-parasite interplay.

The infection dynamic account for two sides, the pathogen and the host. From the pathogen perspective, L3 invade the gastrointestinal mucosa using a combination of mechanical disruption and secreted/excreted factors able to degrade the extracellular matrix and regulate host response, including the extracellular vesicles (EVs). Indeed, comparative transcriptomics studies on specific tissue such as the pharyngeal region containing excretory glands could help in understanding key roles in biological pathways implicated in pathogenesis. Proteolytic enzymes, molecules encoding anesthetics, inhibitors of primary hemostasis and virulence factors, anticoagulants and immunomodulatory peptides were found enriched *Anisakis simplex sensu stricto* and *Anisakis pegreffii* L3 pharynx. The pharyngeal region seems to be also involved in the release of EVs, which deliver a cargo of DNA, proteins and non-coding RNAs as microRNA in a protected state. In this regards, the first miRNAs catalogue of *A. pegreffii* L3 and of its released EVs revealed a degree of sequence conservation with related parasitic nematodes and other pathogenic helminths. Moreover, gene targets prediction in human genome suggested their potential involvement in infection, with several immune/inflammatory related targets. Such data will be used to investigate in detail also the other side of the infection dynamic, namely the host immune reaction. In fact, a human-derived intestinal organoid models will be used to analyze the effect of contact with *Anisakis*-derived EVs on the intestinal cells. So far, EVs have been used to treat human epithelial colonic cancer cells (Caco-2) revealing a decrease in pro-inflammatory cytokines level (IL-6 and IL-8), with a possible regulatory post-transcriptional mechanisms acting after an early exposure, potentially due to proteases or miRNAs.

HOW DO SCHISTOSOMES AFFECT THE GUT MICROBIOME? AND WHY DOES IT MATTER?

Cantacessi C.*

University of Cambridge, Cambridge, United Kingdom

The pathophysiology of schistosomiasis is mainly related to the inflammatory granulomatous response triggered by parasite eggs trapped in host tissues. Over the last few years, evidence has emerged that the host gut microbiota might be (at least partially) involved in the immunological cascade that culminates with the formation of schistosome egg-induced intestinal granulomas. This presentation covers our recent findings on the impact of *Schistosoma mansoni* (*Sm*) infection on the gut microbial composition and predicted function of microbiome-humanised vs. schistosome-infected wild type mice. *S. mansoni* infection induces profound gut microbiome alterations in both rodent hosts. In spite of substantial differences in microbiome composition at baseline, selected pathways are consistently affected by parasite infection, which points towards a likely connection between *S. mansoni* infection and the host gut microbiome. Such pathways include enhanced production of tryptophan metabolites and butyrate, and subsequent activation of AhR (aryl hydrocarbon receptor) signaling and additional butyrate-regulated pathways, that might be involved in prevention of excessive injuries caused by migrating parasite eggs. Together, data from this and previous studies suggest that the host gut microbiome may play a dual role in the pathophysiology of schistosomiasis, where intestinal bacteria may contribute to egg-associated intestinal pathology while, in turn, protecting the intestinal epithelium from uncontrolled tissue damage.

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ENDOPARASSITI DEI RUMINANTI: UNA VIA ITALIANA ALLA PREVENZIONE DELL'ANTIELMINTICO-RESISTENZA

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EUROPEAN STRATEGIES FOR THE CONTROL OF ANTHELMINTIC RESISTANCE AND ITALIAN EXPERIENCES

Bosco A.*, Rinaldi L., Cringoli G.

Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli "Federico II", Naples, Italy

Helminth infections are ubiquitous in grazing ruminant production systems and are responsible for significant costs and production losses (Charlier et al., 2020. Prev Vet Med, 182: 105103). Frequent anthelmintic use to control these infections has resulted in the selection of drug resistant helminth populations. Anthelmintic Resistance (AR) in parasites is now widespread throughout Europe and it is a major threat to the sustainability of modern ruminant livestock production (Rose et al., 2015. Vet Rec, 176:546). A last meta-analysis study to record the distribution of AR revealed that the phenomenon was widespread in Europe, being reported in all economically most important genera of gastrointestinal nematodes (GINs) and across many European countries. Results revealed an average farm level prevalence of AR to benzimidazoles (BZ) of 48 and 51%, to macrocyclic lactones (MLs) except moxidectin (MOX) of 29 and 44%, to levamisole (LEV) of 32 and 20%, in sheep and goats, respectively and to MOX in sheep of 17%. In cattle, farm level prevalence of AR across the European continent were 8% for BZ, 32% for ML, 12% for LEV and 27% for MOX (Rose Vineer et al., 2020. Parasite, 27:69). Combatting AR requires intensified and integrated research efforts in the development of innovative diagnostic tests to detect helminth infections and AR, sustainable anthelmintic treatment strategies and the development of complementary control approaches such as vaccination and plant-based control. It will also require a better understanding of socio-economic drivers of anthelmintic treatment decisions, in order to support a behavioral shift and develop targeted communication strategies (Charlier et al., 2022. Adv Parasitol, 115:171-227).

These control strategies were undertaken during the European COST Action "Combatting Anthelmintic Resistance in Ruminants - COMBAR" (www.combar-ca.eu) that emerged from activities of the Livestock Helminth Research Alliance (LiHRA) and aims to advance research on the prevention of anthelmintic resistance in helminth parasites of ruminants in Europe.

In Italy, few reports of AR in sheep against levamisole, ivermectin and benzimidazoles have been published but mainly in northern and central regions (Traversa et al., 2007. Parasitol Res, 101:1713–6; Lambertz et al., 2019. Vet Rec, 6: 332). On the contrary, in southern Italy some concrete actions appear to be effective in maintaining the efficacy of anthelmintics and slowing the development of AR, e.g. the monitoring of GIN infection in sheep and other livestock by regular diagnosis, use of targeted treatments, rotation of different drugs, correct drenching, and low movement of animals between farms (Rinaldi et al., 2014. Vet Parasitol, 203:139–43). However, a recent study has highlighted two cases of AR to BZ in sheep farms of the Campania region (Bosco et al., 2020. Parasit Vectors, 13:457); therefore, its development is inevitable and its occurrence is not a matter of "if" but "when".

USE OF BODY CONDITION SCORE AS A POSSIBLE MARKER FOR THE TARGETED SELECTIVE TREATMENT OF DAIRY SHEEP AGAINST GASTROINTESTINAL NEMATODES

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Targeted selective treatments (TSTs) have become increasingly popular in the fight against anthelmintic resistance. TSTs entail solely the treatment of the most affected animals (usually a small percentage of the flock), thus leaving most of the animals in the flock untreated, ensuring the presence of larvae in refugia on pastures. Such treatments can be based on various parameters including Fecal Egg Counts (FEC), Hematocrit (HCT), degree of anemia (ocular mucosa coloration, FAMACHA method), faecal consistency score, milk production, weight loss and Body condition score (BCS).
As infection with non-bloodsucking nematodes, frequently encountered on sheep farms in the central Mediterranean basin, commonly causes bodyweight reduction, the aim of our research was to evaluate the effectiveness of BCS as a parameter for the implementation of TSTs in lactating dairy sheep with subclinical gastrointestinal nematodes (GIN) infections from Sardinia, Italy.
Fecal samples were collected from two different populations: 1012 ewes divided into 2 groups (third and fifth month of lactation) and 346 replacement ewe lambs. The BCS was recorded for all enrolled sheep by the same veterinary and each animal was given a score ranging from 1 to 5 (1 = emaciated; 5 = obese) with a precision of 0.25. FEC was assessed using McMaster technique (NaCl solution s.g.=1.2) and coprocultures were performed for identification of present species trough the morphometric keys available in the literature (van Wyk and Mayhew, 2013. Onderstepoort J Vet Res, 80:539).
In lactating sheep an overall GIN prevalence of 85.4% with a mean eggs per gram (EPG) of faeces of 210.1 ± 347.3 was found. Overall, animals with the lowest BCS had the highest EPG values and a negative correlation (r=-0.163) between the EPG values and BCS of the studied animals was found, which was most significant for older sheep. In replacing ewe lambs an overall GIN prevalence of 98% with a mean EPG of faeces of 517.2 ± 447.9 was found. The 79.8% of examined samples were positive for *Nematodirus* spp. eggs. The stratification of animals based on the egg excretion showed also in replacement ewe lambs that the average of BCS decrease with increasing EPG classes with a significant negative correlation between BCS and EPG (r=-0.285). Based on larval examination, *Trichostrongylus* spp. and *Teladorsagia* spp. were the most identified GIN genera in both animal categories.
This research confirmed BCSs and EPG values for GIN in sheep to be negatively correlated, particularly in older ewes. Application of TSTs for lactating sheep with a BCS < 2.25, especially to older ewes, could be beneficial in case of subclinical GIN infections. For the ewe lambs the threshold could be increased at a BCS of 2.75 and this suggests that the threshold for treatment should be more dynamic and adapted to the physiological and productive situation on a farm, similar to the use of FEC.

QUANTITATIVE APPROACH TO TARGET WHOLE-GROUP TREATMENTS IN SMALL RUMINANTS

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Endoparasites threaten small ruminant farming on a global scale, especially in grazing settings. The reliance on anthelmintic drugs for the control of parasitic infections has led to the development of resistance, which is now spreading at alarming pace worldwide. Diagnostic procedures have been consistently overlooked but the risk of anthelmintic resistance (AR) imposes now a change of approach. Convincing farmers to embrace this new attitude towards parasite control is not easy, and whole-group targeted treatments (TT) likely represent the most acceptable first step among the currently recommended strategies for treatment. To define the need for a whole-group treatment, Faecal Egg Count (FEC) techniques (e.g., McMaster and mini-FLOTAC) are well investigated and widely adopted in diagnostic laboratories. However, there is still limited knowledge on the burden determination of parasites in a group of animals (i.e., in a flock) in terms of required sample size and results interpretation. A quantitative approach was developed based on a mathematical formula, which calculates the optimal sample size to achieve a good accuracy in the burden estimation (Maurizio et al., 2021. Vet Sci, 8: 69). The sample size is based on the expected mean FEC, aggregation and on the farm size. According to this approach, samples are analyzed individually and subsequently the mean Eggs Per Gram (EPG) of faeces are calculated for each farm. Testing individual samples rather than pools allows for the calculation of the 95% confidence interval (CI), which provides useful information on the distribution of parasites in the host population. This monitoring approach is currently being tested in sheep and goat farms of North-eastern Italy using McMaster technique, comparing FEC results to the number of treatments actually performed per year in each farm. The first results showed that unnecessary use of anthelmintics is likely common and often easily preventable if proper monitoring is implemented. They also highlighted the advantages of using individual samples: for instance, a relatively wide CI (high parasitic aggregation) could suggest when treating only the few animals with high burden is preferable, thus addressing towards a more selective approach. To conclude, this monitoring approach represents a statistically sound tool when planning a TT based on parasitological parameters but divulgation activities are needed to raise awareness among farmers on the importance of monitoring for a more appropriate use of anthelmintics.

ALTERNATIVE APPROACHES TO THE USE OF SYNTHETIC DRUGS: *IN VITRO* EVALUATION OF THE ANTHELMINTIC ACTIVITY OF NATURAL COMPOUNDS

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Gastrointestinal nematodes are one of the major threats to the welfare and productivity of ruminant production. The control of these nematodes has mainly relied on the use of synthetic anthelmintic drugs. However, the anthelmintic resistance developed by gastrointestinal nematodes has now become a serious problem worldwide. Therefore, parasite management should include alternative approaches to the use of synthetic drugs aimed at an integrated and more sustainable control (Charlier et al., 2022. Adv Parasitol, 115: 171-227). Among these approaches, the use of natural bioactive compounds for their anti-parasitic properties is now considered a possible new option (Hoste et al., 2015. Vet Parasitol, 212: 5-17). The scientific evaluation of the anthelmintic properties of natural products is a necessary step prior to their adoption for parasite control. This can be performed by using *in vitro* and *in vivo* methods. Most of these methods are adaptations of the *in vitro* and *in vivo* tests usually used to evaluate the efficacy of anthelmintics and to detect the anthelmintic resistance of gastrointestinal nematodes. The main advantages of the *in vitro* assays (Githiori et al., 2006. Vet Parasitol, 139: 308-20) are their relatively low cost and the possibility of allowing the screening of many compounds. The *in vivo* evaluation of the same number of compounds would in fact require the use of a high number of animals with consequent high financial and time expenditure. Furthermore, the use of research animals may be reduced to the study of the most promising and less toxic substances. A further advantage of the *in vitro* assays is the possibility to enable the identification of the fractions/pure compounds to which the anthelminthic properties of a natural substance can be attributed. The different *in vitro* methods available may allow evaluating the efficacy of a compound on different stages of gastrointestinal nematode lifecycle, such as eggs (egg development and hatch inhibition tests), larvae (larval development, motility/migration, and exsheathment inhibition tests), and adults (adult motility inhibition test). These *in vitro* methods are reproducible, standardizable, and sensitive, and some of them may allow both qualitative and quantitative (dose-effect) evaluations. However, due to differences between the *in vitro* conditions and the site of the parasite within the hosts, and host animal factors that can affect the bioavailability of the active compounds, often the results observed *in vitro* and *in vivo* may differ (Githiori et al., 2006. Vet Parasitol, 139: 308-20). Therefore, no definitive conclusions should be drawn from *in vitro* studies before the *in vitro* results are validated under *in vivo* conditions, and *in vitro* assays should be considered a preliminary screening aimed to select compounds that deserve further studies and establishing biologically realistic concentrations to be used on animals in future *in vivo* evaluations.

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FOODS OF NON-ANIMAL ORIGIN AND PARASITES: FROM A NEGLECTED TO AN EMERGENT ISSUE

SOIPA

NAPOLI 2022 PADOVA

FOODBORNE PARASITES: THE NEED FOR A EUROPEAN FOCUS

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It is no secret that among foodborne pathogens, parasites are neglected, and this is particularly so in wealthier countries. In general, parasites are an ugly subject associated with travel to other countries, often tropical. However, ignoring unpleasant truths does not mean that they will go away, and transmission of foodborne parasites occurs in Europe – probably on a daily basis.
Although both meat (e.g., *Trichinella*) and fish (e.g., *Anisakis*) can be important transmission vehicles, and consumers often tend to consider them more "dangerous" regarding foodborne disease, probably the most common transmission vehicles for foodborne parasites in Europe are fresh produce eaten raw – salads and fresh fruit – otherwise known as the healthy choice. Such fresh produce may be locally grown, but also many European countries import fresh produce, opening up the possibility of bringing unexpected parasites with which European doctors have relatively little diagnostic experience (e.g., *Cyclospora* or even *Trypanosoma cruzi*).
A risk-ranking of foodborne parasites in Europe conducted as part of a COST Action (Bouwknegt et al., 2018. Euro Surveill, 23(9): 17-00161), and divided by region (Northern, Western, Eastern, South-Western, South-Eastern) found that in all five European regions the top two parasites were ones that could be transmitted by contaminated fresh produce – although not all regions had the same parasites topping the list. Among the parasites listed as the top two were: *Echinococcus granulosus*, *Echinococcus multilocularis*, *Toxoplasma gondii*, and *Cryptosporidium* spp. Although *T. gondii* has various transmission routes, that of contamination of fresh produce may be both very relevant and under-recognised. However, the other three parasites, when foodborne, will be associated largely with fresh produce.
It is of interest to note that another ranking exercise, this time of foodborne pathogens (not just parasites) conducted in Norway (VKM, 2021. https://vkm.no/english/thenorwegianscientificcommitteeforfood andenvironment.4.2375207615dac0245ae1dd4d.html) also had *T. gondii* topping the list, *E. multilocularis* in 3rd place (after *Campylobacter* spp.), and *Cryptosporidium* at number 9 (below 3 more bacterial pathogens and two viral).
In this presentation I want to highlight further why foodborne parasites that can be transmitted to consumers via fresh produce deserve our attention, and why attempts to address this issue should be grounded in a One Health perspective. Using concrete examples from surveys and investigations, my intention is to highlight that although foodborne parasites are indeed, of importance, in faraway places – they are also important here, in Europe. If we continue to consider them largely as being a problem of somewhere else, then this means being complicit in putting the health of consumers in Europe at risk.

HUMAN AND ZOONOTIC INTESTINAL PARASITES IN VEGETABLES AND FRUITS: THE ITALIAN EXPERIENCE

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In recent years, the consumption of fresh vegetables and fruits has increased due to the rising demand for healthy dietary options. Despite being an excellent source of nutrients, these products can be contaminated with several foodborne parasites (FBPs) at any point in the farm-to-fork chain.
In the European ranking of FBPs based on the Euro-FBP criteria, the specific chart that refers to Italy, includes five out of the 10 parasites that can be transmitted via contaminated fresh produce, i.e., *Echinococcus granulosus*, *E. multilocularis*, *Toxoplasma gondii*, *Entamoeba histolytica* and *Cryptosporidium* spp.
Despite the key role played by fresh produce in the transmission of pathogenic parasites, only a few studies have assessed the extent of parasite contamination on fresh produce sold in Italy. The first report produced on the subject in 1968 documented *Giardia duodenalis* cysts in fresh produce from local markets in the city of Rome and, almost 40 years later, the presence of *Giardia* cysts was observed in ready to eat (RTE) salads collected in Palermo. Afterwards, *Cyclospora cayetanensis* DNA was detected in environmental samples and vegetables grown and harvested in Italy, i.e., fennel, tomato, cucumber and celery. Furthermore, a study aimed at investigating the presence of protozoan contamination of RTE salads on sale in Italy revealed the presence of the following protozoan parasites: *C. cayetanensis*, with the highest prevalence, followed by *Cryptosporidium* spp., *T. gondii* and *G. duodenalis*. *Blastocystis hominis* and *Dientamoeba fragilis* were also detected. The most recent data concern the contamination of RTE salads and both local and imported berries sold on the Italian market. Several *Cryptosporidium* species and *G. duodenalis* assemblages as well as *E. histolytica* were identified in both matrices. In addition, *C. cayetanensis* was detected in blueberries imported from an endemic country (Peru) and *E. multilocularis* in RTE salads that had been cultivated and processed in Italy.
Parasitic foodborne diseases represent an important public health problem throughout the world and several outbreaks of disease have been linked to the consumption of contaminated fresh produce which only undergo minimal or no processing and are rarely subjected to heat treatment before being consumed. Furthermore, robust parasite transmission stages are usually not inactivated during food preparation and the chemical sanitizers routinely employed do not seem to be effective against them. In Italy, foodborne outbreaks associated with consumption of contaminated fresh vegetables have not been reported but the data available show that the contamination of fresh produce by pathogenic parasites is a cause for concern given the potential implications for human health. It is of vital importance to keep gathering data on the spread and impact of FBPs in Italy and to implement closer monitoring of both locally produced and imported fresh produce sold on the Italian market.

CYCLOSPORA AND CYCLOSPORIASIS: SHOULD EUROPE BE WORRIED TOO?

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Cyclospora cayetanensis is a coccidia that causes gastrointestinal disease in humans. Until now, *C. cayetanensis* is considered an anthroponotic parasite. It is frequently associated with foodborne outbreaks. Since its discovery, this parasite has been implicated in outbreaks associated with the consumption of berries and vegetables, mainly raspberries, lettuce and herbs produced in endemic areas. *Cyclospora* is frequently found in regions with tropical and semi-tropical climates. In the USA, since the 1990s, outbreaks were mostly associated with plant products imported from endemic areas including Guatemala, Mexico, Colombia, and Mexico. However, in the last five years, several outbreaks have been associated with products produced and processed in the United States. Environmental studies indicated that irrigation water contained *Cyclospora* oocysts and vegetables collected from adjacent farms to the implicated one have also been positive for *Cyclospora*. Therefore, seems that agriculture in a developed country like the USA where good agricultural practices are followed are susceptible to contamination with *Cyclospora*. These results also question whether *Cyclospora* is endemic in the US population and that agricultural water is becoming contaminated with human feces. In Europe, most cases of cyclosporiasis are associated with travelers returning from *Cyclospora* endemic areas; however, surveys of vegetables for sale have tested positive for *Cyclospora*. Outbreaks have also been reported associating products produced and processed in the continent. Surveys of water and soil have also been positive suggesting that fecal contamination is more extensive than previously thought. Surveys of fresh produce and berries in Italy suggest that various regions of this country may also be affected by *Cyclospora*.

PROGRESS TOWARDS VALIDATION OF MOLECULAR DETECTION METHODS

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The protozoan parasites emerge as important foodborne zoonotic pathogens leading to several outbreaks worldwide. Depending on the parasites and the parasite cycle stage, different types of food can act as vehicles of transmission: fresh produce and fresh juice (vegetables and fruits), meat, milk products or shellfish.
For *Cryptosporidium* spp. oocysts and *Giardia duodenalis* cysts, even if an international standard ISO 18744 exists since 2016 for their detection in leafy green vegetables and red berry fruits, it is not widely used, mainly due to the detection by microscopy which is not suitable with food routine analyses. For *Toxoplasma gondii*, no standardized method is available for the detection of oocysts in foods. Therefore, the assessment of human exposure to these pathogens in foods (prospective studies), as well as the identification of contaminated food in case of outbreaks (retrospective studies) remain currently difficult due to the lack of standardized, validated and/or applicable methods for the routine detection of parasites in foods. Besides, validation data on the methods used in prospective studies are rarely provided, hence limiting the understanding of occurrence results and the comparison of studies worldwide.
At the present time, there is no standardized procedure for the development and the validation of detection methods of parasites in foods; neither there are performance criteria defined for these methods. However, some recommendations from the ISO 17468 and ISO 16140 serie, that were initially established for the detection of bacteria in food, can be transferred to parasites and used to develop a validation scheme.
A general procedure for the development and the validation of molecular methods for the detection of protozoan parasites in foods will be exposed and the critical steps will be discussed. Relevant performances criteria will be suggested. The application of such standardized procedure is crucial to be able to assess and to compare the reliability of the many molecular methods now available in the literature, and which are increasingly privileged for the detection of the protozoan parasites in food.

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Boehringer Ingelheim

NEW ITALIAN RECOMMENDATIONS FOR PARASITE CONTROL OF DOGS AND CATS: THE ITALIAN PERSPECTIVE

SOIPA

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ISOXAZOLINES: A ONE HEALTH APPROACH

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Isoxazolines represent the most recent chemical group of insecticides-acaricides developed for use in domestic animals. Their mode of action is systemic, after oral administration or through transcutaneous absorption. Isoxazolines are highly bound to plasma proteins (i.e. 99%), have a long half-life (i.e. 9-14 days for afoxolaner), and provide a high activity against hematophagous arthropods, i.e. fleas and ticks, but also other arthropods. Additionally, isoxazolines are effective against mites (i.e. *Sarcoptes scabiei*, *Demodex canis*, *Otodectes cynotis*). Afoxolaner has been used to treat swine infested with *Sarcoptes scabiei* var. *hominis* in a model of human scabies, but no formulation for human used is currently known to be under development. More interestingly for One Health purposes, isoxazolines have demonstrated insecticidal activity against mosquitoes and sandflies.

In one study, after having fed on treated dogs, the afoxolaner insecticidal efficacy against *A. aegypti* varied from 98% (Day 2) to 75.3% (Day 29) based on arithmetic means. There is no direct impact on the transmission of pathogen agents during the blood meal, but the results of this study are in favour of an indirect protective effect in households by reducing the vector population. In another study, a single administration of afoxolaner to dogs was enough to kill 100% of sandflies within 48h after exposure on Day 1 to 86.4% sandflies within 72h after exposure on Day 28. Recently another isoxazoline, fluralaner, has also demonstrated efficacy against *P. papatasi* sandflies after oral administration to dogs.

Recently afoxolaner and fluralaner insecticidal effect in treated dogs has also been demonstrated against kissing bugs (*Triatoma infestans*), the vector of Chagas disease, with a possible impact of treating dogs to control the vector population and decrease the rate of transmission to humans. Similarly, a study was conducted to determine the insecticidal efficacy of NexGard® against bed bugs (*C. lectularius*) feeding on treated dogs. The percent feeding in dogs ranged from 95% to 100%, demonstrated the capacity to feed on dogs. The reduction of live fed *C. lectularius* in the afoxolaner treated dogs ranged from 63.5% to 85% during the month. It is hypothesized that monthly treatment of dogs with afoxolaner could help in preventing a bed bug population to install in a household (similar data have been obtained with cats, unpublished).

All these results open the door for new possibilities to control vectors or human biting insects by regularly treating dogs and/or cats living in households with isoxazolines.

PARASITES OF DOGS AND CATS IN ITALY: CHANGING EPIDEMIOLOGICAL PATTERNS AND NEW RECOMMENDATIONS FOR VETS

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Parasites of dogs and cats are widespread in Italy. Infection with them can have serious consequences for pet health and well-being and several can also cause disease in humans. The epidemiology of canine and feline parasites is in continuous evolution and there is a need for updating where, when and how to prevent parasitic infections. Indeed, climate change, animal movement and greater attention by veterinary practitioners has led an increase in the number of reports of infection with different parasites in previously unaffected areas. Furthermore, different lifestyles and outdoor activity puts pets at greater risk for infection. Effective control relies on a good knowledge of parasite distribution and the risk factors for infection.

In order to update the current epidemiological scenario, an advisory board made up of 10 leading experts in veterinary parasitology was set up to review the most recent data and to develop national recommendations for the control of parasitic diseases in dogs and cats throughout the country. Here, we focus on some of the main species of parasites of dogs (i.e. *L. infantum*, *D. immitis* and canine lungworms) and cats (i.e. feline lungworms), in Italy.

NOT A "NORTHERN ITALY PARASITE" ANYMORE: *DIROFILARIA IMMITIS* PREVALENCE IN SOUTHERN ITALY

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Dirofilaria immitis is the causative agent of heartworm (HW) disease in dogs; the parasite is vectored by several mosquito species and may cause zoonotic infection in humans as well. Historically, *D. immitis* has been considered endemic in northern Italy, being the Po valley showed as the hyperendemic area par excellence. However, starting from the beginning of the century a changing distribution pattern of the infection has been reported with an increasing report of autochthonous canine cases in central and southern regions of Italy. The colonization by new invasive species of mosquitos (i.e., *Stegomyia albopicta*), the increased movement of animals across the country, and the lack of adoption of preventive strategies such as chemioprophylaxis have been proposed as the main drivers for this changing pattern. Endemic foci of *D. immitis* infection in sheltered and owned dogs have been described in Apulia and in the island of Linosa (Pelagie Archipelagos), respectively; being the latter the southernmost focus of HW in Europe.

Very recently, a cross-sectional multicentric survey on canine filarioses was carried out in central and southern Italian regions. Owned and sheltered dogs from six southern Italian regions (i.e., Lazio, Campania, Apulia, Basilicata, Calabria, and Sicily) were included in the survey regardless their breed, attitude and/or gender. Included dogs were elder than 1 year and had no history or were not under preventative treatment against filarioses. Blood samples were analysed with Knott's test and, if positive, further tested with *D. immitis* specific ELISA antigen test.

One-thousand-eighty-seven dogs were enrolled in the survey. The overall microfilaremia prevalence was 17% (338/1987) being single-species infection (92.6%) more common that mixed (7.4%). Surprisingly, *D. immitis* was the most frequent species with an overall prevalence of 11.4% (no. 251), followed by *D. repens* (no. 98, 3.7%) and *A. reconditum* (no. 14, 0.6%). Sheltered dogs were significantly more at risk for *D. immitis* (χ^2 =163.3427, $p < 0.00001$), and, in the same manner, mongrel dogs (χ^2 = 92.7365, $p< 0.00001$) and animals housed in rural areas (χ^2 = 80.7429, $p< 0.00001$) were more frequently infected by *D. immitis*.

These findings add more evidence to the spread of *D. immitis* infection through southern Italian regions and paradigms as the parasite can colonize new territories and threaten animal and human health slyly. Southern Italian regions should not be considered anymore as non-endemic for *D. immitis*; practitioners and dogs' owners must be aware of this risk, and the adoption of efficient strategies to protect dogs and control new infections should be promoted.

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MALATTIE PARASSITARIE IN SPECIE ANIMALI AFFINI: DIFFERENZE E ANALOGIE PER UNA CORRETTA DIAGNOSI E GESTIONE

SOIPA

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HORSE AND DONKEY PARASITOLOGY: HEAD-TO-HEAD

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It is commonly believed that donkeys are just horses with long ears, however, these two animal species are quite different. The ancient Equidae dates from the Eocene epoch 25 million years ago of the Cenozoic era (the Age of Mammals). The genus *Equus* (equids) include horses, donkeys, and zebras and have its origin in North America. Through adaptation, the modern horse (5 Myr ago) lives on grasslands and can be found on all continents. About 3 Myr ago Zebras and donkeys have diverged from the original *Equus*, adapting to regional conditions. Horses (*Equus caballus*) and donkeys (*Equus asinus*) had their domestication 6 and 4 thousand years ago, respectively (Cucchi et al., 2017. R Soc Open Sci, 5:4). It is considered that undomesticated horses show fluctuating numbers worldwide with a somewhat similar gastrointestinal parasite population to domesticated horses. In general, the animals can be parasitized by a high number of helminths, without showing major clinical symptoms. Although equids demonstrate some level of tolerance to parasites, they may have distinct levels of parasite distribution and intensity (Buono et al., 2021. J Helminthol, 95:1–10). Moreover, if donkeys are parasitized with a high helminth burden clinical signs such as diarrhea or poor body condition may be less common than in horses (Matthews and Burden, 2013. Equine Vet Educ, 25:461-67) showing a healthier look (Maestrini et al., 2020. Vet Sci, 7:195). The most common nematodes are gastrointestinal strongyles (Buono et al., 2021. J Helminthol, 95:1–10; Molento and Vilela, 2021. Bras J Vet Res Anim Sci, 01:1-36), and the re-emergence of encysted cyathostomin larvae can cause the larval cyathostominosis in both species. Even though the fatality rate of this syndrome may be high in horses, the data is scarce for donkeys. In horses, *Parascaris* spp. is reported mainly in young animals while in donkeys they are frequently reported also in adults. Donkeys are the permissive hosts of the lungworm *Dictyocaulus arnfieldi* whereas, in horses, it rarely develops into adults. Both species can act as reservoirs for each other, however, the intense parasite control in horses may affect donkeys that live in the same area by transmitting drug-resistance parasites. Knowing this challenge, we advocate the use of selective treatment in animals that exceed a fecal egg count (FEC) threshold, leaving a susceptible parasite population in untreated animals. In horses, a cut-off of 500 eggs has been proposed for adult mares (Coles and Molento, 2008. EIDC, Denmark) but in donkeys, there are no precise indications and a value of 300 eggs was suggested (Matthews and Burden, 2013. Equine Vet Educ, 25:461-67). Considering the dynamics of parasitic infections between horses and donkeys, parasite control programs must evaluate the differences between the two species. More research should be done to cover the lack of efficacy and safety of anthelmintic in donkeys, as well the rate of transmission of resistant parasites.

CATTLE AND BUFFALO, ONE FACE - TWO SPECIES: FROM SIMILARITY TO PARASITOLOGICAL DIVERSITY

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The large ruminants represent an important economic reality throughout the world. In Italy the cattle population amounts to more than 5 million onto 135,549 farms, while that buffalo amounts to a total of 425,018 animals from 2,580 farms (NDB at 15th January 2022). Parasitic infections of ruminants are one of the major constraints for profitable dairy industry in different areas of the world including Italy, causing considerable global economic losses as a consequence of reduced weight gain, digestive disturbance, lowered production, impaired reproductive performance, condemnation of affected organs, and mortality in infected animals (Marskole et al., 2016. Vet World, 9:1214-17). In addition, the physiology, diverse agroclimatic conditions, animal husbandry practice, and pasture management largely determine differences in the incidence and severity of various parasitic diseases in cattle (*Bos indicus* and *B. taurus*) and water buffaloes (*Bubalus bubalis*).
Regarding protozoal infections, more than 20 *Eimeria* species are described in cattle, and among them, 12 species can affect also water buffaloes although coccidia are usually host-specific parasites. *E. zuernii*, *E. bovis*, and *E. auburnensis* are the most pathogenic species in both hosts worldwide, while *E. bareillyi* is a pathogenic species specific only for water buffaloes (Dubey et al., 2018. Vet Parasitol, 256: 50–7). Despite higher seroprevalence rates of *Neospora caninum* infection in buffalo population co-habiting with cattle ones, naturally abortion events observed in buffalo appear to be less frequent, suggesting this species as more tolerant to the infection (Reichel et al., 2015. Vet Parasitol, 212:75-9).
In Italy the helminth infections have had a decreasing trend in buffalo farms up to a helminth free condition, contrary to what happens in cattle farms (unpublished data), suggesting a higher innate and/or acquired resistance in buffaloes compared to cattle as previously observed for strongyles infection (Aken et al., 2000. Vet Par, 89:133-7).
In studies conducted in southern Italy on cystic echinococcosis it has been demonstrated the absence of fertile cysts of *Echinococcus granulosus sensu stricto* in cattle examined, while they were present in water buffaloes (13%) (Rinaldi et al., 2008. Parasitol Res, 103:175-9). These differences in fertility of *Echinococcus* cysts in ruminant are very interesting and could be due to the closer proximity of water buffaloes to sheep than to cattle (Gasparrini et al., 2002. Theriogenology, 57:237–56).
Finally, also the infestations by ectoparasites are different in the two animal species; the buffalo have high infestation by *Haematopinus tuberculatus* (Bosco et al., 2018. Large Anim Rev, 24: 73-9), while the cattle have a greater presence of *Damalinia* spp. and ticks.
In conclusion, despite the morphological similarity, the two large ruminants present many parasitological differences, certainly due to the different physiology and type of breeding.

CHALLENGING THE 9 LIVES: PARASITES OF CATS AND WILDCATS

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The domestic cat (*Felis catus*) and the European wildcat (*Felis silvestris*) are two small felids that belong to the subfamily Felinae. They are genetically closely related (e.g. they can interbreed and have fertile kittens), and in some areas of Europe they may live in sympatry (e.g. rural and suburban areas) (Kitchener et al., 2017. Cat News, 11: 80). Thus, they share some similar physiological traits and pathogens. Most parasites can equally infect cats and wildcats, though recent studies have shown that there are also some differences between these two feline species, linked to their genetic diversities and different lifestyle and habitat.

Both cats and wildcats may be infected with *Dirofilaria immitis*, the “dog heartworm”, although they are less permissive hosts than dogs (Morelli et al., 2021. Clin Microbiol Rev, 34: e00266-20). Nevertheless, only wildcats are suitable hosts of another cardiovascular nematode, *Angiostrongylus chabaudi*, which is quite frequent in these animals but is not a parasite of domestic cats.

Among lungworms, *Aelurostrongylus abstrusus* and *Capillaria aerophila* can be found in both animals, while *Troglostrongylus brevior* was considered an exclusive wildcat lungworm, until recently. Now we know that it also infects domestic cats, mainly in the Mediterranean basin and in areas of cat-wildcat sympatry.

Cylicospirura spp. is a nematode of the gastric wall, found in wildcats, while it is quite rare in cats. Similarly, trematodes such as *Alaria alata* and *Opisthorchis felineus* as well as the cestodes *Taenia taeniaformis*, *Spirometra* spp. and *Diphillobothrium* spp. are more frequent in wildcats because of their lifecycle that includes common prey of wildcats as intermediate or paratenic hosts (Diakou et al., 2021. Pathogens, 10: 594).

Another difference encountered between cats and wildcats is the level of multiparasitism, which is by far higher in wildcats. Mixed cardio-pulmonary and gastrointestinal parasitosis, in combination with ectoparasites infestations (mites, ticks, fleas, hyppobosciid flies) and infections with blood (e.g. *Hepatozoon* spp., *Cytauxzoon* spp.) and eye (*Thelazia callipaeda*) parasites, often exceed a sum of 10 different parasite species per wildcat, a phenomenon not so common in domestic cats (Diakou et al., 2020. Vet Parasitol Reg Stud Rep, 19: 100357). Although domestic cats seem at lower risk of being infected with some of these parasites and less prone to co-infections than wildcats, it is important to keep in mind that land-use change, urbanization of wild habitats and the overall degradation of natural environments force wild animals to closer proximity to human activities, and consequently to domestic animals. These anthropo-ecological dynamics lead to an increased risk of bridge infections between wildcats and cats, which could bring veterinarians in front of rare parasitic diseases in domestic cats.

SITTING ON THE SAME BRANCH OF THE TREE: PARASITES IN HUMANS AND NON-HUMAN PRIMATES

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The COVID19 pandemic made clear the significance of the human-animal interface in cross-species transmission of zoonotic diseases. Within the one health concept, a multi-disciplinary approach encompassing animal, human, and environmental health is therefore needed to assess the ecology of emerging zoonotic diseases, to engage a risk assessment, and to strengthen strategies for response and control.
It has been shown that the taxonomic proximity of humans and Non-Human Primates (NHPs) make them play an important role as sentinel animals for zoonotic pathogens; while NHPs can be sources for such pathogens, also can be infected with human pathogens threatening NHPs biodiversity.
Multiple steps need to be accomplished to better understand these dynamics: an enhanced understanding of the processes involved in the organization and adaptation of the human genome, including the resistance or susceptibility to infectious diseases, need to a precise evolutionary context and phylogenetic hierarchy of primate species. On the other hand, a better resolution of the parasite's phylogeny should represent a necessary step to deal with host specificity and to comprehend parasite sharing in primates. Limitations on unambiguous parasitic species identification still represents a crucial challenge in the diagnosis, and the correct understanding of the epidemiology and transmission patterns of the diseases.
Lastly, host ecology, geographic distribution and environmental factors including anthropogenic activities represent key additional issues that influence contacts among hosts to facilitate sharing of parasitic diseases.
Different protozoa and metazoa species have been detected in humans and NHPs with possible interspecies exchanges. Understanding how parasites are transmitted across species is of great importance for conservation, wildlife and human health and in relation to the emergence and spread of novel pathogens and pandemics.
Starting from the awareness that we are sitting on the same branch of the tree, examples of different helminths and protozoa infecting NHPs and humans will be hereby discussed as well as the concerning diagnostic challenges.

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INVASIVE ARTHROPOD VECTORS AND EMERGING VECTOR-BORNE DISEASES

PAST AND PRESENT INVASIONS BY MOSQUITO VECTORS OF DISEASES

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I will discuss primarily the history of *Aedes aegypti*, the major vector of mosquito-borne viral diseases including yellow fever, dengue, Zika, and chikungunya. Our genetic studies, coupled with epidemiological records, reveal a reasonably complete understanding of this mosquito. Populations of this widespread species (throughout the tropics and subtropics) are remarkably genetically distinct such that we can identify the sources of new introductions that occur on a regular basis; examples will be given. Populations also vary considerably in their vector competence, so understanding origins of new introductions is important in assessing their threat to public health. Also, our ongoing work has revealed evidence that some populations reported as extinct (e.g., Black Sea) were in fact always present. Similarly, “new” populations (e.g., California) may have been present much before officially reported. This indicates vectors may remain cryptic for some time. Finally, comparisons to a more recent invader, *Aedes albopictus*, will be made and significant differences discussed.

OCCULT LYME BORRELIOSIS, A PUBLIC HEALTH THREAT?

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Lyme disease is probably the most common vector-borne infection in humans, in Europe and in North America. The classic clinical picture of this disease is characterized by clinical symptoms and signs that include the erythema migrans, forms of carditis, joint inflammation, and neurological disorders. However, the occurrence of the different symptoms is variable, and part of the patients are expected to develop the disease in asymptomatic or paucisymptomatic forms, with a non-pathognomonic clinical presentation. In addition, seropositivity for *Borrelia burgdorferi s.l.* in healthy subjects, as revealed in epidemiological studies, suggests that non-diagnosed infections are rather frequent, at least in some areas, in tick-exposed subjects. In summary, the fact that the tick bite is not perceived by all patients, and that part of the infections by *B. burgdorferi s.l.* are likely asymptomatic or paucisymptomatic, imply the possibility that occult forms of Lyme disease are more widespread than commonly thought (which would explain the seroprevalence values recorded in some areas). Considering the propensity of Lyme spirochetes to determine long-lasting infections, a major question is whether some forms of occult infections by these bacteria might be implicated in the onset of chronic-degenerative pathologies. Some authors have indeed discussed the idea that chronic forms of Lyme disease might represent a risk factor for the development of neuroinflammatory or neurodegenerative disorders. However, data supporting these hypotheses are not conclusive. On the other hand, data about the association between occult forms of Lyme disease and dilated myocardiopathy are more convincing. Indeed, the detection of *Borrelia burgdorferi s.l.* in the myocardial tissue of patients with dilated myocardiopathy suggests that the chronic presence of the spirochetes might cause inflammatory phenomena, associated with the development of the pathological alterations in the myocardial tissue. In conclusion, while it is reasonable to assume that many cases of Lyme disease are not diagnosed (and are therefore not treated), it is not clear whether occult infections by *B. burgodorferi s.l.* might represent a risk factor for the development of chronic-degenerative disorders. In view of the increasing exposure of humans to tick parasitism, the issue of the possible association between Lyme disease and degenerative pathologies would deserve more attention, and adequate support to intensify research in this area.

EXOTIC MOSQUITO SPECIES IN ITALY: STORY OF AN INVASION

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INTRODUCTION: Invasive mosquito species (IMS) belonging to genus *Aedes* are repeatedly recorded out of their native places. They can survive during passive transport thanks to diapausing eggs and can adapt easily to a new environment. Invasive *Aedes* species are proven or potential vectors of important arboviruses and their establishment in new areas pose a threat to human and animal health. Several invasive *Aedes* species are now established in Europe, and Italy is one of the most infested European countries. The Asian tiger mosquito, *Aedes albopictus* represents the best example of the success of colonization in Europe. It arrived in Italy in 1990 and the first established population was recognized in 1991; it is now present throughout the country and other European countries.

MATERIALS AND METHODS: Mosquito sampling was carried out in different areas of North Italy and all possible breeding sites for larval development were monitored. In addition, ovitraps, adult mosquito traps (CDC-CO2 and BG-Sentinel) and direct aspiration were used to collect eggs and adults. The mosquitoes (larvae and adults) were identified morphologically and molecularly.

RESULTS AND CONCLUSIONS: Another IMS, *Aedes koreicus* was found in 2011 in north-eastern Italy. It is spreading westwards from the original infested area and is now present in hilly and mountainous areas of six Italian Regions in north Italy. To date (2022), northern Italy is the widest area in Europe where *Ae. koreicus* is established.
Lastly, a third IMS was detected in 2015, again in north-eastern Italy: *Aedes japonicus japonicus*. Larvae were found in July 2015 in different sites in Udine province. To date, *Ae. japonicus* occurs in Friuli Venezia Giulia, Veneto, Trento Province, Lombardy and Piedmont.
The three species develop in the same breeding sites and are often found in artificial containers sharing the breeding sites with other mosquito species. *Aedes koreicus* and *Ae. japonicus* were found both in natural and urbanized areas where vegetation was present. All these IMS bite humans. *Aedes albopictus* is a proven vector of several pathogens while few studies exist regarding the vectorial role of the other two species; however, they are potential vectors of arboviruses such as chikungunya, Zika and, limited to *Ae. japonicus*, of dengue virus.
North-eastern Italy is now colonized by three IMS. Also in the past, in the same area, other invasive species, *Ae. atropalpus* (1996) and *Ae. aegypti* (1972) were recorded but they were not established.
It is interesting to note that Veneto is the region with the most frequent experience of invasive mosquito introduction in Italy. This is likely a consequence of the intensive trade of goods as well as of the intensive mosquito surveillance.
The establishment of IMS complicates the current surveillance system and requires well-trained personnel for identification. New competent vectors of pathogens may represent a challenge for the Health System.

MOSQUITO ALERT: A CITIZEN SCIENCE PROJECT FOR *AEDES INVASIVE* MOSQUITO SPECIES SURVEILLANCE

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Mosquito Alert Italia is a citizen science initiative aiming to involve citizens in monitoring of mosquito species and of human-mosquito contact, and to raise awareness on public health threats associated to mosquito vector of diseases and on individual actions useful to prevent their multiplication. The main pillar of this initiative is an application for smart-phones (Mosquito Alert, MA) created in Spain and available in Italian and other 18 languages since October 2020. The Mosquito Alert Italia task-force (including Universities, natural history museums and health institutions) is taking care of promotion and exploitation of MA in Italy, under the coordination of Medical Entomology group of Sapienza University. We here report results from the first year of the project's implementation with particular reference to: 1) actions implemented for MA promotion; 2) descriptive analysis of data obtain from October 2020 to December 2021; 3) identification of photographic mosquito reports by EntoLab Italian community thanks to a dedicated online software platforms; 4) preliminary data exploitation by mathematical and statistical models.

1) Different communication campaigns have been adopted depending on the user: a) courses for primary and secondary school teachers and lectures for university student of biology and natural history. More than 50 school teachers have been engaged to implement the MA-protocol into the next mosquito season with their alumni. University lectures in Rome Universities succeeded in engaging students in the project for long periods, as well as in collecting mosquito specimens associated to MA records. Moreover, a specific communication campaign focused in tracing mosquitoes in winter months allowed to maintain high participation (9.263 total participants of which 843 during winter season 2020-21). The education activity engaged the MA Italian users: more than one third of the total of users that downloaded the app sent at least one mosquito report. In average a user sent at least 3 reports in 1 year time span.
2) A total of 7,670 reports were received from Italy, of which 4,431 are "reports mosquito" with attached photo, 3 239 are "reports of bite". These represents 15% of MA worldwide reports, posing Italy in third most participating community after Spain, where MA was first launched in 2014.
3) The MA Italy Entolab team succeeded in validating thousands of insect photos and to provide real-time feedback to users and constantly updated of MA web map. A total of 869 photos were identified as Aedes and 651 as Culex. Moreover, an emergency mode was put in place in order to provide immediate field confirmation of unusual mosquito records, thanks to a network of medical entomologists across Italian regions.
4) Statistical models based on Bayesian analysis were carried out to predict species presence and build maps describing the probability of human-vector contact, a crucial parameters to organize the pest control interventions.

UNNOTICED ALIEN MOSQUITO SPECIES IN PRE-ALPS AREAS

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In the last decade, *Aedes koreicus* and *Aedes japonicus japonicus* mosquitoes, which are competent vectors for various arboviruses of public health relevance, colonised Italy and other European countries. Nevertheless, information about their current and potential distribution is partial. Accordingly, in this study four regions of Northern Italy (Lombardy, Liguria, Piedmont and Aosta Valley) were surveyed during 2021 for the presence of these two invasive species. We found evidence for a widespread presence of *Ae. koreicus* in pre-Alpine territories of Lombardy and Piedmont. Larvae from the invasive subspecies of *Ae. j. japonicus* were also collected in the same geographic areas. Occurrence data from this study and results from previous monitoring campaigns were used to generate a Maxent model for the prediction of habitat suitability for *Ae. koreicus* mosquitoes in Northern Italy and the rest of Europe. Peri-urban areas located in proximity to forests, pastures and vineyards were revealed as highly suitable environments for colonisation by this invasive species. Maps of the potential distribution also suggest the presence of further suitable areas in currently uncolonized countries. We conclude that this invasive mosquito species has the potential for a broad expansion at the European level in the coming decades.

PHORTICA OLDENBERGI: A POTENTIAL NEW VECTOR OF ORIENTAL EYEWORM *THELAZIA CALLIPAEDA* IN ITALY

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The oriental eyeworm *Thelazia callipaeda* (Spirurida: Thelaziidae) is an emerging zoonotic parasite that is quickly spreading in several countries of Europe, starting from its original Asiatic areal. Indeed, records of infections from this nematode have increased enormously in the past 20 years and is now widely diffused throughout Europe, being recently reported also in the United States.
According to literature, four drosophilid species of the genus *Phortica* (*Phortica variegata*, *Phortica okadai*, *Phortica magna* and *Phortica kappa*) have been identified as intermediate hosts and natural vectors of *T. callipaeda*. The transmission of the parasite occurs through the lachryphagous activity of the male flies, which transfer the L3 larvae in the conjunctiva of the mammal host (mostly dogs, cats, foxes but also humans) during their feeding activity. However, little is known about the panorama of other vectors potentially present in different countries that may contribute to the transmission of thelaziosis.
The only *Phortica* species until now described to be naturally infected in Europe by *T. callipaeda* is *P. variegata*, which is largely diffused in several countries, with common ecological requirements and population dynamics throughout the continent. However, three other species, *Phortica semivirgo*, *Phortica erinacea* and *Phortica oldenbergi*, have been seldomly detected in Europe but no information is available about their ecology, distribution and behaviour. In particular, *P. oldenbergi* was reported only once in Europe in 1977 without any other detection on the European continent since then.
During samplings conducted during past five years in an area of Central Italy (Manziana, Lazio region) we described, for the first time in this country, the stable presence of *P. oldenbergi* in sympatry with two other *Phortica* species: *P. variegata* and *P. semivirgo*. The current distribution range of the species is unknown but, given the Afrotropical distribution of the other species belonging to its subgenus (Allophortica), Africa would appear to be the original areal of *P. oldenbergi*.
The record of this species in central Italy, observed again in Europe after more than 40 years, is suggestive of an accidental, possibly recent, introduction. Moreover, *P. oldenbergi* has been recently experimentally demonstrated as intermediate host of *T. callipaeda*, indicating a potential role as a vector of the oriental eyeworm. From our preliminary studies, *P. oldenbergi* shows a mostly frugivorous behaviour. However, given the small numbers of male specimens collected, its feeding behaviour is still unclear and deserves further investigations.
The finding for the first time in Italy of a *Phortica* species of potential African origin draws attention to the possible entry routes of other alien species in our country. This also highlights the need to carry out surveys to identify potential pests or disease vectors in ecological contexts not commonly monitored for these purposes.

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PROGETTI DI RICERCA IN AMBITO NAZIONALE ED EUROPEO RIGUARDANTI LE INFEZIONI CAUSATE DA *ECHINOCOCCUS GRANULOSUS S.L.* ED *ECHINOCOCCUS MULTILOCULARIS*

EPIDEMIOLOGY OF CYSTIC AND ALVEOLAR ECHINOCOCCOSIS IN EUROPE

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Cystic and alveolar echinococcosis (CE and AE) are different diseases that require different approaches to clinical management of patients. This is also true for prevention and control, as the underlying lifecycles require fundamentally different epidemiological conditions. AE, although widespread in the Holarctic region, is caused by a single species, *Echinococcus multilocularis*, that depends on wild canids – usually foxes – and small arvicoline rodents as hosts. Its geographical spread is therefore strongly ruled by the presence and frequencies of suitable hosts and the landscapes that support them. However, the obvious association of the parasite with cooler and wetter environments suggests that other factors are involved as well. Human CE, in contrast, can be caused by at least four *Echinococcus* species with diverging animal host ranges. All four occur in Europe, where they are mainly transmitted in domestic lifecycles involving dogs and different species of livestock. Presence and frequency are thus largely ruled by human practices, e.g. livestock husbandry, slaughter supervision and dog management. All CE agents have strongly declined in Europe. Most frequent remains *E. granulosus sensu stricto*. Due to its association with sheep as intermediate hosts it retains strongholds in areas of extensive sheep farming and the ongoing practice of home slaughter - largely in rural areas of southern and southeastern Europe. The cattle-adapted *E. ortleppi*, once widespread across Europe, seems to have disappeared from many countries, but is still sporadically reported across the continent. The pig-adapted *E. canadensis* (G6/7), genetically highly diverse, persists in domestic transmission in various parts of eastern and southern Europe where domestic and semi-feral pigs are kept under smallholder conditions with home slaughter; in parts of its range, goats could also be important hosts. The fourth species, *E. equinus*, whose pathogenicity for humans was only recently recognized, remains the most data deficient, but sporadic reports from horses and donkeys across Europe suggest widely spread persistence of transmission at low level. Although domestic dogs and livestock are still the main hosts for all CE agents in Europe, an increasing number of reports of wildlife infection e.g. in wolves highlights the potential of these parasites to escape from the controllable domestic environment into sylvatic transmission cycles, a situation that needs being monitored.

ECHINO-SAFE-MED: AN INTERNATIONAL PROJECT TO CONTROL CYSTC ECHINOCOCCOSIS IN THE MEDITERRANEAN AREA

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ECHINO-SAFE-MED (New sustainable tools and innovative actions to control cystic ECHINOcoccosis in sheep farms in the MEDiterranean area: improvement of diagnosis and SAFEty in response to climatic changes) is a three-year project started in May 2021 to control cystic echinococcosis (CE) in the Mediterranean area. The main aim of the project is to implement the pasture-based livestock farming systems by delivering sustainable and cost-effective tools, as well as innovative strategies to control CE in sheep farms with the final goal to improve the health, welfare, and productivity of the small ruminant livestock sector in the Mediterranean regions. This will be obtained using high throughput diagnostic, surveillance and control strategies in order to establish guidelines for sustainable CE control to be further extended to other endemic Mediterranean areas.
With this as the main tenet, ECHINO-SAFE-MED has 3 macro-objectives as summarized below:

- To develop novel diagnostic tools for early detection of cystic echinococcosis in sheep in Mediterranean countries of Europe (i.e. France, Greece and Italy) and transfer these methodologies to the Mediterranean areas in North Africa (i.e. Algeria and Tunisia).
- To improve surveillance and control activities for CE in Mediterranean areas using innovative sustainable strategies to be applied in highly endemic areas.
- To strengthen capacity for CE diagnosis, surveillance and control in both Africa and Europe through training & effective communication of project outcomes to project partners and relevant stakeholders, policy-makers and end users.

The objectives of this project will be achieved through the construction of an international network for sharing practices, methods and data to promote in a concerted and organized way, efficient approaches to help animals and farming systems to adapt to climate change. Furthermore, a multi-level approach will be adopted, involving local participating vets and national sheep farmer's organizations to collect information on common practices per country/region and assess the farmers' attitude towards sustainable helminth control and their potential adoption for novel diagnostics and novel concepts of CE control.

Funding source: ECHINO-SAFE-MED is supported by funding from the Partnership on Research and Innovation in the Mediterranean Area (PRIMA) – Call 2020 Farming System.

MEME AND PERITAS INTERNATIONAL RESEARCH PROJECTS ON CYSTIC AND ALVEOLAR ECHINOCOCCOSIS

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INTRODUCTION: MEME is an international multicentre collaborative project which aims to fill research gaps highlighted by international agencies for the detection and control of zoonotic parasites *Echinococcus multilocularis* (*Em*) and *Echinococcus granulosus sensu lato* (*Eg*).
PERITAS is an international collaborative project which aims, to elucidate the pathways of transmission of *Eg* which are poorly understood and have never been systematically investigated.

MATERIALS AND METHODS: MEME focuses on standardization validation of existing parasitological and molecular methods, and the development and comparative assessment of innovative molecular tools to detect *Em* and *Eg* in the food chain. Production of epidemiological data on the presence of *Em/Eg* eggs in the food chain will focus on vegetables for human consumption and on canine faeces in selected endemic countries.
PERITAS is designed as a two subsequent stages project conducted in selected areas of Argentina, Chile and Peru. Stage 1 is a Cross-sectional ultrasound-based prevalence study for the identification of high endemic clusters with active cyst stages of human CE where the subsequent case-control study was implemented. Stage 2 is a village-based case-control study in positive households, negative households (controls) and village common areas where sampling of environmental matrices was conducted for the molecular identification of *Eg* and other helminths.

RESULTS: MEME results on detection methods have already been published: i) Comparison of two DNA extraction methods and two PCRs for the detection of AE in stool samples; ii) Bayesian Analysis of three methods for diagnosis of CE in sheep; iii) Microsatellite investigations of *Eg* cysts; iv) Species detection of *Eg* by novel probe-based real-time PCRs; v) Validated method based on PCR-RFLP and multiplex PCR assay for the identification of *Eg* species; vi) Identification of *Eg* G1/G3 by SNPs assays. MEME is now analysing biological samples for producing epidemiological evidence on the presence of these parasites in different matrices.
Within PERITAS, 2,439 people were screened in the regions of Coquimbo (Chile), 581 in Rio Negro (Argentina), and 790 in Junin (Peru). Prevalence of human CE was 1.6%, 5% and 5%, respectively. Despite COVID-19 pandemic, sampling of matrices were successfully conducted in 2020-2021. The analysis of the data will allow the identification of matrices contaminated by *Eg* eggs and of at-risk behaviours/habits associated with odds of CE infection.

CONCLUSION: MEME and PERITAS will provide a comprehensive set of integrative activities to harmonize procedures, improve the detection and produce epidemiological data on potential pathways of transmission of *Em* and *Eg*.

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ECOLOGY AND SURVEILLANCE OF *ECHINOCOCCUS MULTILOCULARIS* AT THE SOUTHERN BORDER OF ITS DISTRIBUTION IN EUROPE

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The Alps represent the southern border of the European distribution of *Echinococcus multilocularis*. This small tapeworm, affecting wild carnivores and voles in a typical prey-predator life cycle, is the agent of a severe zoonosis, the alveolar echinococcosis. For this reason, in the European Union (EU), it is mandatory to report its detection to national authorities, as well as its surveillance, prevention and control are closely regulated for pets, livestock and wildlife. Collection of relevant metadata is also highly recommended by both the Directive EU 2003/99/EC (Monitoring of zoonoses and zoonotic agents) and the Regulation EU 2016/429 (Animal Health Law).
Here we report and discuss:

- the results of seven years (2012-2018) of monitoring on *E. multilocularis* in its main definitive reservoir, the red fox (*Vulpes vulpes*), in Northeastern Italian Alps (Triveneto), describing on a large scale the changes occurred over time and among areas showing different environmental and ecological features, so highlighting variations among different eco-regions and trends in prevalence across the study years. All *E. multilocularis* isolates consistently came from Alto Adige, where it had been found since 1997, interestingly showing an apparent increasing trend across the last few years (Citterio et al., 2021. Parasit Vectors, 14:29);
- a reassessment of the prevalence of *E. multilocularis* in foxes of the Alto Adige focus. In particular, the performances of different diagnostic tests on faeces (a coproscopy-multiplex PCR method vs a real-time quantitative PCR) have been evaluated across two years (2019-2020), by comparison to an intestinal scraping technique as the reference standard. Such an evaluation allowed to calculate a true prevalence of 14.3% in foxes from Alto Adige, markedly higher than reported in 2012-2018 (consistently <5%). In addition, qPCR showed a much higher sensitivity compared to coproscopy-multiplex PCR (21%), with a far higher agreement with the reference standard (0.816 vs 0.298), meaning that a smaller sample size would be required to detect the disease by qPCR (Obber et al., 2022. PLoS ONE, 17(5): e0268045).

Surveillance of *E. multilocularis* at the edge of its range is hindered by fragmented distributional patterns and low prevalence in definitive hosts. Based on our results, we recommend to apply molecular diagnostic tools directly to host faeces sampled from small geographic areas, in order to increase the probability of detection and to provide more accurate information to estimate the exposure risk for humans.

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A ONE HEALTH APPROACH TO CYSTIC ECHINOCOCCOSIS CONTROL IN A HYPO-ENDEMIC AREA IN ITALY

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Cystic echinococcosis (CE) is considered hyper-endemic or endemic in many parts of Central, Southern and insular Italy, whereas it's sporadically found in northern Italy. However, it has recently gained attention in North Italy, due to the recent demonstration of the autochthonous circulation of the parasite in animal populations. An integrated model, based on a One Health approach, was implemented for the estimation of the epidemiological and economic outcomes of CE and the costs for its prevention in Veneto region.
The model was populated with data retrieved from published literature, official statistics, or actively searched through data mining (i.e., Hospital and slaughterhouse data). When fundamental data were not available, expert opinions were collected through a transparent and interactive process.
The overall impact of CE in the study area was estimated in a yearly cost of about 0.5 million €, due to an average of 19.5 human hospitalized cases and about 200 infected animals among cattle and sheep, per year. The loss of productivity for the human cases accounted for the most of the costs of the human component, which were estimated to be eightfold higher than the animal component. Most of the infected animals were autochthonous, while the identification of an autochthonous source of the infection for the human cases was extremely difficult, and unlikely in most cases. No specific action resulted to be in place for human surveillance, while veterinary surveillance accounted for a yearly cost of about 22,000 €. Sheepherders were found to pay privately an overall amount of around 2,000 € for the preventive treatment of their dogs every year, but the applied protocol proved to be sub-optimal.
The source of most of the human cases was likely external to the study area, and their economic impact accounts for a cost that is far exceeding that of surveillance and preventive actions in place in the veterinary sector. Although autochthonous human cases appeared to be very rare at present, the strengthening of preventive actions and surveillance systems can reduce the risk of their increment.

FIGHT THE PARASITE, AN EDUTAINMENT PROJECT FOR CYSTIC ECHINOCOCCOSIS (CE) AWARENESS CAMPAIGN IN PRIMARY SCHOOLS

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INTRODUCTION: Cystic echinococcosis (CE) is a zoonotic parasitic disease caused by the species complex *Echinococcus granulosus sensu lato* (*s.l.*); CE represents a public health challenge and a socio–economic issue worldwide. Despite the long-term application of prevention and control measures that are primarily targeted to dog deworming, health education (He) and meat inspection, human CE remains a serious neglected zoonotic disease in many resource-poor pastoral regions (Craig et al., 2017. Adv Parasitol, 96: 55-158). Successful CE eradication campaigns in other regions of the world have included He as a core component. Additional public engagement (PE) measures, including novel health educational tools, are required for more sustained integrated control of CE. Fight the parasite is a One Health PE pilot project, carried out by research groups from Cambridge, UK, and Sassari, Italy, involving primary schools of Sardinia (Italy), considered endemic region for CE. The aims of this project are to develop edutainment engaging resources explaining the cause of CE, routes of transmission and best control practices, assess current understanding of Sardinian schoolchildren regarding CE; deliver an interactive parasite awareness program using the developed interactive resources and evaluate the understanding of children involved.

MATERIALS AND METHODS: The study was conducted from March 2022 and May 2022, in Grade 6-10 schoolchildren (tot. 1,032) of 13 primary schools of the Municipalities of Sassari, Alghero, Bono, Benetutti, Nule, Bitti, Orune, Lula, Sorgono, Tortolì, Lotzorai, Girasole, Cagliari. The program involved the design, implementation and evaluation of a participatory awareness program on CE and PE activities. The schoolchildren's previous knowledge regarding CE risks factors, were assessed in questionnaires pre- and post-intervention. The educational digital tool to prevent CE included a comic multilingual booklet with activities and games, a cartoon video (https://youtu.be/XTf3fTtmfA8), a teacher's guidebook, participatory lessons and PE hands on activities. Results and conclusions The Fight the parasite project represents a tool of health communication that can readily be applied to a range of He activities. Authors assert that this strategy represents a solid complementary approach to existing CE control measures in Sardinia considering that was carried out in one of the areas with the highest incidence of CE (AIh11.9/105 inhabitants) (Brundu et al., 2014. Acta Trop, 140:91-6). In the seminar, more detailed results will be delivered from the ongoing He campaign.

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DEFINITION OF ENDEMIC AREA FOR CYSTIC ECHINOCOCCOSIS: THE CASE OF SARDINIA

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For several years, the Reference Center for Echinococcosis (CeNRE) has made considerable efforts to know the epidemiology of echinococcosis in Italy. The data obtained so far focused on the occurrence of Cystic Echinococcosis (CE) in humans (Brundu et al., 2014. Acta Trop, 140: 91-6). By using the Hospital Discharge Records provided by the Ministry of Health, related to the hospitalizations for CE from 2001 to 2015, the economic burden of hospitalization and treatment costs of CE and the Disability Adjusted Life Years have been estimated (Piseddu et al., 2017. PLoS Negl Trop Dis, 11: e0005771). Focusing on Sardinia, out of 2,475 hospitalizations, 1,559 were patients resident in Sardinia, with an average annual rate of 9.5 hospitalizations concerning 6.5 patients/100,000 inhab, compared to a national average of 1.4/100,000 inhab.
Surveys on the prevalence of *Echinococcus granulosus* complex (*EG*) in Italian sheep farms from 2010 to 2015 have been estimated with Regional Veterinary Epidemiology Observatories. A Bayesian simulation model based on the evaluation of the CE prevalence, by region and province, was also developed (Loi et al., 2019. PLoS One, 14: e0214224). The focus on Sardinian data highlighted that 19% of the sheep farms that moved animals to the slaughterhouse were positive (95% CI: 18.82-20.02). Otherwise, considering that not all Sardinian farms move heads to slaughterhouses, these results lead to an underestimation of the real burden of the disease (Loi et al., 2019. PLoS One, 14: e0214224).
To investigate the prevalence of CE in Italian sheep farms, the IZSSA RC01/19 project promoted a public health strategy based on the active surveillance for controlling the outbreak of *EG* in ovine, by increasing the detection of CE positive cases at the time of slaughtering. The results, obtained with the support of the Locals Public Health Veterinary and Food Safety Services of the Department of Health, confirmed the disease trend within each province. The hypothesis of underestimation (less than half) of the CE prevalence at the slaughterhouse was confirmed.
In a recent study (Serra et al., 2022. Vet Sci, 9: 143) concerning the diagnostic methods and operating protocols for controlling the CE spread in environmental contamination by *EG* eggs in 30 educational farms (EF) located in a high-risk area, no statistical differences between EF tested positive or negative were obtained. Otherwise, positive samples were collected in areas previously defined as endemic.
To date, the data collected in Sardinia and related to humans, dogs, and sheep, have allowed us to obtain epidemiological considerations about the definition of endemic area. For CE, a neglected tropical disease, the definition of an endemic situation strictly depends on the accuracy of surveillance systems and a robust method for prevalence estimation in the targeted populations. The obtained results have confirmed the persistent presence of the disease in the study area, which can be defined endemic area for CE.

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CLINICAL FEATURES OF FELINE LUNGWORMS AND THE USE OF EMODEPSIDE

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Aelurostrongylus abstrusus and *Troglostrongylus brevior* are the most important feline respiratory nematodes. Aelurostrongylosis causes upper and lower respiratory tract signs, e.g. sneezing, cough, dyspnea, tachypnea, abdominal/open-mouth breathing. Heavy infections in young animals and cats with other conditions can lead to pneumothorax, pulmonary hypertension, respiratory failure and death. Clinical features of troglostrongylosis are similar, though the infection is rare in adult cats and more frequent and life-threatening in kittens and young cats. In fact, *T. brevior* can be vertically transmitted from the queen to the litter, may obstruct bronchi and bronchioles, and cause lung hemorrhages, congestion and edema, leading to fatal clinical signs.

In the past years, studies proved the efficacy of emodepside for treating parasitologically and clinically cats infected by *A. abstrusus* and *T. brevior*.

Experimental and field studies proved that 1 or 2 administrations, 2 weeks apart, of a spot-on containing emodepside/praziquantel are highly effective in the treatment of aelurostrongylosis, based on parasitological and clinical cure of infected cats (Traversa et al., 2008. Parasitol Res, 105 Suppl 1: S83-89; Bohm et al., 2015. Parasitol Res, 114 Suppl 1: S155-64; Crisi et al., 2020. Vet Rec, 187: e34). Topical products containing emodepside are also efficacious in the parasitological and clinical treatment of cats infected with *T. brevior*, even when they are co-infected with other lungworms (Di Cesare et al., 2015. J Feline Med Surg, 17:181-85; Traversa et al., 2018. J Parasitol, 104: 418-23). Recent trials showed that a spot-on formulation containing emodepside (+ praziquantel) is highly effective after 1 or 2 administrations, 2 weeks apart, in natural (reduction of larval shedding) and experimental (reduction of adult worm burden) conditions (Traversa et al., 2019. Parasit Vectors, 12: 97; https://www.ema.europa.eu/en/documents/assessment-report/felpreva-epar-public-assessment-report_en.pdf). Also, most naturally infected cats with evident clinical signs fully recover after the 1 administration of this product (Traversa et al., 2019. Parasit Vectors, 12:97). A novel spot-on formulation containing emodepside (+ praziquantel and tigolaner) proved to be highly efficacious in treating *T. brevior* infection, assuring 100% elimination of adults and L1 in experimentally and naturally infected cats, respectively (https://www.ema.europa.eu/en/documents/assessment-report/felpreva-epar-public-assessment-report_en.pdf; unpublished data). This efficacy was proved with 1 administration of the novel formulation followed, 2 weeks after, by a 2nd administration of the spot-on containing emodepside + praziquantel.

In conclusion, emodepside is a molecule with high efficacy and safety for treating cats infected by feline lungworms. Therefore, there is a high merit to evaluate its efficacy also for the prevention of aelurostrongylosis and troglostrongylosis.

EFFICACY OF FELPREVA® - A NOVEL TRIPLE COMBINATION OF TIGOLANER (97.90 MG/ML), EMODEPSIDE (20.35 MG/ML) AND PRAZIQUANTEL (81.40 MG/ML) FOR PARASITE CONTROL IN CATS

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Ecto – and endoparasites play a significant role in many feline patients.
Major concerns of parasite exposure to cats are the potential of causing disease in cats but also their zoonotic potential to cause disease in humans.
Felpreva® is a novel spot-on solution for cats and was developed as a broad-spectrum endectocide specifically for cats, containing tigolaner (97.90 mg/mL), emodepside (20.35 mg/mL) and praziquantel (81.40 mg/mL).
Tigolaner is a novel acaricide and insecticide and acting as a potent inhibitor of the neurotransmitter gamma-aminobutyric acid (GABA). The receptor inhibition disrupt's the function of the parasites nervous system leading to death. It is not an isoxazoline but belongs to the chemical class of bispyrazoles.
Field and laboratory studies have been conducted to confirm the safety and efficacy of Felpreva® in the treatment and prevention of ectoparasite infections in cats.
A laboratory study evaluated the speed of flea kill of tigolaner, being the novel insecticidal compound, in cats experimentally infested with fleas (*Ctenocephalides (C.) felis*). For this, the onset of efficacy against existing and following reinfestations was determined.
A first field study has evaluated the safety and three-month preventive efficacy of Felpreva®, a novel spot-on endectocide containing tigolaner (97.90 mg/mL), emodepside (20.35 mg/mL) and praziquantel (81.40 mg/mL). It was administered to privately owned cats infested by fleas (*C. felis*) and/or ticks (*Ixodes ricinus*, *Ixodes hexagonus*, *Rhipicephalus* spp.). The efficacy of Felpreva® to reduce the clinical signs associated with flea allergy dermatitis was also evaluated.
Second, a multicenter, multi-regional field study was conducted in two European countries and confirmed the safety and high efficacy of the triple combination against natural infections with *Otodectes cynotis* in cats. The cats included in the study were either once treated with the Felpreva® spot-on solution containing tigolaner/emodepside/praziquantel or a control product with selamectin/sarolaner.
Additionally, a field study with client-owned cats was conducted for safety and efficacy evaluation against natural infections with *Notoedres cati* (feline scabies). Here, cats of the study treatment group received the Felpreva® spot-on solution (Tigolaner/Emodepside/Praziquantel), whereas cats of the study control group were treated with a placebo.
In this Key-Note, efficacy data from studies of Felpreva® on fleas, ticks and mites will be presented. Studies have demonstrated that a single dose of the novel triple combination of Tigolaner 97.90 mg/mL, Emodepside 20.35 mg/mL and Praziquantel 81.40 mg/mL (Felpreva®) is highly efficacious and safe in the treatment of existing flea and tick infestations in cats and prevents for up to three months. Furthermore, the high efficacy of the novel triple combination in the treatment of head and ear mites has been demonstrated.

https://www.ema.europa.eu/en/medicines/veterinary/EPAR/felpreva

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AQUATIC ANIMAL PARASITES IN THE ANTHROPOCENE ERA

EMERGING AND RE-EMERGING PARASITES IN MEDITERRANEAN MARINE PISCICULTURE

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In the last 25 years, marine fish aquaculture production in the Western Mediterranean has grown steadily and significantly, mainly based on cultivation of European sea bass, gilthead sea bream, and turbot. In addition, notable research and industrial efforts have been made towards production diversification with strategically interesting species, achieving an already consolidated and growing production of species such as Senegalese sole, lemon fish, meagre and assorted sparids. During this time, the quest to increase production yields and the exploration of different production models has evidenced the potential of several parasitic infections to reach epizootic level and compromise the viability of numerous aquaculture facilities. Many of these episodes were caused by species of parasites unknown until their emergence in the industry, as catastrophic events in some cases. However, the subsequent study and characterization of these parasitosis has shown that, in many cases, they are ubiquitous parasites or new species from well-known problematic parasitic taxa, and their emergence is associated with certain production models and circumstances which in some cases are avoidable.

Once certain level of maturity of production models for a given fish species have been reached, coexisting with parasites is the norm, and particularly so in open systems without possible control of environmental parameters and water sources. In this context, several ecto- and endoparasites are responsible of constant and significant drains in production yields, which often run unnoticed. Together with increased operation costs and a potential to generate epizootic clinical episodes, the pressure of parasitic diseases on aquaculture facilities needs to be faced with a higher effort for their prevention and control.

This presentation overviews some of the most relevant parasitic infections in fish species and culture systems in Spain and Western Mediterranean countries, and highlights the complications generated by some of them in specific culture systems and real-world scenarios involving anthropogenic effects. The main knowledge gaps and research needs to mitigate the impact of these infections will be pointed out.

AQUATIC ANIMAL PARASITES IN THE ANTHROPOCENE ERA - HOW ENVIRONMENTAL FACTORS MODULATE PARASITE ECOLOGY

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Despite the progress we have made in recent years regarding both the ecological importance and the ecological implications of aquatic parasites, we are still far from fully understanding the interactions between different environmental conditions and the occurrence and impact of parasites. Given the fundamental ecological changes taking place on a global scale, an integrative approach is therefore needed to understand the ecological implications of aquatic parasites. Parasites can respond to a stressful environment in different ways, resulting in increased or decreased abundance depending on the nature and intensity of the stressors and the complexity of their life cycles. For example, heteroxenic parasites may suffer if the intensity of stressors in the environment increase while monoxenic parasites may even benefit from a more stressful environment. Additionally, there is also an increasing number of papers showing how parasitism and pollution can interact with each other and how this might affect their hosts. In addition to synergistic negative effects of both stressors, there is also evidence of antagonistic interactions. The latter are related to the reduction of pollutant levels in infected hosts compared with uninfected conspecifics. As reduced contaminant concentrations are usually correlated with less adverse effects, it might even be advantageous to harbour parasites if hosts are confronted with environmental pollution. On the other hand, possible pathological effects might reduce a potentially beneficial effect of parasites. There are also examples, however, which show that parasites may enhance toxic effects of pollutants by interfering with the host's protection mechanisms. In these cases, parasites would have exclusively negative effects on the physiological homeostasis of their hosts. In the present talk selected key issues are highlighted that illustrate the current knowledge in the field of environmental parasitology and the implications of aquatic parasites within food-webs by using selected examples from aquatic ecosystems.

FAST-CHANGING ENVIRONMENT AND SLOW-MOVING HOSTS: THE CASE OF SEA TURTLES AND PARASITIC DISEASES IN THE MEDITERRANEAN SEA

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The extant species of sea turtles (ST) have witnessed several large-scale climatic and sea level changes throughout millions of years, persisting since the Jurassic, facing periods of ocean warming similar in magnitude to that expected in the next 100 years. Apparently, ST have successfully coped with climatic changes but the speed rate of the actual environmental modifications challenges their ability to adapt to the new conditions. Studies on the future dynamics of infectious diseases are still in their early beginning, but no clear increasing or decreasing trend in disease reports was observed in the last two decades (Tracy et al., 2019. Proc R Soc B, 286: 20191718). Most of the recent literature focuses on a newly emerging fungal disease, the “sea turtle egg fusariosis” (STEF). Fungi of the *Fusarium solani* species complex have been isolated from egg clutches with up to 100% mortality worldwide (Sarmiento-Ramirez et al., 2014. PLoS ONE, 9(1)). The saprotroph fungi generate infection when humidity levels in the egg chamber rise, i.e. in intertidal zones or after nests flooding, firstly colonizing non-viable eggs, secondarily spreading to the adjacent eggs, leading to necrotic lesions and embryo death (Sarmiento-Ramirez et al., 2010. FEMS Microbiol Lett, 312). An increasing incidence of STEF is expected due to frequent extreme storming events and increased tidal wash, and is therefore addressed as an important threat to species conservation.

One coping strategy with increasing global temperature for ST is the modification of migration pathways and relocation of nests. Nesting activity on the Western Mediterranean coasts witnesses with any probability the third colonization event of Mediterranean Sea by Atlantic *Caretta caretta* since Pleistocene (Carreras et al., 2018. Sci Rep, 8:1435). In this scenario, the increasing rate of contact between the two ST populations will necessarily affect the dynamics of transmissible diseases, including the impactful infection by cardiovascular flukes (Digenea: Spirorchiidae), which is highly prevalent in Western Atlantic loggerhead turtles and sustained by high parasitic diversity. Genetic typing of the two Spirorchiidae isolates from Mediterranean ST demonstrated their high homology with the Atlantic ones (Marchiori et al., 2017. Parasit vectors, 10:467). Health surveillance should be put in place on Mediterranean turtles to unveil infections by new spirorchiid species, included the neurotropic *Neospirorchis* genotypes.

The poor knowledge of the life cycles of most parasites of ST prevents us to predict how global warming will finally affect the ecology of most of them. Though we can speculate that generalist parasites such as the nematode *Sulcascaris sulcata* will be less affected by food web alterations (Cizauskas et al., 2016. R Soc open sci, 4: 160535), identification of specific intermediate hosts for many other taxa will help us to unveil how global changes will affect parasites distribution in ST populations.

MOLECULAR SYSTEMATICS AND ECOLOGICAL ASPECTS OF TRYPANORHYNCH TAPEWORMS IN THE MEDITERRANEAN SEA

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Trypanorhynch tapeworms (Platyhelminthes: Cestoda) are among the most diverse and abundant metazoan parasites of elasmobranchs with more than 270 recognized species (Palm et al., 2009. Mol Phylogenet Evol, 52: 351-67). Their life cycle is complex and involved various organisms at different levels of trophic webs of the marine ecosystem. Adults live in the gastrointestinal tract of sharks and rays, while their larval stages infect a wide range of marine invertebrates and teleosts (Campbell and Beveridge, 1994. Order Trypanorhyncha Diesing, 1863, Jones and Bray, Wallingford, 51-148). Trypanorhynch tapeworms are widely distributed; however, to date, the highest species diversity is reported in coastal tropical-equatorial waters of the Indo-Australian region (Palm et al., 2009. Mol Phylogenet Evol, 52: 351-67). Published literature on the distribution of Trypanorhyncha in other seas of the world, including the Mediterranean Sea is scarce. Scattered records of larval stages of trypanorhynchs in Mediterranean fish have been so far reported. In this frame, the trypanorhynchs biodiversity so far recorded in the Mediterranean Sea by using an integrative taxonomic approach will be presented, with a focus on the ecological aspects. Their potential use as biological and ecological indicators and their implications for the quality and safety of fisheries products will be also discussed.

PARASITE DIVERSITY IN CEPHALOPODS

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Members of the class Cephalopoda are of great ecological and economic importance: many species are relevant to commercial fisheries, to the point that several stocks worldwide are currently overexploited; some species are considered excellent candidates for aquaculture due to their fast growth and high market value, while others represent important experimental models for biomedical research. Cephalopods inhabit a variety of marine habitats, displaying great differences in morphological traits, sociability and ecological niches; as a consequence of such biological and ecological variability, they host a wide range of protozoan and metazoan parasites. By playing an essential role in marine food webs, cephalopods represent important “bridges” for trophically transmitted parasites, allowing them to be maintained in the aquatic ecosystem.

The effects of human activities on cephalopod parasites have begun to be assessed only in the recent past, however such efforts are hindered by the scarcity of historical quantitative data for most groups of parasites and the lack of parasitological information for the majority of cephalopod species known to science.

Parasitological data for selected groups of parasites (e.g. Anisakid nematodes, Trypanorhynch cestodes) have proven useful for the discrimination and management of wild cephalopod stocks; in fact, parasites can be used as biological tags for specific and intraspecific characterization of their cephalopod hosts, and to determine interspecific trophic and ecological relationships.

Moreover, the maintenance of cephalopods in captivity for commercial and research purposes, and their addition to the list of animals regulated for use in scientific procedures within the European Union (Directive 2010/63/EU), generated a need to better understand their parasites and other transmissible agents, for the prevention and early diagnosis of disease outbreaks. Infections with Apicomplexans of the genus *Aggregata* are among the most important parasitic diseases in captive cephalopods, and have been associated with malabsorption syndrome and mortality in farmed octopuses. Furthermore, the disease can severely weaken the affected animals, making them more susceptible to other biotic and abiotic stressors.

A better understanding of cephalopod parasites and diseases in is therefore of critical importance, not only to optimize the welfare of captive animals and their aquaculture production, but also to understand whether human activities can impact parasitic infections in wild cephalopod populations, potentially threatening their successful management and conservation.

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IN-NTD:
IL NETWORK ITALIANO PER LE NTD

AN ITALIAN NETWORK FOR THE NEGLECTED TROPICAL DISEASES (NTDS)

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Negletcted tropical diseases account for a 1.7 billion people still affected in low income countries, but not only. Most of them are communicable, being bacterial, viral, mycotic but over all parasitic in origin. Very recently a non-communicable disease was added to the list, i.e. that caused by snake venoms. In the wake of the launch of the new roadmap 2021-2030 for the control of NTDs by the World Health Organization on January 28th 2021 it was organised a group of scientific Societies (Società Italiana di Parassitologia-SoIPa, Società Italiana di Medicina Tropicale e Salute globale-Simet, Società Italiana di Malattie Infettive e Tropicali-SIMIT), the Italian network for malaria, foundations (Fondazione Ivo de Carneri onlus), University (Corso Mach-Università di Milano), institutions (Istituto Superiore di Sanità and Istituto Farmacologico Mario Negri) no-profit associations (Associazione Italiana amici di Raoul Follereau-AIFO, Sightsavers onlus) and the Public Health Laboratory I. de Carneri situated in Pemba, Tanzania.

Networks focused on NTDs are present in several Countries like Australia (https://www.leprosymission.org.au/leprosy/neglected-tropical-diseases/), Germany (https://dntds.de/the-network.html), France (https://twitter.com/ntdsnetwork_fr), Canada (https://cnntd.org/), United Kingdom (https://unitingtocombatntds.org/), U.S.A. (https://www.ntd-ngonetwork.org/ -and https://www.cor-ntd.org/) and Africa (https://arntd.org/).

After the first approach aimed to join the efforts to sustain the new roadmap it was decided to establish an Italian network for NTDs to which during the time other institutions such as the I.R.C.C.S. Don Calabria Hospital in Negrar (Vr), the I.N.M.I. L. Spallanzani in Rome and the UNESCO Chair in the University of Brescia (Prof. Castelli) have joined.

What is the purpose of the network? The Italian Network for NTDs (Italian Network on NTDs, IN-NTD) proposes itself as a network that facilitates interactions between the various Institutions in Italy that deal with NTDs, both nationally and internationally. The network does not want to set up a new scientific society, to which individual scholars / researchers can belong. The purpose of the network is to coordinate the work of many to achieve the synergy and authority necessary to propose and organize interventions useful to combat NTDs in our country and in countries where NTDs are endemic.

The network has been disseminated in different national Congresses (SoIPa, SIMIT and SIMET) and a two page document has been realised.

All requests of information can be sent to: IN_NTD@iss.it

THE WORLD HEALTH ORGANIZATION AND THE NEGLECTED TROPICAL DISEASES

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The primary role of the World Health Organization is to direct international health within the United Nations' system.

WHO's Department of Control of Neglected Tropical Disease was established in 2005 with the aim of leading the global response against "neglected tropical diseases" (NTDs), a diverse group of conditions caused by parasites, bacteria, viruses, fungi and toxins whose burden mainly lies among disadvantaged communities in tropical countries.

WHO's work on NTDs is focused along three main axes:
- Normative functions
- Technical support
- Advocacy and partners coordination

WHO's action on NTDs is guided by high-level directives and policies:
- NTDs are included in Sustainable Development Goal 3 (the "health SDG"), notably in Target 3.3 ("By 2030, end the epidemics of […] neglected tropical diseases […])
- NTDs are mentioned in the "Political declaration of the high-level meeting on universal health coverage" (A/RES/74/2, adopted by the 74th session of the United Nations General Assembly (UNGA74) in 2019)
- Several disease-specific or cross-cutting World Health Assembly resolutions and decisions

WHO's first road map on NTDs was adopted in 2012. It proposed a set of targets to be achieved by 2020. Member States expressed commitment towards the implementation of the road map through WHA resolution 66.12 (2013), while the global partners community did so through the London declaration on neglected tropical diseases.
The first road map included 17 diseases. Responding to requests from Member States, in 2016 WHO established a process to consider addition of new conditions to the NTD portfolio.
The current (second) road map 2021-2030 was endorsed by the World Health Assembly through decision WHA73(3) and launched in January 2021. It encompasses 20 diseases and proposes a set of overarching, cross-cutting and disease-specific targets meant to guide global efforts towards eradication, elimination of transmission, elimination as a public health problem and control of NTDs.
The global partners community has reaffirmed their support to the fight against NTDs through the Kigali declaration (2022).

Over the past years, progress in the fight against neglected tropical diseases has been significant:
- For 5 consecutive years (2015-2019), over one billion people have been treated for at least one NTD; in 2020, because of COVID-19 disruptions, this number fell to 761.3 million
- As of May 2022, 45 countries have eliminated at least one NTD

A renewed effort is required to meet the 2030 targets set by the road map. In line with the consultative approach followed to develop the road map, WHO also envisions a concerted approach for its implementation. Partners, public and private donors, academia, as well as networks such as the Italian Network for NTDs are called to play an important role and contribute to moving forward the NTD agenda at national and international levels.

THE ACTIVITIES OF THE WHO COLLABORATING CENTERS FOR NTDS IN ITALY

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WHO collaborating centres (WHOCCs) are institutions such as research departments/laboratories of universities/academies/institutions, which are designated by WHO to carry out activities in support of the World Health Organization's programmes. WHOCCs serve WHO and the Member States as sources of information, services and expertise, as well as capacity for training, research and collaboration. The designation of a WHOCC both recognizes a history of collaboration with WHO and provides a formal framework for future joint activities. It is a time-limited agreement of collaboration between WHO and the designated institution, through which the latter agrees to implement a series of concrete activities specifically developed or designed with WHO in close collaboration with the WHOCC Responsible Officers.
Currently, there are over 800 WHO collaborating centres in over 80 Member States working with WHO (https://www.who.int/about/collaboration/collaborating-centres) on different areas of health issues including Neglected Tropical Diseases (NTDs). Italy accounts for nearly 30 collaborating centers on different topics and the four listed below are dedicated to parasitic NTDs:

- WHOCC on Strongyloidiasis and other Neglected Tropical Diseases (ITA-102), Ospedale Sacro Cuore Don Calabria, Negrar, Verona
- WHOCC for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis (in humans and animals) (ITA-107), Istituto Superiore di Sanità, Rome
- WHOCC for Clinical Management of Cystic Echinococcosis (ITA-115), IRCCS S. Matteo Hospital Foundation, Pavia
- WHOCC for Diagnosis of Intestinal Helminths and Protozoa (ITA-116), University of Napoli Federico II, CREMOPAR, Napoli

The activities and the main achievements of the WHOCCs dedicated to NTDs in Italy will be presented in relation to their terms of reference (TOR) aiming at promoting collaboration among the centers and the Italian Network on NTDs.

THE RELEVANCE OF ZOONOTIC TRANSMISSION IN STHS

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Geohelminthiases are the most widespread parasitic diseases in the world, accounting for over 1.5 billion infections, caused by soil transmitted helminths (STHs). The incidence of STHs is particularly high in tropical and sub-tropical conditions where poverty and low hygiene conditions may favour their persistence. Many parasitic nematode species are co-endemic and mixed infections are frequently observed in a single individual, due to similarities in the transmission routes, environmental, socio-economic conditions and geographical distribution. Here, several issues related to the zoonotic transmission of STHs are discussed.

The genus *Ascaris* contains two species, namely *A. lumbricoides*, a parasite of humans and *Ascaris suum*, which infects pigs. Although mainly found in different hosts, the two species are morphologically very conservative, with little or no variation in morphological traits. Evidences from molecular studies suggest the presence of gene flow between the two species and their different occurrence on the base of endemicity of the human species. A large environmental contamination from both species correspond to separated transmission cycles; on the contrary, where *A. lumbricoides* is not endemic, most of the human infections are zoonotic and *A. suum* is the causative agent of ascariasis.

Similarly to *A. lumbricoides*, *T. trichiura* has worldwide distribution, and its occurrence and incidence are directly correlated with poor sanitation and the use of human feces as fertilizer. Humans are commonly infected by *T. trichiura* and, occasionally, by zoonotic species such as *T. vulpis* and *T. suis*. Molecular studies have revealed the existence of more than one taxon able to infect humans and other primates, including captive individuals, and new species have been described thus suggesting the status of *T. trichiura* as a complex of species, counting different cryptic taxonomic units with different ability to infect one particular or several species.

Parasites of the human and dog gut mucosa, the species *Strongyloides stercoralis* displays intriguing strategies in the plasticity of their life cycles. Human infections due to *S. stercoralis* tends to be acute, sometimes evolving to chronic and, in immunocompromised patients, may become largely disseminated up to be fatal. The evolutionary history of *S. stercoralis* has not been extensively studied. Having *S. procyonis* as a closest extant relative to *S. stercoralis* may indicate that both species share a common ancestor, and it might be assumed that *S. stercoralis* evolved as a parasite of canids originally, and later spread into humans as a result of dog domestication. Epidemiological data suggest that *S. stercoralis* type A can infect both humans and dogs while type B has not adapted to infect humans, and this might be the ancestral phenotype of this species.

NTDS IN ITALY

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Neglected tropical diseases (NTDs) are a group of diverse communicable diseases which are highly prevalent in tropical countries, causing a significant burden of morbidity, mortality, suffering and economic loss. Population movements, as well as international travels, are making these conditions more and more frequent nowadays even out of endemic areas.
Although a systematic surveillance for NTDs in Italy is not performed, with the exception of imported arboviroses, rabies, leishmaniasis, and scabies, which require compulsory notification, available data show that NTDs have a not negligible burden in our country. According to a survey recently conducted in nine Italian sentinel centers, schistosomiasis, strongyloidiasis, and Chagas disease were the most common NTDs seen in a 7-year period (Zammarchi et al., 2020. J Travel Med, 27 (1): taz100). A study aimed at providing an insight of the epidemiology of several NTDs at national and regional level in Italy, on both foreign and Italian citizens, using Hospital Discharge Records (HDRs) showed that the most common diagnoses were leishmaniasis (34%), schistosomiasis (29%), strongyloidiasis (12%), Chagas disease (8%), and dengue (8%) (Tilli et al., 2020. Infection, 48: 695-713). Data collected on burden of human cystic echinococcosis based on HDRs from 2001 to 2014 in Italy evidenced the presence of 12,619 patient (10,901 Italian patients and 1,718 foreign patients), 3,634 surgical and 8967 medical, with a case-fatality rate of 0.36% (Piseddu et al., 2017. PLoS Negl Trop Dis, 11: 1-18).
Education at university and post-graduate levels to increase the awareness of healthcare professionals on NTDs is needed, as well as targeted public health interventions (such as screening or presumptive treatment in high-risk groups) to improve clinical management and control of such diseases. For example, screening asymptomatic adult Latin American migrants for Chagas disease in Europe and treating *T. cruzi*-sero-positive individuals with antiparasitic therapy has been found a cost-effective strategy (Requena-Méndez et al., 2017. Lancet Glob Health, 5: 439-47), as well as serological screening of *Strongyloides stercoralis* infection to migrants from Sub-Saharan countries arriving in Italy (Zammarchi et al., 2020. Travel Med Infect Dis, 36: 1-7).

ENDEMIC NTDS IN EUROPE: DO WE REALLY KNOW THE BURDEN OF HUMAN CYSTIC ECHINOCOCCOSIS?

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INTRODUCTION: The neglected zoonosis cystic echinococcosis (CE) affects worldwide poor pastoral and rural communities but also those of medium-high income countries, including Europe, where it should be managed as an orphan and rare disease. Even if human CE caused by *Echinococcus granulosus s.l.* complex is a notifiable infectious disease in some European countries, in practice it is largely underreported by health systems.

MATERIALS AND METHODS: Data on the burden and molecular identification of CE in Europe was extracted by means of systematic review approach from both scientific and grey literature, accounting for the period of publication 1997-2021. Different grade of evidence was collected from national health reports, the European surveillance system data, single/multi-centre case-series, single-centre case-reports. Data source providing the utmost quantitative effect of the burden at country level was selected. The second inclusion criterion for this study was the molecular confirmation of *E. granulosus s.l.* at genotype/species level as causative agent of human CE cases in Europe.

RESULTS: According to the EU case definition of "echinococcosis", a yearly average of 818 confirmed human cases of both cystic and alveolar echinococcosis were recorded in Europe by EFSA/ECDC during the period 2016-2019; of which a yearly average of 431 cases were confirmed as CE. Regardless of the previous picture, data extraction from this study identified around 70,000 human CE infections from 40 European countries in a variable period within 1997-2021. Bulgaria, Italy, Romania and Spain accounted for around 78% of this health numerical burden. Mean annual incidence at European countries level during the considered period was 0.7/100,000. Highest mean annual incidence during the period 2017-2019 was reported by Albania, Bulgaria and Romania. An estimate of around 2,500 new cases per year are expected at European countries level, according to the trend for the years 2017-2019.
This study also identified 599 humans molecularly confirmed echinococcal cysts: 460 (76.8%) identified as *E. granulosus sensu stricto* (*s.s.*), 130 (21.7%) as *E. canadensis* cluster (G6/7 and G10), 7 (1.2%) as *E. ortleppi* (G5), and 2 as *E. vogeli* (0.3%). Three geographical hot-spots of human CE caused by different species of the *E. granulosus s.l.* complex were identified: 1) *E. granulosus s.s.* in Southern and South-eastern Europe (European-Mediterranean and Balkan countries); 2) *E. canadensis* (G6/7) in Central and Easter Europe; 3) *E. ortleppi* in Central and Western Europe.

CONCLUSION: This study provides for the first time an estimate of the burden and the molecular identification of CE at large scale European level. These findings should be useful to support the planning of surveillance and control of CE in Europe according to the WHO 2021-2030 roadman on NTDs.
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UPDATE ON LEISHMANIASIS

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Leishmania infection occurs worldwide with at least twenty-one species able to infect humans. Actually, it is considered endemic in 97 countries with cutaneous leishmaniasis (CL) observed in 88 countries and visceral leishmaniasis (VL) in 78 countries. In 2018, 253.435 new cases of CL and 17.223 new VL cases were reported to the WHO (Ruiz-Postigo et al., 2020. Weekly Epidem Rec, 95: 265-80). Outside endemic countries leishmaniasis is an emerging health problem as a consequence of war conflicts and increasing human mobility (Vandeputte et al., 2020. Travel Med Infect Dis, 38: 101885). *Leishmania* RNA virus (LVRs) has been described either to infect *L. guyanensis* and *L. braziliensis* in the New World (subgenus LVR1) and *L. major*, *L. aethiopica* and *L. infantum* in the Old World (subgenus LVR2) (Rossi et al., 2018. Curr Opin Microbiol, 46: 65-72). Traditionally in the Mediterranean basin leishmaniasis is considered a zoonosis with dogs being the main reservoir host for *L. infantum* but there are evidence that other mammals including lagomorphs and wild and domestic carnivores can be responsible of human cases observed outside well known endemic areas (Rugna et al., 2018. Plos Negl Trop Dis, 12: e0006595). HIV infection and the related immunodeficiency was an important trigger of VL in Europe during the ‘90s of the last century but actually several conditions such as solid organ transplantation, rheumatologic diseases treated with various immunosuppressive drugs are responsible of increasingly observed cases of CL, VL and mucocutaneous leishmaniasis (MCL) (van Griensven et al., 2014. Clin Microbiol Infect, 20: 286-99). Interestingly, a recent European clinical report regarding tegumentary leishmaniasis showed that CL acquired in *L. infantum*-endemic Mediterranean areas caused an unexpected high rates of mucosal involvement comparable to those of CL acquired in Latin America (Guery et al., 2021. Plos Negl Trop Dis, 15: e0009863). Liposomal amphotericin B remains the most frequently used drug for CL and VL but a recent systematic review highlighted the role of pentamidine isethionate for the treatment of any form of human leishmaniasis when first-line option has failed (Piccica et al., 2021. J Travel Med, 28: taab065).

LEPROSY TODAY: WORKING WORLDWIDE TO END THE DISEASE

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Leprosy or Hansen disease is a chronic infectious disease caused by *Mycobacterium leprae*, which develops in susceptible individuals, and predominantly affects the skin and peripheral nerves. The disease is curable, but if not adequately treated it can cause impairments and permanent disabilities. World data are published annually in the Weekly Epidemiological Record (WER) of the WHO. The annual total number of new cases, after a significant decrease in the first five years of this century, remains stable, about 200.000 new cases each year, except for 2021 in which the cases were about 127.000 due to the impact due to the impact of the Covid-19 pandemic. The three countries with the largest annual number of new cases are India, followed by Brazil and Indonesia, which account around 74% of the new caseload globally. Other countries with a high number of new cases (> 1.000) are Bangladesh, R. D. of Congo, Ethiopia, Madagascar, Myanmar, Nepal, Nigeria, Philippines, Sri Lanka, and Tanzania. According to the WHO estimates, in the world there are more than three million people who, despite being treated clinically, have permanent disabilities caused by the disease and need physical rehabilitation. At the same time, the stigma still associated with leprosy remains a barrier to ending transmission, and have an important impact on peoples' lives, long after they have been cured. Often, they are vulnerable people, without the possibility of social inclusion. Considering the factors stated before, it can be said that the disease is still a public health problem in various countries of the tropical and sub-tropical belt. Currently, the main objectives of the leprosy control programs intend to stop the transmission of the disease, prevent disabilities and promote social inclusion by ending discrimination. But referral systems to deal with the disabling complications of leprosy are often weak, and most countries are doing little to tackle the ongoing physical, mental, social and economic consequences of leprosy. Leprosy in included as one of the NTD diseases, addressed through direct case management (CM-NTD). WHO's NTDs Roadmap 2021-2030 is the framework within which leprosy-endemic countries should organize integrated plans for disease control or elimination. In conclusion, we can say that the physical and social consequences of leprosy must be considered in the operative plans, which cannot have a dimension exclusively focused on epidemiological parameters but must become an expression of a work that intends to defend the rights and revitalize the dignity of the persons affected.

Bibliographical references:

• WHO Weekly Epidemiological Record (WER), No. 36, 2021, 96, 421-444
• Towards zero leprosy. WHO Global leprosy (Hansen's Disease) strategy 2021–2030
• WHO NTDs Road Map 2021-2030, "Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030"

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NUOVE FRONTIERE E PROSPETTIVE DELLA LEISHMANIOSI NEL BACINO DEL MEDITERRANEO

LEISHMANIA TARENTOLAE AND *LEISHMANIA INFANTUM*, FROM THE EPIDEMIOLOGY TO POSSIBLE IMPLICATIONS IN VETERINARY MEDICINE

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In the Mediterranean basin, *Leishmania infantum* is the main species causing zoonotic cutaneous and visceral leishmaniasis (Akhoundi et al., 2016). In the same area there are other species of *Leishmania*, such as *Leishmania donovani* in Cyprus and *Leishmania tropica* in Greece from the subgenus *Leishmania* and *Leishmania chameleoenis* and *Leishmania tarentolae* from the subgenus *Sauroleishmania* (Ntais et al., 2013; Akhoundi et al., 2016; Mendoza-Roldan et al., 2021a). *Leishmania tarentolae* has a sympatric distribution with *L. infantum* in the Mediterranean basin (Akhoundi et al., 2016; Klatt et al., 2019) and is associated with saurians being sand flies *Sergentomyia minuta* the vectors. This species has been identified in *Tarentola mauritanica* and *Mediodactylus kotschyi* in Italy (Gramiccia et al., 1985) and *Tarentola annularis* in Sudan (Elwasila, 1988). Recently, this species has been molecularly diagnosed in *Podarcis filfolensis* and *Podarcis siculus* (Mendoza Roldan et al., 2021b, c). Furthermore, the molecular and serological detection of *L. tarentolae* in blood donors from central Italy (Pombi et al., 2020), in inhabitants of the Pelagie archipelago (Iatta et al., 2021) and in sheltered dogs (Mendoza- Roldan et al., 2021b), has increased the scientific interest on *L. tarentolae*. The high abundance of *S. minuta* sand flies, together with the molecular identification of human and dog blood meal in this species (Abbate et al., 2019; Pombi et al., 2020), suggest the possibility of exposure of mammals to *L. tarentolae*. Experimental studies have corroborated the hypothesis that *L. tarentolae* can develop in *P. perniciosus* and *Phlebotomus papatasi* (Ticha et al., 2021). The possibility of mammalian exposure to *L. tarentolae* has been suggested by the fact that *S. minuta* is the most abundant species in the endemic areas of canine leishmaniasis and can be fed on human and dog blood (Abbate et al., 2019; Pombi et al., 2020; Mendoza-Roldan et al., 2021b). In the italian epidemiological context, reptiles, sand flies and canids, as well as humans, living in common environments, can be exposed to both species of *Leishmania*. At the same time, some reptilian specimens were also found to be positive to *L. infantum*, and this is the first molecular evidence of protozoan infections in reptiles in Europe. Also, the exposure to *L. tarentolae* in mammals has been demonstrated, both through serological and molecular tests. There is, however, the possibility of serological cross-reactivity towards both *Leishmania* species in mammals, with diagnostic and clinical implications.

LEISHMANIA TARENTOLAE: FROM THE CELL TO THE VACCINE

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Due to its characteristics, the protozoan parasite *Leishmania tarentolae* is regarded as a promising tool for biomolecular studies and biotechnological applications, from protein production to its use as a model for drug discovery. Interesting features of *L. tarentolae* include: i) its maintenance and growth are achieved in acellular media, at a low cost; ii) growth characteristics are suitable to scale the production to industrial levels, by growing the parasites in bioreactors; iii) *L. tarentolae* presents a protein glycosylation pattern that overlaps that of pathogenic Trypanosomatidae but is also similar to that of mammals. As a tool for bio-molecular applications, *L. tarentolae* was firstly exploited as a model trypanosomatid to investigate gene amplification and RNA editing in the mitochondrion. In parallel, *L. tarentolae* was developed as a platform for protein production, commercialized by Jena Bioscience as the LEXSY system. *L. tarentolae* is also an interesting tool for protein studies (e.g., X-ray Crystallography) and for the production of antigens for sero-diagnostic applications; in this context, our recent study showed that a recombinant protein from SARS-CoV-2, produced in *L. tarentola*e, allows reliable serological diagnosis of COVID-19. Regarding its potential application as a vaccine, this parasite was explored as surrogate pathogen for anti-*Leishmania* vaccines, with the aim to protect against human pathogenic *Leishmania* species. Moreover, engineered strains of *L. tarentolae* were also assayed in animal models as anti-viral vaccines with application against the viruses HIV-1, HPV, HCV and, recently, against the SARS-CoV-2 virus, obtaining promising results.

LEISHMANIOSIS IN THE MEDITERRANEAN BASIN: CLINICAL AND DIAGNOSTIC CHALLENGES IN VETERINARY MEDICINE

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Diagnosis of canine leishmaniosis has always been a challenge for the veterinarian. The interpretation of clinicopathologic, serologic, and molecular tests should take into account the history, signalment, and clinical presentation. In addition to some typical clinical findings, laboratory abnormalities uncovered by routine hematology, clinical chemistry, or urinalysis may support the clinical suspicion of canine leishmaniosis. that is characterized by a wide spectrum of clinical presentations, ranging from infections characterized by the absence of overt clinical findings in the presence of obvious laboratory abnormalities. In canine leishmaniosis, nonregenerative normocytic normochromic anemia, thrombocytopenia, or leukogram changes may be present. Clinical chemistry and urinalysis may indicate renal dysfunction and an inflammatory/immune response. Although a potential gammopathy is usually polyclonal, it may also appear oligo- or monoclonal, especially in dogs coinfected by other vector-borne pathogens. Tests for an etiologic diagnosis are used in clinical practice to confirm the presence of the parasite or its components (direct tests) or of the host's response to the parasite (indirect tests). The most common techniques used to detect antileishmanial antibodies are based on 3 analytic principles: IFAT, ELISA, and ICT. When lesions are accessible to fine-needle aspiration, cytology is strongly advised, as the presence of *Leishmania* amastigotes in a pattern of pyogranulomatous inflammation or lymphoplasmacytic hyperplasia is diagnostic. If the cytologic pattern is inconclusive, the parasite should be identified by histology/immunohistochemistry or PCR on surgical biopsies. Alternatively, cytology and PCR may be performed on bone marrow samples where amastigotes can be observed. Dogs with overt leishmaniasis generally have high antibody titers, while low titers predominate in immunologically resistant infected dogs or in exposed dogs with no parasite confirmation. Quantitative serology is recommended in clinically suspect dogs as high antibodies titers may confirm the clinical diagnosis.
The guidelines for diagnosis and staging of canine leishmaniosis, released by the Canine Leishmaniosis Working Group (CLWG), suggest a combination of clinical and laboratory criteria for the classification of dogs into exposed, infected, or actually sick animals:

1) Exposed dogs: have a low-titer positive serology, and are negative by PCR and/or cytology.
2) Infected dogs: clinically unremarkable, with normal hematology and clinical chemistry variables, but positive PCR and/or cytology in bone marrow, lymph node, spleen, skin, or peripheral blood.
3) Sick dogs: typical clinical or clinicopathologic changes.
4) Severely sick dogs: severe clinical condition, and with concurrent problems that may or may not be related to leishmaniosis, and which may require immunosuppressive treatment; are included also animals with concomitant conditions such as coinfections.

CLIMATE AND ENVIRONMENTAL CHANGES AFFECTING THE BIOLOGY OF SAND FLIES AND THE PATHOGENS THEY TRANSMIT IN EUROPE

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Phlebotomine sand flies are the vectors of human pathogens, including *Leishmania* parasites and phleboviruses, some of which cause deadly diseases when left untreated.
In Europe, two entities of leishmaniasis are endemic: i) zoonotic visceral (VL) and cutaneous (CL) leishmaniases caused by *L. infantum*, endemic in southern countries, having dogs as reservoirs and, ii) anthroponotic CL caused by *L. tropica*, which occurs sporadically in Greece. Autochthonous VL and CL caused by *L. donovani* were recently reported in Cyprus. Among the phleboviruses, the Toscana phlebovirus can cause meningitis and encephalitis, while other phleboviruses are responsible for causing influenza-like syndromes.
The emergence and re-emergence of these neglected pathogens in Europe pose a growing threat to public and animal health. Potential triggers include the movement of humans and dogs, increasing numbers of immunosuppressive conditions, climate change, and other human-mediated environmental changes.
Most proven or suspected vector species are largely confined to the Mediterranean countries; however, future climate trends are predicted to help the northward spread in their geographical range. Generalized warming could also impact sand fly seasonality, density, and survival, and shorten the extrinsic incubation period of the pathogens they transmit.
In this talk, an overview of the main climate and environmental changes affecting the biology of sand flies and transmitting pathogens in Europe will be discussed.

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L'ECOGRAFIA COME AUSILIO DIAGNOSTICO NELLE MALATTIE PARASSITARIE

THE ROLE OF ULTRASONOGRAPHY IN HUMAN PARASITIC INFECTIONS: WHEN, WHAT AND WHY

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Imaging techniques have to be considered an integral component of the possible diagnostic tools for parasitic infections of humans. Especially point-of-care ultrasound has today a well-defined role in the diagnosis and clinical management of parasitic infections. Depending on the parasite involved, ultrasonography can be the diagnostic reference standard, such as in the case of cystic echinococcosis, or aid in the clinical staging of the infection and in morbidity assessment, such as in the case of urinary and hepatosplenic schistosomiasis, or can provide important information towards the correct diagnosis if applied in the context of a differential diagnosis workup. Again, depending on the parasite involved, ultrasonography might be useful for population screening, such as in the case of echinococcal infections, or for diagnosis and/or follow-up in the clinical setting. This presentation will provide an overview of the current role of ultrasonography in the screening, diagnosis and clinical management of selected parasitic infections of humans, with a particular focus on point-of-care ultrasound, to provide practical concepts for the request and interpretation of imaging exams in this field.

INFECTION WITH *SCHISTOSOMA* SPP: THE ROLE OF ULTRASONOGRAPHY IN AN EMERGING ZOONOSIS

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Infections by *Schistosoma* parasites are among the most important zoonoses worldwide, the ecology of which is seeing important changes, from the identification of new foci of transmission in areas previously unaffected, to the ever-increasing distribution of hybrid species. In Europe, mainly as the consequence of intense immigration, schistosomiasis has been defined as “a hidden epidemic”, with about 30% of migrants from sub-Saharan Africa having signs of infection, about 50% of whom having patent infection. Since chronic schistosomiasis can have serious, even fatal consequences, prompt diagnosis, treatment, and follow-up of both active infection and clinical complications is paramount. To this, ultrasonography is included in the toolkit available to the clinician for morbidity assessment and follow-up of hepatosplenic and urinary schistosomiasis. This presentation will provide an overview of the role of ultrasonography in the clinical assessment of the patient with schistosomiasis, with a special focus on point-of-care ultrasound.

ULTRASOUND IN VETERINARY PARASITOLOGY: STATE OF THE ART AND FUTURE PROSPECTIVES

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This presentation is a summary about the current knowledge on the application of ultrasonography in diagnosis, staging and monitoring of helminthic diseases in domestic animals. Only peer reviewed papers written in English language were reviewed and all the papers concerning unicellular parasites, wild animals, non-domestic experimental animals, and *ex vivo* or *in vitro* applications of ultrasonography were excluded from the review. A total of 122 papers met the inclusion criteria. Among them 47% concerned nematodes, 37% cestodes, and 16% trematodes with the genus *Dirofilaria*, *Echinococcus*, and *Fasciola* the most represented, respectively. Helminths can be recognized in ultrasound images by their morphology, size, and location. In some cases, the parasite stages are not directly seen by ultrasound, but the lesions caused by them can be easily visualized. The scientific literature examined shows that ultrasound is a valid diagnostic tool for helminthic disease in domestic animals. The versatility of the method and the constant technological improvement of the equipment are allowing a wide diffusion of this diagnostic imaging technique in the field of veterinary parasitology. Ultrasound should be considered an auxiliary method that can be integrated with epidemiological data, clinical findings, and laboratory diagnosis of parasitic diseases in domestic animals.

ULTRASOUND IN THE DIAGNOSIS AND EVALUATION OF THE CLINICAL PICTURE IN HEARTWORM DISEASE AND ANGIOSTRONGYLOSIS IN DOGS AND SYMPTOMATIC FORMS OF *DIROFILARIA REPENS*

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Ultrasound is a pivotal tool in the field of internal medicine and the technological evolutions that have led to the possibility of using small, high-tech and low-cost US machines continuously multiply its applications. In the field of clinical parasitology, in particular, it is very useful for proper diagnostic purposes (visualization of the parasites) but also in the staging of the patient and in his follow-up to evaluate the damage induced by the parasites themselves. Regarding Heartworm Disease, the same kind of investigation has 2 different applications in relation to the affected species, dog or cat. In dogs, although it is possible and sometimes easy visualize the parasites into the pulmonary arteries and in the right heart chambers in case of Caval Syndrome, the investigation is mainly aimed at staging the patient helping in the estimate of the worm burden, fundamental for the choice of the therapy, but also through some parameters (dilation of the pulmonary arteries, velocity of tricuspid and pulmonary regurgitation, RPAD Index) in detecting and quantifying the states of pulmonary hypertension which are the main noxa induced by *D. immitis* in this species. In cats, given the low reliability of the serological tests used for parasitological diagnosis in dogs, it is the diagnostic tool of choice combining high sensitivity and specificity.
In canine angiostrongylosis, pulmonary ultrasound does not allow the visualization of parasites but in predisposed symptomatic dogs (under 2 years of age) it allows to identify an almost pathognomonic “pathological US pattern” as well as to identify frequently associated pulmonary hypertensive states.
Finally, in dogs and cats with skin nodular manifestations caused by *D. repens*, allowing the direct visualization of the adult worms leads directly to the diagnosis allowing immediately to rule out the neoplastic pathology (mast cell tumor) versus which for the clinical aspects (Darrier’s sign) differential diagnostics is imposed and guiding the minimally invasive removal of parasites.

USE OF LIVER ULTRASONOGRAPHY FOR THE DIAGNOSIS OF CYSTIC ECHINOCOCCOSIS IN SHEEP: PRACTICAL APPLICATIONS IN THE FIELD

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Echinococcus granulosus (*E. granulosus*) is a widespread zoonotic parasite having negative effects on human-animal health and livestock production. The economic impact of the parasite on the entire chain is relevant, therefore the *in-vivo* and in-field techniques to support an early diagnosis in intermediate-hosts as the sheep represent the key points to controlling and reducing the infection in endemic areas. Until a few years ago, the diagnosis of *E. granulosus* infection was based on dogs 'coprological exams and *post-mortem* identification of cysts. Other innovative diagnostic techniques such as the active and passive geospatial surveillance of the flock, molecular analysis methods, and ultrasonography are recently proposed for the early diagnosis.

Ultrasonography can be considered a reliable *in-vivo* technique for cystic echinococcosis (CE) assessment (Borriello et al., 2021. Animals, 11(2):452). Examining the entire organ represents the best diagnostic option (gold standard) under field conditions. However, the environment, the time needed, and the type of ultrasound scan might represent some limits, especially for screening in large flocks grazing. Further strategies to reduce the effects of these logistic difficulties should be developed to improve the widespread of this technique for CE diagnosis in field conditions.

ACUTE VISCERAL CYSTICERCOSIS CAUSED BY *TAENIA HYDATIGENA* IN LAMBS: ULTRASONOGRAPHIC FINDINGS

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INTRODUCTION: Cysticercosis caused by *Cysticercus tenuicollis* is a metacestode infection that affects several species of ungulates. It is caused by the larval stage of *Taenia hydatigena*, an intestinal tapeworm in dogs and wild canids. In the intermediate host, the mature cysticerci are usually found in the omentum, mesentery, and peritoneum, and less frequently in the pleura and pericardium. The migrating larvae can be found mostly in the liver parenchyma causing traumatic hepatitis in young animals. Most infections are chronic and asymptomatic and are diagnosed at the abattoir. The acute form of infection is unusual in sheep and reports of death in lambs are rare.

MATERIAL AND METHODS: In March 2018, fifteen female lambs presented anorexia, weakness, lethargy, and death secondary to acute visceral cysticercosis. Twelve of them underwent hepatic ultrasonography.
Examinations were performed on standing or left lateral recumbent animals. Morphological and molecular identification was performed in order to confirm the diagnosis.

RESULTS: All the examined lambs showed ultrasonographic evidence of liver lesions. On the basis of the ultrasonographic classification, the infection was scored as severe in six lambs, moderate in five, and mild in only one animal. The severity of clinical symptoms was directly related to the extent and severity of liver injuries. Hepatic lesions were characterized by heterogeneous echotexture and mixed echogenicity. The injured areas were crossed by several irregular hypo- or anechoic tracts ranging from 1 to 2 cm in length and 0.1 to 0.3 cm in width. Five severely infected animals presented cystic structures (from 0.5 to 0.7 cm in diameter) localized along the hepatic surface and characterized by a thick and hyperechoic wall, containing a hyperechoic mural branching component surrounded by anechoic fluid. They also presented intraparenchymal cysts (from 0.3 to 0.4 cm in diameter) containing a point-like hyperechoic structure surrounded by anechoic fluid. A Doppler color examination confirme.
The presence of lesions was confirmed by anatomopathological examination, and *T. hydatigena* cysticerci was identified by morphological and molecular characterization of isolates.

Conclusion: The present report demonstrates that ultrasonography is a useful diagnostic tool to identify and define the extent and severity of hepatic lesions caused by *C. tenuicollis* migration in lambs. This technique could be used as an *intra vitam* diagnostic test for *C. tenuicollis* parasitosis in small ruminants.

ULTRASONOGRAPHIC DIAGNOSIS OF PERITONEAL CESTODIASIS CAUSED BY *MESOCESTOIDES* SPP. IN DOG

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Canine peritoneal larval cestodiasis (CPLC) is a parasitological infestation of the peritoneal cavity of wild and domestic carnivores with *Mesocestoides* spp. larvae. *Mesocestoides* spp. are considered zoonotic, although many aspects of the biology of these worms still remain unknown and despite hypothesizing a foodborne origin, no evidence for the route for human infection has been shown (Fuentes et al., 2003. Am J Trop Med Hyg, 68:566–67). Several recent surveys have shown evidence of new genetic variants within this genus, thus requiring further investigations (Varcasia et al., 2018. Parasit Vectors, 11: 619).

Adult *Mesocestoides* tapeworms reside within the small intestine of carnivores, while their larvae occasionally penetrate the host's intestinal wall, causing a potentially life-threatening peritonitis. Severity of infection as well as the host age and response influences the prognosis significantly, and early diagnosis and treatment are essential. However, due to the lack of specific symptoms, this condition is underdiagnosed.

Abdominal ultrasonography (US) had a crucial role in the CPLC, since it allows a suspected diagnosis that can be confirmed trough the parasitological or molecular identification, but there are just few studies including US examination of affected dogs (Venco et al., 2005. Vet Radiol Ultrasound, 46: 417-22; Carta et al., 2021. Parasitol Res, 120: 1727-35). The findings during abdominal US include often the presence of abdominal effusion characterized by echogenic and particulate fluid and several rounded to cylindrical anechoic, cystic structures measuring from 1–3 mm in diameter up to 3-4 centimeters. Several forms of metacestodes can be observed free-floating in the abdominal fluid or enclosed in small cysts and/or adherent to the serous surfaces, which appeared roughened and irregular. US allows to distinguish on the serosal surface the well-formed tetrathyridia, clearly hypoechoic with enlarged heads and a slender tail, or the so-called acephalic larvae that appear as rounded anechoic fluid-filled cystic structures. The omentum and the mesenteric fat can appear hyperechoic with a coarse and irregular echostructure.

Visualization of these features allowed a presumptive diagnosis, but all the diseases causing abdominal distension and ascites must be considered as differential diagnosis. US is also used for the ultrasonographic-guided collection of abdominal fluid and specimens for confirmation, and for therapy management and follow-up. In conclusion, it is essential to promote the development and application of early diagnostics, for which US is considered crucial, in order to prevent severe clinical manifestations and thus for the probability of recovery from disease, since anthelmintic treatments may result in a rapid resolution of clinical manifestations and a reduction of parasitic infestation, even not fully effective in eradicating the infection and preventing the disease recurrence.

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LE INFEZIONI FUNGINE DELL'UOMO E DEGLI ANIMALI: "PENSA AL FUNGO E SALVA LA VITA"

VETERINARY RELEVANT AND ZOONOTIC FUNGAL GROUPS

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The importance of fungal infections in both human and animals has increased over the last decades. Of the over 100,000 described species of fungi, about 100 are known to be regularly involved in human and animal mycoses, and another 620 are occasionally observed as opportunists.
Fungal infections in animals are widely diverse. Animal species may carry their own specific fungi. Groups of animals carry specific fungi, which have a different chance to cause infection in humans.
Fungal infections that can be encountered in animals originating from environmental sources without transmission to humans. These environmental pathogens are wide variety of saprophytic fungi that can cause opportunistic infections across most animal species when they become immunocompromised; these infections are normally not transmissible.
However, pathogenic fungi have evolved specific mechanisms to infect animals and are able to use the host to amplify their infectious propagules; these are known as zoophilic fungi that can be directly transmitted from animals to humans.

MEDICAL AND VETERINARY MYCOLOGY: THE STATE OF ART

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The study of Medical Mycology goes back to 2000 a.c. in the Indian Atharva Veda, conversely, the first mycoses in higher animals were reported in 1800. During the 1930s several studies laid the groundwork for Modern Mycology, when “medical fungi” were recognized as pathogenic for humans and animals. The concept of “true pathogens” was enhanced and most of recognized species were found to be opportunistic organisms, allowing to outline Medical Mycology. In 1950s first active antifungal, opening the field of antifungal therapy, then in the early 1960s renal transplantation or leukemia and consequent aspergilloses in neutropenic hosts were reported. In the “years of expansion of medical mycology” (1970s) the increase of the spectrum of available antibiotics, along with the application of immunosuppressive and cytotoxic drugs, produced a high number of immunosuppressed patients, prone to opportunistic fungal infections. At this phase commercial tests for the detection of mycotic agents in clinical specimens were distributed and the demand for mycological training was increasing. In the early 1980s, following AIDS pandemic, when both pathogenic and opportunistic fungi were recognized as able to induce severe and fatal disease in immunocompromised people, antifungal therapy studies were enhanced, together with available diagnostic tools and primitive vaccines. Veterinary mycology was favored by these novel trends. In the early 2000s, when climate changes became a global emergency, new agents of human and animal infections occurred, mainly drug resistant, amplified by host shifts consequent to globalization, urbanization, trade, habitat disruption. Geographical niches of these organisms (i.e. *Candida auris*, *Emergomyces* sp., *Cryptococcus deuterogattii*) were brought in contact with naïve hosts (Spallone and Schwartz, 2021). A marked loss of biodiversity due to novel fungal pathogens was observed in several animal classes (amphibia, reptiles, chiropters), affected by chytridiomycetes, *Fusarium* sp., *Nannizziopsis* sp., *Paranannizziopsis* sp., *Ophidiomyces ophiodiicola* and *Geomyces destructans*. The overuse of antifungal agents in agricultures led to selection of drug resistant environmental pathogens (*Aspergillus* spp.), with heavy troubles for the treatment and outcome of affected neutropenic patients. Lastly, the microsporidia group has been included within the kingdom Fungi in 2008, expanding the field of study of medical and veterinary mycology to at least eight genera. Microsporidioses are, in fact, most likely zoonotic or waterborne infections adapted to nearly every animal phylum, including protists and humans. Microsporidia are not only important emerging human pathogens in immunocompetent and immunocompromised individuals, but also major threats to sericulture, apiculture, and aquaculture. Currently, there are only a few commercially available antimicrosporidial drugs and therapeutic agents are needed for these infections (Wei et al., 2022).

ANTIFUNGAL THERAPY IN VETERINARY MEDICINE: A CALL FOR EXPERTISE

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A systematically increasing prevalence of fungal infections in animals has been noted worldwide over the past two decades (Gant et al., 2022. J Appl Microbiol, 131: 2095-113). Among these infections, the superficial ones are characterized by high frequency of recalcitrant and recurrent phenomena thus requiring a specific knowledge of the features of the etiological agents. Whereas, invasive fungal infections are characterized by a high morbidity and mortality thus requiring a rapid diagnosis and an appropriate antifungal therapy for the animal recovery. To date, the management of these infections in animals includes systemic or topical treatment and if necessary, environmental decontamination (Segal and Elad, 2018. J Fungi, 4:135). Despite recent advances in antifungal pharmacology, therapeutic options against fungal infections in animals are limited due to the low number of licensed products for animals (Seyedmousavi et al., 2018. Med Mycol, 56:165-87). However, off-label use of antifungals is quite common, and many of the antifungal drugs employed in human medicine are used in animals with evident limitations associated to variable pharmacokinetics, adverse effects, and drug interactions (Seyedmousavi et al., 2018. Med Mycol, 56:165-87). Here, we provide an overview on current knowledge and experience in the treatment of selected fungal infections in different animal species, focusing on the infections characterized by high prevalence and social-economic interest. Overall, our data show that prolonged administration of the drug is usually necessary to treat all fungal infection of animals, thus often resulting cost-prohibitive. In addition, therapeutic guidelines are present only for dogs and cats. In the literature, different protocols of intervention are present and in some cases, they are ineffective most likely due to the appearance of drug resistance phenomena of the etiological agents. Even if fungal susceptibility testing has been standardized for some fungi of human origin, the predictive value of such tests for fungal strains coming from animal has never been demonstrated, and thus their use for veterinary fungal infections is questionable. For *Malassezia* infections, new therapeutic guideline for dogs/cats has been recently published but the recommendations are not robust in their validations representing insightful guidance using clinical cases/reviews and expert clinician opinions (Bond et al., 2020. Vet Dermatol, 31:28-74). No treatment is recommended for horses or farm animals (i.e., bovine and rabbits), and the management of fungal infections might require specific knowledge of the epidemiological features of the pathology. Clinical studies aiming at implementing effective treatment protocols dedicated to different animal species should be encouraged for the control of fungal infections in animals

CRYPTOCOCCUS IN WILDLIFE SPECIES: FRESH INSIGHTS AND THEIR ROLE AS SENTINELS FOR HUMAN DISEASE

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Cryptococcosis is a sporadic but uncommon fungal infection encountered throughout the world. It is more prevalent in certain geographical areas e.g. Australia, the Pacific North West of North America. The range of animals susceptible to infection is perhaps greater than for any other pathogen. It includes a wide diversity of wildlife, companion and production animals, and people. As well as koalas and companion animals, clinical infections and/or asymptomatic carriage are reported in many terrestrial and aquatic placental mammals, marsupials, monotremes, birds, reptiles, amphibians, fish and amoeba. The overall picture of cryptococcal disease in wildlife is complex. Published information is fragmentary, ranging from a single case due to opportunistic necropsy examinations after accidental death (e.g. foxes, elk) to more detailed case studies where patients live in a captive or controlled environment (i.e. zoo or Wildlife Park e.g. 'the captive koala story') or are involved in an outbreak event (e.g. Vancouver Island; goats in Spain).

The *Cryptococcus neoformans* and *C. gattii* species complexes have been identified as the main causes of infection in wildlife, with higher prevalence in aquatic mammals and parrots from endemic areas (Western Canada, Australia and Brazil, respectively), and especially in koalas. These arboreal marsupials live in eucalyptus trees containing hollows strongly associated with *C. gattii*. Additional epidemiological information is provided by monitoring of many wild and synantropic animal populations, usually performed in concert with other public health actions (e.g. avian influenza surveillance in birds and approved 'culling' of a overabundant species).

In general, results from such studies suggest that wild animals are more likely to be asymptomatic carriers (i.e. subclinical disease) than clinically affected by cryptococcosis. Overall, migratory birds show lower prevalence of *C. neoformans/C. gattii* isolates compared to less virulent species such as *C. albidus* and *Papiliotrema laurentii* (previously *Cryptococcus laurentii*). Routine use of modern molecular techniques should be used to assess the genetic diversity of cryptococcal organisms in any epidemiological or clinical setting in wild animals, ideally performed in a mycology reference laboratory or a lab with a special interest in *Cryptococcus* spp.

We contend that surveillance of wildlife can provide an early warning system for outbreaks of new or emerging variant pathogen strains, helping to inform an accurate risk assessment. Standardisation of protocols and tools is needed to make a global comparison possible. Stated another way, wildlife species can be very important sentinels for the *Cryptococcus neoformans/gattii* species complexes and provide new and penetrating insights into the epidemiology and pathogenesis of disease caused by these organisms.

DIAGNOSIS OF FUNGAL INFECTIONS: COMBINING THE OLD AND THE NEW TO MAXIMIZE RESULTS

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In recent years, opportunistic fungal infections in human medicine have increased. The main reason is the rise of people with immunosuppression of various origins (AIDS, chemotherapy, immunosuppressive therapies in organ transplant) (Kozel and Wickes, 2014. Cold Spring Harb Perspect Med, 4: a019299). Moreover, the spectrum of fungi causing infections is expanding, which constitutes an identification challenge for even the most experienced mycologists. To achieve an even earlier and more precise diagnosis, new methods for the detection of fungal elements in tissue samples (e.g. PCR based techniques, serological tests) and fungal identification (e.g. MALDI-TOF technology) are now available in adjunction to traditional methods (microscopic examination of clinical samples, histopathology, and culture). Cases of deep mycosis are more rarely reported in animals because the situations leading to immunosuppression in human patients are not mirrored in veterinary medicine. However, there is an increasing interest in these cases involving companion, zootechnical and wild animals. Thus, new diagnostic procedures are being applied more and more to animal infections (Elad and Segal, 2018. Front Microbiol, 9:1303).

New diagnostic tools likely will reveal animal infection cases that the traditional methods would have missed. Thanks to the improvement of the identification methods, it has been possible to describe new cryptic species responsible for specific diseases (e.g. the species included in the *Aspergillus viridinutans* complex, agents of the sino-orbital Aspergillosis in cats) (Talbot and Barrs, 2017. Med Mycol, 56: 1:12). The use of serological tests (e.g. the search for wall fungal components, such Beta-Glucan) may be a precious tool to diagnose and monitor the therapy response in a variety of diseases (e.g. disseminated Aspergillosis in dogs; avian Aspergillosis) (Burco et al., 2012. Avian Dis, 56: 183-91).

These innovations also regard the infections caused by the dermatophyte fungi (Peano, 2019. Veterinaria, 33: 125-39). Dermatophytosis is less severe than the diseases mentioned above since it is a skin infection, but it is relevant since widely diffused all over the world. Some dermatophytes are transmitted from animals to humans; therefore, these infections represent a public health problem. The diagnosis of dermatophytosis is an excellent example of the usefulness of applying new methods in support (not in substitution) of the older ones. The microscopic hair examination retains its importance as a simple, rapid and inexpensive method. On the other hand, a Real-Time PCR exists now and can detect the infection from clinical samples with very high sensitivity. Further molecular analyses (PCR- and MALDI Tof- based) on the fungus grown in culture allows a definitive identification, sometimes revealing new species (e.g. the recently described species within the *T. benhamiae*-complex) (Čmoková et al., 2020. Fungal Diver, 104: 333-87).

VETERINARY MYCOLOGY IN EUROPE AND IN ITALY

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Mycology is certainly one of the fields of study that fully falls in the concept of one health. In fact, in addition to few zoonotic fungi (i.e. zoophilic dermatophytes, microsporidia, dimorphic fungi), many fungal infections originate from the environment and can be common to animals and humans.
In 1954, a group of European mycologists founded in Paris the "International Society for Human and Animal Mycology (ISHAM)" which gathers mycologists from all over the world and comprises various working groups, including the one in veterinary mycology. Subsequently, at the European level, the European Confederation of Medical Mycology was instituted in 1993, which works towards the unification of all scientists interested in human and veterinary medical issues related to mycology. The ECMM serves as a confederating organization for the National Medical Mycology Societies throughout the European continent. For Italy, the confederate company is Fimua (Federazione Italiana di Micologia Umana e Animale), founded in 1992, with the aim of bringing together various professionals who deal with medical mycology: microbiologists, biologists, dermatologists, hematologists and veterinarians.
Who are the vets dealing with mycology? In European Universities, in which courses of veterinary degree is mycology taught? In general, veterinary mycologist are usually colleagues from universities or research laboratories, who have directed their interest, as part of their activities, to the study of fungi. They include microbiologists, parasitologists, clinicians but also zootechnicians and inspectors, due to the possible occurrence of mycotoxins in animal feed and in food of animal origin. In Europe the teaching of mycology in veterinary medicine degree programs is often not clearly defined. Observing the curriculum of the various EAEVE accredited degree courses in Veterinary Medicine, it can be noted that, in most cases, mycology falls within the basic subjects within the generic term "microbiology", while parasitology is included in clinical science, occasionally associated with dermatology. Most of the veterinarian working in mycology are also microbiologist and the connection between veterinary mycology and parasitology is evident essentially in Italy and France. In the latter country, in June 2022 a joint congress of the Parasitology and the Medical Mycology French societies is scheduled. In this report, the main aspects of research and study of veterinary mycology in Europe and in Italy will be illustrated.

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NEW FRONTIERS IN VECTOR CONTROL

CONTROL OF MOSQUITO-BORNE PATHOGENS USING SYMBIONTS

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Inherited (vertically transmitted) microbial endosymbionts are common in insects and are found in many disease vector species. *Wolbachia* are bacteria able to invade mosquito populations using patterns of crossing sterility known as cytoplasmic incompatibility (CI). Following lab transfers of various *Wolbachia* strains into *Aedes aegypti*, some can block transmission of a number of arboviruses. Strain wAlbB, originating in *Aedes albopictus*, blocked dengue transmission efficiently in *Ae. aegypti* and unlike some other strains was stable and maintained dengue transmission blocking when larvae were reared at high temperatures. In a collaborative study in Malaysia, releases of wAlbB-carrying *Ae. aegypti* were used to introduce it into wild populations, and it spread to and remained at high frequency in a number of locations. In sites that were previously hot-spots for dengue transmission, dengue has been brought under control very effectively, with up to 80% reductions when comparing pre- with post-intervention incidence. It is now being used operationally in Malaysia, with no evidence for loss of efficacy over time. Using backcrossing to introduce wAlbB into other *Ae. aegypti* lines, we have found variation in the fitness costs it causes, and this will affect field spread dynamics and population stability. We also found significant differences between *Wolbachia* strains in the cellular perturbations induced that impact virus transmission blocking. Meanwhile an inherited symbiont found in *Anopheles gambiae* species, Microsporidia MB, is able to block *Plasmodium* transmission, and has potential for developing novel malaria control interventions.

AEDES KOREICUS AND *AE. JAPONICUS*: GENOME SEQUENCING AND SYMBIOSIS CAN HELP THEIR CONTROL?

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The emerging distribution of new alien mosquito species was recently described in Europe. In addition to the invasion of *Aedes albopictus*, several studies have focused on monitoring and controlling other invasive *Aedes* species, as *Aedes koreicus* and *Aedes japonicus*. Considering the increasing development of insecticide resistance in *Aedes* mosquitoes, new control strategies, including the use of bacterial host symbionts, are proposed. However, little is known about the bacterial communities associated with these species, thus the identification of possible candidates for Symbiotic Control is currently limited. The characterization of the natural microbiota of field-collected *Ae. koreicus* mosquitoes from North-East Italy through PCR screening, identified native infections of *Wolbachia* in this species that is also largely colonized by *Asaia* bacteria. Since *Asaia* and *Wolbachia* are proposed as novel tools for Symbiotic Control, our data support their use for innovative control strategies against new invasive species. While the presence of *Asaia* was previously characterized in *Ae. koreicus*, our study characterized this *Wolbachia* strain, also inferring its phylogenetic position. The co-presence of *Wolbachia* and *Asaia* may provide additional information about microbial competition in mosquito, and to select suitable phenotypes for the suppression of pathogen transmission and for the manipulation of host reproduction in *Aedes koreicus*.
In addition to this, the information that can be obtained from the genome screening of these species may offer a decisive step in the knowledge of the biology of these vectors. The first data from genomic sequencing analysis are here presented.

ARTHROPOD-BORNE PATHOGENS OF DOGS AND CATS: PATHWAYS AND TIMES OF TRANSMISSION AND DISEASE CONTROL

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Vector-borne pathogens have exploited a number of ways to infect vertebrate hosts by “synchronizing” their biology and ecology with that of arthropod vectors. The majority of VBD-causing pathogens affecting animals, including dogs, are transmitted by hematophagous arthropods (e.g., mosquitoes, ticks, sand flies and fleas) through the blood meal of females. Other ways of pathogen transmission by arthropods are through their faeces (e.g., *Trypanosoma cruzi* by triatominae bugs), ingestion (*Hepatozoon canis* and *Hepatozoon americanum* with ticks; *Dipylidium caninum* with fleas and lice) or when they feed on lachrymal secretions (*Thelazia callipaeda* by zoophilic secretophagous flies) (Otranto et al., 2006; Baneth, 2011). The length of arthropod feeding and knowledge of their physiology and behaviour is probably the most important driver for explaining pathogen transmission times and for exploiting different control strategies. Indeed, according to pathogen transmission mechanisms and times, a fast ‘speed of kill’ and residual efficacy are pivotal for trying to block pathogen transmission, and therefore for controlling and preventing VBDs. However, depending on the compound used, alone or in combination, their action may be oriented to i) prevent attachment (before it starts – repellence through contact); ii) disrupt contact between the arthropod parasite and the host (also referred to as “expellence”); iii) cause the death of the arthropod parasite after the blood begins (killing effect); iv) interfere with egg fertility and subsequent development of off-host stages (growth inhibition) (reviewed in Halos et al., 2012). Since their introduction in the early 1990s, synthetic pyrethroids (e.g., deltamethrin, flumethrin, and permethrin) have been successfully used in veterinary medicine, alone or in combination with other compounds (Beugnet and Franc, 2012). Due to their capacity to block pathogen transmission, isoxazolines in oral formulation, afoxolaner (Beugnet et al., 2014), fluralaner (Taenzler et al., 2016) and lotilaner (Cavalleri et al., 2017), were successful for the prevention of the transmission of many CVBDs (afoxolaner, Beugnet et al., 2014; fluralaner, Taenzler et al., 2016). Considering the toxicity of synthetic pyrethroids, except for flumethrin, to cats, the efficacy of an association of isoxazoline (e.g., selamectin plus sarolaner) has been recently very welcomed by the market for use to prevent FVBD (Otranto and Little, 2017 and articles in the same special issue). According to the presence of different species of arthropods in a given area and to the epidemiological situation a combined treatment approach (repellent + insecticide) may be advocated. Nonetheless, endosymbionts and vaccines targeting arthropod or pathogen antigens should be further investigated as control strategies towards the goal of achieving an effective integrated strategy for vector-borne diseases.

NOVEL *WOLBACHIA* TRANSINFECTIONS IN *AE. ALBOPICTUS* FOR ARBOVIRUS CONTROL

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The global incidence of arboviral diseases transmitted by *Aedes* mosquitoes, including dengue, chikungunya, yellow fever, and Zika, has increased dramatically in recent decades. The release of *Aedes aegypti* carrying the maternally inherited symbiont *Wolbachia* as an intervention to control arboviruses is being trialled in several countries. However, these efforts are compromised in many endemic regions due to the co-localization of the secondary vector *Aedes albopictus*, the Asian tiger mosquito. *Ae. albopictus* has an expanding global distribution following incursions into a number of new territories. To date, only the wMel and wPip strains of *Wolbachia* have been reported to be transferred into and characterized in this vector. A *Wolbachia* strain naturally infecting *Drosophila simulans*, wAu, was selected for transfer into a Malaysian *Ae. albopictus* line to create a novel triple-strain infection. The newly generated line showed self-compatibility, moderate fitness cost and complete resistance to Zika and dengue infections.

ALTERNATIVE AND ECOLOGICAL METHODS FOR MOSQUITO LARVAL CONTROL IN URBAN ENVIRONMENT

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INTRODUCTION: Mosquito control is one of the main preventive measures of mosquito-borne diseases transmission. Following the observation of mosquito resistance to insecticide, scientists suggest to optimize current control strategies by developing innovative and alternative strategies. In this study, we present the results of the field tests of two alternative methods for mosquito larval control: ZanzaStop® and X-Larv®.

MATERIALS AND METHODS: ZanzaStop® (ZS) is a device placed in catch basins, preventing adult mosquitoes to reach the water and lay eggs. X-Larv® (XL) is a vegetal oil, which forms a film on the water that prevent mosquito larvae and pupae from breathing. It has the same principle of action of Aquatain®, a silicon-based oil, whose effectiveness has already been demonstrated in several studies. ZanzaStop® and X-Larv® have been tested in Ponte San Nicolò (PD) and Ponte di Piave (TV), respectively. In these municipalities, we selected areas where catch basins have been treated with alternative method and control areas where catch basin have been treated with a chemical larvicide, Diflubenzuron. To monitor the efficacy of the two control methods, ovitraps were placed in the treated (ZS and XL area) and control areas (CA).

RESULTS AND CONCLUSIONS: In Ponte San Nicolò, the average number of eggs per collection was higher in ZS area (192.18 eggs/ovitrap) compared to CA area (151.70 eggs/ovitrap), although difference was not significant. We observed larvae in catch basins treated by ZS when they were slightly open because of the presence of leaves or debris, while the clean ones were negative, demonstrating that ZS can be effective for mosquito larval control only if constantly checked.
In Ponte di Piave, the average number of eggs per collection was higher in XL area (305.64 eggs/ovitrap) compared to CA area (245.03 eggs/ovitrap). After we applied the product more frequently and at a higher dosage than recommended by the manufacturer, we observed greater effectiveness. Thank to these observations, the manufacturer has improved the product and updated the use indications. In general, alternative methods seem to be efficient if they are correctly managed. This requires that people involved in control activities, as well as citizen living in areas treated with alternative methods, change their habits in terms of type and frequency of control activities.

AT THE INTERFACE BETWEEN THE HOST, THE PATHOGEN AND THE VECTOR: THE MOSQUITO SALIVA

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This study unravels the multifunctional role of mosquito saliva and of the mouth's cuticle, which is not merely an external exoskeleton forming strong structures allowing the insect, for example, to feed or sting, but it is also a site of key molecular interactions governing the animal biology. Apart form deepening our knowledge of the feeding mechanism in mosquitoes, it opens new ways to target and alter the mosquito feeding process, with the promise to limit mosquito-borne pathogen transmission.
The mosquito proboscis, indeed, is an efficient microelectromechanical system, which allows the insect to feed on vertebrate blood quickly and painlessly. Its efficiency is further enhanced by the insect saliva, although through unclear mechanisms. We describe the initial trigger of an unprecedented feedback signalling pathway in *Aedes* mosquitoes affecting feeding behaviour. We identified LIPS proteins in the saliva of *Aedes* mosquitoes that promote feeding in the vertebrate skin. LIPS show a new all-helical protein fold constituted by two domains: the N-terminal domain interacts with a cuticular protein (Cp19) located at the tip of the mosquito labrum. Upon interaction, the morphology of the labral cuticle changes and this modification is most likely sensed by proprioceptive neurons, controlling, in turn, intradermal probing.

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GESTIONE SANITARIA DELLA FAUNA: QUALI SPAZI PER PARASSITI E MALATTIE PARASSITARIE

WILDLIFE HEALTH MANAGEMENT AT THE INTERFACE WITH LIVESTOCK: AN EUROPEAN PERSPECTIVE

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Massive changes in habitat and human population growth have had significant effects on European wildlife communities. Rural abandonment and growing woodland and scrubland habitats, along with agricultural intensification, favor the population growth of a few successful species, including several carnivores, most ungulates and relatively few highly adaptable bird species. These are the main wildlife species to consider at the European wildlife-livestock interface. Driven by the changes in habitat and animal populations, as well as in human behavior, there is an emergence or re-emergence of infections shared between wildlife and livestock, and considering that some of them are zoonotic, an increased impact of wildlife health on human health. This presentation describes the characteristics of the potential interactions between wildlife and domestic animals in the European context (with special emphasis to what is known in Spain), the problems related to those interactions that can facilitate disease emergence, and introduces the possible impact of climate, environmental or socio-economic change on our capacity to successfully mitigate the sanitary consequences of wildlife/livestock interactions.

CONSERVATION AND DEMOGRAPHIC TREND OF WILD RUMINANTS IN CENTRAL-SOUTH ITALY

Nicoloso S.

D.R.E.Am. Italia soc. Coop

The ruminant wild ungulates conservation of in Central-South Italy has had a recent evolution in terms of geographical as well as numerical distribution. After the almost total extinction to which it was witnessed between the end of the 19th and the beginning of the twentieth century, in northern Italy spontaneous colonization from relics and the neighboring states saw a timid evolution of the Areals already from the years' 20 and 30 of the last century. Since the 1950s, some reintroduction projects have accelerated these processes for the Alpine arch. Reintroduction projects for the center and southern Italian peninsula have a more recent history than the previous ones. The reintroduction projects carried out since the 70s of the last century for red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) have not always been based on accurate feasibility studies. In particular for the roe deer, founders belonging to autochthonous strains of southern Italy have always been used and classified by some as belonging to the Italian subspecies. In some cases, the diffusion in the area was the result of accidental escapes from ornamental fences and with species considered not indigenous for Italy as in the case of the fallow deer (*Dama dama*) and the mouflon (*Ovis aries*). In the new in progress, some reintroduction projects carried out with greater attention to their opportunity have allowed wild ungulates to occupy spaces of considerable importance in the territory in question. The most recent status available is presented here which highlights how the presence of these animals is no longer an exclusive prerogative of the Alpine areas, where it also boasts a long hunting tradition. The current status of ungulates wild ruminants in the center and southern Italy opens numerous scenario for their conservation, hunting bags and health aspects.

TOWARD THE HARMONIZATION OF WILDLIFE POPULATION MONITORING AND DISEASE HEALTH SURVEILLANCE

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In order to effectively manage the emergence of zoonotic pathogens deriving from wildlife, or for the management of enzootic pathologies common to domestic and wild animals, the knowledge of the numerical consistency of wild populations, their territorial distribution, and temporal trends (numerical and geographic distribution) have become fundamental. European authorities recognized that wildlife health must be managed at a higher level by including, in addition to active and passive surveillance, also consistency values (abundance and density) and distribution of animal populations. The Enetwild Consortium (www.enetwild.com), is a network of wildlife professionals that work together, on EFSA's mandate, to provide the basis for integrated wildlife health monitoring. To respond to the African Swine Fever (ASF) outbreak, which has been involving Europe and Asia since 2007, Enetwild has focused its attention primarily on the collection of wild boar abundance and distribution data, as on other groups of sensitive species such as ungulates and carnivores. The project has set up the standards of harmonized data collection in Europe by (i) building the inventory of available data through direct contact with local/national stakeholders in EU and non-EU European Countries, (ii) developing a data collection model (standards), (iii) data collection through the network and collaborators, and (iv) integration in a common database. To fill the gaps in data availability, Citizen Science tools have also been developed and promoted (www.mammalnet.net). Mobile-phone applications, camera-trap interfaces, and platforms have been developed and are currently fully operative to promote citizens' involvement and to extend data collection. These tools have been particularly helpful in ASF-affected areas (i.e. Balkans) to report wild boar carcasses and to promote timely carcass testing and removal. To make the harmonization and collaboration effort implemented by Enetwild permanent, the first European Observatory for Wildlife (www.wildlifeobservatory.org) has been recently established. Overall, Enetwild and the European Observatory for Wildlife have produced a number of tools and resources available to public veterinary and health authorities, as well as to wildlife managers that can be used for population and health management: i) standards for harmonized high-quality data collection, ii) density models for wild boar at a continental scale based on hunting-bag data iii) validated methods for density estimation of wildlife through camera-trapping and iv) tools for citizen science data collection. Mapping specific wildlife/livestock interfaces at a European scale is also among the next steps. Wildlife health surveillance is moving toward shared management at the European level. Enetwild provided a number of tools and a functioning network of professionals to implement an integrated approach to demographic and epidemiological wildlife data.

MANAGEMENT OF PARASITIC ZOONOSES IN WILDLIFE: THE CITIZEN SCIENCE APPROACH AS THE WAY FORWARD

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Parasitic zoonoses include a wide range of diseases, caused by viral, bacterial, fungal and parasitic infections, affecting animals and humans worldwide (Otranto et al., 2021. Parasitol Res, 120: 4073-4). The recent COVID-19 pandemic has re-focused the attention on the spill-over and transmission pathways of zoonotic agents along the wildlife-human interface (Meurens et al., 2021. Animal, 15: 100241). Considering that over 70% of emerging zoonoses origin from wildlife (Morse et al., 2012. Lancet, 380: 1956-65), the increasing density of synanthropic animals in peri-urban areas may enhance the spread of pathogens to pets, farm animals and humans (Otranto et al., 2015. Vet. Parasitol, 213: 24-37; Pittiglio et al., 2018. PLoS One, 13: e0193295). However, in the last decade, the involvement of citizens in scientific research (citizen science approach, CS) provided a network of large-scale and cost-effective surveillance programs of wildlife populations and their zoonotic agents (Lawson et al., 2015. Ecohealth, 12: 693-702). For instance, a national project based on the collection of red fox (*Vulpes vulpes*) carcasses by citizens in sub-urban areas of Sweden, revealed the occurrence of *Echinococcus multilocularis* in these canids and the transmission risk of the zoonotic tapeworm to forestry workers (Osterman Lind et al., 2011. Euro Surveill, 16: 19836). The CS has been also employed as an alternative to the classic wildlife census methods (e.g., telemetry), via sightings reported by citizens to establish density and distribution of (i) foxes and Eurasian badgers (*Meles meles*) in peri-urban settlements of the UK (Scott et al., 2014. PLoS One, 9: e99059), as well as (ii) invasive wild birds in poultry breeding areas of Australia (Greening et al., 2021. Prev Vet Med, 190: 105327). CS has been proved to be useful also for food safety, as demonstrated in a survey from Italy which indicated a high molecular prevalence (39.6%) of *Toxoplasma gondii* in home-made wild boar (*Sus scrofa*) meat products and the infection risk by this protozoan for consumers (Sgroi et al., 2020. Zoonoses Public Health, 67: 1-9). Also, the sampling by hunters of hard ticks and fecal samples from wild boars revealed, respectively, a high (i) exposure to tick-borne bacteria (*Borrelia lusitaniae*, *Coxiella burnetti* and *Rickettsia raoultii*) (Sgroi et al., 2021. Transbound Emerg Dis, 68: 2111-20; Sgroi et al., 2021. Transbound Emerg Dis, 00:1-8) and (ii) transmission risk of emerging zoonotic yeasts (e.g., *Candida albicans*, *Candida krusei*) to people frequenting rural areas (e.g., hunters, hikers and mushroom pickers) in Italy (Rhimi et al., 2022. Mycopathologia, 00: 1-14). Following the one health paradigm, collaborative and multidisciplinary models for wildlife health surveillance and zoonoses control strategies should be furtherly developed by harnessing the potentiality of the CS approach.

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EFFETTI DI CALAMITÀ ED EVENTI CLIMATICI ECCEZIONALI SULLA TRASMISSIONE DI PARASSITI

SOIPA

NAPOLI 2022 PADOVA

CLIMATE CHANGE AND PARASITIC CONTROL: THE USE OF GIS SYSTEMS FOR AN INTEGRATED MANAGEMENT OF ANIMAL HEALTH AND THE ENVIRONMENT, IN A "ONE HEALTH" PERSPECTIVE

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Parasitosis are one of the main obstacles in the management of extensive farms and cause significant economic losses worldwide (Cabaret et al., 2002. Vet Parasitol, 105:33-47; Ola-Fadunsin et al., 2021. Acta Parasitol, 66: 1094). The recent climatic emergency has significantly increased the risks associated with parasites; the increase in rainfall and temperature and climatic extremes favor the host-parasite contact during grazing activities. If not mitigated, climate change will very likely impact the length of the transmission season and the geographical range of a significant proportion of infectious and parasitic diseases (Woodward et al., 2014. Lancet, 383:1185–89), with negative consequences on hosts and farm management practices and implications for animal health and welfare (Skuce et al., 2013. Animal, 7 (S2): 333-45). Therefore, a thorough understanding of the potential impact of the climatic factors on the epidemiological components of a parasitosis is an essential requirement for the proper management of extensive farming practices. In this context, Geographic Information Systems (GIS) can be useful tools to develop sustainable strategies for the control of parasites transmission. The aim of the work is to show that several types of analyses routinely used implemented in GIS, associated to new electronic devices, can provide active support in the assessment of the impact of the climate change on parasites transmission.

GIS facilitate the determination of climatic risk factors and the delimitation of the areas at risk of parasitosis through the implementation of predictive models based on the identification of any spatial and temporal patterns of the infection. In this work the possible correlation between exceptional events, an expression of climate change, which we know will be increasingly frequent and more violent with the identification of possible new areas of epidemiological risk, was evaluated. In addition, the combined use of GIS software and electronic wearable devices (GPS) which allows the continuous tracking of the movements of the animals and the identification of the micro-epidemiological channels of the spread of parasitosis in the grazing areas, was shown.

Extreme weather and climate events can alter parasite replication rates within animal/human vectors and hosts. In the case of earthquakes or floods, the necessary sudden sharing of new confined spaces and raw materials such as water and food could inevitably favor the spread of infectious agents, therefore, knowing a priori using GIS, the territory and the epidemiology, for example, of parasites could limit their spread.

This work aims to show the importance of geospatial technology in supporting pest control strategies and demonstrate that a combined use of different GIS-based techniques can provide effective solutions aimed at increasing the resilience of animals and humans to parasitic infections and their ability to recover from natural hazards or climate change.

ANALYSIS OF THE INFLUENCE OF EXCEPTIONAL CLIMATIC EVENTS IN SARDINIA ON THE EPIDEMIOLOGY OF ENDOPARASITOSIS IN GRAZING ANIMALS

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Extreme weather events such as floods and subsequent flooding of large areas or prolonged drought episodes, accompanied by high temperatures and particularly windy days, can lead to the onset of fires of vast proportions.
Unfortunately, in Sardinia these events have occurred more frequently in recent years, often catching unprepared the livestock sector, and causing considerable economic losses. In this context, the epidemiological trend of parasitic diseases of animals undergoes important influences as well.
Fire consumes the vegetation and the soil layer where many parasites spend part of their life cycle, thus reducing the availability of their habitat, with consequent potential benefits for the hosts (Álvarez-Ruiz et al., 2021. Proceedings of the Royal Society B, 288(1954).
Here we report some considerations concerning the possible effects of such extreme climatic phenomena on the epidemiology of endoparasitosis of grazing animals, through the examination of two different extreme environmental/climatic situations that have occurred in Sardinia in recent years. The first was a flood in November 2013 in the north-eastern part (Torpè and Lodè) and the second one was a massive fire that occurred in the summer of 2021 in Montiferru (central-western area).
We can hypothesize different effects determined by these two “opposite” phenomena: widening of the spread of some parasitoses in the flood (e.g.: Cryptosporidiosis, Fasciolosis, etc.); drastic reduction of some forms of propagation (e.g.: eggs of cestodes, L3 larvae of gastro-intestinal nematodes), of intermediate hosts (e.g.: oribatid mites, xerophilous gastropod molluscs) and ixodids, possible vectors of piroplasmosis in the areas affected by a fire.
In addition, the death of numerous animals grazing and/or reared in the areas affected by floods, especially in Sardinia, may be the source of a possible extension of the spread of important metacestodes, including those of zoonotic interest, such as cystic echinococcosis.
An important indirect influence on the epidemiological trend of some parasitoses could also be determined by the fact that animals removed from the original grazing areas affected by the fire and transferred to other pastures with different pedoclimatic characteristics, could acquire “new” parasitic forms.

IMPACT OF NATURAL DISASTERS ON PARASITE-HOST EQUILIBRIA; REFLECTIONS ON A DROUGHT EVENT'S IMPACT ON INTESTINAL PARASITE ABUNDANCE IN A SHEEP FARM SITUATED IN THE APENNINE MOUNTAINS (MARCHE REGION)

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Since a few decades, global warming has become a major concern and a research subject also among parasitologists. The rise of mean temperatures is expected to speed up the development of environmental parasite stages (oocysts, eggs, larvae), the abundance of poikilothermic intermediate hosts (insects, snails) and accelerate parasite maturation in them. However, the expected consequent increase in transmission intensity might be tempered by decreased survival rates of the same parasite stages and intermediate hosts at higher temperatures (Cable et al., 2017. Phil Trans R Soc B, 372: 20160088).
Comparatively, limited attention is being paid to the effects of extreme weather events on parasite diffusion and infection risks, although catastrophic events such as floodings, droughts and fires are increasing in frequency and intensity.
In 2021, the Marche Region experienced an exceptionally dry summer (40% less precipitations than in the 3 decades 1981 – 2010), forcing sheep breeders in the Apennine Mountains to modify grazing practices and integrate feeds with hay. Conducting monitoring of gastro-intestinal parasites in a farm in Valfornace municipality, we noted an increase in GIN egg counts and absence of *Moniezia* eggs in autumn of the same year. Mean faecal egg counts of GIN reached 624 (CI95: 458-790) EPG in Oct. 2021, compared to 420 (CI95: 297-543) in Feb. 2021, 409 (CI95: 278-540) in Jan. 2022 and 249 (CI95: 177-321) in Apr. 2022. *Moniezia* abundance resulted zero in Oct. 2021, compared to 83 (CI95: 39-127) in Feb. 2021, 105 (CI95: 6-204) in Jan. 2022 and 16 (CI95: 0-38) in Apr. 2022. The temporary absence of *Moniezia* might be explained by an impact on survival of the intermediate hosts, the oribatid mites. Regarding GIN, the temporary abundance increase after the dry period is more difficult to interpret: a relevant proportion of L3 might have survived the drought stress by moving into deeper, moist soil layers; and/or the prolonged permanence of the flock on a restricted pasture plot around the farm might have led to high L3 densities on that plot in early autumn; and/or host immunity to GIN may have been compromised by nutritional stress during the drought period, entailing a longer survival of GIN adults and higher egg production capacity by females.
This example illustrates how extreme weather events can exert exceptional stress on parasite host equilibria with possible detrimental effects either to the host or to the parasite. Furthermore, anthropogenic factors may play a pivotal role, as, e.g., event-related modifications of husbandry practices may significantly contribute to rise infection risks and undermine host resilience to parasites.
In conclusion, the increasing frequency and intensity of natural catastrophes calls for special attention of parasitologists in their role as practitioners, researchers, and teachers, to raise awareness, preparedness and capacity to prevent epidemics of parasitic diseases in both animals and humans.

IMPACT OF NATURAL DISASTER ON ANIMALS AND THE ROLE OF VET IN THE MANAGEMENT OF EMERGENCY AND PREVENTION

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According to the World Health Organization (WHO) an emergency can be define as “any situation in which the personnel and means available in a given area are insufficient for the implementation of an effective health intervention. These are sudden events that require immediate and effective action and which may be due to epidemic, natural and technological causes “. The role of vets is not always understood by the category and only following important telluric events its importance was defined. In this context I would like to propose disaster in which parasites or parasitologists with different roles was involved.

Technological (man made) disaster:
- Sahrawi refugee camps In Algeria (1975 - and for ever). *Echinococcus* and *Toxoplasma*, Giovanni Poglayen, Fabrizio Bruschi (Castagna et al., 2012. SOIPA XXVII Congress pag. 319)
- The diossina accident in Seveso (Milan province) in 1976. Bruno Romboli from Pisa University and with a different role a young student Giorgio Traldi a future parasitologist (Poglayen e Traldi, pers.com).
- In 1984 in central India: an accident at the Union Carbide chemical plant in Bhopal led to the release of more than 42 tons of methyl isocyanate, a chemical compound used for the production of pesticides. Ettore Biocca and Giuseppe Saccà (Poglayen, pers.com).
- Nuclear Cernobyl episode in1986. Teratogenic ostertagine (Manfredi & Genchi; ICASEP I -1991 pag.256 -257).
- During the balkanic wars (1991 - 2001) there was a dramatic collapse of public health systems. *Trichinella* and Fascioloides, Albert Marinculich (per. com).
- Episode of dramatic drought in 2017 in Sicily. Infection by nasal leches (Arfuso et al., 2019, J Med Vet Sci, 1 -10).

Telluric disaster:
- Irpinia earthquake in 1980. Disastrology born by Adriano Mantovani and first parasitologic approach. Giovanni Poglayen (Poglayen et al 1981; Parassitologia, 23, pag. 225 -226).
- In 1998 the first guidelines was born on veterinary action in disaster, Adriano Mantovani. They were updated in 2002 and currently it has merged into the National Prevention Plan, Raffaele Bove (Bove, 2018, Il Cervene, 111).
- Earthquake of L’Aquila in 2009. Veterinary Urban Igiene, *Echinococcus* and ticks. Giovanni Poglayen (per.com.).
- Earthquake in Emilia in 2012. Fight against arthropods and eco friendly rebuilding, Antonio Gelati (Gelati et al. 215; www.asoer.org). Manual for the future emergencies, Maurizio Ferraresi (Ferraresi et al. 216; Dipartimento Sanità Pubblica Veterinaria ASL Modena, UO Mirandola).

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ANTIPARASITIC DRUGS: EFFICACY AND RESISTANCE

FUNCTIONAL TARGETING OF *TOXOPLASMA GONDII* 14-3-3 PROTEIN: A PROMISING STRATEGY AGAINST TOXOPLASMOSIS

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Keywords: *Toxoplasma gondii*, 14-3-3, protein-protein interaction inhibition

INTRODUCTION: The protozoan *Toxoplasma gondii* is a zoonotic obligate intracellular parasite responsible for either acquired or congenital toxoplasmosis in humans. Currently, neither an approved vaccine to prevent human infection nor drugs able to eradicate tissue cysts in those chronically infected is available. Drug treatment limits to clinical toxoplamosis by combination of sulfonamides and pyrimethamine. Reported drug-resistant and severe side effects to current drugs, calls for the development of new treatments. Druggability of protein–protein interactions is emerging as an innovative therapeutic approach. 14-3-3s are a family of small dimeric proteins evolutionarily conserved in eukaryotes that bind to specific pSer/pThr motifs in the target proteins and act as conformation clamps to modulate enzyme activity, target intracellular localization or promote protein-protein interactions. Among the four 14-3-3 forms annotated in ToxoDB, Tg14-3-3-Api plays as central role being its knock-out in RH strain (type I) lethal for the parasite. Moreover, Tg14-3-3 has been related to pathogenesis by enhancing the migratory capability of immune cells and parasite dissemination to various host tissues.

MATERIALS AND METHODS: This work aims to characterize in depth Tg14-3-3-Api and assess the effect of selective inhibition of 14-3-3 binding on parasite development. We used ME49 strain (type II) allowing the analysis of three different invasive stages (sporozoite, tachyzoite and bradyzoite) both *in vivo* and *in vitro*. Tg14-3-3-Api expression and intracellular localization were investigated using mouse and rabbit pAbs raised against recombinant Tg14-3-3-Api. Affinity purification and LC-MS/MS analysis were carried out to characterize post-translational modifications and gain information on Tg14-3-3 interactors. To study the effects of Tg14-3-3-Api inhibition *in vivo* we exploited i) the CRISPR/CAS9 technique to drive the inducible expression of the 14-3-3 inhibitory peptide difopein in transgenic parasite line and ii) a natural small molecule binder of 14-3-3s.

RESULTS AND CONCLUSIONS: We demonstrate that Tg14-3-3-Api is expressed in all invasive stages. In extracellular tachyzoites the protein also accumulates in the cortex, whereas during replication it appears to be associated with the inner membrane complex of daughter cells. MS analysis shows Tg14-3-3-Api is phosphorylated, with phosphorylation affecting Tg14-3-3-Api binding activity. Putative parasite interactors of Tg14-3-3-Api were identified, including surface antigens and proteins involved metabolic pathways, signalling, maintenance and modification of the parasitophorous vacuole. Selective inhibition of Tg14-3-3-Api by either difopein or by addition of a natural molecule impairs tachizoyte replication (decreased capability to form lysis plaques).
In conclusion, our work supports druggability of Tg14-3-3 function as a promising anti-*Toxoplasma* strategy and provides evidence on the possibility to select molecules interfering with Tg14-3-3-target interactions.

EFFICACY OF TOPICAL ADMINISTRATION OF PRALLETHRIN-PERMETHRIN-PIPERONYL BUTOXIDE COMBINATION FOR THE TREATMENT AND CONTROL OF FLIES AND OTHER NUISANCE INSECTS IN HORSES

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Keywords: Flies and insects control, horses, prallethrin, permethrin, piperonyl butoxide combo

INTRODUCTION: Pyrethrins and pyrethroids have been widely used for many years to control insect pests (Franc et al., 2012. Vet Parasitol, 189: 333-37; Matsuo and Mori, 2012, "Pyrethroids: From Chrysanthemum to Modern Industrial Insecticide", Springer, 1-220). Their effectiveness against arthropods affecting different animal species is well known, but there is a lack of data regarding their use in horses. The aim of the study was to evaluate the repellent activity of a spray formulation based on prallethrin (0.033%) and permethrin (0.10%), synergized with piperonyl butoxide (0.50%), against annoying and harmful insects for horses in field conditions

MATERIALS AND METHODS: A horse stable in the Tuscany region, Italy was chosen for the trial. Twelve horses infected with a minimum of 15 flies were selected and divided into two groups (control and treated). Insect counts were performed on Day 0 (before product administration), and for three subsequent days, at 1, 10, 20 and 30 minutes and at 1, 2, 3, 4, 5 and 6 hours after the administration. The product (Bronco® Equine Fly Spray, Farnam) was applied on all parts of the horses, including mane, head and tail. Insect counts were carried out at different time intervals post-treatment, by three different operators.

RESULTS AND CONCLUSIONS: One minute after the administration of the product, all the horses were negative for the presence of insects. The repellent efficacy for *Hippobosca equina*, tabanid flies and *Simulium* spp. remained higher than 93% for all 4 days pt. Efficacy against *Musca domestica* and *M. autumnalis* was 100% after 1-minute and remained at this level for *M. autumnalis* till 6 hours. The efficacy against *M. domestica* decreased to 89.1% at 10 minutes pt and only reached 53.5% at 6 hours pt.
The treatment is safe and effective in killing and repelling insect pests in horses. Residual activity lasted four consecutive days after treatment.

TRANSPORTER GENE EXPRESSION AND *WOLBACHIA* QUANTIFICATION IN ADULTS OF *DIROFILARIA IMMITIS* TREATED *IN VITRO* WITH IVERMECTIN OR MOXIDECTIN ALONE OR IN COMBINATION WITH DOXYCYCLINE FOR 12 HOURS

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Keywords: *Dirofilaria immitis*, ABC transporters genes, *Wolbachia*

INTRODUCTION: Macrocyclic lactones (MLs) are widely used anthelminthic drugs. Due to their marked larvicidal activity, their use in the mainstay of *Dirofilaria immitis* prevention in dogs. They have been also shown to eliminate adult parasites after long-term administration, with a so called “slow-kill” effect (McCall, 2005. Vet Parasitol, 133: 197-206). In addition, recent studies have established that a combination of doxycycline, targeting the endosymbiont *Wolbachia*, with MLs has superior adulticide effects when compared to MLs alone (Bazzocchi et al., 2008. Int J Parasitol, 38: 1401-10). The apparent synergism between doxycycline/MLs may be due to interaction with drug efflux transport proteins. The aim of the study was to evaluate gene expression of several transport proteins in *D. immitis*.

MATERIALS AND METHODS: Adult parasites were treated *in vitro* either with doxycycline, ivermectin, moxidectin, doxycycline + ivermectin, doxycycline + moxidectin in RPMI medium for 12 h at 37°C, 5% CO2. Each treatment was performed in triplicates for both sexes. RNA and DNA were isolated from each worm for subsequent analyses. A quantitative Real-time PCR was used for relative gene expression of the following ABC-B transporters genes: Dim-pgp-10, Dim-pgp-11, Dim-haf-1 and Dim-haf-5 and the gene coding for a GluCl channel (Di-avr-14). The total amount of *Wolbachia* was defined by an absolute quantification amplifying a fragment of the ftsZ gene of *Wolbachia* and of the genomic 18S ribosomal subunit (18S rDNA) of *D. immitis*. Copy numbers of the two genes were subsequently normalized and bacterial loads were presented as ftsZ/18S rDNA (*Wolbachia*/nematode) ratios (Bazzocchi et al., 2008. Int J Parasitol, 38: 1401-10).

RESULTS AND CONCLUSIONS: Quantitative PCR analysis showed a sex-dependent response to treatments. In female worms, Dim-pgp-10 and Dim-haf-5 were upregulated compared to controls with doxycicline alone and when combined with ivermectin. In males, moxidectin administered alone induced a slight increase in Dim-pgp-10, Dim-pgp-11, Dim-haf-1 and Di-avr-14, while ivermectin in combination with doxycycline produced significant upregulation of the ML receptor Di-avr-14.
These results suggest possible synergism between the two drug classes and different susceptibility of males vs. females to adulticide effects.

EFFICACY OF TOPICAL EPRINOMECTIN (EPRINEX® MULTI) AGAINST *PRZHEVALSKIANA SILENUS* INFESTATION IN GOATS

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Keywords: *Przhevalskiana silenus*, goat warble fly, eprinomectin

INTRODUCTION: *Przhevalskiana silenus* is a fly causing a myasis known as goat warble fly infestation. In Italy, the parasitosis is distributed mainly in southern regions, and although the parasite does not induce significant mortality, it causes animal distress and economic losses mainly due to reduced milk production and skin lesions. Data on drug efficacy against *P. silenus* larval infestation in goats is limited. Eprinomectin 0.5% w/v topical solution (EPRINEX® Multi) was recently authorized in several countries in Europe at a dosage of 1 mg per kg body weight in goats and sheep as anthelmintic with zero milk withdrawal. Because of the known activity against bovine *Hypoderma* larvae, a negative controlled, masked clinical study was designed to assess its therapeutic efficacy against *P. silenus* larvae in naturally infested goats.

MATERIALS AND METHODS: Forty-five indigenous Sicilian mixed-breed dairy goats from an area known to be endemic for *P. silenus* were included in the study. An ELISA test for the detection of anti-*P. silenus* antibodies was carried out on Study Day (SD) -9 to determine the exposure to *P. silenus* and stratify the animals into three groups of 15 goats each: Group (G) 1, untreated animals; G 2, goats treated with Eprinomectin (0.5% w/v) on SD 0 (October 2019); G 3, treated on SD 167 (April 2020). The goats were physically examined prior to inclusion into the study and EPRINEX® Multi was administered pour on at the backline at 1 mL/5 kg according to the individual weight of the goats. Goats were examined for warbles on SDs 89, 103, 117, 131, 163, 174 and 181, and mature larvae were collected on SDs 174 and 181. On SD 186, the animals were euthanized and skin and carcass were examined for the remaining larvae.

RESULTS AND CONCLUSIONS: No warbles were detected in the G 2 goats at any examination. On SD 163, warbles were detected in seven and eight goats of G 1 and G 3, respectively. Following treatment of G 3 animals, mature larvae (alive or dead) were collected from G 1 and G 3 animals on SDs 174 and 181. Overall, goats treated once with EPRINEX® Multi on Day 0 or Day 167 had zero live *P. silenus* larval counts, while live *P. silenus* larvae were collected from six of the 15 untreated animals (1 to 3 larvae per animal). No adverse experience related to the treatment was observed in any goat. Topical EPRINEX® Multi treatment provided excellent efficacy against L1/L2 subcutaneous instars before damage to carcass and skin occurs, and against L3 when warbles were already present. Thus, treatment of goats during the clinically undetectable L1/L2 stages of infestation will prevent losses typically associated with *P. silenus* myiasis.

ANIMAL HEALTH AND LIVESTOCK PRODUCTION: ETHNOPHARMACEUTICAL VETERINARY MACERATE BASED ON *PUNICA GRANATUM* FOR THE CONTROL OF GASTROINTESTINAL NEMATODES AND IMPROVEMENT OF MILK PRODUCTION IN SHEEP

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Keywords: *Punica granatum*, animal health and production, *in vivo* anthelmintic efficacy

INTRODUCTION: Resistance to anthelmintic drugs in sheep gastrointestinal nematodes (GINs) has spread widely also in Europe (Rose et al., 2015. Vet Rec, 176: 546). This phenomenon negatively affects the health and sheep welfare, and consequently their productions. Therefore, it is a priority to identify alternative solutions for the control of GINs in sheep (Castagna et al., 2021. Vet Sci, 8: 237).

MATERIALS AND METHODS: Anthelmintic efficacy (AE) of a Calabrian etnoveterinary aqueous macerate based on *Punica granatum* (whole fruits), compared with Albendazole drug, was evaluated in Comisana pregnant sheep. In addition, qualitative and quantitative analysis of milk were performed. For the study, 45 sheep naturally infected with GINs and homogeneous for parasitic intensity, expressed in eggs per gram of feces (EPG), were selected. The sheep were divided into 3 groups of 15 animals: PGG, treated with *P. granatum* macerate, as single dose administered orally at 50 mL/sheep; AG, treated with Albendazole, administered orally at 3,75 mg/kg/BW and CG, untreated. The timing was: D0, allocation to groups, fecal sampling, and treatments; D7, D14, D21, fecal sampling and AE evaluation. The individual GIN fecal egg count (FEC) was determined with the FLOTAC technique, using a sodium chloride flotation solution (specific gravity=1.20) (Cringoli et al., 2010. Nat Protoc, 5: 503-15). The formula used to evaluate FEC reduction, 100×(1-[T2/C2]), was that recommended by the World Association for the Advancement of Veterinary Parasitology (Coles et al., 1992. Vet Parasitol, 44: 35–44). For measurement of productions, milk was collected after the weaning of the lambs, in these days (DL): DL35, DL42, DL49, DL56, DL63, D70, DL77, DL84. Quantitative assessments were carried out by measuring the individual milk production (expressed in ml) after each milking. The quality of the milk (protein content, casein, lactose, and fat, expressed as a percentage) was evaluated using MilkoScan™ FT+Foss Electric, Denmark.

RESULTS AND CONCLUSIONS: The results are reported in image below.
The ethnopharmaceutical macerate showed a significant AE (51.8%), thus improving the health and animals' welfare. Furthermore, its use has increased the quantitative (15.5%) and qualitative (5.5% protein, 4.1% casein, 4.3% lactose and 8.3% fat) milk productions. These improvements are presumably linked to the synergistic action of the components of phytocomplex (such as ellagic and gallic acid) identified using LC/HRMS, ESI (-). In conclusion, it can be said that better productions are obtained from animals in best health, therefore, considering the results of this study, it is recommended to consider the use of this green ethnopharmacological preparation for the control of GINs in sheep.

Groups	D_0	D_7		D_{14}		D_{21}	
	epg mean	epg mean	FECR %	epg mean	FECR %	epg mean	FECR %
PGG	460	264	55.7	298.8	53.8	387.6	46.1
AG	460	98	-	1.2	99.8	282	94.7
CG	496	1077.3	-	647.6	-	282	-

Groups	Milk production (ml)									
	DL35	DL42	DL49	DL56	DL63	DL70	DL77	DL	Mean	Increase (%)
PGG	1360	1340	1320	1300	1100	1000	760	560	1092*	15.5
AG	1480	1380	1380	1340	1200	1100	860	660	1183**	25.1
CG	1180	1160	1150	1080	970	880	670	460	945	

** p< 0.05 ** p<0.001*

Milk components (%)	Groups	Day lacting								
		DL 35	DL 42	DL 49	DL 56	DL 63	DL 70	DL 77	DL 84	Mean
Protein	PGG	4.9	5.1	5.1	5.1	4.7	5.3	5.2	5.7	5.11*
	AG	5.4	4.9	5	5	4.7	4.9	5.6	5.5	5.12**
	CG	4.4	4.7	4.8	4.7	4.7	4.8	4.9	5.3	4.9
Casein	PGG	3.6	4.1	4.1	4.1	3.8	4.3	4.2	4.8	4.12*
	AG	4.3	3.9	4.1	4	3.9	4.1	4.7	4.7	4.21*
	CG	3.4	3.6	3.9	3.8	3.8	4	4.2	4.6	3.91
Lactose	PGG	4.8	3.4	4.5	4.3	4.4	4.2	4.3	3.8	4.35
	AG	4.7	4.4	4.3	4.3	4.3	4.2	4.4	4.4	4.36
	CG	4.9	3.6	4.4	4.5	4.1	4.1	3.2	3.6	4.21
Fat	PGG	6.5	8	8.2	8.3	8.1	8.6	8.3	9.5	8.31*
	AG	7.7	8.5	9.5	8.5	8.5	8.6	9.2	9.9	8.84**
	CG	5.4	6.8	7.6	7.3	8.5	8.1	8.3	8.3	7.59

MONOVALENT IONOPHORES AS POTENTIAL ANTILEISHMANIAL AGENTS IN HUMANS AND DOGS

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Keywords: ionophores, *Leishmania*, antileishmanial agents

INTRODUCTION: Leishmaniasis is a tropical protozoal disease affecting thousands of people, especially in poor countries. Infected dogs are one of the primary reservoir in endemic regions and canine leishmaniasis is diffused in many regions of Italy, especially in the south but with wide diffusion also in the northern regions. Despite the presence of a vaccine available for the canine species, chemotherapy remains the most important strategy available. However, the increased drug resistance urgently requires the identification of new molecules. We have previously demonstrated that monovalent sodium and potassium ionophores, largely used in veterinary medicine and proposed as human anticancer agents, have antimalarial activity against asexual and sexual intraerythrocitic stages of *Plasmodium falciparum* (Pf). Monensin also showed anti-leishmanial activity on amastigotes of *Leishmania donovani* and *L. major*. Salinomycin is active against promastigotes of *L. donovani*. Here, we extended these observations to different *Leishmania* spp. and evaluated the activity against amastigotes in human and dog macrophages.

MATERIALS AND METHODS: *Leishmania* spp. promastigote viability and cytotoxicity on different cell lines (THP-1 Human monocytes; Human dermal fibroblasts; Human Microvascular Endothelian Cells; Immortalized Murine Bone Marrow Derived Macrophages) were assessed by MTT assay. Amastigotes were obtained by infecting with *L. infantum* promastigotes (1:10 cell promastigote ratio) human THP-1 or dog macrophages obtained by dog whole blood by Ficoll separation and differentiated to macrophages with PMA. Macrophage infection was measured by Giemsa staining and microscopic observation.

RESULTS AND CONCLUSIONS: The activity of monensin and salinomycin was confirmed on the promastigotes of different *Leishmania* spp. (*L. infantum*, *L. tropica* and *L. brasiliensis*) and the study extended to nigericin, which showed an inhibitory activity against promastigotes comparable to monensin (IC50 < 1μM), and higher than that of salinomycin (IC50 ~5μM). Experiments on *Leishmania* amastigotes demonstrated that all the ionophores had an IC50 of about 1.5-2 uM, with a selectivity index higher than 30 for monensin and nigericin. When dog macrophages were infected with *Leishmania* spp., higher percentages of infection were obtained compared to human macrophages, but the effect of the ionophores was lower. Although further studies are needed, the monovalent ionophores are promising anti protozoal agents for humans and dogs.

TRADITIONAL KNOWLEDGE AND USE OF THE ANTHELMINTIC FORAGE PLANT *ONOBRYCHIS VICIIFOLIA* (SAINFOIN) BY SHEEP BREEDERS IN THE APENNINE MOUNTAINS (MARCHE REGION)

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Keywords: sainfoin, bio-forage, gastro-intestinal nematodes

INTRODUCTION: Sainfoin (*Onobrychis viciifolia*) is a forage plant used for small ruminants. Beneficial responses of animals fed on sainfoin include improved growth, milk and wool production, fertility, and reduced methane emissions. Various *in vitro* and *in vivo* studies demonstrate the plant's anthelmintic effects related to condensed tannins, namely prodelphinidins and procyanidins (Mueller-Harvey et al., 2019. Crop Science, 59:1-25).
This study explores sheep breeders' knowledge and use of sainfoin in the Apennine Mountains (Marche Region) and its possible contribution to the control of gastro-intestinal nematodes (GIN) in local farms.

MATERIALS AND METHODS: A questionnaire was administered to 12 sheep breeders of advanced age. Open-ended questions addressed knowledge, perceptions and practices on sainfoin cultivation, properties and use.
Faecal samples were collected from 6 sheep farms in 2018 and 12 farms in 2021-22. One farm was surveyed from 2020 to 2022. Faecal samples (15-50 per farm) were analysed by the mini-FLOTAC technique with ZnSO4 flotation solution. The number of eggs per gram (EPG) of faeces were recorded and abundance values calculated as arithmetic means and 95%CI.

RESULTS AND CONCLUSIONS: Sheep breeders recognize and appreciate the nutritional benefits of sainfoin, stating that sheep 'like it very much', 'produce more milk' and 'the milk has a pleasant smell'. In contrast to lucerne, fresh sainfoin does not provoke meteorism. Some breeders give sainfoin preferentially to lambing ewes, physically decayed subjects, or lambs with growth retardation. However, none of the interviewees declares using sainfoin for parasite control or has heard about effects on sheep parasites. Farmers employ various forages, including lucerne and trefoil, according to soil characteristics and altitude of their cultivatable land. Therefore, the quantity of potentially anthelmintic sainfoin (hay or fresh) assumed by flocks varies considerably from one farm to another and according to the season. Breeders' parasite control strategy in the area mainly relies on yearly mass treatments with anthelmintic drugs. One farm was identified in which sheep are traditionally kept on sainfoin hay or pasture all year round, sainfoin making up about 50% of the total forage feed. In this farm GIN abundance in lambing ewes, assessed twice a year from spring 2020 to spring 2022, resulted as low as 22 (95%CI: 1-43) EPG. Anthelmintic drug treatment cannot explain this low value, given that the last treatment was administered in spring 2020 to 25% of the animals. For comparison, GIN abundance measured in 6 farms of the area in 2018 and in 12 farms in 2021-22 amounted to 294 (95%CI: 68-519) and 223 (95%CI: 125-321), respectively. On these farms sainfoin isn't used at all or is given just sporadically to the sheep.
Adopting a research action approach, sainfoin feeding schemes are currently elaborated with interested sheep breeders to promote farm-tailored, bio-forage integrated parasite control in the area.

PLATINUM-MEFLOQUINE COMPLEX IS AN ANTIMALARIAL AGENT AGAINST ASEXUAL AND SEXUAL BLOOD STAGES BY BLOCKING THE HEME DETOXIFICATION PROCESS

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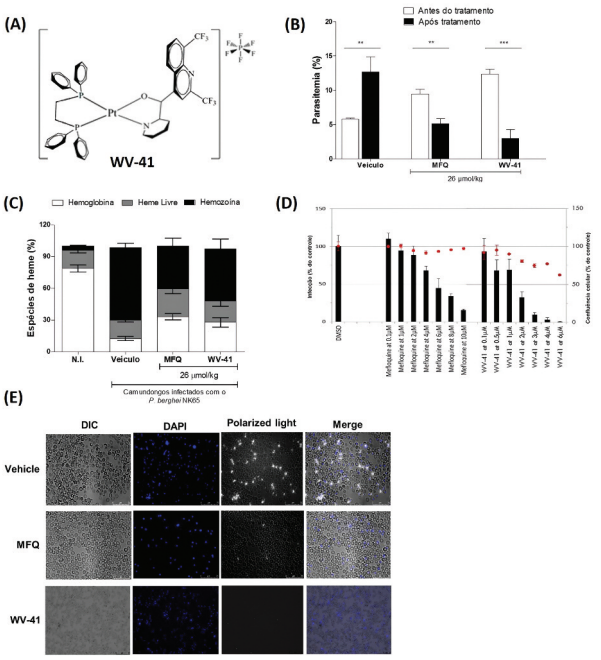
Keywords: malaria, antiparasitic drug development, WV-41

INTRODUCTION: Malaria, a disease caused by the unicellular pathogen *Plasmodium* spp., represents a major public health problem worldwide (OMS, 2021). Due drug-resistant parasite strains and limited efficacy of medicines in clinical use, the development of new antiparasitic drugs are necessary, especially with activity spanning multiple stages of the *Plasmodium* life cycle (Pereira et al., 2020; Quadros et al., 2021) and of susceptible for the circulating drug-resistant parasite strain. Thus, this study aimed to investigate the *in vitro* and *in vivo* pharmacological properties of the platinum-mefloquine complex named WV-41 against asexual and sexual *Plasmodium* blood stages.

MATERIALS AND METHODS: Initially, the mechanism of action of WV-41 was evaluated *in vitro* and *in vivo* through of inhibition of β-hematin and blockage of hemozoin biosynthesis assays, respectively. After, we assessed ultrastructural alterations in *Plasmodium* parasite using the transmission electron microscopy (TEM) technique and evaluated hemozoin blockage and parasitemia reduction using polarized light microscopy. The antiplasmodial activity was assessed against 3D7 or D10 chloroquine-sensitive and W2 chloroquine-resistant *P. falciparum* strains, gametocytes, and sporozoites. To determine the selectivity index, WV-41 was tested in HMEC-1 and BMDM cells lines in a cytotoxicity assay. Lastly, it was investigated the WV-41 potency in inhibiting the ring maturation of *Plasmodium*.

RESULTS AND CONCLUSIONS: From TEM analysis, WV-41 was able to inhibit the nucleation of hemozoin crystals as well as presented a similar profile to mefloquine in inhibiting the formation of β-hematin *in vitro*. *In vivo*, WV-41 was as potent as mefloquine in suppressing parasitemia, but mefloquine was twice more potent in inhibiting hemozoin biosynthesis. In cytotoxicity assay, WV-41 did not demonstrate cell toxicity to HMEC-1 and BMDM cell lines, being selective to parasite. When evaluating the inhibition of ring maturation, WV-41 was potent in arresting the ring development in trophozoites in all tested concentrations, while with mefloquine there was an increase in the number of trophozoites, and consequently a minor potency in inhibit the asexual blood stage; however, both drugs did not able to inhibit the maturation of trophozoites to schizonts. This is consistent to the peak of antiplasmodial activity and mechanism of mefloquine in the ring/trophozoites of the parasite cycle. For antiplasmodial and gametocidal activities, both WV-41 and mefloquine showed similar potencies against *P. falciparum* strains. Taken together, the results showed that the complexation of platinum metal with mefloquine culminated in WV-41 derivative, which presented a multistage profiling and a similar potency and efficacy to mefloquine against *Plasmodium*.



(F)

Compounds	*P. falciparum*, IC_{50} ± S.D. [µM]			Cells, CC_{50} ± S.D. [µM]		Selective Index[a]		
	CQ-sensitive 3D7	CQ-sensitive D10	CQ-resistant W2	HMEC-1	BMDM	3D7	D10	W2
MFQ	0,027 ± 0,01	0,023 ± 0,01	0,008 ± 0,001	9,86 ± 2,22	18,22 ± 3,50	675	795	2376
WV-41	0,024 ± 0,003	0,040 ± 0,01	0,01 ± 0,005	3,42 ± 0,85	11,78 ± 1,30	486	296	1096
CQ	0,019 ± 0,002	0,020 ± 0,005	0,37 ± 0,04	-	-	-	-	-

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COMPANION ANIMAL PARASITIC DISEASES

KILLING ME SOFTLY: SUCCESSFUL ERADICATION OF CANINE HEARTH WORM DISEASE IN THE ISLAND OF LINOSA, SOUTHERN ITALY

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Keywords: Flies and insects control, horses, prallethrin, permethrin, piperonyl butoxide combo

INTRODUCTION: Autochthonous endemic foci of *Dirofilaria immitis* infection in dogs have been recently described in southern Italian regions further supporting the southwards spread of this parasite. In the Linosa Island (Pelagie archipelago, Sicily) most of the dog population (58.9%) scored positive to *D. immitis* infection. Because of the high rate of infection and thanks to the epidemiological peculiarities, the island provided an exceptional opportunity to perform the first heart-worm disease (HWD) eradication campaign using the so called soft-kill protocol in all infected dogs, combined with monthly administration of 10% w/v imidacloprid and 2.5% w/v moxidectin as preventative strategy in non-infected animals.

MATERIALS AND METHODS: In October 2020 (T0) dogs of the island were physically examined, and blood samples were collected and analysed using Knott's test, a duplex real-time PCR for differentiation of *Dirofilaria* spp., and an ELISA rapid assay test for the detection of *D. immitis* circulating antigens. Infected dogs (G1), those that scored positive at least in one of the diagnostic tests employed, were treated with doxycycline (10mg/kg BID PO for 4 weeks) in combination with a monthly application of a spot-on formulation containing 10% w/v imidacloprid and 2.5% w/v moxidectin for 12 months. Non-infected dogs (G2) were monthly treated with the same spot-on product administered to dogs of G1 group for 12 months. All dogs were monthly followed-up (T1-T12), dogs in G1 were bleed and samples analyzed by Knott's test at T1 and T2 and/or by the ELISA antigenic test at T1, T2, T3, T6, T9 and T12. Dogs in G2 were tested for antigenemia at T3, T6, T9 and T12.

RESULTS AND CONCLUSIONS: On T0, 54 dogs (33 males and 21 females) were screened, of which 28 (51.85%) scored positive for *D. immitis*. Circulating microfilariae were found in 17 (60.7%) dogs, while *D. immitis* specific antigens were detected in 27 dogs (96.4%). On T1 a reduction of 76.5% of the number of microfilaremic dogs was recorded being only 4 still positive to Knott's test. On T2 and onwards, no microfilariae were found in the infected dogs. From T3 the number of antigenic positive dogs decreased progressively being 20, 10 and 2 on T3, T6 and T9, respectively. At the one-year follow-up (T12) all dogs in the G1 group tested negative for *D. immitis* circulating antigens.
Dirofilaria immitis has been successfully eradicated form the Linosa Island by treating infected animals with a protocol alternative to melarsomine and the adoption of chemoprophylaxis in non-infected animals. The epidemiological peculiarities of this HWD focus (i.e., geographically confined population, small number of dogs and absence of other wild hosts/reservoirs) enhanced the powerful of the study eradication program. The soft-kill therapeutic approach proved to be effective in eradicating HWD in a highly endemic area.

EFFICACY OF "MOXI-DOXY" AS ADULTICIDE TREATMENT IN DOGS NATURALLY CO-INFECTED BY *DIROFILARIA IMMITIS* AND *D. REPENS*

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Keywords: *Dirofilaria* spp., injectable and oral moxidectin, topical moxidectin

INTRODUCTION: *Dirofilaria immitis* and *D. repens* are vector-borne filarial nematodes that causes canine heartworm disease (HWD) and subcutaneous dirofilariosis respectively in many areas of the world (Genchi and Kramer, 2020. Vet Parasitol, 280: 108995). Several studies in both experimentally and naturally infected dogs have reported the adulticide effect of a combination of macrocyclic lactones (ML) and doxycycline against *D. immitis*, showing that these protocols are safe and effective (Bazzocchi et al., 2008. Int J Parasitol, 38: 1401-10; Savadelis et al., 2017. Parasit Vectors, 10: 245; Genchi et al., 2019. Vet Parasitol, 273: 11-16). Therefore, the present study aimed to evaluate the adulticide effect of different formulations of moxidectin (Moxi) (oral and injectable solution formulations), combined with doxycycline (Doxy) in dogs naturally infected with *D. immitis* (as mono-infection or co-infection with *D. repens*) in southern Italy. The efficacy of the oral and injectable formulations of Moxi were compared with the topical formulation of the same ML.

MATERIALS AND METHODS: Thirty dogs (28 co-infected with *D. immitis* and *D. repens* and two infected only by *D. immitis*) were enrolled (Day -30) from a shelter in southern Italy. Three treatment groups (10 dogs each) were formed: G1 (two doses of injectable Moxi at Days 30 and 180), G2 (Moxi per os, once a month), G3 (Moxi topical once a month). All the dogs were treated for 9 consecutive months together with Doxy, orally, once a day for the first 30 days. All the dogs were monitored monthly from Day 30 to Day 330 for *Dirofilaria* spp. circulating microfilariae (mff) and antigen (ag) of *D. immitis*. Thoracic radiography and cardiac ultrasound (CU) exams were performed at Days -30 and 180.

RESULTS AND CONCLUSIONS: In the groups G1 and G3, eight dogs resulted negative at the *D. immitis* antigen from Day 180 to Day 330. In G2, five dogs were negative for *D. immitis* antigen at Day 330. At Day 30, all the dogs of the three groups showed mff in the blood; at Days 60 and 90 only two dogs from the G2 were still positive for mff of *D. immitis* and *D. repens*, respectively. At Day 120, all the dogs were negative for *Dirofilaria* mff. All the dogs that presented mild interstitial lung pattern or diffuse and interstitial lung pattern before treatment, improved after 6 months of therapy. At the CU, only three dogs had slight/moderate alterations before treatment, but no change in CU was observed after therapy. Conclusions-The combination of moxidectin and doxycicline is effective in eliminating mff and adults of *Dirofilaria* spp. However, injectable and topical formulations resulted to be more effective than the oral one.

COMPARISON OF IHC AND QPCR METHODS FOR *LEISHMANIA* DETECTION FROM CANINE GRANULOMATOUS DERMATITIS OCCURRING IN CANL ENDEMIC AREAS

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Keywords: granulomatous dermatitis, immunohistochemistry, qPCR

INTRODUCTION: In canine leishmaniosis (CanL) endemic areas, pathologists often receive skin biopsies with histopathologic findings suggestive but not specific of CanL cutaneous lesions and, in the absence of data on the infective status of animals, the diagnosis can be challenging. Aim of the present retrospective study was to evaluate the ability of immunohistochemistry (IHC) and qPCR methods to detect *Leishmania* infection in formalin-fixed and paraffin-embedded (FFPE) skin sections from dogs living in an endemic area for CanL and having a histopathologic diagnosis of lymphoplasmacytic/histiocytic (LHD) and/or granulomatous (GD) dermatitis without any previous suspicion of CanL.

MATERIALS AND METHODS: The database repository of the Pathology Service of the Veterinary Teaching Hospital (OVUD) of Perugia was searched for histopathological records of skin biopsies of dogs living in areas recently estimated to be at medium-high risk for CanL (Di Muccio et al., 2012. J Clin Microbiol, 50:2651–9). Thirty FFPE blocks with features of LHD and/or GD were included and evaluated by hematoxylin-eosin (H&E), IHC (Tafuri et al., 2004. J Immunol Methods, 292:17–23), cPCR (Francino et al., 2006. Vet Parasitol, 137:214–21) and qPCR (Solano-Gallego et al., 2007. Vet Parasitol, 147:315–9). The severity, pattern and depth of the dermal inflammation and parasite load were graded. The frequencies of positive results obtained through H&E staining, IHC, cPCR, and qPCR were calculated and compared ($\chi 2$ test). The K coefficient between tests was determined. The correlation between the histological variables and grading of the parasitic load detected by H&E staining, IHC and qPCR was assessed using the Pearson test and the Spearman test.

RESULTS AND CONCLUSIONS: *Leishmania* was detected by H&E-stained sections in 8/30 samples (26.66%) and in 14/30 (46.66%) by IHC. Parasite DNA was detected in 14/30 cases (46.66%) by cPCR and in 21/30 cases (70%) by qPCR with a parasite load within lesions extremely variable (1.32-62.700 copies). The level of agreement between H&E and cPCR was fair (κ =0.32) and was moderate between H&E and IHC (κ =0.58); the agreement between IHC and cPCR was good (κ = 0.65), between IHC and qPCR was moderate (κ= 0.41) and between cPCR and qPCR was fair (κ = 0.28). A significant association was found between the severity of dermal inflammation and the parasitic skin load (IHC), although with weak linear correlation.
No single test is suitable alone for the diagnosis of *Leishmania* cutaneous lesions. In moderate/severe LHD and GD, IHC it has proven to be the only technique able of clearly defining the pathogenesis of lesions. However, when mild lesions occur, IHC loses its discriminatory power; in these cases, qPCR could be the more suitable test, given its greater detection ability, although the results should be interpreted with caution.

SEROEPIDEMIOLOGICAL INVESTIGATION ON *TOXOPLASMA GONDII* INFECTION IN DOGS

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Keywords: toxoplasmosis, dog, seroprevalence

INTRODUCTION: Dogs, as well as a wide variety of other warm-blooded animals, act as intermediate host of *Toxoplasma gondii*. In this species, most cases of toxoplasmosis are subclinical, although clinical disease has been sporadically reported, even in Italy (Migliore et al., 2017. BMC Vet Res, 13: 10–13); generalised toxoplasmosis can be seen mostly in young dogs and is characterised by fever, tonsillitis, dyspnea, diarrhea and vomiting (Greene, 2012. Infectious Diseases of Dog and Cat. Elsevier Saunders, St. Louis. 806-820). Hunting dogs may have a higher level of exposure to *T. gondii* bradizoite than other canine populations for their closer contact with wild intermediate hosts (Machačová et al., 2016. Folia Parasitol, 63: 0-12), while truffle dogs may have a higher level of exposure to oocysts contaminated areas. The aim of the present study was to evaluate the seroprevalence of *T. gondii* infection in dogs in Central-North Italy and to investigate the related possible risk factors.

MATERIALS AND METHODS: Sera were collected from 120 dogs from 20 municipalities located in Emilia-Romagna e Marche Regions in Italy. A questionnaire was submitted to the owners in order to obtain information about age, gender, function, lifestyle, cohabitation with cats, and coprophagy habits. The presence of IgG antibodies to *T. gondii* was detected by indirect fluorescence antibody test (IFAT), the sera were diluted with PBS starting at titre 1:40; a titre of 40 was considered positive. Statistical analysis was performed using STATA 12.0. Logistic and ordered multiple logistic regression was realized with prevalence and serum titre as dependent variables, respectively. Significance was set at $p<0.05$.

RESULTS AND CONCLUSIONS: The age of the 120 dogs ranged from 12 to 168 months (average 71.45); 44.2% were female and 55.8% male. As regards the function, 54.17% were hunting dogs, 24.17% were companion dogs, 14.17% were truffle dogs and 7.5% were watchdogs. Out of 120 dogs, 39.2% cohabitated with at least one cat, while 27.73% were coprophagic; none of the dogs lived always indoor, while 19.17% lived exclusively outdoor.
Thirtyfour dogs tested positive for *T. gondii* IgG. The maximun titre detected was 1:1280 in a 71 months old female hunting dog, living always outdoor with at least one cat, coprophagic.
The prevalence and titre were not related to dog sex and age. The lack of relation with age suggests a possible decrease of antibody titre with time. Coprophagy, cohabitation with cats and living always outdoors were significant risk factors, with titre-odds-ratios equal to 3.3, 2.8 and 6.8 respectively. The titre-odds-ratio of hunting dogs infection was 4 times higher than truffle dogs ($p=0.055$), although not fully significant. All the detected risk factors suggest that both the ingestion of oocysts (living with cats and coprophagy) and the predatory behaviour (living outdoor and hunting) are likely source of dog's *T. gondii* infection.

A SEVERE AND UNUSUAL CASE OF *SARCOPTES SCABIEI* INFECTION IN A CAT

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Keywords: feline sarcoptic mange, mites, skin lesions

INTRODUCTION: *Sarcoptes scabiei* causes sarcoptic mange in dogs and other mammals, while very little is known on the epidemiology, clinical features and treatment of feline sarcoptic mange (Morelli et al., 2021. Clin Microbiol Rev, 34: e00266-20). Past reports have shown that clinical signs of *S. scabiei* infection in cats may vary from non-pruritic crusted lesions to itchy and mild lesions, while severe signs are sporadic (Malik et al., 2006. J Feline Med Surg, 8: 327-39; Sivajothi et al., 2015. Comp Clin Path, 24: 1031-32). This report describes a case of severe sarcoptic mange in a cat.

MATERIALS AND METHODS: An adult male domestic shorthair cat was referred to a veterinary practice in Rome due to poor body conditions and a severe dermatopathy. At the clinical examination, the cat showed bilateral alopecia in the thigh regions, and hyperpigmented, crusty, erythematous, scaled and exfoliated lesions at the base of the tail. Other lesions, though less severe, were present on the head. The cat was hospitalized and subjected to complete blood count, serum biochemistry, fecal floatation, rapid tests for the detection of FIV, FeLV and Parvovirus (SNAP FIV/FeLV Combo test®, SNAP Parvo test® Idexx Laboratories), urinalysis, scotch test and skin scraping from head and tail regions.

RESULTS AND CONCLUSIONS: Mite eggs, larvae and adults were found in both scotch test and skin scraping. The mites were morphologically and morphometrically identified as *S. scabiei* based on morphometrical and morphological keys (Burgess, 1994. Adv Parasitol, 33: 235-73). The cat tested positive for FIV and *Dipylidium caninum* proglottids were detected in the feces. The cat was treated off label with a topical formulation containing esafoxolaner, eprinomectin and praziquantel (Nexgard combo® Boehringer Ingelheim), amoxicillin-clavulanic acid (20 mg/kg q12 per os) for bacterial superinfection and probiotics. One month later, the skin lesions improved and hair regrowth was observed. No further clinical examinations were performed due to lack of compliance of the owners and to the death of the cat for causes unrelated to sarcoptic mange. This case indicates that *S. scabiei* can reproduce and cause severe clinical signs also in cats, which are considered non-permissive hosts for this mite (Malik et al., 2006. J Feline Med Surg, 8: 327-39). It could be argued that the FIV infection had impacted on the immune system of the animal, thus favoring the clinical infestation as discussed elsewhere (Malik et al., 2006. J Feline Med Surg, 8: 327-39). In conclusion, feline sarcoptic mange may be underestimated due to lack of awareness and diagnostic constraints, i.e. false negative results or misidentification of mites. It is advisable that *S. scabiei* is included in the differential diagnosis of feline skin diseases especially in those cats with an impaired immune system.

ENDOPARASITES IN STRAY CATS AND DOGS FROM VENETO REGION: PRELIMINARY RESULTS

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Keywords: endoparasites, cats, dogs

INTRODUCTION: Intestinal parasites of dogs and cats are cosmopolitan pathogens with zoonotic potential for humans. In Italy, several taxa have been reported in Italy, with variable prevalence estimates (29.1% - 35.9%) depending on the study area, the category of animal (owned or stray) and the coprological exam technique. The epidemiology of endoparasites is continuously influenced by the movements of pets (unfortunately even illegal), so the risk of introducing new pathogens into currently free areas must be routinely monitored. In this study, we report the preliminary results of 1 year of coprological investigations on stray cats and dogs from shelters and kennels in the Veneto region.

MATERIALS AND METHODS: Over a 12-month study period (February 2021- February 2022) stool samples from feral colonies (cats no. = 101) and kennels (dogs no. = 137) of several provinces in the Veneto region were analyzed by copromicroscopic by flotation technique with zinc sulfate solution (specific gravity = 1.3). Additionally, all samples were tested using a filtration/sieving technique modified to enhance the likelihood to detect taeniid eggs (Mathis et al., 1996. J Helminthol, 70(3): 219–22). The cestode species was determined through multiplex PCR, targeting and sequencing ND1 and 12S genes. Before processing, all fecal samples were kept frozen at − 80 °C for at least 72 h to inactivate possible *Echinococcus* eggs.

RESULTS AND CONCLUSIONS: The overall prevalence of gastro-intestinal parasites was 69.3% (70/101) and 19.7% (27/137) in cats and dogs respectively (Table 1). *Toxocara cati* was the most common species among cats (45.5%) and *Trichuris* spp. among dogs (8.0%). *Taenia taeniaeformis* eggs were reported in three cats. All other animals were negative for taeniid eggs, thus we can exclude presence of *Echinococcus granulosus* and *E. multilocularis* in these animals. These results suggest that endoparasites are still common in pets and many of them are of zoonotic concern and vectors of pathogens for humans. As intestinal parasite positivity was found throughout the study period, year-round treatment should be considered according to the ESCCAP Guidelines 01 Sixth Edition 2021. Furthermore, in a One-Health concept, these preliminary data confirm the need to protect the human-animal bond by using appropriate endo- and ectoparasiticides to reduce the risk of human infection. This study was supported by the Italian Ministry of Health (IZSVe RC 12/19).

Table1. Prevalence (%) of endoparasites in shelter cats and dogs from the Veneto region.

Cats			Dogs		
Parasite	**Positive animals (n)**	**Prevalence (%)**	**Parasite**	**Positive animals (n)**	**Prevalence (%)**
Ancylostoma spp	16	15.8	Ancylostoma spp	5	3.6
Cystoisospora	6	5.9	*Cystoisospora*	5	3.6
Toxocara cati	46	45.5	*Toxocara canis*	6	4.4
Capillaria spp.	2	2.0	Trichuris spp	11	8.0
Positive	**70**	**69.3**	**Positive**	**27**	**19.7**
Total	101		Total	137	

COMPARATIVE PERFORMANCE EVALUATION OF METHODS FOR DIAGNOSING *GIARDIA* SPP. IN DOGS AND IDENTIFICATION OF ZOONOTIC ASSEMBLAGES

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Keywords: *Giardia*, zoonosis, diagnosis

INTRODUCTION: *Giardia duodenalis* (syn, *G. intestinalis* or *G. lamblia*) is a unicellular protozoan parasite that infects the upper intestinal tract a broad range of hosts including humans and domestic animals. Thus, it has raised concerns about the public health risk due to companion animals. Recently, with the improvement of living standards and increasing contacts with pets, the zoonotic transmission of *Giardia* has been dramatically increased. Molecular studies have shown that the species includes eight different genetic assemblages (A-H); C and D are canine specific, while A and B are most frequently found in humans, but they can also infect other animals (Cacciò et al., 2018. Infect Genet Evol, 66:335-45). The laboratory diagnosis of *Giardia* is mainly based on the finding of microscopic cyst in stool samples by direct smear or after fecal flotation. Other methods include the detection of antigens (RDT), indirect immunofluorescence (IFI) and PCR-based assay, which allows the identification of the different assemblages.

MATERIALS AND METHODS: With the aim to compare the performance of methods for diagnosing *Giardia* in dogs and to assess the assemblages circulating in canine population, we collected 200 faecal samples from owned dogs admitted at the CDVet Research laboratory for routine controls. Samples were submitted to microscopic examination after flotation, RDT (*Giardia* IC,Agrolab), IFI (Merifluor, Meridian Diagnostics) and PCR protocols (Tan et al., 2016. Par Res, 115: 2045-50). Assemblages were further identified from PCR-positive samples (Vanni et al., 2012. PLoS Negl Trop Dis, 6: e1776).

RESULTS AND CONCLUSIONS: Overall 60 out of 200 examined dogs scored positive for *Giardia*, with a prevalence of 30%. Microscopic examinations were positive for 46 (23%) faecal samples. The diagnostic performance of the methods compared here using microscopy as gold standard test, are summarized in Table 1. A total of 15 isolates were assigned to assemblage B while the remaining 45 ones to the dog-specific assemblage C. Microscopy is the reference standard for routine laboratory diagnosis in faecal parasitology but there is growing interest in alternative methods to overcome its limitations, i.e. time-consuming and highly dependent on an operator's skills and expertise. In this study, microscopy showed low sensitivity as detected *Giardia* cysts in 23% of the samples. Conversely RDT showed the best performance but it cannot distinguish between active or previous infection. PCR-based method displayed limitations maybe due to the difficulty of parasitic DNA extraction and the presence of PCR inhibitors in stool samples. IFI showed high specificity and good sensitivity. Therefore, it can be recommended as a complementary test to the traditional microscopy or RDT technique in the routine medical laboratory. The high prevalence of *Giardia* and the identification of potentially zoonotic assemblages underlines the potential risk for public health and suggests a frequent cross-species transmission between human and dog.

Diagnostis test	Positive/examined (%)	Sensitivity	Specificity	K value
RDT	58/200 (29)	84.70	87.32	86.71
IFI	56/200 (28)	71.73	86.09	84.26
PCR	60/200 (30)	54.32	76.82	71.52

EVALUATION OF THE *IN VITRO* ANTHELMINTIC ACTIVITY OF NATURAL COMPOUNDS AGAINST *TOXOCARA CANIS*, *ANCYLOSTOMA CANINUM* AND *EUCOLEUS AEROPHILUS*

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Keywords: dog nematodes, natural compounds, *in vitro* anthelmintic activity

INTRODUCTION: *Toxocara canis*, *Ancylostoma caninum*, and *Eucoleus aerophilus* are included among the most frequent endoparasites of dogs and in infected animals they may be responsible for severe clinical forms. Furthermore, all these parasites have zoonotic potential (Riggio et al., 2013. Vet Parasitol, 193: 78-84; Paoletti et al., 2015. Parasitol Res. 114: 2135-41; Sauda et al., 2018. Parasite, 25: 2; Traversa et al., 2019. Acta Trop, 193: 227-35; Tamponi et al., 2020. Am J Trop Med Hyg, 103: 1143-49). Infections caused by these nematodes are mainly controlled with the use of synthetic anthelmintic drugs. However, (multi)drug resistant strains of *T. canis* and, mainly, of *A. caninum* have been reported in some countries (von Samson-Himmelstjerna et al., 2021. Int J Parasitol Drugs Drug Resist, 17: 36-45). In addition, the interest of pet owners in therapies based on natural rather than synthetic substances has greatly increased in recent years.

MATERIALS AND METHODS: The aim of this study was to evaluate the *in vitro* anthelmintic activity of natural compounds against the egg development of *T. canis*, *E. aerophilus* and *A. caninum*. More specifically, a sea buckthorn berry juice (*Hippophae rhamnoides*) and different aqueous extracts from three multiflora bee pollen samples whose main chemical components are known, were selected. The activity of each tested compound was compared with negative (PBS, saline solution) and positive controls treated with a reference drug (thiabendazole). Data were statistically analysed.

RESULTS AND CONCLUSIONS: All bee pollen extracts demonstrated a significant inhibition of the *in vitro* egg development of all selected dog nematodes compared to negative controls, showing an anthelmintic activity comparable to that of reference drug ($P<0.05$). The sea buckthorn berry juice inhibited completely the development of *T. canis* eggs but was significantly less effective on *E. aerophilus* and *A. caninum* eggs. Obtained results encourage further studies aimed at identifying the main compounds potentially responsible for the anthelmintic activity of the sea buckthorn berry juice and bee pollen extracts tested in this study, also broadening the evaluation of the anthelmintic activity to different target stages of these parasites.

PREVALENCE OF *BARTONELLA HENSELAE* AND *BARTONELLA CLARRIDGEIAE* IN FREE ROAMING CATS IN NORTH EAST ITALY

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Keywords: *Bartonella henselae*, *Bartonella clarridgeiae*, cats

INTRODUCTION: *Bartonella* spp. is a zoonotic vector-borne haemotropic bacterium affecting a broad spectrum of mammals including humans. *Bartonella henselae* is the main causative agent of cat scratch disease (CSD) in humans, a self-limiting regional lymphadenopathy and cats are its main reservoir host. *Bartonella* spp. are vector-borne bacteria, and transmission of *B. henselae* by cat fleas occurs mainly through infected flea faeces, although new potential vectors (ticks and biting flies) have been identified (Chomel et al., 2010. Appl Microbiol, 109:743-50). Several studies have been conducted worldwide to determine the importance of cats as reservoir of this bacterium. Prevalence of infection varies considerably among cat populations (stray or pets) with an increasing gradient from cold climates (0% in Norway) to warm and humid climates (71.4% in Spain) (Boulouis et al., 2005. Vet Res, 36:383-10). This study evaluated the prevalence of *B. henselae* and *B. clarridgeiae* in stray cats in Veneto region, comparing molecular and serological detections.

MATERIALS AND METHODS: K3EDTA blood samples from 250 cats were collected from catteries of several provinces of Veneto region during the 2019-2021 period to be screened for *B. hanselae* seroprevalence and *B. henseale*/*B. clarridgeiae* by SYBR® green-real time PCR (Staggemeier et al., 2014. Rev Inst Med Trop Sao Paulo, 56: 93-5). Positive amplifications were confirmed and identified by sequencing.

RESULTS AND CONCLUSIONS: According to previous results, the present study confirmed that *Bartonella* spp. is widespread among cats in Northern Italy, showing a higher PCR positivity for *B. henselae* than for *B. clarridgeiae* (Table 1). The indirect immunofluorescent antibody test (IFAT) was used for the detection of *B. henselae*-specific antibodies in 174 out of 250 cats (69.9%), reporting serum titres ≥1:256 in more than 30% of positive cats. In addition, since serological tests are usually the gold standard methods, cross-reactivity in *B. henselae* serology has been reported (Vermeulen at al., 2010. J Med Microbiol, 59:743-45). Our preliminary results reported that 16 out of 60 (28.33%) cats serologically positive for *B. henselae*, were identified PCR positive for *B. clarridgeiae*. Cats have been identified as a notable reservoir of *Bartonella* species and may play an important role as source for human infection and environmental sentinels. This preliminary study highlights the need for further data on the molecular prevalence of *Bartonella* spp. and serological positivity in cats, in order to better understand the zoonotic risk in a One-Health view. This study was partially supported by the Italian Ministry of Health (IZSVe RC 12/19).

Table1: Feline *Bartonella* spp. prevalence (PCR) in North Italy.

	Prevalence (PCR)		
	N° of cats (pos/tot)	This study	Other studies
B. henselae	35/250	14,00%	27,1%
B. clarridgeiae	28/250	11,20%	1,5 – 6%
Coinfection	11/250	4,40%	nr
Overall prevalence	63/250	25,20%	1,3 - 17%

Nr = not reported

THE FIRST CLINICAL CASE OF HEPATOZOONOSIS IN A DOMESTIC CAT IN ITALY

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Keywords: cat, *Hepatozoon silvestris*, Italy

INTRODUCTION: *Hepatozoon* spp. is a vector-borne protozoa affecting several animal species all over the world. Hepatozoonosis in felids is almost unknown, but recently three species (i.e. *Hepatozoon felis*, *Hepatozoon canis* and *Hepatozoon silvestris*) were molecularly isolated from European domestic and wild felids (Giannelli et al., 2017. Ticks Tick Borne Dis, 8:721–24; Hodžić et al., 2017. Parasitology, 144:650-61). Infected felids are usually asymptomatic, and some clinical cases have been newly reported in domestic cats from Central Europe (Kegler et al., 2018. Parasit Vectors, 11: 428; Basso et al., 2019. Parasitol Int, 72:101945). We describe the first clinical case in Italy of hepatozoonosis in a domestic cat with a peculiar clinical picture.

MATERIALS AND METHODS: An 11-years old European short-hair cat, living in a hilly area of the Piedmont region, was hospitalized for a severe intestinal intussusception caused by a sessile endoluminal nodule in the jejunum. Blood samples were collected for haematology and clinical biochemistry; the intestinal nodule was surgically removed and histologically evaluated. In addition, molecular investigations targeting *Hepatozoon* SSU-rDNA were performed on surgical samples. Haematology was normal and the biochemical profile showed increased creatine phosphokinase (CPK: 2371 U/L; reference range: 52-542 U/L). Rare *Hepatozoon* gamonts were observed in granulocytes in the blood smear, then molecularly confirmed. Histological sections of the intestinal nodule revealed a severe inflammatory reaction characterized by chronic ulcerative enteritis with a polypoid proliferation and severe lymphangiectasia. Many inclusions similar to protozoan replicative forms were observed in enterocytes near the lumen with a high burden of infection in all histological sections. Molecular investigations in tissue samples confirmed *Hepatozoon silvestris* infection. After surgery, the patient was treated with doxycycline at 5 mg/kg/q24h for 30 days. The cat progressively improved and was fully recovered after two weeks with normalization of CPK.

RESULTS AND CONCLUSIONS: This is the first case of hepatozoonosis in a domestic cat in Italy. The unique manifestation of the infection makes this cat particularly interesting. Clinical signs are usually related to the tropism of *H. silvestris* for skeletal muscles and myocardium. In this case, the intestinal nodule was probably due to the inflammatory local reaction of the host around the site of protozoa penetration; the increased CPK might suggest subclinical myositis. Excision of the intestinal nodule and resolution of the intussusception was life-saving in this cat. Doxycycline treatment might have contributed to clearing the *Hepatozoon* infection.

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FARM ANIMAL PARASITIC DISEASES

OUTBREAK OF BOVINE BESNOITIOSIS IN A DAIRY CATTLE HERD IN NORTHERN ITALY: EARLY DETECTION OF THE INFECTION THROUGH SEROLOGY AND MOLECULAR TESTING

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Keywords: *Besnoitia besnoiti*, dairy cattle, acute infection

INTRODUCTION: Despite its economic impact on cattle breeding, bovine besnoitiosis (BB) is still a neglected and misdiagnosed parasitic disease. An early diagnosis of infection is essential to set up an effective control strategy (Gutiérrez-Expósito et al., 2017. Int J Parasitol, 47: 737-51), however often BB is not included in the differential diagnosis, particularly in the acute phase of the infection when the clinical signs (fever, respiratory problems) are nonspecific. An outbreak of BB in an intensive dairy cattle herd from northern Italy is reported with the main objective to depict diagnosis strategies and effects on bovine productions.

MATERIALS AND METHODS: In May 2021 (T0), four cows with clinical signs resembling *Besnoitia besnoiti* acute infection and distress as reported by individual animal tracking tags were observed. Detection of specific IgG (ELISA) and IgM (IFAT) and circulating DNA (Real Time PCR) (Cortes et al., 2007. Vet Parasitol, 146: 352-56) were performed. After the diagnosis of the first cases of infection, the farmer started a control program based on i) the progressive reform of seropositive animals, and ii) the control of vector insects. All animals in the farm (both cows and heifers) were screened combining the three tests both in July (T1) and November (T2). Productive data (monthly milk production) and individual records relating to distress (e.g., decreased rumination) as reported by tracking tags were collected during the study period (January-December 2021) and analyzed in relation to *B. besnoiti* seropositivity.

RESULTS AND CONCLUSIONS: At T0, all suspected cases of BB were confirmed: three cows positive to IgG, and one cow to IgM and Real Time-PCR. On the whole, 11.1% infected animals (n: IgG=21, IgM=9, PCR=1) were detected at T1; the seroprevalence at T2 showed a slight increase (12.8%), with six new cases of infection (n: IgG=24, IgM=3, PCR=0). During the study period, a total of 30 cows tested positive (at least at one sampling), while heifers always tested negative for *B. besnoiti*. Except from a cow with cutaneous clinical signs and three animals with scleral pearls and/or cysts on teets, after the febrile acute stage of infection no other animal developed detectable clinical signs during the study period. The analysis of the productive and physiological data of the bovines showed a greater number of distress events in infected animals compared to seronegative (40% vs. 21.7%), with a decrease in rumination in 36.6% vs. 13% of cases, respectively.
In the context of BB control strategies, 11 infected animals were reformed due to a drop in milk production during or after the study period. However, a statistically significant effect of BB infection on milk production could not be demonstrated.
The combination of different diagnostic tests allowed an early diagnosis of the outbreak, recognizing also animals still in the acute phase of infection. The promptly implementation of a control program effectively limited the spread of the infection within the farm.

ESTIMATION OF PREVALENCE OF ACUTE INFECTION FROM SEROLOGICAL DATA THROUGH A MATHEMATICAL MODEL: THE CASE OF *TOXOPLASMA GONDII* IN HERBIVORE HOSTS

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Keywords: acute toxoplasmosis, mathematical modelling, incidence

INTRODUCTION: Infection status in animals is commonly assessed by testing for antibody presence, thus obtaining seroprevalence estimates. However, a definition of the number of individuals that are in the acute phase of infection is crucial for surveillance, assessment of risk of transmission and efficacy of control strategies. *Toxoplasma gondii* represents one of the most diffused parasites on earth, infecting a broad range of host species, including humans. Most of the studies on *T. gondii* presence are carried through serological testing of chronic infection. Although this may provide insightful information on the risk of infection through meat consumption, these data can hardly inform on the incidence of new infections. Mathematical models, by simulating dynamics and mechanisms underlying the spread of an infection are a tool that can help us in understanding those dynamics leading to chronic infection. Thus, the aim of this research was to obtain an easily applicable tool to predict the prevalence of acute infections from serological data, through a simulation of the *T. gondii* infection in herbivore hosts.

MATERIALS AND METHODS: The acute prevalence of *T. gondii* was estimated through a mathematical model describing the infection in a goat population. The model is composed by three compartments representing infectious status of individuals (Susceptible, Acute and Chronic infected). The number of Susceptible individuals increases with hosts' birth rate (b) and decreases with their death rate (d) and the rate they acquire infection (λ). The number of individuals with Acute infection increases with the rate of new infection (λ) and decreases with their death rate (d) and recovery rate (σ). The number of chronically infected individuals increases with the recovery rate (σ) of acute infected individuals and decreases with their death rate (d).
Definitive hosts were not included in this model by considering a catalytic modelling where the rate of infection of an intermediate host is represented by the force of infection (i.e. the rate at which susceptible individuals become infected per unit time). Equilibria of the model were calculated and used to estimate the acute prevalence as a function of known prevalence of chronic infections, birth and host death rates and force of infection.

RESULTS AND CONCLUSIONS: In a goat flock with life span of five years, *T. gondii* seroprevalence of 30% and acute infection lasting for 14 days, the force of infection was estimated to be λ=0.0002 new infections/day and the equilibrium prevalence of acute infection was 0.23%.
The above-presented approach can be applied to estimate the number of new infections for risk assessment and for experimental design of new investigations where expected acute prevalence is needed. Furthermore, this model can be easily adapted for other infection routes (meat-borne) and more complex data sources containing age-structured seroprevalence data.

TOXOPLASMA INFECTION IN GOATS IN PAKISTAN: PRELIMINARY RESULTS OF A SEROPREVALENCE SURVEY

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Keywords: *Toxoplasma gondii*, goats' seroprevalence, Pakistan

INTRODUCTION: *Toxoplasma gondii* is a parasitic protozoan infecting all warm-blooded mammals, including humans. Toxoplasmosis has been associated with fetal mortality in both small ruminants and humans, as well as a spectrum of other symptoms. The immunocompromised are particularly vulnerable. Infection mostly occurs by the ingestion of contaminated vegetables/water and undercooked or raw animal products (meat/milk) from infected animals. Goats are the most common livestock in South Asian countries and consumption of unpasteurized goat milk is widespread.
Due to the lack of epidemiological data in Pakistan, this study shows the first data of a broader project aimed at investigating: a) the prevalence of *T. gondii* infection in goats based on antibody detection in serum and milk in Layyah District (Punjab Province, Pakistan); b) the potential for milk borne transmission of *T. gondii* through DNA detection in milk and sequencing; c) the risk factors associated with infection in goats and the public health significance of *T. gondii* in goats for consumers. Here we present the seroprevalence data.

MATERIALS AND METHODS: The seroprevalence of *T. gondii* in goats was estimated using a two-stage sampling design. The seroprevalence was assumed to be 40% (±10%), with an intra-farm coefficient of variation 0.01. All the farms in the study area were stratified according to their size and among them, one hundred and ten farms were randomly selected. From each flock, 12 goats (>1 year) were sampled between September 2021 to January 2022, resulting in a total of 1320 blood and 1320 milk samples. Sera samples were refrigerated at collection then transferred to Italy for testing for anti-*T. gondii* antibodies using a commercial ELISA multi-species kit (ID Screen, ID-VET. Montpellier, France) according to the manufacturer's instructions. A questionnaire was also administered to farmers to obtain information about conditions and management practices at each sampled farm.

RESULTS AND CONCLUSIONS: To date, 444 sera samples from 37 farms have been analyzed. *T. gondii* seroprevalence at the individual level was 32%, and over 90% of flocks had at least one seropositive animal. The percentage of infected goats within each positive flock ranged between 8.3% and 83%. Sample to Positive (S/P%) values ranged from 51.1 to 1,332.5 (mean: 133.63). Although both young and adult animals were seropositive, goats of 2-3 years were more likely to be seropositive (46.5%) than younger (1-2 years) goats (37.67%). All farmers replied to the questionnaire: 52.6% reported cats have access to goat houses, 82.4% recorded abortion history in goats, 90.3% of them and their family members drink raw milk. Although the data obtained at submission are preliminary, our results indicate considerable exposure of goats to *T. gondii* in this region. Overall, our results will provide data on the prevalence of *T. gondii* in goat farms, circulation of *T. gondii* subtypes, and the *Toxoplasma* risk associated with goat milk consumption in the study area.

ON-FARM BIOSECURITY: THE BOOTS AS CARRIERS OF PARASITES IN SHEEP FARMS

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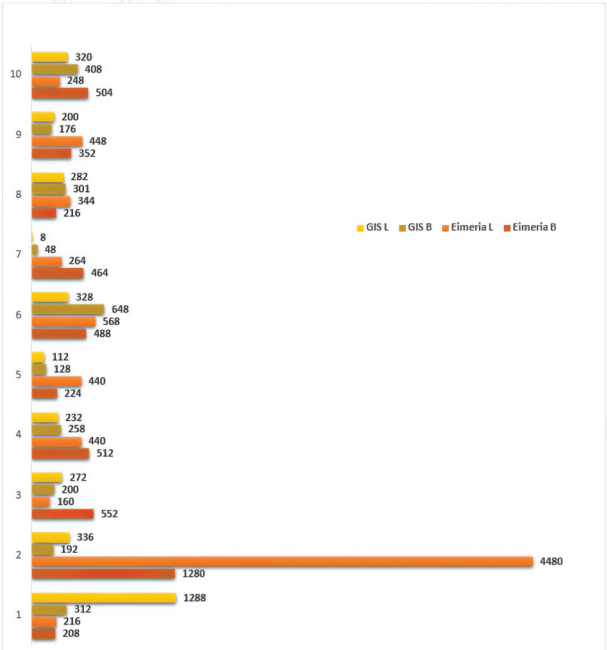
Keywords: sheep, biosecurity, parasites

INTRODUCTION: To date, there are no scientific studies about the principles of biosecurity in small ruminant breeding as in other species. From this consideration arises the need to deepen the knowledge of the main dynamics considered more at risk in the spread of diseases and to identify strategic actions (Kelly, 2005. ILAR journal, 46: 62-4; Robertson, 2020. Engineering, 6: 20-5).
This study is part of a project (RC19/2018) that has set itself the specific objective of determining the degree of contamination of the soil / litter on the farm by some of the most important etiological agents of sheep to evaluate the possibility that contaminated footwear, used by farm workers, can act as passive carriers of disease. The boots with litter under the sole that we used in the study were processed for parasitic and bacteria first, and only for germs after cleaning and disinfection. In this paper we report the results about the parasites research.

MATERIALS AND METHODS: We selected 10 sheep farms in Umbria region, those where information about parasites presence were already available. We sampled the litter in the box of ewes during the lambing period, divided into homogeneous sample units of 60 mq and three of these units were randomly chosen. A "walk" with 4 pairs of boots was carried out within each sample unit, following a continuous and uninterrupted path, on approximately 50% of the surface. Soil and feces from the sole of the boots were collected and examined with a quantitative parasitological examination (FLOTAC® Dual technique) (Cringoli et al., 2010. Nat Protoc, 5: 503-15). Five fresh stools ware also taken from the litter in the same parcel and processed in pool with the same technique.

RESULTS AND CONCLUSIONS: We found *Eimeria* spp. oocysts and unidentified gastrointestinal strongyles eggs in all the samples. *Trichuris* spp. eggs were in 2 boots and one litter, while *Nematodirus* spp. eggs only in one litter. Larvae of pulmonary nematodes were in 3 boots and 5 litters, and *Strongyloides* spp. eggs in 7 boots and 3 litters. We also found *Dicrocoelium dendriticum* eggs in 5 boots and 5 litters, and *Taenia* spp. eggs in 5 boots and 3 litters.
The results obtained clearly highlight how the footwear can act as a collector of pathogens.
In the case of fecal oocyst of coccidia and eggs of gastrointestinal strongyles the count was greater in almost all boots compared to the amount in the feces taken directly from the litter (Tab.1). It was not possible to assess in what concentration the pathogens are released into the environment starting from a contaminated boot, but assuming that the infectious dose of the pathogens investigated is rather small, the mere presence of the same must represent an element of risk for their diffusion. The knowledge of the spread dynamics of some pathogens could certainly help to define strategic action for proper animal husbandry of the future where biosecurity will be a keystones in the management.

Tab1. Fecal oocyst count media of *Eimeria spp.* (opg) and egg count of Gastrointestinal Strongyles (upg) (GIS) in boots (B) and litter (L) samples.



SEROLOGICAL SURVEY ON *SARCOPTES SCABIEI* IN WILD BOARS HUNTED IN AN ANTHROPIZED AREA AND IN SWINE FROM INTENSIVE FARMS IN NORTHERN ITALY

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Keywords: Sarcoptic mange, biosecurity, ELISA

INTRODUCTION: Sarcoptic mange is caused by the mite *Sarcoptes scabiei*, an obligate arthropod ectoparasitic, responsible for significant morbidity in both domestic and wild animals. Transmission between hosts can occur both directly (by prolonged skin to skin contact) and indirectly, since mites may apparently survive for some time off their hosts remaining infective in humid and cold environments. Data on the distribution of this ectoparasite in pig farms and in wild boars' populations are scarce and fragmentary worldwide. In this regard, the application of serological diagnostic methods allows to conduct epidemiological surveys to monitor the status of both domestic and wild animals.
Therefore, the aim of the study was to investigate the serological exposure to *S. scabiei* both in wild boars hunted in an anthropized area and in swine from intensive farms in northern Italy.

MATERIALS AND METHODS: 219 fattening pigs and 151 sows from 23 conventional farms in Lombardy were sampled. Data on farm management were collected and a "biosecurity score" (ranging from 1=poor, 2=moderate, to 3=optimal) was determined for each farm.
Blood samples were collected from 128 wild boars hunted in an anthropized area of the province of Cremona. Individual epidemiological data regarding estimated age, gender and killing place were collected.
Serum samples were analyzed using the commercial indirect *SARCOPTES*-ELISA 2001® Pig (AFOSA GmbH, Germany), according to the manufacturer's instructions.

RESULTS AND CONCLUSIONS: At the farm level, 69.6% (16/23) of the selected farms, 90.9% housing sows and 40% fattening pigs, scored positive. At the individual level, 43 animals (43/370, P = 11.6%) were positive to *S. scabiei* antibodies; a higher seroprevalence was detected in sows (35/151, P = 23.2%) if compared to fattening pigs (8/219, P = 3.6%). Moreover, a higher seroprevalence was recorded in farms with low and moderate sanitary score (P = 100% and P = 64.3%, respectively) if compared to farms with high sanitary score (P = 44.4%).
Considering wild boars, 9 animals (7.03%) had *S. scabiei* antibodies. Positive wild boars were mainly young animals (2 < 1 year old, 5 between 1 and 2 and 2 between 2 and 3 years old), including both male (no.= 3) and female (no.= 6) exemplars.
This study evidenced that *S. scabiei* circulates both in domestic swine from intensive farms and in wild boar populations in northern Italy. The infestation by this mite causes intense itching with skin irritation and lesions, resulting in a decrease in growth rate and feed efficiency, and therefore in economic losses for the intensive pig industry. Besides, since wild boar populations are nowadays in expansion in terms both of number of animals and habitat range, the increased frequency of contacts among wild boars and livestock could also influence the transmission of animal-specific pathogens. Further, zoonotic risks for humans derived from pig handling or carcasses managing should be considered.

FIRST RECORD OF *LIPOPTENA CAPREOLI* RÓNDANI, 1878 (DIPTERA: HIPPOBOSCIDAE) FROM ITALY

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Keywords: *Lipoptena capreoli*, goat, first record in Italy

INTRODUCTION: Louse flies (keds) of the family Hippoboscidae are a small group of obligate hematophagous ectoparasites of birds and mammals. The members of the subfamily Lipopteninae are either wingless or shed their wings after arrival on a host. As reviewed recently (Rehbein, 2021. Med Vet Entomol, 35: 254-56), it appears that in Europe there are records available for four *Lipoptena* species of which two, *L. cervi* and *L. fortisetosa*, primarily parasites of deer, have reliably been recorded in Italy. Already in 1879, Róndani suggested that *L. capreoli*, described on a single female fly collected at Cyprus island, may occur in southern Italy (Róndani, 1879. Bull Soc Ital Entomol, 11: 3-28). However, to the best of the authors' knowledge, *L. capreoli*, which is primarily a goat parasite, has not yet been reported from Italy.

MATERIALS AND METHODS: During activities for a *Przhevalskiana silenus* study in a farm in northeastern Sicily (Napoli et al., this SoIPa conference) in October 2019, keds were observed on one goat, which were collected and subsequently identified using a binocular microscope based on morphological characters. In 2020, 44 and 47 goats of the farm were examined in late April and late October, respectively, attempting to remove all keds by total body search. Keds were collected in dry tubes, counted, sexed and speciated. Pupae developed from prepupae deposited by female flies during storage after collection from the goats in October 2020 were incubated at ~28-30 °C. DNA was extracted from flies and molecular markers (partial COX1 gene and 16S rRNA gene sequences) were established.

RESULTS AND CONCLUSIONS: Morphological examination of the collected keds (all wingless) revealed the presence of one species, *L. capreoli* Róndani, 1878. Keds were collected from 4/44 goats [9.1%] examined in April 2020 (4/29 goats [13.8%] not recently treated for *P. silenus* myiasis) and 34/47 goats [72.3%] examined in October 2020. Intensity of infestation ranged from 1 to 3 keds in April 2020 (1.75±0.96) and 1 to 21 keds in October 2020 (3.41±3.73). The male to female ratio of *L. capreoli* varied among the animals; however, the combined male to female ratio of the keds collected in 2020 was close, 1÷1.02, indicating that no sex predominated. Winged young adult *L. capreoli* were obtained after about 30 days of incubation. Sequencing of the partial COX1 revealed 99% similarity to another GenBank® entry for *L. capreoli*. There was no *L. capreoli* 16S rRNA sequence deposited in GenBank®; however, the sequence established did not match with any 16S rRNA entry for hippoboscid flies. This investigation demonstrates a new record of a species of hippoboscid fly for Italy, *Lipoptena capreoli* Róndani, 1878.

AN AUTOCHTHONOUS OUTBREAK OF BOVINE BESNOITIOSIS IN CENTRAL ITALY

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Keywords: *Besnoitia besnoiti*, bovine besnoitiosis, outbreak

INTRODUCTION: Bovine besnoitiosis is a parasitic disease caused by the *Besnoitia besnoiti* (genus *Besnoitia*, family Sarcocystidae, phylum Apicomplexa). Besnoitiosis in cattle is considered a chronic debilitating disease characterized by weight loss, decrease in milk production, skin lesions, occasional abortion and sterility in males. Consequently, it causes serious economic losses in livestock farms (Alvarez-Garcia et al., 2013. Trend Parasitol, 29: 407-15). In Europe, bovine besnoitiosis was described as an emerging disease (EFSA Journal, 2010. 8: 1499); in Italy, probably it is introduced by the importation of animals from France and Spain, but recently some endemic outbreaks have been confirmed in different regions (Neve et al., 2022. Pathogens, 11: 122). This study examines an endemic outbreak of bovine besnoitiosis recently occurred in Tuscany, Central Italy, for to contribute to the epidemiological knowledge of this protozoan infection, in order to prevent its diffusion and to reduce the related economic losses.

MATERIALS AND METHODS: In September 2021, in a herd of beef cattle bred in Tuscany, a bull born and raised in Italy was found to have lesions referable to bovine besnoitiosis (mild alopecia and scleroderma more evident in muzzle, eyelids, scrotum and perineal region). During slaughtering operations, skin samples were collected from eyelids and perineal region to attempt a direct histological diagnosis by hematoxylin-eosin staining. While blood samples were collected from adult cattle over 12 months of age and serology for *B. besnoiti* diagnosis by ELISA was performed using ID Screen *Besnoitia* indirect 2.0 by IDvet, France.

RESULTS AND CONCLUSIONS: Histology confirmed the presence of many dermal and submucosal cists of 100- to 500-µm, composed by thick walls, containing banana-shaped bradyzoites (7.0/8.0 x 2.0 µm) and surrounded by an inflammatory reaction characterized by the presence of macrophages and eosinophils. Histological findings confirmed the presence of protozoa belonging to *Besnoitia besnoiti*. While, on a total of 225 serum samples collected, 93 tested animals are seropositive by ELISA (41,3%). These results confirm that bovine besnoitiosis is endemic in Italy, even in autochthonous cattles. The high seroprevalence suggests the necessity of being attentive to clinical signs, of using early laboratory diagnosis, of performing epidemiological investigations to evaluate the real distribution of this parasitosis and to provide control and prevention systems.

PRELIMINARY SURVEY ON BOVINE ECTOPARASITES IN NORTHERN-CENTRAL ITALY

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Keywords: livestock, ectoparasites, *Chorioptes*

INTRODUCTION: Cattle ectoparasitosis can be asymptomatic, but in some cases severe infestations can lead to critical health issues or cause economic losses. Continuous agitation and reduced food consumption due to itching lead to a decrease in animal production, and some ectoparasites can cause permanent skin damage, declining their value (Pangui, 1994. Rev sci tech Off int Epiz, 13: 1227-47). Recent works describing the prevalence of bovine ectoparasites in Italian farms are lacking. The aim of this research was to investigate this issue by carrying out a survey in northern-central Italy.

MATERIALS AND METHODS: In this study, 7 dairy or mixed farms of the provinces of Bologna and Modena and one beef farms of Florence province were visited over the period of May 2021 to October 2021. Whenever possible, samples were taken from all age groups: adult cattle, heifers and calves. A total of 315 animals were examined, both with and without lesions. Fifty ml Falcon type tubes and scalpel blades were used to perform a 5x5 cm scraping area. For each animal one sample from the base of the tail e one from the withers area were collected, with a total amount of 630 skin scraping samples examined. In addition, residual material from 8 mechanical brushes present in the dairy farms were collected. The samples were dissolved in 10% NaOH for two hours at 37°C, processed with flotation technique and observed at microscope. The keys of Séguy (1944. Faune de France- Insectes ectoparasites, Office Central de Faunistique, Paris) and Baker et al (1967. A Manual of parasitic mites of medical or economic importance. Henry Tripp, NY) were used for identification. Chi square tests were used to evaluate possible associations between the presence of ectoparasites and age, environmental factors, and husbandry conditions. Significant results were considered when $P \leq 0.05$.

RESULTS AND CONCLUSIONS: A total of 40 cattle out of 315 examined (12.7%) were positive for ectoparasites in at least one of the sampled skin areas, while all samples obtained from the brushes were negative. The presence of lesions was significantly greater in subjects with ectoparasites compared to negative ones ($\chi 2 = 59.16$; $P<0.0001$). The most widespread ectoparasite in dairy cows was *Chorioptes* sp. found in 30 (9.5%) animals from 5 out of 7 farms (71.4%) of the provinces of Bologna and Modena. Adult cattle were significantly more positive than younger subjects. Sucking lice were detected less frequently: *Haematopinus eurysternus* (6 cattle = 1.9% from 2 farms) and *Solenopotes capillatus* (3 cattle = 0.9% from 2 farms). *Bovicola bovis* was detected only in Romagnola breed cattle, 4 from the farm in province of Florence and in a mixed breed farm of the Bologna province; finally, dermatophytes (2 calves = 0.6% from one farm). The method used has made possible to detect ectoparasites even in asymptomatic animals and can therefore be a valid support to verify their presence in absence of macroscopic lesions.

TOXOPLASMA GONDII INFECTION IN SLAUGHTERED CATTLE FROM NORTHERN ITALY: MEAT-JUICE SEROLOGY AND MOLECULAR DETECTION

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Keywords: toxoplasmosis, foodborne zoonosis, cattle

INTRODUCTION: Toxoplasmosis represents an important public health issue, with the consumption of raw or undercooked meat a major way of human infection. In particular, the habit of consuming raw beef has been increasing in recent years, and the risk posed by *Toxoplasma gondii* infection in cattle should not be overlooked.
Since the antibody detection in cattle does not strictly correspond to the presence of parasite tissue cysts (Opsteegh et al., 2019. Int J Parasitol, 49: 515-22), the use of both serology and molecular methods allows to better assess the risk of *T. gondii* infection transmission from the consumption of meat originating from infected animals (Sroka et al., 2020. Parasit Vectors, 13:223). Therefore, to update information on *T. gondii* in cattle reared in Italy, an epidemiological survey combining serological and molecular tests was planned in steers slaughtered in northern Italy.

MATERIALS AND METHODS: Between January 2020 and March 2021, 144 steers were sampled from a slaughterhouse in Northern Italy. Both dairy and dual purpose breeds or crossbreeds from 37 farms located in northern Italy were sampled; age of animals varied from six months to three years. Data on gender, age, breed and movements were noted. Approximately 50 g of diaphragm was collected to obtain meat juice and muscle homogenate samples. Meat juice samples were analyzed with a commercial ELISA to detect specific anti-*T. gondii* antibodies. DNAs extracted from muscle homogenate samples were subjected to B1 real-time PCR. Statistical analysis of obtained data was performed by means of generalized linear models (GLMs).

RESULTS AND CONCLUSIONS: Anti-*T. gondii* antibodies were found in 17 (11.8%) examined animals, whereas parasitic DNA was detected in 41 diaphragm muscle samples (28.5%). Only nine samples scored positive in both test: a fair agreement between ELISA and real-time PCR results was achieved (κ value = 0.172). Nevertheless, higher ELISA S/P% values were recorded in diaphragm samples scoring positive to PCR (80.9 S/P%) vs. those scored negative to PCR (61.03 S/P%). Higher number of positive samples were found in younger than older animals considering both ELISA and B1 real-time PCR results. Similarly, animals that have been acquired from other holdings scored more frequently positive to both ELISA and real-time PCR compared to animals that have never left the farm of origin until slaughter. Statistical analysis showed an effect of ELISA S/P% values on real-time PCR results, increasing the risk of parasitic DNA detection when increasing the S/P% values. Animal trade was also statistically associated with positivity (ELISA or real-time PCR), with animals purchased from other Italian farms more at risk than animals that have never been moved from the farm of origin. The study confirmed the role of beef meat as a potential source of *T. gondii* infection for humans. Considering the consumption of raw beef preparations in many regions of northern Italy, the zoonotic importance of *T. gondii* from beef should not be neglected.

A REVERSE TRANSCRIPTASE-QUANTITATIVE PCR STUDY ON HONEYBEES CO-INFECTED WITH *NOSEMA CERANAE* AND CHRONIC BEE PARALYSIS VIRUS

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Keywords: honeybees, *Nosema ceranae* and chronic bee paralysis virus, reverse transcriptase - quantitative PCR

INTRODUCTION: The Microsporidian parasite *Nosema ceranae* and Chronic Bee Paralysis Virus (CBPV) are two important pathogens of honeybees (*Apis mellifera* Linnaeus) that share the ability to infect the digestive tract of the host (Porrini et al., 2016. PLoS ONE, 11: e0155411). In this regard, there is a lack of studies that clarify the possibility of a synergistic effect. In order to detect and quantify the presence of CBPV in the host, it is necessary to resort to molecular techniques like Reverse Transcriptase - quantitative PCR (RT-qPCR). With regard to *N. ceranae*, the commonly used molecular methods target the genomic DNA contained in the spores and are not validated for the analysis of RNA extracted from vegetative forms. The purpose of this study was to verify the existence of a possible correlation between the viral load of CBPV in the intestine of honeybees, sampled from colonies showing symptoms of chronic paralysis, and active *N. ceranae* infection measured through the development of a suitable methodology based on RT-qPCR.

MATERIALS AND METHODS: In spring 2021, sampling was carried out in two apiaries, located respectively in Settala (MI) and Lodi (LO), in which the presence of symptoms apparently attributable to CBPV in some colonies was detected. In these colonies, both bees on honeycombs with apparent symptoms of CBPV at different intensity and foraging bees were sampled; the latter were analyzed by optical microscopy for the search for spores of *Nosema* spp. (Fries et al., 2013. J Apic Res, 52: 1-18). RNA was then extracted from the digestive tract of 23 bees sampled on honeycombs. Once extracted, the RNA samples were quantified, back-transcribed to cDNA and analyzed by RT-PCR Real Time with the TaqMan method for CBPV (Blanchart et al., 2007. J Virol Methods, 141: 7-13) and to the housekeeping gene Rpl32 (Reim et al., 2013. Apidologie, 44: 342-50). For the analysis of *N. ceranae* RNA, a new RT-qPCR protocol was designed based on a pair of primers for the 16S ribosomal RNA gene, spanning a TaqMan probe target site. The amplification performance of this new protocol was determined by constructing standard curves starting from serial dilutions of both genomic DNA at known concentration and cDNA from field samples. The data relating to the viral and *N. ceranae* load were then normalized with reference to expression level of the housekeeping gene and were then subjected to statistical correlation analysis.

RESULTS AND CONCLUSIONS: An RT-qPCR protocol for the analysis of *N. ceranae* starting from RNA was designed and validated, with an amplification efficiency equal to 104% and a linearity (R2) equal to 0.99 in the range from 108 to 100 copies of target cDNA/amplification well. In honeybees subjected to the quantitative determination CBPV and *N. ceranae* load, the statistical analysis did not show any significant correlation between the level of infection of the two pathogens (R = 0.08; R2 = 0.007).

A SURVEY ON ENDOPARASITES OF ALPACAS (*VICUGNA PACOS*) BRED IN ITALY

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Keywords: Alpaca (*Vicugna pacos*), endoparasites, Italy

INTRODUCTION: Alpacas (*Vicugna pacos*) are camelids native to the Andean region of South America where they are mainly bred for meat and wool. Due to their high adaptability and the increased interest in this animal species, alpacas have recently become popular in Europe mainly for wool production and as pet animals. Endoparasites are considered a major health concern in alpacas often resulting in clinical diseases and economic losses (Ballweber, 2009. Vet Clin North Am Food Anim Pract, 25: 295-310; Rosadio et al., 2010. Vet Parasitol, 168:116-20; Thomas and Morgan, 2013. Vet Parasitol, 198: 244-49; Hilbe et al., 2015. Vet Pathol, 52: 1202-09; Frezzato et al., 2020. J Vet Med Sci, 82: 1655-61). Few data are available on parasites of alpacas bred in Italy.

MATERIALS AND METHODS: The evaluation of gastrointestinal parasites in Italian alpaca farms represented the main objective of this study. To this aim, 157 alpacas of different age and sex from three extensive farms in central and southern Italy (Florence, Grosseto, and L'Aquila) were examined. In all farms, gastrointestinal parasite control is based mainly on six-monthly anthelmintic treatments. Individual fecal samples were collected from all animals and examined by qualitative (flotation, sedimentation-flotation with low/ high-density flotation solutions) and quantitative parasitological techniques (a McMaster method with a sensitivity of 50 eggs per gram of feces-EPG/oocysts per gram of feces-OPG). *Eimeria* species were identified based on the morphology of sporulated and unsporulated oocysts.

RESULTS AND CONCLUSIONS: Results showed that 107 out of 157 examined samples (prevalence 68.1%) were positive at least for a parasite species. Among the 107 positive samples, 86 (80.4%) tested positive for two or more parasites. Different prevalence rates were found in the three examined farms, i.e., Grosseto 54%, Florence 66.7%, and L'Aquila 100%. Gastrointestinal strongyles and coccidia were found prevalent in all farms, mostly in young animals and adult females. Overall, gastrointestinal strongyles were identified in 91/107 (85%) positive samples with EPG levels ranging from 50 to 800 and included *Nematodirus* sp. infections (4.7%, 5/107), while 86/107 samples (80.4%) tested positive for coccidia (OPG ranging from 50 to 4000) and the species *Eimeria alpacae*, *Eimeria lamae*, *Eimeria punoensis*, and *Eimeria macusaniensis*, were identified. Some adult animals of the Florence farm were found positive also for *Capillaria* sp. (2.8%), *Trichuris* sp. (2.8%), and *Dicrocoelium dendriticum* (3.7%). The high prevalence of endoparasites found in the examined alpacas, especially of gastrointestinal strongyles and coccidia, highlight the need to deepen studies on the economic and health impact of these parasites and their diffusion in alpaca farms in Italy. Moreover, the use of effective control measures based not only on the regular use of anthelmintic drugs should be implemented.

THE DIVERSITY OF ARTHROPOD ECTOPARASITES OF GOATS IN SICILY, ITALY

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Keywords: goat ectoparasite, multiple ectoparasite infection, *Lipoptena capreoli*

INTRODUCTION: Although a range of parasites is known that inhabit the skin and/or hair coat of goats, limited information is available for goats from Italy; especially studies assessing the diversity of ectoparasites of goats (parasites infesting goats concurrently) are lacking. In conjunction with a study to evaluate the efficacy of topical eprinomectin treatment against *Przhevalskiana silenus* myiasis (Napoli et al., this SoIPa conference), goats of one farm in northeastern Sicily were surveyed for ectoparasites at two time points of the year 2020.

MATERIALS AND METHODS: Forty-four adult animals (7 male, 37 female) were examined at slaughter in April, and 47 goats (4 male, 43 female) were examined in October. In all goats, skin and hair coat of the entire body were thoroughly searched for ectoparasites using flea combs brushing and deep ear swabs from both ears were collected. In addition, pieces of skin cut from the back of the pasterns on both hind legs were collected from the slaughtered goats. Ear swabs and skin pieces were digested in hot 10% potassium hydroxide solution, and all parasites collected or recovered in ear or skin samples were identified to genus and/or species based on their morphology.

RESULTS AND CONCLUSIONS: 82% of the goats examined in April and 76% of those examined in October were found infested with ectoparasites. Ten species of arthropod ectoparasites were recovered: 3 species of mites (ear swabs: *Psoroptes* sp.; pastern skin: *Chorioptes bovis* and *Demodex caprae*), 2 species of ticks (*Ixodes ricinus*, *Rhipicephalus sanguineus s.l.*), 3 species of lice (*Bovicola caprae*, *Linognathus africanus*, *L. stenopsis*), and 1 species each of ked (*Lipoptena capreoli*) and flea (*Ctenocephalides felis*). All the 10 arthropod parasite species were recorded in the goats examined in late April while no species of arachnids but the five species of insect parasites were recovered in the goats examined in October. Ectoparasite positive goats examined in April hosted between 1 and 7 species of ectoparasites concurrently, while positive goats checked in October were hosts for 1, 2 or 3 species of ectoparasites concurrently. Substantial differences in the frequency and intensity of ectoparasite infestation were observed between goats examined in April or in October, and between sub-groups of goats included in the *P. silenus* study relative to their treatment record (April examination). This investigation shows that native goats in Sicily, when systematically examined, can be demonstrated to host a diverse fauna of ectoparasites of several taxa of arachnids and insects. Each of the ectoparasites has been reported previously from goats; however, at least one constitutes a new record for Italy (*L. capreoli*) and one other a new record for Sicily (*L. africanus*).

FIRST REPORT OF *ASCARIS SUUM* SPECIMENS IN THE BILIARY DUCTS OF A PIGLET

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Keywords: *Ascaris suum*, pig, bile ducts

INTRODUCTION: The present study reports an unusual localization of *Ascaris suum* specimens in the bile ducts of a piglet. To the best of our knowledge, this is the first case in literature reporting this particular localization of *A. suum* in a piglet.

MATERIALS AND METHODS: A 30-days-old piglet slaughtered in February 2022 in the Oristano province presented *Ascaris* spp. specimens in the hepatic bile ducts. The piglet was the seventh of a litter born from a 3-year-old *Sarda multiparous* sow in which eggs of *Ascaris* spp. were found through the copromicroscopical examination. At the time of the home slaughtering, according to the Legislative Decree 2 February 2021 n.27 which provides the inspection in the 10% of the home slaughtering, the official veterinarian did not attend, and the intestine was discharged. Liver was subjected to an ultrasonography and selected tissue samples of the liver showing ascarid lesions were fixed in 10% buffered formalin for the following histological examination. DNA was extracted from the ascarids collected and used for the molecular identification carried out according to Zhu et al. (1999. Int J Parasitol, 29:169-78).

RESULTS AND CONCLUSIONS: The presence of ascarids in the bile ducts was detected only in one piglet of the whole litter. A total of 43 preadult nematodes were found occupying the gallbladder and the small and large bile ducts, with a mean length of 8 cm and a mean width of 1.7 mm. Parasites were molecularly identified as *A. suum*. Macroscopically, the liver was moderately enlarged, with accentuated lobular pattern and scattered pales areas admixed with multifocal haemorrhages. Multifocally, the parasitized bile ducts were severely enlarged. Expanding portal areas and compressing adjacent sinusoids and hepatic cords was a severe, chronic inflammatory infiltrate often centred around up to 5 mm in diameter ectatic bile ducts lined by hyperplastic epithelium (biliary epithelial hyperplasia) and filled by sections of nematodes. Within the lumen of bile ducts, sloughed epithelial cells and cellular debris were longitudinal up to 4 mm and transverse (up to 1.5 mm) sections of nematodes with a smooth cuticle, coelomyarian musculature, prominent lateral alae, intestinal tract lined by columnar uninucleated cells within a pseudocoelom. For *in vivo* diagnosis, the liver ultrasonography could be suggested as observed in this study it allows the detection of the parasites in the bile ducts. Unfortunately, the absence of the intestine does not allow to define the pathogenesis of the infestation. However, as reported in infestation by *Ascaris lumbricoides* in humans, the *A. suum* specimens may have migrated to the liver from the intestine through the choledochus (Anup, 2014. J Glob Infect Dis, 6:65-72). Alternatively, the worms may have passed through the intestinal wall and have migrated through the portal system to the liver and lung (Husin et al., 2021. Malays Fam Physician, 16).

EPIDEMIOLOGICAL SURVEY ON GASTRO-INTESTINAL HELMINTH INFECTIONS IN DONKEYS IN SARDINIA

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Keywords: donkeys, helminth infections, cyathostomins

INTRODUCTION: In Sardinia there are 4676 registered donkeys located in 1653 farms (Banca Dati Nazionale, 2015). Increased use of donkey products and employment of the species in pet therapy, call for a revision of health and farm management practices (Veneziano et al., 2011. Vet J, 190: 414–15). The present study aims to assess the prevalence and distribution of main gastro-intestinal nematodes (GIN) in donkeys in Sardinia.

MATERIALS AND METHODS: The study was carried out in Sardinia from January 2021 to April 2022 and it involved 400 donkeys from 35 farms. Each faecal sample was accompanied by a form filled in with the donkey's information and a questionnaire on management and parasite control practices in the farm. Coprological examination was carried out using the modified McMaster technique as described by Raynaud (1970. Ann Parasitol, 45:321-42) using a sodium chloride (NaCl) supersaturated solution (specific gravity = 1.2) for flotation and a detection limit of 15 eggs per gram (EPG) of feces. Pooled fecal cultures were set up for each farm using samples with a fecal egg count (FEC) greater than 300 EPG. The third-stage larvae (L3s) were identified using the available morphometric keys (Cernea et al., 2008. The Atlas of equine strongylidosis. Ed Academic Pres. Cluj-Napoca, Romania).

RESULTS AND CONCLUSIONS: Gastrointestinal Strongyles appear to be the most widespread parasites (84.75%), followed by *Parascaris* spp. (4.5%), *Oxyuris equi* (0.25%). In addition, oncospheres of the cestode *Anoplocephala* spp. were found in 0.25%. The average EPG of GIN was 1421.2 ± 1280 (min 0–max 7035). The 68% of examined donkeys showed EPG values ≥300 EPG, the suggested 300 EPG selective therapy cut-off (Matthews and Burden, 2013. Equine Vet Educ 25: 461–67). More specifically, 16.7% of donkeys had a FEC < 300 EPG (low contaminators), 10% had a FEC ranging between 300 and 600 EPG (moderate contaminators) and 58% had a FEC > 600 EPG (high contaminators). The infection rate for females, intact males and geldings was 85.6%, 81.5 % and 85.7%, respectively. Analysis of data based on animal age showed the higher prevalence (89%) in animal aged between 4 and 10 years ($\chi 2$ trend=4.835, P=0.027). Larval examination revealed in all samples exclusively the presence of cyathostomins (100%). The data from the questionnaire show that all donkeys live on pasture (100%), while several (65.2%) have at least one covered paddock. Most of the donkeys were mainly involved for reproductive purposes (56.5%), followed by other uses such as recreational outdoor activity and onotherapy (39.2%), while only two farms produced donkey's milk (4.3%). The highest percentage of owners (56.5%) dewormed their donkeys once a year (56.5%) but only few of them (21.7%) performed previous coprological examinations. The EPG values observed in this study are compatible with considerable pain state related to this parasites burden, highlighting the need of appropriate control measures.

ISOLATION AND MORPHOLOGICAL OBSERVATIONS ON *LOTMARIA PASSIM* (EUGLENOZOA, TRYPANOSOMATIDAE)

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Keywords: *Lotmaria passim*, isolation, morphology

INTRODUCTION: The presence of monoxenous trypanosomatids in the digestive system of honey bees has been increasingly recognized and it has been suggested their potential contribution to honey bee health decline, although the details of their pathogenic effects are still not fully understood. Until a few years ago, these trypanosomatidae were attributed to one recognized specie *Crithidia mellificae*. Nevertheless, after its recent description (Schwarz, 2015. J Eukaryot Microbiol, 62: 567–83), the species *Lotmaria passim* is presently acknowledged as the most prevalent *Apis mellifera* trypanosomatid in all parts of the world (Europe, USA and South America) where its prevalence has been evaluated at large scale (Ribani et al., 2021. J Invertebr Pathol, 184: 107628). In this report, we describe a simple method for isolation of *L. passim* from bee gut and the morphological characteristics observed in isolates from apiaries of Emilia Romagna region.

MATERIALS AND METHODS: Each isolation was performed on single honey bee guts. The bee was immobilized at - 20 ◦C for 4–5 min, briefly washed in 99% ethanol, and decapitated. The intestine was removed with sterile tools, submerged in 0.5 mL of supplemented DS2 medium (CorningTM Insectagro DS2 serum free/protein free medium without L glutamine) plus 5% fetal bovine serum and 1% Antibiotic/Antimycotic solution in a 1.5-mL microtube, gently grinded with a sterile pestle and incubated at 26 ◦C. Ten µL of each culture were observed on wet mount slide, after 3 and 7 days to verify the presence of free active flagellates. The cultures were maintained by subculture steps every 4–10 days in fresh medium (ratio 1:5). Cultures of different age were prepared for microscopic observation of wet and stained slide (May Grunwald Giemsa, MGG) and for Scanning Electron Microscopy (SEM).

RESULTS AND CONCLUSIONS: Wet slides from young cultures showed the presence of active moving elongate flagellates and of cell aggregates, known as “rosettes”, described also for *Leishmania* spp. (Iovannisci et al., 2010. J Eukaryot Microbiol, 57: 405–14). In older cultures, the presence of spheroid forms was observed more frequently in MGG stained slides, the predominant forms were elongated and tear-drop shaped cells typical of a promastigote morphotype that narrowed posteriorly to a short caudate (tail-like) extension. Morphological polymorphism was observed, with some transitional variants or spheroid stage. Average promastigote length of the dominant elongate morphotype (no. = 50) were 11.22 µm (s.d. + 1.92 µm, range 6.76–14.59 µm). Observation of SEM specimens allowed a more detailed morphological characterization of the different forms. The evidenced morphology is clearly different from *C. mellificae* (ATCC 30254) and consistent with the description of Schwartz et al., (2015 l.c.). Morphological examination could therefore be useful to simply differentiate these two species and its study in different culture conditions can help to understand the life cycle of the parasite within the bee intestine.

A PICTURE OF THE PREVALENCE OF *ASCARIS SUUM* IN SLAUGHTERED PIGS IN THE NORTH OF ITALY

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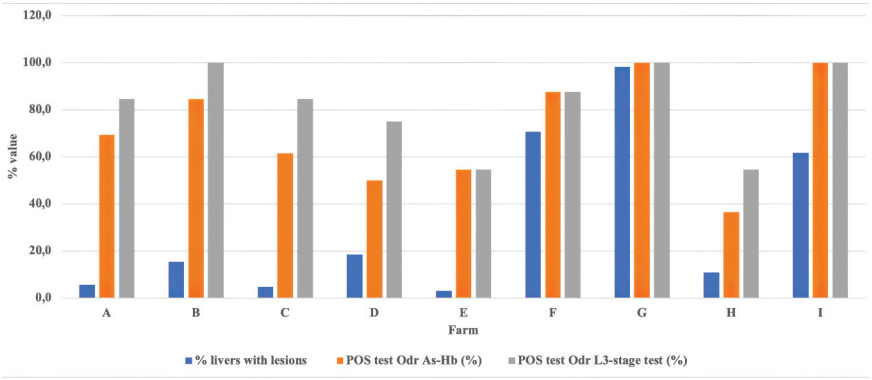
Keywords: *Ascaris suum*, north of Italy, serology

INTRODUCTION: *Ascaris suum* is one of the most important parasites of pigs. The migration of adult worms in liver causes fibrous lesions, commonly called “milk spots”, clearly recognizable at the slaughterhouse during meat inspection. The presence of livers with lesions leads to elimination of the organ, with important economic losses (Stewart et al., 1988. J Anim Sci, 66: 1548–54). Apart from liver condemnation, *A. suum* infections can also compromise weight gain, feed conversion efficacy, as well as meat quality (Hale et al., 1985. J Anim Sci, 60:220–25).
There are no recent data about the prevalence of the *A. suum* in pigs in northern Italy and for this reason the aim of the present study was to evaluate prevalence of this parasite in our area using both serological data and meat inspection exam at the slaughterhouse.

MATERIALS AND METHODS: The study was conducted in a slaughterhouse near Parma where about 300 pigs/hour are slaughtered at approximately 10 months of age and 180 kg.
For each batch, all the livers were observed for the presence of evident lesions (“milk spots”) and blood from 10% of animals was collected. Data about farms, treatments in the previous 90 days, number of pigs in each batch, numbers of liver lesions were registered.
Blood samples were conserved at 4°C till the arrival at the laboratory of Parma University (which it took place within 12 hours from collection) where they were centrifuged at 2500 rpm for 10 min to obtain serum. Serum samples were then conserved at -20°C till the shipment to Ghent (Belgium).
A total of 100 sera were analysed by two ELISA tests based on the recognition of a haemoglobin antigen, which is typically secreted by the intestinal stages (Odr As-Hb), and another test that is based on antigens from the 3rd stage larvae present in the lungs (Odr L3-stage) by Laboratory of Parasitology of Ghent University (Dr. Peter Geldhof).

RESULTS AND CONCLUSIONS: A total of 113 blood samples were collected from pigs coming from nine farms located in several provinces of northern of Italy. Due to poor quality only 100 sera were sent to Ghent University and analysed.
Liver lesions were found in all the batches, with percentage of positivity ranging from 3.8% to 98.3%. ELISA test for Odr As-Hb reported positive signals in 71%, while those for Odr L3-stage were 83%. Fig. 1 reports data for each farm.
The presence of *A. suum* in pigs farms of northern Italy was evident. Considering data from meat inspection the average percentage of positive livers was 32.1%.
The sensitivity of both ELISA tests was higher than the observation of liver “milk spots”, indicating underestimation of the actual prevalence of the parasite, with important negative implications from an epidemiological point of view. Moreover, the sensitivity of the ELISA tests could represent an important tool for fattening farms that want to significantly reduce the infection level with *A. suum*.



GASTROINTESTINAL PARASITES OF AUTOCHTHONOUS AND ORNAMENTAL CHICKENS IN NORTHERN ITALY: RISK FACTORS ANALYSIS IN SMALL-SCALE BREEDING FACILITIES

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Keywords: gastrointestinal parasites, chickens, northern Italy

INTRODUCTION: Nowadays poultry industry is based on highly selected commercial lines. Small-scale breeders are becoming more aware of the interest not only for autochthonous breeds for backyard farming but also for breeds reared as pets and selected for aesthetics. The management and health status of small-scale breeding facilities rearing autochthonous and ornamental chickens are not well known. The present study aimed to investigate the features of these facilities and correlate them with the most prevalent endoparasites detected by a copromicroscopic survey

MATERIALS AND METHODS: From November 2019 to February 2020, 32 breeders adhered to the survey. In their facilities, they reared 47 autochthonous/ornamental breeds. Out of 32 facilities, 9 were "small" (rearing up to 75 chickens), 13 "medium" (76-150), 10 "large" (more than 150). In 14/32 facilities, other rural breeds/hybrids of chicken were reared; 18/32 breeders reared only autochthonous/ornamental breeds; 18/32 reared other Galliformes species, 15/32 Anseriformes. In the 32 surveyed facilities, pooled faecal samples were collected from all the chickens' groups composed of autochthonous/ornamental breeds. Overall 185 groups of chickens were sampled; 114 were "small" groups (<10 animals), 71 were "large" (≥ 10). Young animals (<6 months old) were present in 34/185. Groups composed of males, females, and mixed sexes groups were 26, 24, and 135. Information about cleaning practices and disinfection was collected to produce a score. The 185 pooled faecal samples were analyzed by FLOTAC dual technique. Statistical analysis was implemented by SPSS (IBM, Chicago, IL).

RESULTS AND CONCLUSIONS: *Ascaridia*/*Heterakis*, *Capillaria* sp., Trichostrongylidae, *Strongyloides* sp., *Raillietina* sp., and *Eimeria* sp. were detected in 66.5%, 57.8%, 9,7%, 1.6%, 2.7%, and 81.6% of samples. *Ascaridia*/*Heterakis*, *Capillaria* sp., and *Eimeria* sp. were found with means values of EPG/OPG of 70 (min-max: 0-4,668), 48 (0-1,233), and 968 (0-28,848). Final models showed that *Ascaridia/Heterakis* EPG was higher in "medium" facilities, in groups reared on wood shaving litters, and in farms with worst hygienic conditions. Medium-size breeds excreted significant higher EPG. EPG of *Capillaria* sp. were higher in groups exclusively composed of females and lower in groups housed in chicken cops with an aviary. EPG of *Ascaridia/Heterakis* and *Capillaria* sp. were lower in groups of chicken that received anthelmintic treatments in the past six months. *Eimeria* sp. OPG were higher in larger groups of animals, in groups with young animals, and in medium-size and dwarf breeds; OPG were lower in farms that reared Anseriformes and in animals reared in chicken cops with an aviary. Gastrointestinal parasites are widespread in small-scale breeding facilities of autochthonous and ornamental chickens in northern Italy; EPG/OPG of most common endoparasites can be affected by management and farm and animal features. Control of chickens endoparasites should be much considered in small-scale breeding facilities.

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INNOVATIVE DIAGNOSIS OF PARASITIC DISEASES

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IDENTIFICATION OF MIRNAS OF *STRONGYLOIDES STERCORALIS* L1 AND IL3 LARVAE ISOLATED FROM HUMAN STOOL

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Keywords: *Strongyloides stercoralis*, RNAseq, miRNA

INTRODUCTION: *Strongyloides stercoralis* is a soil-transmitted helminth that can cause a fatal syndrome in immunosuppressed individuals. The infection is often misdiagnosed, thus a better understanding of the molecular biology of the parasite can be useful for the discovery of potential new biomarkers. Recent studies reported the identification of small RNAs in Strongyloididae but no data are provided for *S. stercoralis*.
In this study, we presented the first miRNAs identification in both rhabditiform (L1) and infective filariform (iL3) *S. stercoralis* larvae. Our objectives were: i) to analyze the miRNome of L1 and iL3 *S. stercoralis*, pinpointing on specific miRNAs of this nematode, ii) to obtain the mRNAs profiles in these two larval stages and iii) to predict the possible targeting between miRNAs & mRNAs.

MATERIALS AND METHODS: Total RNA was isolated from L1 and iL3 collected from the stool of 5 infected individuals. RNA-seq libraries were generated from 1000 ng of total RNA using the TruSeq Stranded mRNA, while miRNA libraries were prepared from 300 ng of total RNA using QIAseq miRNA Library Kit. Libraries were sequenced on the Illumina Nextseq500 system (Illumina) and 75bp single-end reads were generated.

RESULTS AND CONCLUSIONS: Bioinformatic analysis revealed 391 miRNA sequences. Among these, 206 showed homology to *S. ratti*, 44 to *Caenorhabditis elegans*, while 141 were labelled as novel specific miRNAs. Differential analysis between the larval stages showed 6 miRNAs differentially expressed (STR-MIR-34A-3P, STR-MIR-8397-3P, STR-MIR-34B-3P and STR-MIR-34C-3P up-regulated in iL3 while STR-MIR-7880H-5P and STR-MIR-7880M-5P more expressed in L1). Comparing the transcripts expression between L1 and iL3, we found 1553 modulated transcripts, of these only 81 genes resulted statistically significant (FDR<0.05; 49 genes more expressed in L1 than iL3; 32 genes more expressed in L3 than L1). mRNA sequences were analyzed in order to predict which of them could be a potential target of miRNAs and we found 33 mRNAs significantly modulated between L1 and iL3. These targets were involved in transcription, translation, metabolism and energy production, cytoskeleton rearrangements and signal transduction.
Overall, these results provide relevant data for further investigations aiming to analyze the potential role of miRNAs as diagnostic markers for strongyloidiasis and to better understand their role in the larval stages development.

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MEDICAL TROPICAL PARASITIC DISEASES

SOIPA

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PARASITOLOGICAL DIAGNOSIS AT TIME OF COVID 19 PANDEMIC

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Keywords: fasciolosis, amebosis, Covid19

INTRODUCTION: *Fasciola hepatica* and *Entamoeba histolytica* diseases are characterized by intermediate stages that involve the invasion of organs and tissues. The purpose of the following work, carried out at the Parasitology Unit of the P.O "D. Cotugno" of Naples, Regional Reference Center for Parasitological Diagnoses, was to demonstrate the importance of diagnosing neglected parasitic diseases especially during the Covid 19 pandemic. In an Italian patient, male, 34 years old, cooperating in Uganda, we found ova of *F. hepatica* from a copro- parasitological sample and in a patient of African nationality, with lung' s abscess, *E. histolytica*.

MATERIALS AND METHODS: For the diagnosis of *F. hepatica* and *E. histolytica*, we examined 3 copro-parasitological samples, collected every other day, by fresh examination and after Ridley' s concentration and, for *E. histolytica*, we carried out Giemsa stain and immunochromatographic test (ICT). We confirmed systemic lung infection with *E. histolytica* by enzymatic immuno- assay (EIA) of specific antibodies.

RESULTS AND CONCLUSIONS: From 3 copro- parasitological samples of the patient suffering from hepatomegaly 2 were positive for the presence of *F. hepatica* ova. From the 3 copro- parasitological samples of the patient with lung abscess, one sample tested positive for *E. histolytica* and was obtained an antibody titer of 33 U/ml from the EIA. In conclusion, *F. hepatica* parasitosis is a serious zoonotic disease causing serious complications, infection by *E. histolytica* can cause invasion of tissues and organs, and give rise to the formation of abscesses; both if not diagnosed correctly and urgently can lead to the death of the patient.

DIAGNOSIS OF CEREBRAL NEUROTOXOCARIASIS IN CAMPANIA REGION, IN SOUTH OF ITALY

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Keywords: neurotoxocariasis, neuroimaging, EIA

INTRODUCTION: *Toxocara canis* and *Toxocara cati* are causative agents of Toxocariasis, a cosmopolitan zoonosis. The purpose of this work, carried out at the UOS of Parasitology of the P.O "D. Cotugno" of Naples, Regional Reference Center for Parasitological Diagnoses, was to demonstrate the infection with *Toxocara* spp. in a patient from Campania, male, breeder, suffering from brain injury (neuroimaging: TC scan without medium contrast).

MATERIALS AND METHODS: For the diagnosis of Toxocariasis, was carried out serum antibody research by immuno-enzymatic assay (EIA) which allowed to calculate the estimated antibody titer in Units/ml.

RESULTS AND CONCLUSIONS: After TC scan without medium contrast with evidence of "brain lesions from parasites" we received a serum sample from the Neurology of the "Federico II" University of Naples for the search of antibodies anti-*cysticercus*, resulted negative. In the face of a strong clinical suspicion motivated by clear risk factors, the parasitology laboratory recommended and performed antibody tests for *Entamoeba hystolitica*, *Echinococcus granulosus*, *Trichinella spiralis*, *Strongyloides stercoralis* and *Toxocara canis*. Of the tests carried out we found positivity for antibodies anti-*Toxocara canis* at high titers. In conclusion, the brain lesion found in the patient on Campania territory was associated with damage to brain tissue following migration of *Toxocara* spp. larvae into the CNS. Of fundamental importance was the fruitful and open collaboration between clinician and parasitologists for a rapid and correct diagnosis.

A GIS-BASED WEBSITE TO MONITOR THE IMPACT OF INTERVENTION AGAINST SOIL-TRANSMITTED HELMINTHS IN ENDEMIC COUNTRIES

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Keywords: soil-transmitted helminths, web-GIS, preventive chemotherapy programmes

INTRODUCTION: Soil-transmitted helminth (STH) infections are among the most common neglected tropical diseases (NTD) worldwide causing high morbidity and mortality rates in endemic areas (Marocco et al., 2020. Infect Dis Poverty 9: 48-58). Preventive chemotherapy (PC) programmes and health education, particularly targeted at pre-school and school age children (pre-SAC and SAC), are recommended by the World Health Organization (WHO) to reduce the impact of STH in endemic countries (Montresor et al., 2020. PLoS Negl Trop Dis, 14: e0008505). Within the activities of the WHO collaborating centre (WHO CC ITA-116) a WebGIS and a dataset (www.whocc.ita116.unina.it) were developed to support PC programmes to monitor the impact of STH control in the six WHO regions.

MATERIALS AND METHODS: Data were collected using the standardized reports provided by the WHO's Department of Control of NTD (i.e., Joint Reporting Form, Epidemiological Data Reporting Form, official reports from countries), scientific publications and other sources.

RESULTS AND CONCLUSIONS: Based on data collected, four different sets of maps (Drug distribution and coverage; Progress of implementation; Impact of intervention on STH prevalence, Impact of intervention on STH morbidity) were developed using the ArcGIS Pro 2.7 software. Each set contains a global dynamic map showing the full view with an interactive display to zoom into the different regions/countries; in addition, static maps with summary tables and graphs are available for each country to provide detailed data.
The progress of implementation of PC for STH is assessed analysing the national drug coverage reported in the last five years available (2016-2020), using a formula which permitted to assign a point corresponding to a precise colour on map for each country.
Endemic countries are invited to evaluate their progress with a survey (after at least five years of PC intervention) which provides the impact of the intervention on STH prevalence and morbidity.
In conclusion, the GIS-based website and the maps are very useful tools to visualize the evolution of impact of PC programmes for following the progress of the control programme globally, improving the coordination and communication among WHO's regional and country offices, Ministries of Health, pharmaceutical industries and other partners.

PROTEOMICS APPROACHES FOR STRONGYLOIDIASIS: PARASITE, HOST AND HUMAN DISEASE

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Keywords: Strongyloidiasis, proteomics, host-pathogen interaction

INTRODUCTION: Strongyloidiasis is a neglected tropical disease affecting an estimated 600 million people. The infection can persist lifelong due to *Strongyloides stercoralis* peculiar auto-infective cycle. The cumbersome diagnosis and the limited knowledge of the mechanisms underpinning this chronic infection are key issues in the management of the disease. Previously, we have shown that long-lasting strongyloidiasis is associated with a dampened immune response. To better explore the host-pathogen interaction, here we applied an untargeted approach to characterize infective larvae (iL3) and infected subjects' serum proteomes.

MATERIALS AND METHODS: Different proteomics approaches were used to investigate the mechanisms of host pathogen interaction in human strongyloidiasis and to highlight novel candidate antigens for the improvement of current serodiagnosis. Indeed, iL3 larvae isolated from a fecal sample were analyzed by high-throughput (HT) qualitative proteomics to establish infective larvae proteome; while serum samples from strongyloidiasis patients (no.= 5) before and after treatment and uninfected controls were quantitatively compared by SWATH mass spectrometry to characterize alterations in serum protein expression in chronic strongyloidiasis.

RESULTS AND CONCLUSIONS: An automatic search strategy combined with manual annotation identified more than 240 *S. stercoralis* iL3 proteins, which to our knowledge is the largest proteome ever published. Gene ontology analysis was used to select candidates for structural characterization and immunogenic epitope prediction, after exclusion of human homologous. Quantitative proteomics identified with high confidence 208 serum proteins. Twenty four proteins were significantly modulated during the infection or following treatment, the most promising of which are undergoing validation as disease biomarker.
The study of both parasite- and host-derived proteins using HT proteomics is a promising strategy to improve our knowledge of the mechanisms of host-pathogen interaction in human strongyloidiasis and to highlight novel candidate antigens for the improvement of current serodiagnosis.
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LEISHMANIOSIS, MALARIA, STRONGYLOIDOSIS: CO- INFECTIONS AT THE TIME OF COVID

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Keywords: leishmaniosis, malaria, strongiloidosis

INTRODUCTION: Leishmaniosis (LVZ), malaria and strongyloidosis are serious parasitic diseases. The purpose of the following study, carried out at the Parasitology Unit of the P.O "D. Cotugno" of Naples, Regional Reference Center for Parasitological Diagnoses, is to evaluate the incidence of co- infections from parasites in patients affected by Covid 19.

MATERIALS AND METHODS: The diagnosis of LVZ was performed through the search for specific antibodies (IFI), observation of amastigotes in bone marrow and cultural examination. Malaria was diagnosed by observation of peripheral blood smears (MGG), thick drop (Giemsa) and LAMP. Strongyloidosis was diagnosed by parasitological examination at fresh and after Ridley's concentration and by assay of specific antibodies by immune-enzymatic assay (EIA).

RESULTS AND CONCLUSIONS: In 2021, from 30 positive samples for the research of *Leishmania* spp. out of a total of 122, 6 tested positive for Sars-CoV-2. From 67 samples for the diagnosis of malaria 21 tested positive for *Plasmodium falciparum* and 2 for *Plasmodium ovale*, of these 6 were affected by Covid 19. For the research of *Strongyloides stercoralis* on 26 total samples 2 were positive for antibody research and of them 1 was also positive for the copro- parasitological examination; of these the first was affected by Covid19, and the second had a co- infection by *Plasmodium falciparum*. In conclusion, LVZ and *Strongyloides stercoralis* are frequent in immunocompromised patients. From the elaboration of the results it was possible to confirm that the antibody titer (*Leishmania* spp., *Strongyloides* spp.) and parasitemia (*Plasmodium* spp.) are influenced by the course of Sars-CoV-2 disease and by the effects of corticosteroid therapy.

A MISSING ROLE OF A NEGLECTED PARASITE: *LEISHMANIA INFANTUM* AS A NOVEL TOOL TO PREVENT THE NLRP3-DERIVED NEUROINFLAMMATION IN MICROGLIA

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Keywords: *Leishmania infantum*, microglia, neuroinflammation

INTRODUCTION: Cycling between insect vectors and vertebrate hosts, ancient *Leishmania infantum* parasites must adapt to diverse microenvironments while suppressing killing microbicidal activities that threaten parasite persistence. In mammals, *Leishmania* hijacks inflammatory immune responses by macrophages (their main cellular niche), including the activation of the NLRP3 inflammasome, a sensor complex of the innate immunity. NLRP3 activation is a hallmark of other immunopathologies including Alzheimer's disease (AD), where amyloid-β (Aβ) peptide accumulation is sensed by microglia (MG), the brain macrophages, resulting in an aberrant inflammatory cascade. Given that AD is an uncurable condition and that the NLRP3 is a key drug target against AD5, we hypothesized that Leishmania subversion tactics might revert MG activation through NLRP3 inhibition, leading to a protective anti-inflammatory effect.

MATERIALS AND METHODS: We utilized an immortalized murine microglia cell line (MMC) and *ex vivo* primary MG (pMG) isolated from postnatal C57BL/6 mice, infected with wild-type or transgenic fluorescent and bioluminescent *L. infantum*. Phagocytosis of *L. infantum* and Aβ was studied by live-cell imaging and immunofluorescence. Inflammatory cytokines in Aβ-treated MG were analyzed by ELISA and qPCR, and neurotoxic nitric oxide (NO) by the Griess assay. NLRP3 modulation was studied through caspase-1, IL-1β and IL-18 release, ASC-Speck formation and pyroptosis by LDH. Finally, gene expression analyses served to identify the underlying *Leishmania* mechanistic insight in Aβ-stimulated MG.

RESULTS AND CONCLUSIONS: Our results demonstrate that *L. infantum* are internalized by MMC and pMG without inducing MG activation, thus resulting in a "silent" parasite entry. Interestingly, both *L. infantum* and Aβ were concomitantly phagocytized by both microglial types. Further, we observed that the Aβ-induced expression of pro-inflammatory and neurotoxic factors including IL-1β, TNFα and NO was inhibited by *L. infantum*, whereas IL-18 levels remained low and/or unchanged upon parasite challenge. NLRP3-mediated pyroptotic cell death was also reduced in *L. infantum*-infected MG. Besides, the anti-inflammatory effects were dose-dependent and specific for *L. infantum* since the use of the NLRP3-inhibitor MCC950 yielded similar results as those elicited by *Leishmania*, and such inhibition was absent in response to inert latex beads. These protective effects were next replicated in *Leishmania*-infected MG by the pharmacological NLRP3 inducer nigericin. Remarkably, we further found that *L. infantum* dampens the NLRP3 by increasing the expression of A20, a cytoplasmic zinc finger protein that blocks the NF-κB-dependent inflammation and a NLRP3-negative regulator in MG6. *Leishmania* has co-evolved fascinating strategies to persist in host macrophages. Intriguingly, in Aβ-treated MG, *Leishmania* infection dampens the NLRP3 inflammasome. These results may represent a potential and unprecedented bioinspired strategy against neuroinflammation in AD.

MODULATION OF MACROPHAGES RESPONSE IN AN *IN VITRO* MODEL OF LEISHMANIASIS AND MALARIA CO-INFECTION

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Keywords: *Leishmania* malaria co-infection, immune response, macrophages

INTRODUCTION: Leishmaniasis and malaria are two protozoal diseases caused by the parasites of the genus *Leishmania* and *Plasmodium*, respectively. In some geographical areas their diffusion overlaps, causing cases of co-infections difficult to diagnose and to treat due to the lack of knowledge and of adequate treatments. Macrophages are the primary host cells for *Leishmania*, and crucial to determine the outcome of the disease. Their killing activity, mediated by nitric oxide (NO), and the induction of a T-helper 1 response lead to a resolution, while a predominance of T-helper 2 response promotes the proliferation of parasites and progression of the disease. Hemozoin (Hz), the malaria pigment deriving from the digestion of hemoglobin by *Plasmodium* parasites, is known to accumulate in macrophages and to interfere with the immune responses. Thus, the aim of this research was to understand *in vitro* whether and how the concomitant presence of *Leishmania infantum* (*L. infantum*) infection and Hz modulates macrophages-mediated responses.

MATERIALS AND METHODS: The human monocytic cell line THP-1 differentiated with PMA into macrophages and immortalized murine bone marrow derived macrophages (BMDM) from C57BL/6 mice were infected with *L. infantum* promastigotes in presence or not of Hz 2-20 microg/mL isolated from cultures of *Plasmodium falciparum*. The localization of parasites and of malaria pigment in macrophages was demonstrated by electron microscopy, while the degree of infection was established through optical microscopy counts. Nitric oxide production was evaluated by Griess assay. The levels of pro-inflammatory cytokines in the supernatants were measured by ELISA.

RESULTS AND CONCLUSIONS: The co-localization of Hz in macrophages infected by *L. infantum* amastigotes was confirmed by electron microscopy and led to a decrease of the percentage of infected macrophages compared to *Leishmania* infection alone. Nitric oxide, a key mediator of leishmanicidal activity, was slightly induced by *L. infantum* infection and further increased by the concomitant presence of Hz. Thus, the decrease of the infection by the simultaneous presence of Hz in *L. infantum* infected macrophages can be explained with a higher leishmanicidal activity, as demonstrated by the increase of NO production induced by Hz.
Similarly to NO, the levels of the pro-inflammatory cytokines TNFalpha and IL-12 were higher in the presence of the co-stimulus Hz compared to *L. infantum* infection alone. Since the increased secretion of TNFalpha and IL-12 is associated with a stronger T-helper 1 response, Hz may favor a better disease outcome. These data demonstrate how the immunological scenario and the disease outcome could be deeply modified by the co-presence of the two parasitic diseases, thus underlying once again the necessity of new investigations for the diagnosis and the treatment of this severe co-infection.

SARS-COV-2 AND *PNEUMOCYSTIS JIROVECII* INFECTION

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Keywords: *Pneumocystis jirovecii*, Sars-CoV-2, infection

INTRODUCTION: *Pneumocystis jirovecii* is a ubiquitous eukaryotic microorganism, capable of infecting the lungs of numerous species of mammals, including humans. The purpose of this work, carried out at the Parasitology Unit of the P.O "D. Cotugno" of Naples, Regional Reference Center for Parasitological Diagnoses, is to evaluate the presence of *P. jirovecii* infection in Sars-CoV-2 positive patients.

MATERIALS AND METHODS: For the diagnosis of *P. jirovecii* infection we carried out: Giemsa stain, Direct Immunofluorescence (IFA) and Molecular Biology (BM). The samples are processed with fluidifier and washing in order to obtain slides for Giemsa stain, for IFA and 2.5-3 ml of sample for BM. PneumocystisELITe Real time PCR (Elitech Ingenius) is the fully automated BM system for the detection and quantification of *P. jirovecii*' s DNA.

RESULTS AND CONCLUSIONS: From 17/09/2020 to 31/03/2022, we received 210 samples: 155 males and 55 females aged between 6 months and 83 years. From these, 12 positive to Giemsa stain, 43 positive to IFA and 61 positive to BM. From 61 patients suffering from P.J. Pneumonia 34 were affected by Sars-CoV-2 Pneumonia, 9 transplanted, 14 HIV+, 1 patient hospitalized in serious condition in Intensive Care of Cardiac Surgery and 1 child of only 6 months. In conclusion, from this study it is evident that SARS-CoV-2 patients have a "immunodepression" induced by the virus and corticosteroid therapy. From the elaboration of the results, we can say that BM proved to be an extremely sensitive and specific test, fundamental for a clinical diagnosis and follow-up of therapy.

LEISHMANIA ISOLATION FROM A SKIN SORE IN A TRAVELER FROM PERU: CHARACTERIZATION USING FIVE DIFFERENT TARGETS

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Keywords: *Leishmania* sp., american leishmaniasis, characterization

INTRODUCTION: Leishmaniasis is a group of infectious diseases caused by parasites belonging to the genus *Leishmania*; more than 20 *Leishmania* species are pathogenic for humans. Leishmaniasis pathology is grouped in visceral Leishmaniasis (VL), cutaneous Leishmaniasis (CL) and mucocutaneous Leishmaniasis (MCL). Italy is an endemic country for *L. infantum* where dogs are the main reservoir and humans are considered accidental host. Despite of this, due to the global movement, we face an increasing number of imported cases of human Leishmaniasis, particularly from the Americas where different species are endemic. The specie identification is crucial for the correct treatment; indeed, it is necessary to be able to identify the specie infecting the patients as prompt as possible, considering all the specie from the old and from the new World.

MATERIALS AND METHODS: A man, who worked during several months in the Peruvian forest, attended at our center with a skin sore of the neck. The patient was treated initially with topical paromomycin but for the extension of the infectious process to local lymph nodes, a course of liposomal amphotericin B has been completed with good answer. Two months after treatment an apparently inactive scare remains on the neck. Clinical follow-up is ongoing.
The patient tested positive for leishmaniasis by an in-house real-time PCR targeting a 61 pb of the gene SSU rRNA, the *LEISHMANIA* ELISA IgG+IgM (VIRCELL, Granada, Spain), and the *LEISHMANIA* Western Blot IgG (LdBio, Lyon, France). Several amastigotes were visualized microscopically after Giemsa coloration. We punched the skin sore, inoculate in Novy-MacNeal-Nicolle medium and incubate at 25°C until *Leishmania* growth. After DNA extraction, we characterized the isolate trough the study of 5 different targets: ITS1, ITS2, HSP70, CytB and G6PDH.

RESULTS AND CONCLUSIONS: The combination of different targets allowed a more specific characterization particularly for specie endemic in South America. This approach gave us the possibility to identify different strains for which a single gene would not be sufficient and indeed personalize the treatment in order to be more efficient.

ARTESUNATE AND DIHYDROARTEMISININ-PIPERAQUINE TREATMENT FAILURE IN A SEVERE *PLASMODIUM FALCIPARUM* MALARIA CASE IMPORTED FROM IVORY COAST

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Keywords: malaria, treatment failure, antimalarial drug resistance

INTRODUCTION: An Italian traveler returning from Ivory Coast -with no antimalarial prophylaxis- was admitted to Pisa University Hospital with fever and chills, diagnosed with severe *Plasmodium falciparum* malaria and treated with intravenous Artesunate followed by oral Dihydroartemisinin-Piperaquine. The patient was discharged after signs and symptoms resolution and negative blood films for malaria parasites. A month later the patient developed a new *P. falciparum* episode and was successfully treated with Atovaquone-Proguanil. Molecular analyses were retrospectively performed on blood samples collected at different time points to investigate reasons for malaria recurrence, in absence of further travel to endemic areas.

MATERIALS AND METHODS: Total DNA was extracted with Qiagen DNA Mini Blood kit. *Plasmodium* species was identified by a nested PCR protocol (Snounou et al., 1993. Mol Biochem Parasitol, 61: 315-20) and by Real Time PCR (Real Star Malaria kit, Altona Diagnostics). Genotyping of *P. falciparum* clones was performed by Pfmsp1 and Pfmsp2 length polymorphism analysis (Soulama et al., 2009. Malar J, 8: 1-8). The presence of mutations associated with antimalarial drug resistance was investigated by PCR and Sanger amplicon sequencing of Pfk13, Pfcrt, Pfmdr1, PfcytB, Pfexonuclease, Pfdhps and Pfdhfr loci. Resistance to piperaquine was further evaluated by analysis of Pfplasmepsin2/3 copy number by breakpoint mutation genotyping (Ansbro et al., 2020. Malar J, 19: 1–10).

RESULTS AND CONCLUSIONS: Both malaria episodes were caused by *P. falciparum* only, excluding the second episode could have been caused by relapsing hypnozoites of *P. vivax* and *P. ovale*. The infection was monoclonal and caused by an identical *P. falciparum* strain in the two episodes, confirming that the second episode was caused by resurgence of the infection. Both microscopy and Real Time PCR showed a slower reduction of parasite density in the first episode compared to the second (first episode day1 vs day0: %iRBC=-75.0%, ΔCt=-1.53; second episode day1 vs day0: %iRBC=-99.5%, ΔCt=-4.01) suggesting a suboptimal efficacy of the first antimalarial treatment. No mutations associated with resistance to Artemisinin derivatives and Piperaquine were detected, whereas resistance associated mutations to Sulfadoxine and Pyrimethamine (SP) were observed.
As results showed no evidence for resistance to the administered antimalarial drugs, different causes should be considered for the observed therapeutic failure, including drug malabsorption or poor drug manufacturing. The observation of SP resistance mutations highlights the importance of surveillance of antimalarial drug resistance in imported malaria cases for the global monitoring of this biological threat to the malaria control efforts (Labbé et al., 2003. Emerg Infect Dis, 9: 33-6; Sibley et al., 2008. Trends Parasitol, 24: 43-8).

INVESTIGATING THE ROLE OF INTRAPERITONEAL INJECTION OF H-IPSE, A MAJOR PROTEIN SECRETED FROM *SCHISTOSOMA HAEMATOBIUM* EGGS, ON IMMUNITY TO *PLASMODIUM BERGHEI* INFECTION IN MICE

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Keywords: malaria, schistosomiasis, immunity

INTRODUCTION: Malaria is the parasitic disease with the heaviest health burden worldwide, with 241 million clinical cases and 627000 deaths reported in 2021, the great majority occurring in Sub-Saharan Africa (WHO, World Malaria Report 2021). Previous studies have suggested that infection with helminths could affect susceptibility to malaria by modulating the immune response towards *Plasmodium* spp. (Salgame et al., 2013. Nat Immunol, 14:1118–26). Since a highly common helminth infection in Sub-Saharan Africa is from *Schistosoma haematobium* (Degarage et al., 2016. Plos Negl Trop Dis, 10:1–18; Mangano et al., 2020. Acta Trop, 205: 105381) we aim at investigating the role of HIPSE, a major egg secretory protein with immunomodulatory activities (Pennington et al., 2017. Infect Immun, 85: e00301-17), on immunity to plasmodium parasites, by taking advantage of a murine malaria model.

MATERIALS AND METHODS: Two experiments were conducted each involving two groups of 5 female CD1 mice weighting 25g, one treated with HIPSE and one with PBS (control). In the first experiment, mice were inoculated intra-peritoneally with either HIPSE (25µg H-IPSE/200µl PBS) or PBS (200µl). One day later, all mice were infected by intra-peritoneal injection with a clone of *P. berghei* (1 x 104 infected Red Blood Cells (iRBC) in 200 µl PBS) expressing green fluorescent protein (GFP). Parasite density was measured by cytofluorimetry and expressed as % of iRBC at day 3, 5 and 7 post infection. In the second experiment mice were inoculated with either HIPSE or PBS 1 day prior to infection as above, but also 6 days after infection, to mimic natural conditions where exposure to *Schistosoma* antigens is sustained over time. Parasite density was measured at day 3, 5, 6, 7 and 10 post infection.

RESULTS AND CONCLUSIONS: Results of experiment 1 showed lower parasite density at all time points and slower growth of parasite density in mice treated with HIPSE compared to PBS, but differences did not reach statistical significance (mixed-effects restricted maximum likelihood model for repeated measures and missing data, p-value=0.0517). Mice started to die on day 7 in both groups. Results of experiment 2 showed lower parasite density in HIPSE treated mice versus controls at all time points except day 7 p.i., and slower growth of parasite density, with statistically significant differences (p-value=0.0048). Mice started to die on day 7 in the PBS group and at day 10 in the HIPSE group.
These data suggest an impact of HIPSE treatment in reducing *P. berghei* parasite density in mice, with more significant effects when treatment is repeated during the course of infection, providing some evidence of a role of this antigen in modulating anti-malarial immune responses. However, these experiments must be repeated in order to draw any solid conclusion. Also, the implication for the impact of *S. haematobium* on immunity to *P. falciparum* malaria in humans remains to be determined.

PREVALENCE OF INTESTINAL PARASITES IN ASYLUM SEEKERS IN SOUTHERN ITALY: SURVEILLANCE ACTIVITIES AT THE WHO CC ITA-116

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Keywords: WHO CC ITA-116, intestinal parasites, migrants

INTRODUCTION: In February 2020, the Centro Regionale Monitoraggio Parassitosi (CREMOPAR) was designated as collaborating centre of the World Health Organization (WHO) for diagnosis of intestinal helminths and protozoa (WHO CC ITA-116). Beyond supporting endemic countries in monitoring preventive chemotherapy programmes, the WHO CC ITA-116 carries out regular parasitological surveillance and health care for migrants in southern Italy. The present study provides figures on the prevalence of intestinal parasites in asylum seekers in this area.

MATERIALS AND METHODS: From July 2020 to March 2022, a total of 310 asylum seekers, from 29 different countries, were tested for intestinal parasites. The spokes for stool collection were set at different migrant assistance centres (ASL-NA1, CAS and SIPROIMI) located in the Campania region. Intestinal parasites were detected using two different copro-microscopic techniques: the wet smear with a drop of iodine solution and the FLOTAC dual technique (Cringoli et al., 2010. Nat Protoc, 5: 503-15). Furthermore, patients were asked to fill in a questionnaire reporting age, sex, country of origin, alimentary habits, health status and length of permanence in Italy.

RESULTS AND CONCLUSIONS: A total of 73 asylum seekers resulted positive for intestinal parasites, revealing an overall prevalence of 23.6% (95% Confidence Interval [CI] = 19.0-28.7). Among helminths, hookworms (24.7%) and *Trichuris trichiura* (21.9%) were the most prevalent and the highest intensity detected was 2112 eggs per gram (EPG) of faeces, related to hookworms. *Capillaria* spp. (4.1%), *Schistosoma mansoni* (4.1%), *Enterobius vermicularis* (1.4%), *Hymenolepis diminuta* (1.4%), *Hymenolepis nana* (1.4%) and *Strongyloides stercoralis* (1.4%) were also found. As regard to pathogenic protozoa, *Giardia duodenalis* was the most commonly detected (8.2%), followed by *Entamoeba histolytica/dispar* (4.1%) and *Blastocystis hominis* (2.7%). Other protozoa were also detected, i.e. *Entamoeba coli* (26.0%), *Endolimax nana* (19.2%), *Iodamoeba butschlii* (4.1%), *Entamoeba hartmanni* (2.7%) and *Chilomastix mesnili* (1.4%). No significant association was found ($P > 0.05$) between the positivity to parasites and the following independent variables: age, sex, country of origin, alimentary habits and health status. In line with other similar studies (Gualdieri et al., 2016. Parasitol Res, 115:1315-23; Mazzitelli et al., 2018. Infez Med, 4:347-55) a statistically significant difference was found between the length of permanence in Italy and the presence of intestinal parasites (63.6% of migrants resulted positive for intestinal parasites were in Italy for less than 1 year; $P = 0.0076$). In conclusion, the results of this study add data to the parasitological scenario of asylum seekers arrived in Italy (Fontanelli Sulekova et al., 2019. Trav Med Infec Dis, 27:46-52) and highlight the importance to perform a regular parasitological screening in migrants as soon as possible after their arrival in order to avoid chronic diseases and complications.

DIFFERENT DIAGNOSTIC APPROACHES FOR THE DETECTION OF MALARIA PARASITES IN CLINICAL SAMPLES FROM BENIN, WEST AFRICA

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Keywords: malaria, diagnosis, Benin

INTRODUCTION: As the majority of sub-Saharan African countries, Benin still bears a heavy malaria burden. The prevalence of malaria in the country is around 20% of the general population (Ministère de la santé Annuaire des statistiques sanitaires du Bénin, 2019) with an average of 39% which interests children under five years of age. With respect to 2012 malaria prevalence has increased significantly, from 28% to 39% in 2018 (The DHS Program. 2020. Malaria Indicator Trends in Benin: Outputs from a DHS Program Workshop on Data Use. Rockville, Maryland, USA). Accurate diagnosis is essential for effective malaria control and treatment management. The present study aimed to compare results obtained by microscopy, loop-mediated isothermal amplification (LAMP), and polymerase chain reaction (PCR) for the malaria diagnosis in Benin.

MATERIALS AND METHODS: Venous blood from 107 consenting patients was collected in Saint Jean de Dieu hospital in Tanguiéta, Benin, to detect the presence of malaria parasites using LAMP (Illumigene®-Malaria, Meridian Bioscience Europe) and microscopy (thick and thin film). Clinical information such as age, gender, symptomatology, and treatment of each participant was recorded. To confirm results obtained by LAMP and or microscopy, molecular diagnosis based on nested-PCR amplification of the 18s rRNA gene of *Plasmodium* spp. has been performed at Istituto Superiore di Sanità according to already published protocols (Paglia et al., 2012. Diagn Microb Inf Dis, 72:175-80), as well as a microscopy confirmation.

RESULTS AND CONCLUSIONS: Out of 107 collected samples, 54 showed positive reaction to *Plasmodium* by the LAMP method. Microscopy detected 56 samples as *Plasmodium* infections, of which the majority (84 %) were identified as *Plasmodium falciparum*. Nested-PCR detected a total of 56 positive samples and 52 these were identified as *P. falciparum*, 1 as *P. ovale*, 1 as *P. vivax* and 1 as *P. malariae*. Finally, 1 sample showed a mixed-species infection of *P. falciparum* and *P. ovale*. A total of 8 discordant results were also identified in our analysis, in particular we found that 6 samples were detected negative for PCR but positive for both LAMP and microscopy and 2 samples were negative for LAMP and PCR but positive for microscopy. In the first case, it can be hypothesized that a low parasitemia and a possible degradation of the DNA have contributed to the negative result in PCR. In the second case, a possible false negative result linked to the use of the LAMP technique, already reported in the literature, can be considered. In conclusion, the three different diagnostic techniques showed a similar ability to identify malaria infections, without a particular advantage in favor of molecular LAMP and PCR techniques over microscopy. The use of a more sensitive molecular technique such as Real time PCR could help, in a further analysis, to better understand the observed discordant cases.

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MYCOTIC DISEASES

SOIPA

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RESPIRATORY MICROBIOTA PROFILES ASSOCIATED WITH *PNEUMOCYSTIS JIROVECII* PNEUMONIA

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Keywords: *Pneumocystis jirovecii*, metagenomics next generation sequencing, lung microbiome

INTRODUCTION: A dynamic relationship involving pathogens, host immune response, and microbiome characterizes the biological framework of many infectious and inflammatory diseases. *Pneumocystis jirovecii* is an opportunistic fungus that may lead to a life-threatening pneumonia (*Pneumocystis jirovecii* pneumonia, PJP) in immunocompromised patients (pts); it may, however, persist also in an asymptomatic form. Taxonomic profiling via metagenomic next-generation sequencing (mNGS) techniques has enabled higher sensitivity and resolution than does conventional culture approaches for better understanding of microbial compositions present in microbiological specimens. The aim of this study was to evaluate the potential of mNGS to determine respiratory tract microbiota profile associated with PJP and different comorbidities.

MATERIALS AND METHODS: In this study, we retrospectively analyzed the data of conventional culture testing and mNGS of the sputum samples (ESP) and bronchioalveolar lavage fluid (BAL) collected from 36 pts (27 BAL and 9 ESP) received at the parasitology laboratory of the Policlinico Tor Vergata - Rome, between September 2020 to June 2021. We focused on the diversity of bacterial communities between ESP and BAL samples and between pts with different underlying diseases, according to 16S rRNA V3V4 gene amplicon sequencing.

RESULTS AND CONCLUSIONS: Among 36 consecutive patients with PJP (22 males, 14 females, median age 62 years, range 22-87), 10 pts had solid tumors, 9 had haematological malignancies, 7 were HIV-positive pts, 6 pts had chronic lung disease and 4 pts had autoimmune diseases. Microbiomes from ESP and BAL differs significantly, as expected, with *Acinetobacter*, *Haemophilus* e *Streptococcus* that were more frequent in ESP samples while Psudomonadaceae and Enterobacteriaceae were prominent in BAL samples. Even more differences were seen among pts with different underlying disease. *Streptococcus* was significantly more present in pts affected by solid tumor, HM, while *Haemophilus* and *Neisseria* were more frequent among HIV pts.
In conclusion, despite the limitation of the absence of negative controls, and the small size of the population, the preliminary results of this study confirm that mNGS is a promising technique to detect co-pathogens in mixed pulmonary infection, with potential benefits in speed and sensitivity of detection.

CHROMOBLASTOMYCOSIS CAUSED BY *CLADOSPORIUM* SP. IN A CAPTIVE BULLFROG (*LITHOBATES CATESBEIANUS*) IN ITALY

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Keywords: chromoblastomycosis, chromoblastomycosis, frog

INTRODUCTION: Chromoblastomycosis (CBM) is a skin disease most commonly seen in tropical and subtropical regions, caused by melanized or brown-pigmented fungi (Queiroz-Telles et al., 2017. Clin Microbiol Rev, 30:233-76). *Phialophora* spp., *Fonsecaea* spp., *Scolecobasidium* spp., and *Cladosporium* spp. are responsible for CBM in amphibians, especially in stressed animals (Bube et al., 1992. J Comp Pathol, 106:73-7). Nonetheless, infection in man and animals is infrequent and remains localized while in amphibians is usually systemic (Hosoya et al., 2015. Med Mycol, 53:369–77). The present study describes the first case of a lethal *Cladosporium* spp. infection in a captive bullfrog.

MATERIALS AND METHODS: One adult male bullfrog was referred for lethargy and the presence of a cutaneous nodule. Fine needle aspiration from the lesion was performed for cytological examination. Tissue samples collected after surgical excision of the nodule were stained with hematoxylin and eosin. A suspected fungal infection was supposed and confirmed by fungal culture. Molecular diagnosis was performed by using formalin-fixed paraffin-embedded tissues and sequencing the rDNA internal transcribed spacer region (ITS). Therefore, antifungal treatment (climbazole twice a day) was started but the frog died after 30 days. A complete autopsy was performed.

RESULTS AND CONCLUSIONS: Cytological samples were poorly cellular but septate fungal hyphae were present. Histologically, pigmented hyphae and structures consistent with muriform bodies were found on a background of diffuse granulomatous inflammation. Fungal culture of samples revealed growth of colonies of pigmented molds, subsequently identified as *Cladosporium herbarum* by MALDI-TOF. ITS sequence analysis identified *Cladosporium* spp. A focally extensive granuloma with intralesional hyphae and muriform bodies effacing the architecture of head tissues was present at the histological examination in association with involvement of the liver, kidneys, lungs, and large intestine.
This study describes the first Italian report of the occurrence of lethal *Cladosporium* spp. infection in a frog and points out the role of *Cladosporium* spp. in Chromoblastomycosis. The treatment herein proposed was not successful most likely due to delay in the diagnosis and drug administration. In the management of this extensive disease, a desirable goal might be early diagnosis and species-specific therapy.

ANTIFUNGAL, ANTIOXIDANT, AND ANTIBIOFILM ACTIVITIES OF *CYMBOPOGON* SPP. ESSENTIAL OILS

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Keywords: *Cymbopogon* spp., essential oils, antifungal and antibiofilm activities

INTRODUCTION: Essential oils (EOs) are considered relevant therapeutic drugs for the treatment of animals and human infectious diseases (Iqbal et al., 2021. Front Plant Sci, 12:297). In particular, EOs may represent sources of bioactive agents (i.e., terpenes) with antimicrobial and antibiofilm activities (Evangelista et al., 2021. Crit Rev Food Sci Nut, 8:1-17). Although the chemical profiles of *Cymbopogon* spp. EOs proved the presence of monoterpenes and sesquiterpenoids (Mahmoud et al., 2022. Egypt J Chem, 65:287-96), there is a lack of scientific evidence regarding their usefulness as antifungal, antibiofilm, and antioxidant drugs. Thus, the present study was designed to evaluate the antifungal, antibiofilm and antioxidant properties of *Cymbopogon citratus* and *Cymbopogon proximux* EOs.

MATERIALS AND METHODS: A total of 59 *Candida* spp. strains from cloaca of domestic and wild animals and 9 *Malassezia furfur* strains from the skin of hospitalized human patients with bloodstream infections were employed. All the strains were biochemically and molecularly identified. The antifungal activity was evaluated by broth microdilution methods according to CLSI protocol for *Candida* and CLSI modified protocol for *Malassezia* (Rhimi et al., 2018, Ind Corps Prod, 113:196-201). The antibiofilm and antioxidant activities were evaluated according to previously reported methods (Brand et al. 1995. LWT-Food Sci Technol, 28:25-30; Pierce et al., 2008. Nat Protoc, 3:1494-1500). The antioxidant activities were expressed as EC50 (µg EO /mL), which is the concentration necessary to obtain a 50% reduction of 2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazy (DPPH) or 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical.

RESULTS AND CONCLUSIONS: Both EOs showed antifungal (minimal inhibitory concentration –MIC-values ranging from 1.25 to 20 µl/ mL) and antibiofilm activities (% of reduction ranging from 27.65±11.7 to 96.39±2.8) against all yeast species. *M. furfur* strains were more susceptible to both EOs than *Candida* spp. Both EOs exhibited high antioxidant activities, with an EC50 ranging from 28.73 to 42.18 µg/ml.
This study suggests that *C. citratus* and *C. proximus* EOs might be considered as an excellent source of antifungal, antibiofilm and antioxidant drugs and might be useful for preventing *Malassezia* infections in both medical and veterinary medicine. The antioxidants properties of these EOs are promising as anti-biofilm agents and might be a potential solution for biofilm-associated infections in the future.

VIRULENCE OF *MALASSEZIA FURFUR* STRAINS CAUSING HUMAN BLOODSTREAM INFECTIONS

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Keywords: *Malassezia furfur*, enzymatic activities, biofilm

INTRODUCTION: In the last 10 years, the incidence of non-*Candida* fungal infections is increasing and among them, *Malassezia* infection represents one of the most common emerging bloodstream infections (BSI) in preterm infants (Rhimi et al., 2020. Front Cell Infect Microbiol, 10:370). However, surveillance studies focused on *Malassezia* isolation from patients with fungemia are scant, thus the pathophysiology of *Malassezia* strains coming from BSIs are not well-explored and all available research focuses on *Malassezia* virulence factors linked to skin. Therefore, the aims of the present study were to evaluate the phospholipase (Pz), lipase (Lz), hemolytic (Hz) activities, and the *in vitro* ability to form biofilm of clinical isolates of *Malassezia furfur* from BSI preterm infants.

MATERIALS AND METHODS: A total of 39 *M. furfur* strains (i.e., 14 from skin and 25 from blood) from preterm infants were employed. Pz, Lz, Hz activities, and biofilm formation were evaluated as previously reported (Muhsin et al., 1997. Mycoses, 40: 465–469; Cafarchia et al., 2004. J Clin Microbiol, 42: 4868–69; Aktas and Yıgıt, 2015. J Med Mycol, 25:25–30; Angiolella et al., 2018. Med Mycol, 56:110–16).

RESULTS AND CONCLUSIONS: All isolates were higher producers of Pz and Lz regardless the origin. The Hz activity of *M. furfur* strains from blood was higher than that registered form skin strains. All the strains were able to produce biofilm but *M. furfur* from blood were higher biofilm producers. This study shows that secreted hydrolytic and hemolytic enzymes seem to be important factors in the life cycle of *M. furfur* and might contribute to its virulence in susceptible hosts. The positive correlation between the haemolytic activity and the biofilm formation might explain the implication of *M. furfur* strains in invasive fungal infections.

BEAUVERIA BASSIANA (ASCOMYCOTA: HYPOCREALES) FOR THE CONTROL OF *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

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Keywords: *Aedes albopictus*, bio-control, *Beauveria bassiana*

INTRODUCTION: *Aedes albopictus* is considered one of the main invasive mosquito species and competent vector of various human pathogens. The most frequently used control strategy against mosquitoes relies on the use of insecticides that can cause environmental contamination and resistance development (Pichler et al., 2018. Pest Manag Sci, 74:1319-27). The use of entomopathogenic fungi and in particular, *Beauveria bassiana* (Bb) for mosquito control has been recently proposed as valid non-chemical alternative approach (Shen et al., 2020. Curr Opin Insect Sci, 40:111-6). However, the employment of these fungi presents some limitations mainly due survival after field application. Consequently, new Bb application methods have been developed to improve field performance (Friuli et al., 2022. Parasit Vectors, 15: 79). The aims of this study are to evaluate the viability of Bb in hydrogels based on alginate or cellulose with proven oviposition attraction capability for tiger mosquitoes and the *in vitro* effect of *Bb* as in conidial aqueous suspension (CIS) or in a biocompatible hydrogels (HBBs) against *A. albopictus* eggs.

MATERIALS AND METHODS: A "native" strain of Bb (CD1123) and a total of 1500 eggs from insectary were employed. CIS of Bb was obtained by culturing 15 strains for 3 weeks at 26°C. Conidia were harvested by washing the plates and turbidity was adjusted spectrophotometrically to 2.8 McFarland. HBBs were prepared by using the same suspension. The viability of Bb (colony-forming unit/ml-CFU/mL) within the HBBs and in the aqueous solution (CIS) was evaluated every 4 days of incubation up to 21 days at 28 °C and 80% RH. For the bioassay, groups of 90 eggs of *A. albopictus* were placed in contact with a suspension containing 107 conidia/ml of BB and with HBBs. The viability of the eggs was assessed after 5, 10 and 14 days of contact by submerging the eggs in 80ml of water. *A. albopictus* eggs without any contact with Bb were used as a control group.

RESULTS AND CONCLUSIONS: The viability of Bb in the HBBs increased from 1-5x106 CFU/ml to 1-5x107 CFU/ml after 5 days of incubation remaining constant until the day 21st. A reduction of Bb vitality from 1-5x106 CFU/ml to 1-5x104 CFU/ml was observed in CIS of Bb after 10 days of incubation at 28°C, 80% RH. The mortality of *A. albopictus* eggs varied according to the contact substrate. The 100% mortality was recorded only in the cellulose matrix in the presence of Bb after 14 days of contact. Bb in aqueous solution caused a mortality of the eggs less than or equal to that of the control. The results show that the combination of Bb with cellulose hydrogel is effective against *A. albopictus* eggs, while the simple suspension in water of Bb did not show any efficacy. The usage of natural hydrogels in combination with Bb represents a promising tool to be used in alternative to chemical compounds currently used for the control of *A. albopictus*.

LIZARDS AS RESERVOIRS OF PATHOGENIC YEASTS: A POTENTIAL HAZARD TO HUMAN AND ANIMAL HEALTH

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Keywords: lizards, yeasts, antifungal profiles

INTRODUCTION: Reptiles have become popular exotic pets, and in some parts of the world, they are used as important source of food, medicines, and materials (Mendoza-Roldan et al., 2019. Parasites Vectors, 12: 35; Mendoza-Roldan et al., 2021. PLoS Negl Trop Dis, 15: e0009090). Synanthropic lizards are recognized as reservoirs of viruses, bacteria (Zancolli et al., 2015. Microbial ecology, 70: 579-84), and parasites (Mendoza-Roldan et al., 2019. Parasites Vectors, 12: 35) but their role in dissemination of zoonotic pathogenic yeasts in the environment was never investigated. Thus, this study aimed to i) investigate the presence of yeasts in the faeces of lizards, ii) evaluate the antifungal profile of the isolated strains and iii) estimate the relationship of yeasts and antifungal profile with lizard environment.

MATERIALS AND METHODS: A total of 177 fecal samples from *Podarcis siculus*, *Chalcides ocellatus* and *Tarentola mauritanica* were collected and yeasts were isolated and identified biochemically and molecularly by sequencing the rDNA internal transcribed spacer region (ITS). The phylogenetical relationship of isolated yeast species and their antifungal susceptibility profiles were also assessed.

RESULTS AND CONCLUSIONS: Sixty samples (33.9%) scored positive for yeasts, with the highest occurrence in *C. ocellatus* (64.7%) and the highest variety of species in *P. siculus*. A total of 364 isolates belonging to *Candida*, *Trichosporon*, *Saccharomyces* and *Geotrichum* genera were molecularly identified. In particular, *Candida albicans* (44%) followed by *Trichosporon coremiiforme* (12.1%), *Pichia kudriavzevii* (8.8%) and *Trichosporon asahii* (7.7%) were the most frequently isolated species. The phylogenetic tree grouped all representative sequence types within the clade including *Candida* spp. strains from different geographical areas and from animal species, including human. All tested strains showed very high susceptibility to the employed drugs. This study suggests, for the first time, the role of lizards as reservoirs and spreaders of pathogenic yeasts in the environment and their possible role in the epidemiological chain of important zoonosis. The absence of resistant phenomena in the isolated yeasts might reflect an environment free of azole antifungal pollution or chemicals and suggests that these animals might represent excellent bio indicators of environment quality.

REPEATED ULTRASOUND CHECKS AS CONTRIBUTE TO EFFECTIVE SURGERY OF FELINE PSEUDOMYCETOMA

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Keywords: pseudomycetoma, ultrasonography, *Microsporum canis*

INTRODUCTION: Feline pseudomycetomas are uncommon but well-known dermal, subcutaneous or intra-abdominal atypical dermatophyte infections, occurring more frequently in Persian cats and most commonly caused by *Microsporum canis* (Nobre et al., 2010. Rev Iberoam Micol, 27: 98-100; Bianchi et al., 2017. Rev Iberoam Micol, 34: 112-15). Surgical excision and concurrent long time antimycotic therapy are required. Nevertheless, the prognosis is guarded, with common relapse even in those cases that respond initially (Nobre et al., l.c.; Moriello et al., 2017. Vet Dermatol, 28: 266–e68). Here we describe a case in which ultrasonography was helpful to targeted surgical approach of cutaneous abdominal pseudomycetoma.

MATERIALS AND METHODS: An 8-year neutered female European cat was presented for an ulcerated mass in the umbilical region, which extended towards the abdominal cavity. At ultrasonography, the mass was well defined, hyperechoic to surrounding tissue and had many small, discrete hyperechoic nonshadowing foci. Fine needle aspiration of the mass and regional lymph nodes revealed a chronic pyogranulomatous inflammation and the presence of fungal hyphae; Mycosel agar culture was positive for *Microsporum canis*. Itraconazole was administered for 125 days and well tolerated; periodic ultrasonographic checks monitored the size reduction of the mass until the surgical procedure was carried out, with excision of the mass and abdominal wall reconstruction without the aid of prosthetic mesh implant. Histopathology confirmed the diagnosis of pseudomycetoma.

RESULTS AND CONCLUSIONS: After sutures' removal, on ultrasound the abdominal wall did not display signs of recurrence. At 6 years follow-up, no relapse was observed. In pseudomycetoma pathogenesis, a direct invasion of the dermal tissues by the organisms through rupture of follicular structures is proposed. Lymphatic involvement or systemic dissemination has not been reported (Chang et al., 2011. Vet Dermatol, 22:181-7). The present case could represent a complication of ovariohysterectomy, which could have been the portal of entry of the mycete, in relation with the detection of mycotic granulomatous lymphadenitis. The ultrasonography was helpful to evaluate the spread of the infection in the tissues and its regression during therapy, allowing to a complete surgical excision of the mass and favouring a lasting remission of the disease.

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PARASITIC DISEASES IN AQUATIC ANIMALS

NOVEL MARKERS IN A MULTILOCUS GENOTYPING APPROACH FOR THE DETECTION OF INTERSPECIFIC HYBRIDIZATION BETWEEN *ANISAKIS BERLANDI* AND *A. PEGREFFII* IN THE SYMPATRIC AREA OF THE SOUTHWESTERN ATLANTIC OCEAN

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Keywords: *Anisakis pegreffii*, *Anisakis berlandi*, hybridization

INTRODUCTION: In recent years, new nuclear diagnostic markers between the species of the *A. simplex* (*s.l.*) complex (i.e. *A. simplex s.s.*, *A. pegreffii*, *A. berlandi*) at the EF-1-α nDNA (Mattiucci et al., 2016. Parasitology, 143: 998-11), nas10 nDNA (Palomba et al., 2020. Parasite, 27: 39), and DNA microsatellite (SSR loci) (Mattiucci et al., 2019. Parasitology, 146: 1387-03) were developed, in the aim to use a multilocus approach for recognition of parental species and hybridization/introgression events in their sympatric areas. The Austral Region (30°-60°S) is known to be a sympatric area of *A. pegreffii* and *A. berlandi* (Mattiucci et al., 2018. Adv Parasitol, 99: 93-263). Hybridization events between the two species were previously discovered in New Zealand waters (Bello et al., 2021. Inf Gen Ev, 92: 104887). The aim of the present study was to use newly developed nuclear gene loci in a multilocus genotyping approach to recognise parental taxa of *A. simplex* (*s.l.*) complex and their mixed ancestry in the sympatric area of the southwestern Atlantic Ocean.

MATERIALS AND METHODS: A total of 144 adults and 156 L3 of *Anisakis*, respectively collected in *Orcinus orca* and *Merluccius hubbsi* from Argentine waters, were previously identified by cox2 and nas10, then genotyped at diagnostic SSRs loci and at two new here developed nuclear loci (i.e. superoxide-dismutase Sod, adenylate-kinase Adk), and Primers for Adk and Sod were designed starting from an available partial genome of *A. simplex*. To investigate instances of gene exchange between *A. pegreffii* and *A. berlandi*, the Bayesian clustering algorithm by STRUCTUREv.2.3.3 (Pritchard et al., 2000. Genetics, 155: 945-59) and NEWHYBRIDS (Anderson & Thompson, 2002. Genetics, 160: 1217-29) to assess the occurrence of hybridization events between them, were performed on nuclear genetic data set.

RESULTS AND CONCLUSIONS: A total of 60 and 4 adults from *O. orca* were assigned, respectively, to *A. pegreffii* and *A. berlandi*, as well as respectively 143 and 13 larvae from *M. hubbsi*, by mtDNA cox2. STRUCTURE analysis of genotypes obtained from the nuclear data set, allowed the assignment (100% probability) to the two “pure parental” taxa, except in the case of 2 adults and 1 larva specimens. These last specimens were identified as admixed between the two species, having a Qvalue=50%. NEWHYBRIDS assigned (100% probability) most of individuals as belonging to the two “pure parental” taxa; those 3 admixed individuals to the F1 hybrid class. They showed *A. pegreffii* maternal inheritance at the mtDNA cox2.
The two novel nuclear loci (Sod and Adk) showing, respectively, 9 and 7 fixed alternative SNPs between *A. pegreffii* and *A. berlandi*, resulted 100% diagnostic. The multilocus nuclear genotyping approach will be useful to study hybridization and/or introgression events occurring between these species in other sympatric areas, as well as in other sibling species of the *A. simplex* (*s.l.*) complex.

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COMPARISON BETWEEN MINI-FLOTAC AND A CONVENTIONAL TECHNIQUE FOR THE DETECTION OF HELMINTH EGGS IN CETACEAN STOOL SAMPLES

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Keywords: Mini-FLOTAC, cetaceans, helminthes

INTRODUCTION: Free ranging cetaceans are sentinels of the marine ecosystem's health, and valuable information about the population health and migratory pathways can be obtained from evaluation of their parasitic community. Non-invasive methods, set up for the collection of fecal samples from whales at sea, allow to bring together parasitological data from necropsies and from healthy, free-living animals (Flores-Cascante et al., 2019. Acta Parasitol, 64:625-37). To evaluate which copromicroscopic protocol describes more reliably the gastrointestinal helminthic communities in cetaceans, we studied the Mini-FLOTAC (MF) (Cringoli et al., 2017. Nat Protoc, 12:1723-32) and a classical sedimentation-flotation technique (SF) comparing them with helminth isolation from the digestive tract.

MATERIALS AND METHODS: Gastrointestinal content and fecal samples were collected during necropsy from 45 stranded cetaceans, including odontocetes (no.= 40) and mysticetes (no.= 5), in the period 2009-2022. Helminths were recovered through a classical filtration-sedimentation technique, preserved in ethanol 70° and morphologically identified at light microscope. Fecal samples preserved in 5% formalin were submitted to a double copromicroscopic examination using a sodium nitrate, sodium thiosulphate and sucrose solution (s.g. =1.450) for both SF and MF. Sensitivity of the copromicroscopic methods was calculated using the isolation of helminths as a reference test.

RESULTS AND CONCLUSIONS: On the whole, helminths belonging to 9 taxa (i.e., the trematodes *Synthesium tursionis*, *Synthesium delamurei*, *Campula palliata*, *Braunina cordiformis*, *Pholeter gastrophilus*, the nematode *Anisakis* sp., cestodes of the family Tetrabothriidae and the acanthocephalan *Bolbosoma* sp.) were isolated with filtration-sedimentation technique. No eggs belonging to additional taxa, nor eggs of cestodes were found at copromicroscopic analyses by any method. Sensitivity of the Mini-FLOTAC method appeared higher for all taxa with respect to SF (range 30-100%) and equal for *Anisakis* sp. (56%). Evaluation of the concordance between the two tests showed a moderate to perfect agreement (K-value = 0.42-1) for the different taxa.
Not excluding the limitations in terms of sensitivity inherent to the techniques themselves, intermittent egg shedding, prepatent infections or under threshold parasitic burdens could account for negative results at copromicroscopy. Dynamics of eggs shedding in these helminth species are largely unexplored, and this is the first study comparing data from copromicroscopy and gastrointestinal helminth isolation in cetaceans. Notwithstanding the limitations linked to all these factors, we conclude that Mini-FLOTAC protocol seems to be a useful technique for estimating the composition of gastrointestinal helminthic community of cetaceans, providing at the same time a quantitative estimation.

NEMATODES OF THE GENUS *EUSTRONGYLIDES* IN THE MASSACIUCCOLI LAKE, TUSCANY, CENTRAL ITALY: PRELIMINARY RESULTS OF AN ONGOING PROJECT

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Keywords: fish-borne parasites, food safety, food quality

INTRODUCTION: The genus *Eustrongylides* includes parasitic nematodes with a worldwide distribution. Freshwater piscivorous birds act as definitive hosts, aquatic oligochaetes as first intermediate hosts and fish species as second intermediate or paratenic hosts (Mazzone et al., 2019., J Parasitol, 105: 882-89). Humans may represent accidental hosts (Eberhard and Ruiz-Tiben, 2014. Am J Trop Med Hyg, 90: 315–17). In Italy, the first record of this genus occurred in 2015 in perch (*Perca fluviatilis*) from the Trasimeno Lake (Central Italy) (Dezfuli et al., 2015. Parasit Vectors, 8: 227). Subsequent records occurred in the same lake (Mazzone et al., 2019. J Parasitol, 105: 882-89; Franceschini et al., 2022. Food Control, 136: 108858) and in other Italian lakes (Guardone et al., 2021. Food Control, 120: 107517; Menconi et al., 2021. Water, 13: 3581), but its distribution in fish species is still poorly known. This work presents the results on the first five months of investigations of a 2-year project, financed by the Italian Ministry of Health, aimed at evaluating the occurrence, infection levels and localization of *Eustrongylides* spp. larvae in commercial fish species in lakes in Tuscany and Latium.

MATERIALS AND METHODS: Ten fish species were sampled from the Massaciuccoli lake (North Tuscany) from August to December 2021: 74 catfish (67 *Ameiurus melas* and 8 *Silurus glanis*); 43 thin lip grey mullets (*Chelon ramada*); 31 gold fish (*Carassius auratus*); 19 common carps (*Cyprinus carpio*); 5 tench (*Tinca tinca*); 3 largemouth bass (*Micropterus salmoides*); 1 pumpkin seed (*Lepomis gibbosus*); 4 pools (consisting of small specimens of around 1 g) of sand smelt (*Atherina boyeri*, no. per pool=290-1500); 3 pools of thin lip grey mullets (no. per pool=10-150) and 1 pool of stone moroko (*Pseudorasbora parva*, no.= 16). All specimens were subjected to visual inspection, followed by artificial digestion. Parasites were counted and morphologically identified to genus level according to Measures, 1988 (Can J Zool, 66: 2223-32).

RESULTS AND CONCLUSIONS: *Eustrongylides* sp. larvae were found in 5 of the 10 examined species: in catfish, with a prevalence of 23.9% (95%CI = 15.3-35.3) in *A. melas* and of 62.5% (95%CI = 30.6-86.3) in *S. glanis*; in 1 of the 3 *M. salmoides* examined, in all the 4 pools of *A. boyeri* (prevalence ranging from 1.4 to 5.8%) and in 2 out of the 3 pools of *C. ramada* (prevalence from 4.7 to 16.0%). Overall, 189 larvae were collected. The highest mean abundance was observed in catfish (0.4-0.63), the lowest in sand smelt pools (0.01-0.06), while the mean infection was always 1, except for *A. melas* (1.69). The study contributes to update the distribution of this nematode in Italy. The observed differences could be attributed to different feeding behaviour and trophic levels, as suggested for other lakes (Menconi et al., 2021. Water, 13: 3581). Moreover, although no human cases have been reported so far in Europe, the presence of this potentially zoonotic species should be carefully monitored and its impact on fisheries evaluated.

COMBINED EFFECTS OF SALINITY AND TREMATODE INFECTIONS ON MUSSELS PERFORMANCE

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Keywords: *Mytilus*, trematode, salinity

INTRODUCTION: In coastal habitats with severe salinity gradients, such as the Baltic Sea, the blue mussel *Mytilus edulis* is a crucial ecosystem engineer, and salinity is a primary abiotic driver of mussel functioning. Biotic interactions, such as parasitism, can also affect the performance of mussels. The interaction between salinity and parasite infection, on the other hand, is poorly understood

MATERIALS AND METHODS: This study investigated on the effects of salinity and metacercarial infections of the trematode *Himasthla elongata* on the filtration capacity, growth, and condition of *M. edulis* from the Baltic Sea. For one month, infected and uninfected mussels were exposed to a large salinity gradient (6 to 30, in steps of 3) in a laboratory experiment.

RESULTS AND CONCLUSIONS: The influence of salinity on mussel filtration was found to be significant, with an initial optimum at salinity 18 shifting to 18–24 towards the end of the experiment. *H. elongata* infection, on the other hand, had no influence on mussel filtration. Furthermore, mussel shell growth was found to be positively associated with salinity, peaking at 18–24 at the end of the experiment, suggesting that freshening had a detrimental impact on shell calcification. While parasite infections had no effect on mussel shell growth, infected mussels had considerably lower tissue dry weight than uninfected mussels. Salinity had only a minimal effect on tissue dry weight, implying that trematode infections are the primary cause of soft tissue damage in mussels. As a result, the mussels were negatively affected by the combination of salinity and trematode infections.

MORPHOLOGICAL AND MOLECULAR STUDY OF THE GILL DIDYMOZOID PARASITE (TREMATODA: DIDYMOZOIDAE) OF *EPINEPHELUS MARGINATUS* (TELEOSTEI: SERRANIDAE) FROM THE MEDITERRANEAN SEA

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Keywords: Didymozoidae, trematodes, dusky grouper

INTRODUCTION: Didymozoids (Platyhelminthes, Trematoda) are poorly known parasites, usually found in wild marine teleost fish worldwide. In the dusky grouper *Epinephelus marginatus*, Lowe 1834, didymozoids have been reported attached to the gills, pseudobranchs and orobranchial cavity (Polinas et al., 2018. J Fish Dis, 41: 1385–93). These parasites, macroscopically characterised by a white-yellow capsule, were morphologically identified as *Didymodiclinus* sp. (De Benedetto et al., 2021. Animals, 11: 2523). The aim of the present study was to perform a morphological and molecular characterisation of *Didymodiclinus* sp. in *E. marginatus* from the Mediterranean Sea.

MATERIALS AND METHODS: From 1998 to 2020, 279 specimens of *E. marginatus* from the Mediterranean Sea were examined for didymozoid gill parasites (Majorca, N=209; Sicily, N=70). For morphological analysis, parasites were observed under a stereo microscope, stained with iron acetocarmine and observed under a light microscope. Molecular analyses were performed by polymerase chain reaction, for which, partial 28S, partial ITS-2 ribosomal RNA regions and mitochondrial COX1 gene were used. The obtained sequences were aligned with available Didymozoidae nucleotide sequences using the MUSCLE algorithm.

RESULTS AND CONCLUSIONS: Ninety-two specimens of *E. marginatus* from Majorca and 13 from Sicily (P=44.9% and 18.6%, respectively) were infected with didymozoid parasites. The morphological characteristics of the examined specimens were not superimposable to any previously described species, indicating their belonging to a new species. Molecular analyses confirmed that the samples collected in Majorca and Sicily were identical. The comparison between the obtained *Didymodiclinus* n. sp. 28S sequences and those deposited in GenBank showed that the new isolates cluster with numerous unidentified didymozoids, and with the Nematobothrinae subfamily sister group. Moreover, the 28S and cox1 phylogenetic analysis showed a clear separation between Didymodiclinae, Didymozoinae and other gonochoric didymozoids.

GENETIC VARIABILITY AMONG *GYRODACTYLUS GERASEVI N.* SP. AND *GYRODACTYLUS SPHINX* (PLATYHELMINTHES: MONOGENEA) SHOWS CRYPTIC SPECIATION AT THE HOST RANGE BOUNDARIES

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Keywords: monogenea, *Gyrodactylus*, speciation

INTRODUCTION: *Gyrodactylus sphinx* (Dmitrieva & Gerasev, 2000) is a gill and skin parasite of the sphinx blenny *Aidablennius sphynx* (Valenciennes), so far reported only in the Black Sea. In the present study specimens of gyrodactylids, morphologically similar to *G. sphinx*, were found on the blenniids *Salaria pavo* (Risso) and *S. basilisca* (Valenciennes) in the Mediterranean Sea off Sardinia, and *S. pavo* and *A. sphinx* in the Black Sea near Caucasus and Crimea. The aims of this study are to describe the morphological and genetic variability of these specimens, to reconstruct their phylogenetic relationships with other species of the same genus and to depict the phylogeographic relationships among the samples collected in the present study.

MATERIALS AND METHODS: In the summers of 2015 and 2018, a total of 78 specimens of blenniid fish from the Mediterranean Sea and the Black Sea were examined for monogeneans. Some specimens were mounted in glycerine-jelly and others fixed in 70% ethanol, for morphological and molecular analyses, respectively. In total, 169 monogeneans were used for morphometric analysis, the measuring scheme followed Malmberg (1970). Molecular analyses were carried out using the nuclear ITS2 and 5.8S rDNA regions on 37 specimens from the western Mediterranean Sea and 44 from the Black Sea. Network, Bayesian phylogenetic tree and species delimitation analyses were performed to infer the number of taxonomic units and the phylogeographic relationships present in the dataset among individuals.

RESULTS AND CONCLUSIONS: Molecular analyses showed the occurrence of two taxonomic entities: the most common was present in individuals of the Black and Mediterranean seas, and it was described as *Gyrodactylus gerasevi* n. sp.; whereas a second one, which was recognised as a *G. sphinx*, was found only in individuals from two localities off Crimea. However, the examined samples of *G. sphinx* and *G. gerasevi* n. sp. from different hosts and localities were morphologically indistinguishable, and the two taxonomic entities found can be considered as sibling species. The occurrence of these two well-differentiated species - *G. sphinx* only in the Black Sea off Crimea and *G. gerasevi* n. sp. on the same hosts in the Mediterranean and Black seas - may be explained as a possible consequence of both the distance between geographical areas and of the non-migratory habits of the hosts, which resulted in a parapatric divergence.

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REPORT OF *TOXOPLASMA GONDII* FROM A DOLPHIN STRANDED ALONG SICILIAN COASTS AND ITS PHYLOGENETIC RELATIONSHIP INFERENCE

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Keywords: *Toxoplasma gondii*, dolphins, gra6

INTRODUCTION: *Toxoplasma gondii*, a protozoan pathogen causing zoonosis, represents a serious threat for aquatic mammals including dolphins, as it causes severe brain lesions in dolphins leading them to stranding and death.
This study aimed to investigate *T. gondii* DNA presence in organs collected from dolphins stranded along Sicilian coasts and to carry out sequence analysis based on gra6 gene.

MATERIALS AND METHODS: During 2021, nine dolphins were analysed, found stranded along the coast of Sicily. Five animals were *Stenella coeruleoalba*, 2 *Delphinus delphis* and 2 *Tursiops truncatus*. Different organs were collected, including brain, spleen, liver, lung, lymph node, muscle and heart, for a total of 45 examined organs. One gram of tissue sample, diluted in 9 mL of saline solution, was homogenized in the Stomacher. Genomic DNA was extracted from the homogenate using a commercial kit. *T. gondii* DNA was amplified by both a nested PCR targeting the B1 gene and a TaqMan Real Time PCR targeting the 529 bp repeat element. A fragment of 773 bp of the gra6 gene was amplified in positive samples by nested PCRs and sequenced. Obtained sequences were analysed using BioEdit and MEGA version 7.0. Phylogenetic analyses were performed by neighbour-joining using the Maximum Composite Likelihood method. Phylogenetic tree was constructed with several valid type I (RH), II (Beverley and ME49) and III (NED, TONT and C56) T. gondii strains.

RESULTS AND CONCLUSIONS: A *S. coeruleoalba* dolphin resulted positive for *T. gondii* DNA. The animal was found in the Western coast of Sicily and all the examined organs (brain, lymph nodes, spleen, hearth, liver and muscle) were positive, including those of choice for this pathogen. The nucleotide sequences of gra6 fragment obtained from all the different organs revealed high percentage of sequence similarity with the published sequences of *T. gondii* gra6, thereby establishing its specificity. In silico analysis of the consensus obtained sequence of gra6, its subsequent phylogenetic analysis and pairwise distance calculations revealed the closest genetic relationship of the *T. gondii* strain from Sicilian stranded dolphin with that of type III strains (NED, TONT and C56).
T. gondii Type II lineage as well as a Type II-atypical isolate have been previously characterized from dolphins stranded along the northern coasts of Italy.
The positive animal belonged to cetacean species living in the open sea, making interesting better understanding the transmission routes of *T. gondii* for such animals in this area. Further studies are necessary to better characterize the genetic configuration of the strain and to clarify its possible origin and transmission routes in aquatic mammals.

PRESENCE OF *CONTRACAECUM RUDOLPHII* IN *PHALACROCORAX CARBO SINENSIS* FROM SOUTHERN ITALY

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Keywords: *Contracaecum*, cormorants, south Mediterranean

INTRODUCTION: Among the Anisakidae family, *Contracaecum* spp. nematodes occur as parasites of birds and pinnipeds. Adult forms of this genus were reported in different piscivorous birds such as pelicans and cormorants. The great cormorant *Phalacrocorax carbo sinensis* is a highly specialized bird feeding on fish, which it captures during the day by diving. It is a polytypic species with a sub-cosmopolitan distribution. Different studies on the parasite fauna of the great cormorant in the Mediterranean confirmed that this species is the main host of *Contracaecum rudolphii sensu lato*, showing very high intensity values and a prevalence of infestation of 100%. In this work, we examined the occurrence of *C. rudolphii s.l.* in cormorants from Southern Italy waters, with molecular characterization of species.

MATERIALS AND METHODS: Four specimens of *P. carbo sinensis* were collected from 2011 to 2022 (2 from Sicily and 2 from Apulia region). A total of 155 nematodes, at larval and adult stage, were collected from the stomach at necropsy and a subsample of 77 parasites were characterized using genetic markers. PCR-RFLP analyses of rrnS and ITS markers were performed to characterize *C. rudolphii* sibling species (D'Amelio et al., 2012. Parasitology, 134:1041).

RESULTS AND CONCLUSIONS: The adult nematodes recovered from *P. carbo sinensis* from Sicily and Apulia regions were morphologically identified as *C. rudolphii s.l.* Thirty-five nematodes infecting cormorants from Sicily (south western Sicily, Mediterranean Sea) belonged to *Contracaecum rudolphii* B, whereas most of the 42 nematodes infecting cormorants from Apulia region (South Adriatic Sea) were identified as *Contracaecum rudolphii* A (93%), according to the RFLP patterns obtained at ITS and rrnS genomic regions. *C. rudolphii* B was reported as well (4.6%), and one putative hybrid (2.4%) was observed at nuclear ITS digestion pattern. This study provides information about the occurrence of *C. rudolphii s.l.* in southern Italy, a poorly investigated area, showing results in agreement with the available literature. The coexistence of *C. rudolphii* A and *C. rudolphii* B has been described in Sardinia, with the rare observation of hybrids (Amor et al., 2020. Parasitology, 147:1538). Evidence from central Italy reported both sibling species (Mattiucci et al., 2020. Parasitol Res, 119:1243), with a life-cycle adapted to brackish and freshwater environments of *C. rudolphii* A and *C. rudolphii* B, respectively. Moreover, similar results were found in cormorants from the pre-mountain area of the Alps in Northern Italy (Carmeno et al., 2022. Vet Parasitol Reg Stud Reports, 27:100674).

COMPARISON OF DIFFERENT DIAGNOSTIC METHODS FOR THE DETECTION OF *ENTEROMYXUM LEEI* (MYXOZOA) IN FARMED GILTHEAD SEA BREAM (*SPARUS AURATA*)

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Keywords: *Enteromyxum leei*, *Sparus aurata*, diagnosis

INTRODUCTION: *Enteromyxum leei* is an enteric Myxozoan parasite causing the so-called “razor blade syndrome” in gilthead sea bream (*Sparus aurata*), a chronic condition characterized by a progressive weight loss up to emaciation in adult fish. Currently no treatments are found to be effective against this parasite, but a recent experimental study showed promising results using a functional feed for mitigating enteromyxosis (Palenzuela et al., 2020. Dis Aquat Org, 138: 111–20). During a field trial aimed to confirm the efficacy of this functional feed in an Italian sea bream facility where enteromyxosis was already known to be present, different methods for the detection of *E. leei* were compared to establish the more reliable diagnostic test also in relation to a non-lethal approach.

MATERIALS AND METHODS: During the aforementioned field trial, different methods were applied to detect *E. leei*: microscopical observation of a drop of intestinal content collected by squeezing the anus followed by 100 random observations per slide (400× magnification) and count of parasitic stages/field; rectal sample collected by probing the rectum with a cotton swab and subjected to qPCR (Piazzon et al., 2017. Microbiome, 5: 164); histological observation of portions of rectal ampulla from the same fish subjected to microscopical examination and qPCR.

RESULTS AND CONCLUSIONS: The comparison of the different diagnostic methods applied confirmed the qPCR as the most reliable and sensitive method for detecting the presence of the parasite, offering also a semiquantitative estimation of the infection intensity based on the cycle threshold (ct). The microscopical observation, despite being easily feasible, costless and reliable to detect *E. leei* in advanced course of infection, can show difficulties in detecting early developmental stages and estimating low infection intensities when compared to qPCR. Histology appears to be a useful complement to qPCR and microscopical observation in assessing pathological alterations due to *E. leei* but shows underestimation issues with regard to the intensity of infection, also with false negative results. In fact, at histology developmental stages of *E. leei* have never been detected when the qPCR ct was ≥31, even if the accepted ct threshold to consider a fish positive for *E. leei* is <38 (Piazzon et al., 2017. l.c.). Furthermore, unlike histology, microscopical observation and qPCR can be performed on non-lethal samples collected from anesthetized fish. In conclusion, the use of qPCR could be the best option for the screening of newly introduced fish and to check the course of infection in farmed fish.

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MORPHOLOGICAL CHARACTERIZATION OF AMOEBAS INVOLVED IN A NODULAR GILL DISEASE (NGD) OUTBREAK IN BROOK TROUT (*SALVELINUS FONTINALIS*) AND RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FARMED IN NORTHERN ITALY

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Keywords: nodular gill disease, amoebas, brook trout *Salvelinus fontinalis*

INTRODUCTION: Nodular Gill Disease (NGD) is an emerging amoebic disease of freshwater salmonids farmed worldwide. Although it is more frequently described in rainbow trout (*Oncorhynchus mykiss*), other co-farmed salmonids may be affected, including arctic char (*Salvelinus alpinus*), Chinook salmon (*Oncorhynchus tshawytscha*), and brown trout (*Salmo trutta*). An outbreak of NGD-related mortality and morphological characterization of involved amoebas is here described in brook trout (*Salvelinus fontinalis*) farmed in Northern Italy.

MATERIALS AND METHODS: The NGD outbreak was monitored in a mixed rainbow/brook trout facility. The mortality reached 30% for brook trout over a 1-month period. Twenty brook trout and twenty rainbow trout presenting clinical signs of NGD were sampled and underwent macroscopic, microscopic, and histological examination with the use of Giemsa stain, with the assessment of 6-grade lesion scores. From the same facility a second sample was carried out after few weeks collecting 2-3 portions of gill arches from 50 rainbow trout. Gills were put on non-nutrient agar (NNA) Petri dishes for amoeba isolation, cultivation, and identification by Transmission Electron Microscopy (TEM).

RESULTS AND CONCLUSIONS: Brook trout showed macroscopic and histological lesions classically associated with NGD. When compared with affected rainbow trout, affected brook trout presented milder amoebic infection associated with larger size and higher severity of the lesions.
Successful amoeba cultivation was possible from 2 samples, resulting in the isolation of 2 amoeba colonies. The first colony was composed of motionless rounded or ovoid cells (8-10 µm) with filopods. The second colony was made of actively motile irregularly shaped cells (10-15 µm). When observed with TEM, the cells of the first colony showed an organic case covering the whole cell surface with the presence of a pseudostoma, while in the cells of the second colony the outer plasma membrane was covered by a glycocalyx. They were morphologically identified as *Rhogostoma* sp., probably *R. minus* (Thecofilosea: Rhogostomidae) and *Vanella* sp. (Flabellinia: Vannellidae), respectively.
The outbreak of NGD in brook trout here described demonstrates that also *S. fontinalis* can be prone to serious gill pathologies associated with the presence of amoebas. The cultivation of amoebae still represents a diagnostic challenge given the high rate of bacterial and mycotic contamination impairing the viability of cultures, which caused the loss of a great number of samples.
The different sizes of the affected brook trout compared to rainbow trout suggests an age-dependent susceptibility, while the presence of a more severe degree of injuries with a relatively mild amoebic infection could suggest a greater susceptibility of brook trout than rainbow trout.
Further studies are necessary to assess the pathogenicity of different species of amoebas and their relation to NGD occurrence in different environments and salmonid species.

APPLICATION OF MINI-FLOTAC TECHNIQUE TO SEA TURTLES FECAL SAMPLES: EVALUATION OF SENSITIVITY AND COMPARISON WITH A TRADITIONAL COPROMICROSCOPIC METHOD

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Keywords: *Caretta caretta*, Mini-FLOTAC, helminthes

INTRODUCTION: The choice of a copromicroscopic technique to be applied on field is leaded on the one side by practical constraints, especially in terms of time and lab equipment, and on the other hand by the need of accuracy, to select proper treatment protocols on the animals and check their efficacy. The Mini-FLOTAC (MF) has already been employed in sea turtles as well as in other wild species, in that it seems to satisfy both needs (Pace et al., 2019. BMC Vet Res, 15:370). In this study, the sensitivity of MF in sea turtles stool samples is assessed using *post-mortem* isolation of gastrointestinal helminths as a reference test and is compared with a traditional copromicroscopic technique.

MATERIALS AND METHODS: Helminths were recovered from the digestive system of 51 stranded loggerhead sea turtles after necropsy, by a filtration-sedimentation process of gastric and intestinal contents. All helminths were counted and identified at light microscope following keys in literature. Samples of rectal content were stored in 5% formalin, and copromicroscopic exam was performed by both MF and a traditional sedimentation-flotation method (SF), using the same solution (specific gravity = 1.450). Concordance between the results of the two copromicroscopic methods was evaluated with k-value, and the sensitivity (Se) of each method assessed through the comparison with helminths isolation. Finally, the correlation among fecal egg counts (FEC) and helminth burden was calculated through Spearman's rank coefficient.

RESULTS AND CONCLUSIONS: An overall number of 8 helminth taxa were collected from the gastrointestinal system, including the trematodes *Rhytidodes gelatinosus*, *Enodiotrema* sp., *Pachipsolus irroratus*, *Orchidasma amphiorchis*, *Pleurogonius trigonocephalus*, *Calycodes anthos* and the two nematodes *Sulcascaris sulcata* and *Kathlania leptura*. Eggs referable to the same taxa were detected at copromicroscopy, together with eggs of cardiocirculatory flukes (Spirorchiidae eggs type 1 and 3). Concordance among the two copromicroscopic techniques was good to excellent for the ten different taxa (k=0.61-1.00) and the Se for the different taxa was also similar (41-75% for SF, 45-75% for MF). Weak correlation was found between FEC and helminthic burden for all taxa.
The Mini-FLOTAC method showed similar performances to the traditional SF technique in terms of sensitivity, proving at the same time faster to perform without specific lab supplies. Previous studies in which MF was applied to sea turtles stool samples, eggs of Spirorchiidae and nematodes had not been detected. Geographical differences in the epidemiology of these helminthiases must be considered (Santoro et al., 2020. Parasite Vectors, 13:52), nevertheless, the efficiency of different types of flotating solutions should also be further investigated.
As a first assessment on the correlation between FEC and helminth burden, this study suggests to consider anti-helminthic treatments in hospitalized turtles regardless of FEC for pathogenic species such as *S. sulcata*.

ACANTHOCEPHALANS FROM MARINE FISHES: MOLECULAR EVIDENCE OF NON-MONOPHYLY OF THE ARHYTHMACANTHIDAE YAMAGUTI, 1935

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Keywords: phylogenetic relationships, *Breizacanthus*, *Euzetacanthus*

INTRODUCTION: The family Arhythmacanthidae Yamaguti, 1935 was erected to accommodate echinorhynchid acanthocephalans from fish with an abrupt or gradual transition from small basal hooks/spines without roots to larger rooted hooks on the proboscis (García-Varela and Andrade-Gómez, 2021. Int Parasitol, 81:102264). Three subfamilies, namely Arhythmacanthinae Yamaguti, 1935, Neoacanthocephaloidinae Golvan, 1960 and Paracanthocephaloidinae Golvan, 1969, are placed in the Arhythmacanthidae (Pichelin and Cribb, 1999. Parasite, 6:293–302). In this study, the relationships of two members of the Paracanthocephaloidinae, i.e., *Breizacanthus* Golvan, 1969 and *Euzetacanthus* Golvan and Houin, 1964, were assessed using a multi-gene phylogenetic analysis.

MATERIALS AND METHODS: Sampling was carried out on commercial trawl landings from two western Mediterranean localities: northern Sardinia and northern Algeria. Specimens of *Breizacanthus ligur* Paggi, Orecchia and Della Setta, 1975 were collected from *Chlorophthalmus agassizi* Bonaparte caught in Sardinia in 2015 and 2020; and specimens of *Breizacanthus irenae* Golvan, 1969 and *Euzetacanthus simplex* (Rudolphi, 1810) were collected from *Mullus surmuletus* L. caught in Sardinia and Algeria in 2021. Partial sequences of the small and large subunits nuclear ribosomal RNA genes (SSU and LSU) and the mitochondrial cytochrome c oxidase subunit 1 (cox1) were generated for the examined specimens. Maximum likelihood and Bayesian inference analyses were performed to evaluate the phylogenetic relationships between different species of the Arhythmacanthidae.

RESULTS AND CONCLUSIONS: A total of three partial 18S sequences (Sardina: *B. ligur*, 1; *B. irenae*, 1; *E. simplex*, 1), three partial 28S sequences (Sardina: *B. ligur*, 1; *B. irenae*, 1; *E. simplex*, 1) and 10 partial cox1 sequences (Sardina: *B. ligur*, 2; *B. irenae*, 3; *E. simplex*, 2; Algeria: *B. irenae*, 3) were generated in this study. Maximum likelihood and Bayesian inference analyses revealed the Arhythmacanthidae, Paracanthocephaloidinae and *Breizacanthus* as non-monophyletic. Furthermore, *B. irenae* should be transferred to *Euzetacanthus* and *Solearhynchus rhytidotes* (Monticelli, 1905) (syn. *Paracanthocephaloides soleae* (Porta, 1905)), currently placed in the Echinorhynchidae Cobbold, 1879, to the Arhythmacanthidae. The present phylogenetic analysis contradicts morphology-based classification of the Echinorhynchida and new arrangement of some taxa is pending.

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GASTROINTESTINAL HELMINTHOFAUNA OF MEDITERRANEAN ELASMOBRANCHS

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Keywords: elasmobranchs, helminths, mediterranean sea

INTRODUCTION: Elasmobranchs represent an important group of fish at the apex of the trophic webs in the marine environment, playing the role of definitive hosts for a wide range of heteroxenous helminth parasites. More specifically, the collection and identification of the gastrointestinal helminthofauna of different elasmobranch species from different geographical areas could provide useful information on their diet and habitat preferences, furnishing useful information for stock discrimination and conservation purposes (Yamaguchi et al., 2003. Fish Sci, 69: 337–42). Parasitological surveys on elasmobranchs from the Mediterranean Sea are scarce despite the huge amount of these fish collected through fisheries by-catch. This study aims to identify at morphological and molecular level the helminth parasites found in the stomach and intestines of six species of elasmobranchs sampled from Mediterranean by-catches, in order to update and provide information on the parasitic fauna of the species under study.

MATERIALS AND METHODS: A total of 54 elasmobranchs, namely 16 sharks (6 common dogfish *Mustelus mustelus*, 8 pointed dogfish *M. punctulatus*, 1 blue shark *Prionace glauca*, 1 dogfish *Scyliorhinus canicula*) and 38 rays (29 starry rays *Raja asterias* and 9 pelagic stingrays Pteroplatytrygon violacea) were collected from Adriatic Sea and the Gulf of Lyon. Stomach and intestine of each fish were subjected to parasitological examination. Parasites were isolated, washed in saline and preserved in 70% ethanol for morphological identification by light and Scanning Electron Microscopy (SEM) and for molecular analyses (PCR and sequencing).

RESULTS AND CONCLUSIONS: The parasitological study carried out on the two species of dogfish of the genus *Mustelus*, allowed the identification by morphology and molecular analyses of a single species of nematode, *Acanthocheilus rotundatus* (Rhabditida: Acanthocheilidae), already reported in literature (Tedesco et al., 2020. Eur Zool J, 87: 616–23). In *P. glauca*, 3 species of tapeworms have been identified: *Scyphophyllidium exiguum* (Phyllobothriidea: Phyllobothriidae), *Anthobothrium caseyi* (Tetraphyllidea *incertae sedis*) and *Nybelinia indica* (Trypanorhyncha: Tentaculariidae), while in *R. asterias* 3 specimens of nematodes of the genus *Pseudanisakis* (Rhabditida: Acanthocheilidae) and a cestode referable to the genus *Nybelinia* were identified. Among all these helminths, *A. caseyi* in *P. glauca* represents a new report in the Mediterranean Sea, while *S. exiguum* and *N. indica* in *P. glauca* and *Pseudanisakis* sp. in *R. asterias* are new host record for these parasite species. The results of this survey represent a contribution to broaden the knowledge of the parasitic fauna of these elasmobranchs in the Mediterranean Sea. This and future studies based on a larger number of fish, will possibly contribute to build up a database on the parasite fauna of Mediterranean elasmobranchs, which could be used as biological tags for conservation and management purposes.

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PARASITIC DISEASES IN WILD AND EXOTIC ANIMALS

A COPROLOGICAL SURVEY ON INTERNAL PARASITES OF WILD BOARS (*SUS SCROFA*) IN CAMPANIA REGION, SOUTHERN ITALY

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Keywords: wild animals, epidemiology, coprological examinations

INTRODUCTION: Wild boar populations increased across Europe in the last decades. This expansion increases in the likelihood of many livestock, wildlife, and human diseases. Due to its zoonotic potential, it is important to evaluate the health status of these wild ungulates, particularly regarding the prevalence and diffusion of parasitic infections (Tamara et al., 2020. Acta Parasitol, 66: 104-15). This study aims to assess the prevalence, distribution and risk factors associated with the main endoparasite infections in hunted wild boars in Campania region.

MATERIALS AND METHODS: The study was performed from October to December 2021 on 369 wild boars culled in Campania region during one regular hunting season. Individual faecal samples were collected from the dissected terminal colon and rectum. Data recorded at sampling time included hunting area, weight, sex, and age of wild boars. Animals were classified into three age group: piglets (<1 year), subadults (1-2 years), adults (>2 years). Individual Faecal Egg Counts (FECs) were performed using Mini-FLOTAC technique with a detection limit of five eggs/oocysts (EPG/OPG) per gram of faeces. The floatation medium used was a saturated ZnSO4 (specific gravity, 1.350). Moreover, sedimentation technique was performed to detect trematode eggs.

RESULTS AND CONCLUSIONS: On 369 wild boars, 183 (49.6%) were females and 186 (50.4%) males; 2.7% (no.= 10) were piglets, 40.1% (no.= 148) subadult, 57.2% (no.= 211) adults. An overall prevalence of 92.4% (341/369) in single or mixed infections was reported. Coccidia (*Eimeria* spp./*Isospora suis*) were the most common parasites (70.2%), followed by gastrointestinal strongyles (65.9%), *Metastrongylus* spp. (48.8%), *Ascaris suum* (9.2%), *Strongyloides ransomi* and *Trichuris suis* (4.6% each), *Capillaria*/*Eucoleus* spp. (4.3%), *Balantidium coli* and *Dicrocoelium dendriticum* (1.6% each), *Physocephalus sexalatus* and *Ascarops strongylina* (0.5% each). Single infections were diagnosed in 23.5% (80/341) of positive animals while mixed infections in 76.5% (261/341). Sex and age were considered predictors for positive status for Coccidia, gastrointestinal strongyles, *Metastrongylus* spp., and *A. suum*. No significant statistical differences related to sex were found. Age was significantly ($p<0.05$) associated with *A. suum* infection (10% piglets; 14.2% sub-adults; 5.7% adults) proving that although wild boars are exposed to *A. suum* throughout life, the infection is more prevalent in younger animals due to a lack of acquired immunity (Katakam et al., 2016. Parasit Vectors, 9: 80). In conclusion, data obtained confirm a high prevalence of endoparasite in wild boar population and the role as potential reservoirs of parasitic infection, especially for domestic pigs when raised in an extensive production system (Moretta et al., 2011. Large Anim Rev, 17: 187-92). Parasitological monitoring of wild boars is crucial to safeguard the wild species and livestock health.

LONG-TERM SURVEY ON *TRICHINELLA* INFECTION IN WILD CANIDS OF CAMPANIA REGION

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Keywords: *Trichinella britovi*, wolf, fox

INTRODUCTION: *Trichinella* spp. are cosmopolitan parasites infecting humans by the consumption of raw or undercooked infected meat and animals by cannibalism and scavenger attitude. Wild animals represent the main reservoir hosts of this zoonotic nematode (Gottstein et al., 2009. Clin Microbiol Rev, 1:127-45). *Trichinella britovi* is the most prevalent species among wild canids of Europe, including Italy (Badagliacca et al., 2016. Vet Parasitol, 231: 124-27). This survey aims to investigate on the difference positivity between of *Trichinella* spp. infections in wild canids from southern Italy during last eight years.

MATERIALS AND METHODS: From 2015 to 2022, as part of the wildlife disease monitoring plan in the Campania region (southern Italy), 349 carcasses of wild canids were collected, divided into 30 wolves (*Canis lupus*) and 319 foxes (*Vulpes vulpes*). Samples of 5 g of diaphragm and tibialis muscles from each animal were analysed individually using the magnetic stirrer method with artificial digestion, in accordance with Regulation (EU) 2015/1375. Larvae from positive samples, were kept in 90% ethyl alcohol and send to European Union Reference Laboratory for Parasites (EURLP) for identification at species level.

RESULTS AND CONCLUSIONS: Out of 349 samples examined, 10/30 wolves and 4/319 foxes were positive, and all isolated larvae have been identified as *T. britovi*. Positives in wolves were 0/1 in 2015, 0/2 in 2016, 0/5 in 2017, 2/5 in 2018, 0/6 in 2019, 2/3 in 2020, 4/5 in 2021 and 2/3 in 2022. The increasing prevalence trend in recent years has been statistically significant ($p < 0.05$). While positives in foxes was 0/40 in 2015, 0/8 in 2016, 1/53 in 2017, 0/25 in 2018, 1/54 in 2019, 1/85 in 2020, 0/32 in 2021 and 1/22 in 2022. Foxes's prevalence did not show a significant difference during the study period. The cumulative prevalence during the eight years was 33.33% in wolves and 1.25% in foxes, and the highest positivity in wolves compared to foxes was statistically significant ($p<0.001$). Table 1 summarizes the results of the investigation on *Trichinella* infection in wild canids. This study confirms the circulation of *T. britovi* and the epidemiological role of wild canids in maintaining sylvatic cycles of *Trichinella* spp. in the investigated area, according to results obtained in central of Italy (Ricchiuti et al., 2021. Int J Parasitol Parasites Wildl, 15:195-98). The increasing trend of positivity in the last eight years are comparable to results of Abruzzi region (Badagliacca et al., 2021. Biol Life Sci Forum, 5: 1). The high prevalence in wolves than foxes is due probably to low wolf's presence in anthropized areas and different feeding habits of these two species of wild canids. Further studies on larval biomass are necessary to confirm this host competence.

Table 1. *Trichinella britovi* positive in wild canids during 2015-2022 in Campania region.

Year	Parameter	Wolf	Fox	Total
2015	n.	1	40	41
	%	0	0	0
2016	n	2	8	10
	%	0	0	0
2017	n	5	53	58
	%	0	1	1,72
2018	n	5	25	30
	%	40	0	6,67
2019	n	6	54	60
	%	0	1,85	1,67
2020	n	3	85	88
	%	66,67	1,18	3,41
2021	n	5	32	37
	%	80	0	10,81
2022	n	3	22	25
	%	66,67	4.55	12

n, number of samples; % prevalence.

LIVER FLUKE INFECTION IN ROE DEER (*CAPREOLUS CAPREOLUS*) FROM CENTRAL ITALY

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Keywords: *Dicrocoelium dendriticum*, hunting, wild animals

INTRODUCTION: Liver flukes (*Fasciola hepatica, Fascioloides magna*, and *Dicrocoelium dendriticum*) are trematode parasites that cause disease in grazing domestic and wild animals. Transmission of pathogens and parasites can occur in both directions in the wildlife-domestic interface (Horcajada-Sánchez et al., 2019. Sci Rep, 9(1):15953). Although liver fluke infections in livestock are well documented, there are few data on wild ungulates that frequently share pastures with domestic animals. Therefore, their surveillance must be considered as important as livestock (Arias et al., 2013. Res Vet Sci, 95(3):1031-35). This study aims to investigate liver flukes' infection in roe deer (*Capreolus capreolus*) in central Italy, where the management of this species is carried out carefully (Meriggi et al., 2008. Hystrix It J Mamm, 19(2):103-20).

MATERIALS AND METHODS: A total of 84 roe deer's livers were collected in Tuscany from June to September 2021. Data recorded at sampling time included hunting area, sex, and age class (fawns <1 year; yearlings 1-2 years; adult >2 years; Verheyden et al., 2020. J Helminthol, 94: e159) considered predictors factors for positive status. Livers were macroscopically inspected, cut into slices, washed with tap water, and then palpated to dislodge flukes within the bile and hepatic ducts to detect the adult stage of liver flukes (Goater et al., 2007. J Parasitol, 93:491-94). The water was filtered with a sieve. Adult parasites were collected, counted, and fixed in 70% alcohol.

RESULTS AND CONCLUSIONS: Of 84 roe deer 42 (50.0%) were females and 42 (50.0%) males; 15 (17.9%) were fawns, 27 (32.1%) yearlings, 42 (50.0%) adults. None of the examined livers showed macroscopic lesions. In total, 23/84 (27.4%) samples were infected by *D. dendriticum*, while no positivity to *F. hepatica* and *F. magna* was detected. Of the positive samples, 11 were male (26.2%) and 12 female (28.6%); 2 fawns (26.7%), 6 yearlings (25.9%), and 12 adults (28.6%). No significant statistical differences related to sex and age class were found. The mean intensity of infection was 6.7 helminths/animal (min 1 – max 41); this low parasitic burden may be due to the sampling season. In fact, according to Manga-Gonzales et al. (2001. Parasitology, 123: S91-S114), the greatest intensity of infection should occur during autumn, at the end of the ants' activity period. The seasonality of the infection needs to be further investigated, as well as how the interaction between roe deer and small ruminants can modify the spread of parasitic infection, affecting the health and fitness of these hosts (Verheyden et al., 2020. J Helminthol, 94: e159). Since the roe deer population is expanding in Italy, it is plausible that there is going to be an increasing overlap between roe deer and small ruminants ranges. In this scenario, it is important to assess if roe deer are reservoirs for small ruminants or vice versa.

INTESTINAL NEMATODE COMMUNITY OF THE RED FOX *VULPES VULPES* IN BOLZANO PROVINCE, ALTO ADIGE

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Keywords: red fox, nematoda, Alto Adige

INTRODUCTION: The red fox *Vulpes vulpes* is a reservoir for a number of major and minor zoonotic parasite species, including the nematodes *Toxocara canis* and those of the family Ancylostomatidae, shared with the domestic dog. Due to the great adaptability of the species to very diverse habitats including the peri-urban areas, sanitary surveillance on this species aims at prevention and control of the spread of infections to domestic animals and humans. In this survey, we investigated the fox intestinal helminthic fauna, focusing on the phylum Nematoda, in 150 carcasses of red fox, recovered in Bolzano province in the period 2019-2020.

MATERIALS AND METHODS: Nematodes were collected from the small intestine using a scraping and filtration technique and all parasites recovered were counted and identified at light microscope using taxonomic keys. A copromicroscopic exam using a Zinc chloride solution (specific gravity = 1,350) was carried out on fecal matter extracted from the same animals, and its results compared with scraping findings.
Prevalence and intensity of infection for each parasite species were then estimated and their association with age and sex was investigated by means of Pearson's chi-squared and non-parametric tests, respectively.

RESULTS AND CONCLUSIONS: Three nematode taxa were detected by intestinal scraping, i.e. *Toxocara canis*, *Uncinaria stenocephala* and *Pterigodermatites affinis*. *Toxocara canis* and *U. stenocephala* occurred with the highest prevalence (P) and abundance (A), i.e. P=44% (95%CI: 36-52) and A=2.6, P=25% (95%CI: 18-32) and A=1.0, respectively. Sex ratio of the three species was skewed towards females (1.25:1 to 2.5:1). Additionally, *Trichuris* sp. and *Capillaria* sp. were detected at copromicroscopic examination at lower prevalence. Young animals were found more frequently infected by *U. stenocephala* and *Capillaria* sp. ($p<0.05$) and abundance of *U. stenocephala* and *T. canis* were higher in the same category ($p<0.05$). Poor concordance was found between copromicroscopic examination and intestinal scraping ($k<0.21$) as already pointed out by other studies (Magi et al., 2016. Helminthol, 53:1-31).
Nematode taxa recovered in this survey are common component of the gastrointestinal helminth communities of red foxes in Northern Italy. Indeed, the same taxa are reported by several other studies across the Alpine region (Capelli et al., 2003. J Mt Ecol, 7: 199-205; Di Cerbo et al., 2008. Helminthol, 45:13-19), with *T. canis* and *U. stenocephala* always being most prevalent, and *P. affinis* detected with lower frequency. Most of these parasites were more abundant in young animals.
A high number of false negative results at copromicroscopy confirms that a single examination may not be reliable, although the relative frequency of the two main nematodes in the overall population was similar with the two techniques. Copromicroscopy can maintain therefore a role in the detection of parasites relevant for public health such as *T. canis* and Ancylostomatidae, whose presence was confirmed in the study area.

FIRST PARASITOLOGICAL DATA OF GOLDEN JACKAL (*CANIS AUREUS MOREOTICUS* I. GEOFFROY SAINT HILAIRE, 1835) IN FRIULI VENEZIA GIULIA REGION (FVG)

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Keywords: golden jackal, *Canis aureus*, parasites

INTRODUCTION: The golden jackal (*Canis aureus moreoticus*) is an expanding mesocarnivore in Europe and its presence in northeastern Italy has been documented since the 1980s (Lapini et al., 2009. Boll Mus civ St nat Venezia, 60: 169-86), consolidating its presence over the time and, therefore, constantly monitored both from the ecological and health perspective. In fact, its biology and behavior create premises for their infection with a broad range of pathogens, including parasites. Therefore, over the past 10 years, road-killed golden jackals in FVG have also been exploited for endoparasites.

MATERIALS AND METHODS: Carcasses of 47 golden jackals were collected throughout FVG, frozen before examination, and submitted to biometric analysis, necropsy and parasite collection by total worm count. Parasites were morphologically identified and, in selected cases, molecularly analysed. Faecal samples were collected from carcasses and parasitological analyses were performed, using ZnSO4 centrifugation flotation. Descriptive statistic, including prevalence, mean intensity, mean abundance, and richness were calculated for each parasite species.

RESULTS AND CONCLUSIONS: Among the examined jackals, 91.5% were infected with at least one parasite species (range 1-6). A total of 17 endoparasite species were found, among which the nematodes *Uncinaria stenocephala* (25.5%), *Toxocara canis* (19.1%), the cestodes *Taenia hydatigena* (21.3%) and *Mesocestoides lineatus* (2.8%), and digenean trematode *Alaria alata* (12.8%) were the most prevalent parasites in the gut, *Angiostrongylus vasorum* (16.3%) and *Crenosoma vulpis* (9.3%) in the lung and *Capillaria plica* (76%) in urinary bladder. Other helminths identified were: *Aonchotheca putorii* (10.6%) in the stomach, *Molineus patens* (8.5%), *Trichuris vulpis* (6.4%), *Pterigodermatitis affinis* (6.4%), *Toxascaris leonina* (2.1%), *Metagonimus yokogawai* (8.5%), *Dipylidium caninum* (2.1%), *Taenia pisiformis* (2.1%) in the gut; *Crenosoma vulpis* (9.3%) and *Eucoleus aerophilus* (2.3%) in the lung. Faeces collected from carcasses (no.= 22) presented generally elements attributable to the identified endoparasites, also 59% were positive for *Sarcocystis* sp..
This study represents the first survey on golden jackal endoparasites in Italy. The parasitofauna of FVG golden jackal population is almost overlapping with that reported in other European countries (Gherman and Mihalca, 2017. Parasit Vectors, 10: 419). The FVG-jackal parasitic biocenosis is good in number of species, even if the prevalence and mean intensity of infrapopulations is generally low. The finding of *M. yokogawai*, to the best of our knowledge, represents the first report in golden jackals in Italy, as well as *A. vasorum* confirming that jackals could be a definitive host (gravid females and larvae there were), as already demonstrated in Europe (Takács et al., 2014. Acta Vet Hun, 62: 33-41; Gavrilović et al., 2017. Acta Parasitol, 62: 880-84). Investigating the parasitofauna of wild canides is needed to monitor the spread of zoonoses.

FIRST DESCRIPTION OF *TRICHOSTRONGYLUS COLUBRIFORMIS* IN CAPTIVE *LEMUR CATTA*

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Keywords: *Lemur catta*, *Trichostrongylus colubriformis*, Zoo

INTRODUCTION: Ring-tailed Lemur (*Lemur catta*) is the only surviving semi-terrestrial diurnal lemur in Madagascar. It is considered vulnerable and are listed on CITES 1 appendix. Easily bred and raised in captivity, it is present in zoos worldwide. There are relatively few helminth parasites known from wild lemurs (Chabaud et al, 1965. Ann Paras, 40: 181 - 214; Irwin & Raharison., 2009. Malagasy Nature, 2: 66-93) and the ethical restrictions have focused on non-invasive sampling of feces, with descriptions and counts of helminth eggs, lacking of parasite identification. Even opportunistic necropsies of animals dying of natural causes or fecal cultures have not frequently been employed to describe adult parasites (Irwin & Raharison, LC.). In this study we describe adult helminths found in the intestine of captive *L. catta* hosted in a natural park of northern Italy and died for natural causes.

MATERIALS AND METHODS: The intestine of a three months old ring-tailed lemur was collected during necropsy, opened lengthwise, the mucosa scraped and the contents examined under a stereo microscope. All helminths were counted and preserved in 70% ethanol. The worms were identified by morphology and by sequencing the ITS rDNA.

RESULTS AND CONCLUSIONS: Overall, 178 adults (117 females and 61 males) bursate Nematoda were collected. By morphology the worms were identified as *Trichostrongylus colubriformis* according to Travassos, 1921 (Mem Inst Oswaldo Cruz, XIII:5-135). The sequences obtained were compared by BLAST and the search gave 99.5% similarity with *T. colubriformis*.
Despite *L. catta* is the most intensively studied of all lemur taxa, only few helminths were described in this species. In particular Chabaud et al. (1965, LC) reported only *Trichuris lemura*. Pinworm and strongylid eggs (provisionally identified as *Lemuricola* sp and *Lemurostrongylus* sp, respectively) and tapeworm eggs were described by Loudon et al., 2006 (Ecol Environ Anthropol, 2: 54-74), while Robles et al., 2010 (Acta Parasitologica, 55: 270–75) found the oxyurid *Lemuricola bauchoti*.
T. colubriformis is reported around the world and, despite being more frequently recovered in ruminants, it was also described in camelids and primates, including human in which it's described as a common zoonotic species in several countries (Hidalgo et al., 2020. Acta Parasitol, 65:790–95). To our knowledge this is the first description of *T. colubriformis* in *L. catta*. Even if there are no indication of its presence in wild populations, this must be considered in the zoos that host them, due to the possibility of interspecies transmission and the zoonotic aspects.

MOLECULAR SURVEY ON PROTOZOA AND MICROSPORIDIA IN FOXES (*VULPES VULPES*) FROM PISA (TUSCANY, CENTRAL ITALY)

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Keywords: *Neospora caninum*, microsporidia, *Hepatozoon* spp.

INTRODUCTION: The red fox (*Vulpes vulpes*) is the most widespread wild carnivore in Italy and a potential reservoir of viruses, bacteria, protozoa, helminths and arthropods (Magi et al., 2015. J Helminthol, 89: 506-11; Otranto et al., 2015. Vet Parasitol, 213: 12-23). This study aimed to evaluate the occurrence of selected protozoan and microsporidia parasites of relevance for public and animal health in foxes from Pisa province (Tuscany).

MATERIALS AND METHODS: A molecular investigation was carried out to search for microsporidia (*Encephalitozoon* sp.), *Neospora caninum*, *Babesia* spp. and *Hepatozoon* spp. Samples of different types of tissue (117 brain, 91 kidneys, 15 spleen) and of blood (24) were then taken from 127 fox carcasses from the province of Pisa. Extracted DNA was submitted to PCR amplification using already described primer pairs and amplification protocols (Fedorko et al., 1995. J Clin Microbiol, 33: 1739-41; Müller et al., 1996. J Clin Microbiol, 34: 2850-52; Inokuma et al., 2002. Vet Parasitol, 106: 265-71; Beck et al., 2009. Int J Parasitol, 39: 843-48). Positive samples were sequenced.

RESULTS AND CONCLUSIONS: All 117 brain samples tested negative for *Neospora caninum*. Analogously, all 117 brain samples and all 91 kidney samples (from 126 foxes) tested negative for microsporidia. On the contrary, of the 28 foxes from which spleen and blood samples were examined, 3 tested positive for *Babesia vulpes* (10.7%, 95%CI= 3.7-27.2), and 8 tested positive for *Hepatozoon canis* (28.6%, 95%CI= 15.2-47.1). The results concerning *N. caninum* agree with the negative results or low prevalence values found elsewhere (Suteu et al., 2014. J Wildl Dis, 50: 713-16; Lukášová et al., 2018. J Wildl Dis, 54: 825-28), although values ~5% have also been reported (De Craeye et al., 2011. Vet Parasitol, 178: 64-69). Very few data are available for microsporidia in foxes. *Encephalitozoon cuniculi* and *E. intestinalis* were found in 2 of the 148 foxes examined in Ireland (Murphy et al., 2007. Vet Parasitol, 146: 227-34). The recently re-described *B. vulpes* (Baneth et al., 2015. Parasit Vectors, 8: 1-7) appears to be widespread in foxes in Europe, with highly variable prevalence rates (Zanet et al., 2014. Parasit Vectors, 7: 1-7; Checa et al., 2018. Vet Parasitol, 252: 22-8). This species was reported as responsible for clinical cases in dogs in Europe (Solano-Gallego et al., 2016. Parasit Vectors, 9: 1-18). Variable prevalence rates were found in Europe also for *H. canis* (Farkas et al., 2014; Ebani et al., 2017. Acta trop, 172: 197-200), which again represents a potential risk for dogs (Pacifico et al., 2020. Parasitol Res, 119: 3023-31). The presented results showed that, referring to the selected pathogens, the most significant risk related to foxes in the area is represented by the presence of *H. canis* and *B. vulpes*, which could be transmitted to dogs. The presence of *N. caninum* and microsporidia seems to be negligible, although a larger study should be conducted to further assess these parasites epidemiology.

ARE TWO COINCIDENCES A PROOF? THE EUROPEAN ROE DEER (*CAPREOLUS CAPREOLUS*) IS A SUITABLE INTERMEDIATE HOST FOR *TAENIA SERIALIS*

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Keywords: *Taenia serialis*, *Capreolus capreolus*, Monti Sibillini National Park

INTRODUCTION: Taeniids are multi-host parasites with an indirect life cycle that strictly depends on a predator-prey relationship. This is referred to as a multi-host trophically-transmitted parasite system, which involves mammalian species in both adult and larval stage. Parasites with a complex life cycle may exhibit different degrees of host-specificity at each life stage. To measure host-specificity it is not sufficient the number of the species that a parasite can infect but also the relatedness to each other. Trophically-transmitted parasites necessitate high predation rates assuring high transmission levels within the food chain. A generalist parasite that uses several intermediate host species has more likelihood to infect its definitive host if this feeds broadly among many prey species. Thus, knowing the host breadth at the different life cycle stage is a fundamental concept of the biology and epidemiology of these multi-host parasites.

MATERIALS AND METHODS: A free-ranging adult female European roe deer died after a fatal car crash in within Monti Sibillini National Park. The carcass was brought in to the IZSUM of Tolentino (MC), and necropsy was performed. Cyst-like lesions were recovered from the epicardial and the endocardial surfaces, and the intercostal muscles. Histological and molecular investigations were carried out for the cysts identification.

RESULTS AND CONCLUSIONS: M-PCR gave an amplicon referable to *Taenia* spp. (267 bp). Sanger sequencing showed 99% query cover, 1e-103 e-value, and 100% identity with *T. serialis*. Histology of the parasitized heart revealed the presence of a metacestode larval stage (coenurus) compatible with taeniid larval stage of *T. serialis*. A large and more tissue-compressing cyst, which contains a single protoscolex was observed, surrounded by dystrophic, vacuolated and compressed cardiomyocytes. No inflammatory cells were observed around the cyst that means, as for *E. multilocularis*, immune tolerance mediated by specialized regulatory T cells and related cytokines as IL-10 and TGF-β. Wildlife plays a prominent role in the *T. serialis* epidemiology. *T. serialis-coenurosis* is already diagnosed in lagomorphs, rodents, and several cases in primates rarely detected also in cats, sheep, and marsupials. Few diagnoses have been confirmed by using molecular analysis and no one by hystopathological approach. Thus, *T. serialis* may be more flexible in the selection of intermediate hosts than previously hypothesized. To our knowledge, few published articles report the presence of *T. serialis* either in definitive or intermediate hosts in Italy. The interesting finding we describe here is that in a relatively short period of time and close to each other, two roe deer were detected positive to *T. serialis-coenurosis* (Morandi et al., 2022. Int J Parasitol: Parasites Wildl, 17:110-13). Studies on the *T. serialis*-dynamic will be conducted. Wildlife surveillance is crucial to monitor for human and animal health as intermediate hosts breadth of critical taeniids may suddenly change.

INTESTINAL PARASITES SUGGEST WILD PREYS PREFERENCE IN *CANIS LUPUS ITALICUS* FROM THE MONTI SIBILLINI NATIONAL PARK

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Keywords: intestinal parasites, italian wolf, Marche region

INTRODUCTION: The Italian wolf (*Canis lupus italicus*) is a subspecies of the grey wolf native to the Italian Peninsula. Although the bottleneck has been overwhelmed by now, the species is still considered as vulnerable in the IUCN's list. The wolf is exposed to a multitude of trophically-transmitted parasites with an indirect life cycle that strictly depends on a predator-prey relationship as being the top consumer of the trophic cascade. Typically, wolves could be definitive hosts of *Taenia* and *Echinococcus* species, which affect a variety of mammals as intermediate or accidental hosts including humans. The aim of this preliminary study was to investigate the frequency of intestinal parasites in wolves coming from within and close to the Monti Sibillini National Park (MSNP).

MATERIALS AND METHODS: All the obtained guts (duodenum), as a safety precaution, were stored for 10 days at −80 °C then at −20 °C until examination. Organs were washed over a sieve under running water for largest helminths; the water passed through the sieve was placed into conical plastic jars to collect smallest parasites. Contents were examined under a stereo-microscope. All recovered helminths were fixed in 70% ethanol. The specimens were mounted on a glass slide, cleared with lactophenol and covered with a cover slip. Helminth identification was morphologically performed and more appropriate stained techniques are still ongoing.

RESULTS AND CONCLUSIONS: A total of 21 intestinal tracts were evaluated, 7 (33.3%) were detected positive for parasites. Among nematodes *Uncinaria stenocephala* (both adult and L4) was detected with a frequency of 14.2%, *Toxocara canis* 9.5%. Noteworthy, there were five adult *Trichinella* sp. detected in a wolf. Among tapeworms, 19% of wolves were positive for *Taenia hydatigena* and 9.5% for *T. krabbei*. *Sarcocystis* sp. was accidentally detected in two wolves. Results confirm the high variability of the helminth fauna that concern the Italian wolf. Anyway, *Echinococcus granulosus s.l.* was not detected in any examined sample. This may indicate that, in MSNP area, the common preys are not represented by sheep, as the main intermediate hosts of *E. granulosus s.s.* in the Mediterranean Basin. These results show that the involvement of wolves in Italy in *E. granulosus* transmission can be considered a downstream phenomenon of the domestic cycle and no wild cycle seems to be active in the area. Furthermore, due to these preliminary results, where involved parasites mainly depend on a wild cycle, such as *T. krabbei*, suggest that the wolf within the MSNP has a good management which targets wolf predation on wild rather than domestic preys. The study of intestinal parasites of wolves from the area is also essential to explore their potential role in the diffusion of Taenia serialis to roe deer, as being recently described (Morandi et al., 2022. Int J Parasitol: Parasites Wildl, 17: 110-13). To perform necropsy on wildlife is essential for the local and global understanding of free-living animal populations as a One Health principle.

TAPE-WOLF: ECHINOCOCCOSIS IN WOLVES RECOLONIZING THE ALPS

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Keywords: *Echinococcus sensu strictu*, wolf, sylvatic cycle

INTRODUCTION: The Eurasian wolf (*Canis lupus*) has been identified as definitive host for *Echinococcus* spp. in several countries in Europe, including Italy.
Since the late Nineties, wolves have spontaneously returned to Northern Italy after a long-time eradication, recolonizing at first the Western Alps. Until then, *Echinococcus granulosus* was hypo-endemically maintained through the domestic cycle, and *Echinococcus multilocularis* was considered absent in Italy.
Nonetheless, *E. multilocularis* was reported in foxes from neighboring areas in Switzerland and France (Combes et al., 2012. Emerg Infect Dis, 18:2059), and a recent report confirmed its presence in wolves from Imperia province (Liguria, Italy) hypothesizing a new endemic area at the southern edge of the Alps (Massolo et al., 2019. Int J Parasitol Parasites Wildl, 7: 309-16).
The aim of this study was to determine the prevalence of *E. granulosus* and *E. multilocularis* in wolves from North-western Italy collected between 2010 and 2022 for passive surveillance campaign in the frame of "Life WolfAlps" project.

MATERIALS AND METHODS: 154 carcasses of wolves from Piedmont, Liguria and Valle d'Aosta were examined at the Department of Veterinary Sciences (Turin, Italy), and about 20 grams of feces were sampled from each wolf.
Extraction of DNA was performed using the kit QIAmp DNA stool Mini kit (QIAGEN), then molecular analysis has been performed through a screening Real-time PCR for *Echinococcus* spp. which employs the primer and TaqMan probe specific for Eg95 gene (Haag et al., 2009. PloS One, 4: e5362). Positive samples were sequenced by National Reference Center for Echinococcosis (CeNRE).

RESULTS AND CONCLUSIONS: Two wolves collected in 2018 and 2021, originating from the provinces of Cuneo and Aosta, resulted positive for *E. granulosus sensu strictu* (prevalence 1.3%), while no one tested positive for *E. multilocularis*.
This finding confirms an hypoendemic occurrence of *E. granulosus* in North-western Italy, in accordance with the low prevalence of cystic hydatidosis in domestic intermediate hosts and its occasional presence in wild intermediate hosts in the same area (Dalmasso et al., 2012. Small Rumin Res, 106: 18-20).
E. multilocularis seems to be absent in the wolf population investigated on the Italian side of the Western Alps, suggesting that further studies are needed to better clarify the extension of the unexpected *E. multilocularis* detection in Imperia province, and its connectivity with *E. multilocularis* endemic areas in France and Switzerland.
Data in this study support a potential role of wolves in maintaining the circulation of *E. granulosus* in hypoendemic Western Italy and highlights the sentinel role played by this top predator for both *E. granulosus* and *E. multilocularis* occurrence in remote mountain areas.

MULTILOCUS SEQUENCE ANALYSIS FOR *TOXOPLASMA GONDII* AND *NEOSPORA CANINUM* IN BIRDS OF PREY

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Keywords: *Toxoplasma gondii*, *Neospora caninum*, birds of prey

INTRODUCTION: *Toxoplasma gondii* and *Neospora caninum* are Apicomplexan parasites of major concern in livestock, as an important cause of abortion, and for *T. gondii* it is also of great public health concern. These protozoa have a wide range of intermediate hosts, including birds. In particular birds of prey may become infected by ingesting infected animals, mostly small mammals and birds (Sato et al., 2021. Parasitol Int, 82). This work aimed to assess the prevalence of these parasites in birds of prey, since they may have relevance in both sedentary and migratory species, respectively, for environmental contamination and parasite genetic variability along the migratory route.

MATERIALS AND METHODS: The peritoneal muscles of 159 birds of prey, belonging to 18 species and recovered across 6 Wildlife Recovery Centers in Central Italy, were sampled and genomic DNA was extracted. The DNA was tested by sequence typing targeting GRA6, 529bp, B1, PK1, BTUB, 5'SAG2, alt.SAG2 and APICO genes for *T. gondii* (Su et al., 2010. Parasitology, 137:1-11) and to end-point PCR targeting NC5 gene for *N. caninum* (Zanet et al., 2014. Vet Parasitol, 199:247-49).

RESULTS AND CONCLUSIONS: Thirty-seven out of the 159 analyzed samples tested positive for *T. gondii* with a prevalence of 23.27% (CI 95%= 17.38-30.48) and 9 for *N. caninum*, with a prevalence of 5.66% (CI95% = 3.10-11.62). Thirty-two sequences were obtained from the 37 isolates of *T. gondii*. Among these, 26 belonged to Type I lineage, 4 to Type II lineage and 2 consisted of atypical strains. Although no significant differences in *T. gondii* prevalence were recorded between migratory and sedentary species ($p>0.05$), in migratory species atypical strains were detected. *T. gondii* genetic variability in birds of prey confirms previous findings of wildlife as reservoirs of atypical strains (Battisti et al., 2018. Vet Parasitol, 253:43-7) and primarily of genotype I. MS-typing from direct sequencing of tissue samples has a lower success rate in amplification of typing markers and could underestimate the true prevalence of atypical strain in wildlife. NGS techniques could help unravel the true structure of the *T. gondii* population in Europe.

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VECTORS AND VECTOR-BORNE DISEASES

MICRO RNAS AND OTHER SMALL RNAS IN *AEDES AEGYPTI* SALIVA AND SALIVARY GLANDS FOLLOWING CHIKUNGUNYA VIRUS INFECTION

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Keywords: *Aedes saliva*, small noncoding RNAs, vector-host-pathogen interactions

INTRODUCTION: Mosquito saliva is a complex cocktail whose main role is to facilitate blood feeding by counteracting host responses to tissue injury through the anti-haemostatic, anti-inflammatory and immunomodulatory properties of salivary proteins. However, the finding that saliva of mosquitoes and ticks also carries miRNAs suggested they may contribute to host manipulation, with possible implications for vector-host-pathogen interactions. To get further insights into miRNAs from mosquito saliva we analysed by RNA-seq small RNAs from *Aedes aegypti* saliva and salivary glands, either uninfected or infected by the chikungunya virus (CHIKV).

MATERIALS AND METHODS: Small RNA fractions (<200 nt) from saliva (S), salivary glands (G), infected saliva (SCK) and infected salivary glands (GCK) were used for library preparation and Illumina sequencing. After quality filtering and trimming, reads were aligned to the *Ae. aegypti* and CHIKV genomes and to a collection of known and predicted *Ae. aegypti* miRNAs. Differential expression analysis was performed by EdgeR. The sRNAtoolbox and the WebGestalt tools were used for target prediction and pathway/GO enrichment analyses, respectively.

RESULTS AND CONCLUSIONS: Infection of *Ae. aegypti* by CHIKV was found to activate in salivary glands both the siRNA and piRNA antiviral pathways, as indicated by the detection of reads of viral origin and by their specific signatures. Overall, a total of 208 miRNAs were identified, with 155 expressed in the saliva samples. Differential expression analysis showed that miRNA expression profiles were only marginally affected by CHIKV; on the contrary, irrespective of infection, miRNAs were differentially sorted between saliva and salivary glands. *Aedes aegypti* saliva appeared enriched in specific miRNA subsets, and comparative studies revealed a good conservation of saliva miRNAs among mosquitoes and ticks, clearly pointing to a non-random sorting and occurrence. Prediction analyses, and searches for experimentally validated targets of identical human miRNAs, provided evidence that miRNAs from *Ae. aegypti* saliva may target human immune and inflammatory pathways. Finally, a 26 nt Gly-GCC 5'-tRNA-derived fragment (Gly-GCC 5tRF) was identified: this previously not reported *Ae. aegypti* tRF was by far the most abundant small RNA across the sequenced libraries and its possible function may deserve future investigations. Overall, we believe that our observations convincingly support a scenario where both proteins and miRNAs from mosquito saliva are injected into vertebrates during blood feeding and contribute to the complex vector-host-pathogen interactions. This would imply that hematophagous arthropod saliva is even more complex than originally anticipated, and that adaptation to blood feeding and effective manipulation of host responses to tissue damage was achieved through the recruitment of both specific salivary proteins and selected salivary miRNAs.

A NOVEL ECOFRIENDLY TOOL FOR THE DELIVERY OF BIOINSECTICIDES TO MOSQUITO LARVAE

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Keywords: mosquitoes control, Bti, bioinsecticides delivery

INTRODUCTION: The control of arthropod pests and disease vectors, such as mosquitoes, still remains a major challenge. Natural methods, combining effective control with environmental safety, have been among the most pursued path during the last decade. Marketed products, in line with this approach, include those based on *Bacillus thuringiensis* var. *israelensis* (Bti), proved to be very effective. These microbe-derived insecticides are biodegradable, not toxic to non-target organisms and do not determine accumulation in the environment. As a drawback, however, they undergo a rapid degradation in the environment, which implies that multiple applications are required, with an increase in the costs, as well as the risk of the emergence of resistance phenomena in the target species. Therefore, in this study we propose a novel method of administration that not only protects and protracts the effect of Bti but also enhances its activity by baiting the immature stages of mosquitoes.

MATERIALS AND METHODS: A new formulation of hydrogel containing Bti, of complete natural origin, was developed, in the form of floating rafts, capable of attracting mosquito larvae and, at same time, kill them into their breeding sites. Attraction and mortality tests were carried out on mosquito larvae of *Ae. albopictus*. The attractiveness of the rafts, due to the inclusion of a phagostimulant was analyzed after treatment of mosquito larvae in Petri dishes with rafts with different composition, both by observation under a fluorescence microscope (ingestion test) and by monitoring larval movements with the DanioVision ethological-analysis tool (behavioral tests). Afterwards, multiple mortality tests were carried out to verify the larvicidal effect of the rafts by laboratory bioassays (in glasses) and semi-field bioassays (in breeders).

RESULTS AND CONCLUSIONS: Our assays showed that larvae are actively attracted by the rafts; in addition, after reaching the rafts, they feed on them, ingesting the matrix of the raft and its content (Bti in our experiments). Furthermore, bioassays in both laboratory and semi-field conditions showed that Bti-containing rafts determine the killing of mosquito larvae, from the first day of treatment. Therefore, the proposed delivery rafts could be a viable strategy to attract mosquito larvae to the bioinsecticide, without dispersing the active ingredients into the water of the breeding site, thus enhancing their effect and minimizing their dilution.

COMPARISON OF TWO TYPES OF OVITRAPS AND STICKY GRAVID TRAP FOR MONITORING *AEDES INVASIVE* MOSQUITOES

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Keywords: *Aedes koreicus*, *Aedes japonicus*, mosquito surveillance

INTRODUCTION: *Aedes Invasive* Mosquitoes (AIM) play an important role in public health, as vectors of pathogens and a source of nuisance for humans and animals. European and national guidelines for the surveillance of AIM suggest several methods for the monitoring of these species. In this study, we compared the performance of three traps for monitoring AIM.

MATERIALS AND METHODS: We compared two types of ovitraps containing 0.25 L and 1 L of water respectively, and a Sticky Gravid Trap (SGT). We named the two ovitraps, Standard Ovitrap (SOV) and AIM-COST Ovitrap (COV), since the latter has been proposed by the AIM-COST Action Team. We selected five sites in five cities in Veneto and Friuli Venezia Giulia regions: Sommacampagna (VR), Padova (PD), Occhiobello (RO), Belluno (BL), and Tolmezzo (UD); the three traps were placed in each site. Every two weeks, we collected the samples (eggs and adult mosquitoes) and rotated the traps, according to a 3x3 Latin square. Adult mosquitoes and eggs were counted and identified in IZSVe Laboratory (eggs were hatched, first and fourth instar larvae were identified).

RESULTS AND CONCLUSIONS: From June 2020 to October 2020, we collected 416 samples by ovitraps (207 SOV and 209 COV) and 218 by SGT. We observed no significant difference between the mean number of eggs collected by the two ovitraps (SOV=138.64, COV=131.36, $p>0.05$). The mean number of AIM females collected by SGT was 6.10 per trap. We observed no correlation between adults and eggs number (r2 SOV=0.32; r2 COV=0.24). In three sites (Sommacampagna, Padova, Occhiobello) we collected only *Aedes albopictus*, as eggs and adults. In Belluno, *Ae. albopictus* was the most abundant species (78.8% of collected eggs and 89.9% of adults), followed by *Ae. koreicus* and *Ae. japonicus*. In Tolmezzo, *Ae japonicus* was the most abundant species (79.8% of collected eggs and 79.7% of adults), followed by *Ae. geniculatus* and *Ae. albopictus*. In conclusion, both types of ovitraps are good in collecting *Aedes* eggs. Entomologists prefer ovitraps containing a larger volume of water, because they are unlikely to dry out during the summer season, avoiding the need to increase the frequency of sample collection. In our study, SOV were not affected by drought, probably because they were placed in a rainy area. SGT are a good method for AIM collection. Unfortunately, the engorged females collected by SGT were not further analyzed (e.g. screening of pathogens or hosts preference) because the specimens were easily damaged during handling. The method could be improved by studying a less aggressive sticky sheet, but equally effective in mosquitoes collection.

ANALYSIS OF IMPORTED MALARIA IN ITALY IN 2015-2021 AND EVALUATION OF THE IMPACT OF THE SARS-COV2 PANDEMIC IN THE TRENDS OF THE LAST TWO YEARS

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Keywords: National malaria surveillance system, epidemiology, COVID-19

INTRODUCTION: In non-endemic countries, imported malaria is one of the most common vector-borne diseases, associated with the number of international travelers and migratory flows from endemic areas. In European Union, malaria cases have increased in recent years, showing Italy as one of the countries with the highest number of imported infections. In this study, the epidemiological features of imported malaria in Italy in 2015-2021 were analyzed, and the impact of the SARS-CoV2 pandemic on the 2020-2021 trends was also evaluated.

MATERIALS AND METHODS: According to the Ministry of Health Circular (December 27, 2016), the demographic, epidemiological and laboratory data of the cases, notified by the Local Health Services (LHS) and confirmed through microscopic diagnosis certification by the Istituto Superiore di Sanità, were entered in a database and analyzed per year.

RESULTS AND CONCLUSIONS: In 2015-2019 period, 3,958 cases of malaria were reported, with an annual average of 790 cases. The male gender (70%) and the 25-44 age group (44%) were the most affected. A case rate of 17% occurred among Italians, traveling for work (46%), tourism (27%) and voluntary/religious mission (23%). Non-Italians accounted for 83% of cases, of which 78% visit friends and relatives (VFR), and 14% on first entry. Infections were acquired for 92% in Africa, then in Asia (7%), Central/South America (0.4%) and Papua New Guinea (0.1%). The *Plasmodium falciparum* species was responsible for 84% of cases, followed by *P. vivax* (9%), *P. ovale* (5%), *P. malariae* (1.5%) and mixed infections (0.3%). Reported deaths were 11. Non-traveled related cases were 12: 2 induced (*P. falciparum*) and 10 cryptic cases (8 *P. falciparum*, 1 *P. ovale* and 1 *P. malariae*). In 2020, the number of cases dropped to 166, 79% less than the average of the 2015-2019 period. Notifications were concentrated for 46% in January, February and March, before the mobility restrictions introduced to limit the spread of SARS-CoV2 infections. There was a slight increase in percentage of cases in the following groups: 25-44 age (48%), *P. ovale* infections (11%), and Italian travellers (31%) (Figure 1). The other epidemiological aspects were like the pre-pandemic years. One death was reported. In 2021, provisional data currently show 365 cases, 54% less than the average recorded in 2015-2019; other analyses will be available shortly.

In 2015-2019, the imported malaria dataset showed similar trend and epidemiological status, while in 2020 and 2021, following the restrictive measures on international travels imposed by the Covid-19 pandemic, a drastic decrease of cases was observed. In the studied period a different distribution of notifications among the Regions was recorded, suggesting underreporting. However, it should be considered that in 2020-2021 the emergency due to the pandemic could have caused a more consistent underreporting by the LHS, further affecting the total number of cases.

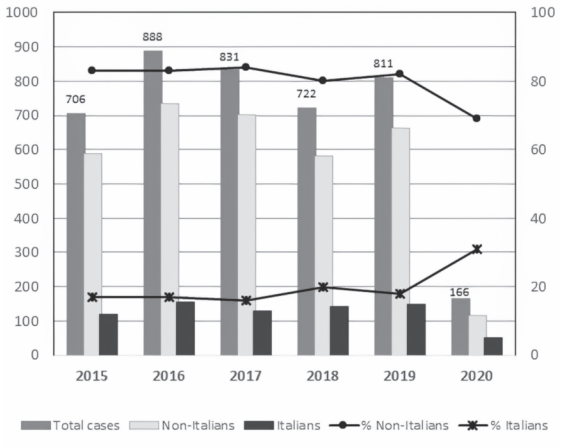

Figure 1. Trend of imported malaria cases in Italy in 2015-2020. The cases are also shown by nationality. The group of non-Italians includes both settled-immigrants resident in Italy, returning from travel to their countries of origin (VFRs), and those on their first entry.

GENOTYPIC AND PHENOTYPIC RESISTANCE TO DIFLUBENZURON IN ITALIAN POPULATIONS OF *CULEX PIPIENS* AND *AEDES ALBOPICTUS*

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Keywords: mosquito, larval control, insecticide resistance

INTRODUCTION: One of the most used products authorized in Italy for mosquito larval control (as well as against some agricultural pests) is Diflubenzuron (DFB), an Insect Growth Regulator which inhibits chitin biosynthesis interrupting the normal development of larvae into adults. Recent studies identified high levels of resistance in *Culex pipiens* from Emilia-Romagna (Italy) with a 128-fold Resistance Ratio (RR). The phenotype was associated with mutations I1043L/M within the Chitin synthase gene (chs1), which were shown to be widespread in the region, with highest frequencies (77%) in coastal sites. We here present results of i) genotypic assessment of DFB resistant alleles in *Cx. pipiens* and *Aedes albopictus* populations in other Italian regions; ii) phenotypic assessment of susceptibility to DFB in *Ae. albopictus* in Italy.

MATERIALS AND METHODS: i) Genotyping of mutations I1043L/M was carried out by PCR in *Cx. pipiens* (Grigoraki et al., 2017. Sci Rep, 7: 11699) and by sequencing a fragment of the chs1 gene in *Ae. albopictus* (Balaska et al., 2020. Parasit Vector, 13: 328).
ii) Phenotypic assessment of susceptibility to DFB was carried out by exposing *Ae. albopictus* larvae from wild populations, as well as one susceptible laboratory strain, to 6 concentrations of DFB, ranging from 0.0004mg/L to 0.02 mg/L. For each population the EI50 and EI90 values, i.e. the concentrations necessary to inhibit emergency of 50% and 90% of larvae, were compared with the susceptible reference strain by computing resistance ratios (RR).

RESULTS AND CONCLUSIONS: i) Genotyping of DFB-resistant alleles in 385 *Cx. pipiens* specimens from Lazio, Liguria, Trentino, Piemonte, Puglia, Veneto revealed presence of allele 1043L in all regions analysed (except Puglia, no.= 4), with frequencies >40% in some of the north-eastern sites while allele 1043M was only observed in Veneto (2%-14%). No mutations in position 1043 of the chs1 gene were detected among the 63 *Ae. albopictus* specimens analysed from Lazio and Puglia regions.
ii) Results from bioassays carried out on 16 wild *Ae. albopictus* populations from Lazio, Toscana, Trentino and Puglia, showed RR50 values ranging from 0.04 to 5.48 with highest values - indicating a possible reduced susceptibility to DFB - in Lazio region.
We here report high frequencies of DFB-resistance alleles in *Cx. pipiens* populations across Italy, with highest frequencies in north-eastern regions. This pattern is not shown in *Ae. albopictus*, despite the two species frequently share larval sites in urban areas. However, first evidence of reduced susceptibility *Ae. albopictus* are suggested by bioassay results. Overall, these data highlight the need to closely monitor the future evolution of resistance to DFB in both species and to introduce insecticide resistance monitoring in mosquito control activities in order to rapidly introduce management plans to maintain the efficacy of available larvicides.

LONGITUDINAL STUDY OF SAND FLY COHORTS FROM SEVEN ITALIAN REGIONS AND MOLECULAR DETECTION OF PHLEBOTOMINE-BORNE DISEASES AS BASELINE FOR RISK-MAP IMPLEMENTATION

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Keywords: sand fly, natural infection, molecular detection

INTRODUCTION: Phlebotomine-borne diseases caused by *Leishmania* and Phlebovirus are considered to be expanding their boundaries and burden in southern Europe as the result of climate and environmental changes, allowing the geographical expansion of sand fly vectors into novel territories and/or increasing their densities in endemic ones. Zoonotic leishmaniasis and summer aseptic meningitis caused by *Leishmania infantum* and Toscana virus (TOSV), respectively, are endemic in our country. In this study, we report results on sand fly identification, their distribution and in-progress examination for natural infection with the above pathogens.

MATERIALS AND METHODS: A longitudinal study in the frame of West Nile virus surveillance was carried out from 2017 to 2021 in seven Italian regions: Emilia-Romagna, Friuli Venezia Giulia, Latium, Piemonte, Sardinia, Tuscany, and Veneto. Sampling was performed twice a month, using CDC and BG-sentinel traps and keeping flies frozen pending subsequent analysis. Collected specimens were morphologically identified and monospecific pools were molecularly tested by RFLP and RT-PCR for pathogen detection, *Leishmania* spp. and TOSV respectively (Sánchez-Seco et al., 2003. J of Med Virol, 71:140-49; Di Muccio et al., 2015. PLoS One, 10: e0134885). To obtain live specimens and be able to dissect them for Leishmania spp. isolation and culture, hand captures were conducted in one Sardinian site (Olmedo, SS).

RESULTS AND CONCLUSIONS: A total of 24,153 sand flies were identified as *Ph. perfiliewi* (91.81%), *Ph. perniciosus* (6.40%), *Se. minuta* (1.61%), *Ph. mascittii* (0.17%), *Ph. papatasi* (0.01%), and one specimen as *Ph. neglectus*. Altogether 314 pools (no.= 9403) were molecularly analyzed so far, of which 25 (0.3%) tested positive for *Leishmania* in Veneto (6.5%), followed by Tuscany (0.2%) and Sardinia (1.9%) (Tab.1), with higher vector species prevalence for *Ph. perniciosus* (1.3%). Focusing on dissection analysis, isolation and culture highlighted presence of 3 *L. tarentolae* (10.0%) and 1 *Trypanosoma platydactyli* (3.3%) strains obtained from *Se. minuta*.
A total of 146 pools (no.= 2,761) were analyzed for TOSV, of which 3 (0.1%) from *Ph. perfiliewi* tested positive, in Latium (25.0%) and, for the first time, Piedmont (1.8%) (Tab.1).
The putative *Leishmania* and Phlebovirus vectors were differently represented in the investigated sites with markedly different densities. These preliminary analyses and further studies will improve knowledge of presence and distribution of phlebotomine-borne diseases in the Italian territory.

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		% Reported positive pools for region (N tested)				
Region	Species	*Leishmania spp.*	*L. infantum*	*L. tarentolae*	*T. platydactyli*	TOSV
Latium	*Ph. perfiliewi*	0.0 (N=429)	NA	NA	NA	25.0 (N=240)
Sardinia	*Ph. perniciosus*	0.9 (N=565)	0.0 (N=61)	1.6 (N=61)	0.0 (N=18)	NA
	Se. minuta	1.9 (N=313)	0.0 (N=96)	4.0 (N=126)	3.3 (N=30)	NA
Tuscany	*Ph. perfiliewi*	0.2 (N=6198)	0.1 (N=730)	0.0 (N=730)	NA	NA
Piedmont	*Ph. perniciosus*	0.0 (N=67)	NA	NA	NA	0.0 (N=67)
	Ph. perfiliewi	0.0 (N=46)	NA	NA	NA	2.2 (N=46)
Veneto	*Ph. perniciosus*	7.0 (N=43)	NA	NA	NA	NA
	Ph. perfiliewi	0.0 (N=1)	NA	NA	NA	NA
	Ph. mascittii	0.0 (N=2)	NA	NA	NA	NA

Table 1. Prevalence of *Leishmania*, TOSV and *T. platydactyli* sand fly infections. NA= Pending for analysis.

TICKS, TICK-BORNE PATHOGENS AND HOST RESERVOIRS IN NORTHEASTERN ITALY

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Keywords: *Ixodes ricinus*, *Anaplasma*, *Rickettsia*

INTRODUCTION: The ecology of tick-borne pathogens (TBPs) involves several vertebrate hosts that could play different role such as blood meal sources, reservoirs and/or amplifiers of pathogens. The aim of this study was to investigate the occurrence and prevalence of TBPs in ticks removed from different wildlife and domestic animals and assess the ecological and reservoir role of these hosts.

MATERIALS AND METHODS: During 2019–2021 period, fed ticks (adults and nymphs) were collected from both wildlife and domestic hosts through passive surveillance. The samplings were carried out in TBPs endemic regions (northeastern Italy). After collection, ticks were identified morphologically and then molecularly screened individually or in pools for TBPs (TBE virus, *Anaplasma* spp., *Babesia* spp., *Borrelia* spp., *Ehrlichia* spp., and *Rickettsia* spp.). Minimum Infection Rate (MIR) was used to calculate prevalence. Statistical analyses were carried out only for *A. phagocytophilum* and *R. helvetica* since the prevalence of other pathogens was too low. A binomial Generalized Linear Model was applied to assess if infection in ticks was influenced by host species and tick stages (R Development Core Team, 2011). The response variable was the occurrence of TBPs, while explanatory variables were host species and tick stages.

RESULTS AND CONCLUSIONS: A total of 367 engorged ticks were collected and four species were identified: no.= 215 *Ixodes ricinus*, no.= 146 *I. hexagonus*, no.= 1 *D. marginatus*, no.= 1 *Rhipicephalus sanguineus*; plus, no.= 2 *Ixodes* spp. and no.= 2 *Dermacento*r spp. Ticks were collected from 71 hosts belonging to 11 species (cattle, chamois, deer, roe deer, wild boar, wolf, fox, golden jackal, badger, hedgehog, and buzzard). *Ixodes ricinus* was collected from all the host species; *I. hexagonus* from badger, hedgehog, and fox; *D. marginatus* and *Dermacentor* spp. from wild boar, and *R. sanguineus* from hedgehog. Eleven TBPs were detected from 321 pools (206 adults, 15 nymphs' pools) analyzed; 30.2% (65/215) of *I. ricinus* was found positive for at least one TBP. The main TBP in *I. ricinus* were *A. phagocitophylum* (MIR=19.1), *R. helvetica* (MIR=9.8) and *R. monacensis* (MIR=4.7). One *Dermacentor* spp. specimen was found positive for *R. slovaca* and five *I. hexagonus* for *Rickettsia* spp. A total of 15 ticks (4.1%) removed from two host species (chamois and roe deer) were found co/multiple infected. *Anaplasma phagocytophilum* prevalence was significantly higher in ticks fed on wolf and jackal ($p<0.001$) and deer and roe deer ($p<0.05$), while *R. helvetica* prevalence was significantly higher in ticks fed on roe deer, wolf and jackal ($p<0.001$) and chamois and deer ($p<0.05$).

SECOND SURVEY OF ITALIAN VETERINARY PRACTITIONER ON *DIROFILARIA IMMITIS* AND *D. REPENS*: PRELIMINARY DATA

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Keywords: *Dirofilaria immitis*, *Dirofilaria repens*, survey

INTRODUCTION: In 2018, an online questionnaire was conducted to evaluate practitioners' knowledge on Dirofilariosis (*D. immitis, D. repens*) in dog and cat. Four years later, we have decided to re-propose the survey in order to update the data and evaluate an improvement in veterinarians' awareness about Dirofilariosis.

MATERIALS AND METHODS: An electronic questionnaire was sent to all Italian veterinary facilities (surgeries, clinics, hospitals and public facilities). In addition, in collaboration with Zoetis Italia S.R.L., the questionnaire was advertised through the network of their sales and social networks (i.e Instagram and Facebook). The 34 questions of the survey focused mainly on where the facilities were located, and on diagnosis, prevention, and treatment commonly used for *D. immitis* and *D. repens* in dogs and cats. In addition, practitioners were asked if they knew of guidelines for Dirofilariosis.

RESULTS AND CONCLUSIONS: Preliminary data showed that 22.7% of facilities reported only infections of *D. immitis*, 5.0% only of *D. repens*, 14.2% mixed infections and 58.2% no cases of either parasite in the last year. *D. immitis* infections were observed especially in the northern and central Italy. However, also many regions of the south and the islands (Sicily and Sardinia) reported heartworm infections. *D. repens* was fairly evenly distributed throughout Italy and mainly as co-infections with *D. immitis*. The most frequent diagnostic method used in dogs was the Ag test (23.8%) followed by the fresh blood smear together with the Ag test (18.4%) and the aid of a diagnostic laboratory 7.5%. Knott's test together with Ag test, thoracic radiology and ultrasound examination were used by 2.13% of the facilities.
The most frequent diagnostic techniques used for *D. repens* in dogs were: external diagnostic laboratory (52.5%), skin biopsy (29.8%), Knott's test (28.0%). The most frequent diagnostic technique used for *D. immitis* in cats were: diagnostic laboratory (26.2%), Ag test (26.6%) and fresh blood smear (4.3%). For treatment of canine heartworm infection, more then 32% used ivermectin + doxycycline or moxidectin + doxycycline (19%), while more than 22% used only melarsomine. Prevention was started in the dog in March (28,0%) - April (38.3%) and finished in November (44.0%) – December (19.1%), while 13.5% treated all year. Finally, more than 52.8% knew the ESCCAP Guideline, 40.4% knew the AHS Guideline, while 35.5% knew the ESDA Guideline. Our preliminary data showed that *D. immitis* and *D. repens* are distributed in most of the Italian provinces. Furthermore, the diagnosis is often underestimated and relies mainly on serology alone. This type of study can be considered a good starting point for the development of clearer guidelines proposed by scientific societies, together with the need of a more widespread diffusion and, not less important, a useful and rapid method to have updated risk maps.

DETECTION OF *DIROFILARIA* DNA AND HOST BLOOD-MEAL IDENTIFICATION IN *CULICOIDES PAOLAE* BITING-MIDGES

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Keywords: *Dirofilaria immitis*, *Dirofilaria repens*, *Culicoides paolae*

INTRODUCTION: *Dirofilaria immitis* and *Dirofilaria repens* (Spirurida, Onchocercidae) are among the most common canine vector borne pathogens (CVBPs) in Europe, being a real threat for animal and human health. During the past few decades, changes in the geographical distribution of *Dirofilaria* spp. have been observed in Europe. To date, more than 70 mosquito species (i.e., genera *Aedes*/*Ochlerotatus*, *Anopheles* and *Culex*) have been recognized as competent or putative vectors for *Dirofilaria* spp. However, other nematodes, belonging to the family Onchocercidae, can be also transmitted by diverse arthropod species. Given the expansion of canine heartworm infection in southern Europe due to *D. immitis* and the report of the infection in Linosa island (Sicily, Italy), we investigated the potential involvement of *Culicoides* spp. in *Dirofilaria* spp. transmission in this specific focus.

MATERIALS AND METHODS: Eight sites in different parts of Linosa Island were selected for an entomological survey. Trapping was performed daily between 5.30 p.m. and 9.00 a.m., from July to November 2020 using light traps; after each sampling, the collection bags were stored at −4°C, until transferred to the laboratory to the end of the sampling period. *Culicoides* spp. were divided from other insects and morphologically classified at species level and the 10% were molecularly processed to confirm the species identification. Genomic DNA was extracted from each female specimen and DNA samples were processed by duplex real-time PCR for the detection and differentiation of *Dirofilaria* spp., using two species-specific primers targeting cox1 and ITS-2, set for *D. immitis* and *D. repens*.

RESULTS AND CONCLUSIONS: Out of 1,791 specimens collected 1,773 were morphologically and molecularly identified as *Culicoides paolae* and 18 as *Culicoides obsoletus* complex. Of the 1,596 females, four (0.26%) parous *C. paolae* tested positive for *D. immitis* and *D. repens* (i.e., two *Culicoides* specimens for each filarial species), of which *D. immitis* positive samples fed on both human and dog and *D. repens* positive samples only on humans.
The detection of *D. immitis* and *D. repens* DNA in *Culicoides* midges suggests their potential involvement in the epidemiology of the filarial nematodes, which may be of medical and veterinary importance in hyper-endemic areas. Findings of this study shed new lights in the epidemiology of *Dirofilaria* spp. infections thought further investigations are needed.

FELINE LEISHMANIOSIS IN THE LAZIO REGION: PRELIMINARY RESULTS

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Keywords: *Leishmania*, cats, diagnosis

INTRODUCTION: Leishmaniosis is a neglected vector-borne disease causing an estimated 300,000 new cases and about 20,000 deaths in humans each year. In Italy, *L. infantum* is the most important species of zoonotic concern, with domestic dogs as the main reservoirs and phlebotomine sand flies as vectors. Where canine leishmaniasis is endemic, cats are often exposed to the parasite. In Italy, an overall cumulative *L. infantum* prevalence of 3.9% was recorded by serology (3.3%) and by qPCR (0.8%), with a higher rate (10.5%) in southern regions as result of the favorable geographical climate conditions that allow the presence and abundance of sand fly vectors (Iatta et al.,2019. PLoS Negl Trop Dis, 13:e0007594). In central Italy feline leishmaniosis was scantly investigated with two reports from Tuscany with 0.9% and 2.5% of seroprevalence (Poli et al., 2002. Vet Parasitol, 106: 181-91; Morelli et al., 2020. Front Vet Sci, 7: 616566).

MATERIALS AND METHODS: The aim of this study was to evaluate the presence of *L. infantum* in a feline population from the Lazio region, assessed performing IFAT, ELISA and qPCR. A total number of 200 serum and blood samples were collected from cats admitted at the CDVet Research laboratory for routine controls. Sera were subjected to an indirect immunofluorescence antibody test (IFAT) for the detection of specific IgG against *L. infantum* (MegaFLUO Leish,Megacor Diagnostik GmbH) using an anti-cat IgG conjugate. The cut-off dilution of 1:40 and 1:80 was applied. Serodiagnosis was also performed using an home-made enzyme immunoassay (ELISA) following the protocol by Alcover et al., 2021 (Parasit Vectors, 14: 178). Genomic DNA was extracted from blood samples and tested by qPCR (genesig® *Leishmania* Standard Kit). Detailed information about sex, age, and lifestyle was recorded for each cat. Consent for sample collection and screening was obtained from the animal owners.

RESULTS AND CONCLUSIONS: Overall, antibodies against *L. infantum* were found in 4 of the 200 (2%) examined cats, with antibody titres ranging from 1/40 (3/200) to 1/80 (1/200). Positive sera were also confirmed by ELISA test. Molecular identifications are still in progress as well as the evaluation of eventual significant associations between exposure to *L. infantum* and possible risk factors.
Our preliminary study highlights the presence of *L. infantum* in 2% of the examined cats in complete absence of historical features and physical signs compatible with the disease. Further evidence might be obtained by molecular analysis still in progress. Most of the sera (3 out 4) resulted positive with an Ab titer of 1/40. Such different cut-off values, also used in previous surveys (Dedola et al., 2018. Vet Parasitol Reg Stud Reports, 13:120-23), is an important critical issue influencing the different results in serodiagnosis. Thus, a standardization of procedures for a prompt diagnosis and screening of cat population is crucial for better understanding the epidemiology and the potential role of cats as reservoirs of feline leishmaniasis.

FELINE LEISHMANIOSIS IN OWNED AND STRAY CATS IN A COASTAL AREA OF CAMPANIA REGION

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Keywords: leishmaniosis, cat, vector borne disease

INTRODUCTION: Feline leishmaniosis (FeL) is an emerging vector-borne disease, with increasing numbers of cases registered in the last years (Pennisi and Persichetti, 2018. Vet Parasitol, 251: 131-37). The aim of this study was to obtain data on FeL and possible co-infection with Feline Immunodeficiency Virus (FIV), Feline Leukemia Virus (FeLV) and SARS-CoV-2 in owned and stray cats in a coastal area of Naples province, Campania region, southern Italy.

MATERIALS AND METHODS: Eighty-two cats were included in the study, aged between 6 months and 22 years. Fifty-one cats were owned, while 31 were stray cats, managed by a group of volunteers. All the animals were subjected to clinical examination at private vet clinics or public veterinary service. Sera samples from all cats were collected and analysed by an Immunofluorescence Antibody Test (IFAT), provided by the National Reference Center for Leishmaniasis (CRENAL, Palermo, Italy) to detect anti-*Leishmania* antibodies, by a commercial immunoassay (Elecsys, Roche) to detect the presence of antibodies against SARS-CoV-2 and by a commercial rapid test to detect FIV antibodies/FeLV antigens (Speed Duo, Virbac).

RESULTS AND CONCLUSIONS: Of the 82 cats, 33 (40.2%; 95% Confidence Interval, 95%CI= 29.7-51.7) resulted positive. The most frequent clinical signs were lymph nodes enlargement, dehydration, ocular discharge, vomiting, weight loss, cutaneous lesions, anorexia, apathy and sneezing. Four cats (4.9%; 95%CI= 1.6-12.7) were positive to FIV (one positive also for leishmaniosis) and two (2.4%; 95%CI= 0.4-9.4) resulted positive to FeLV. Moreover, two stray cats (2.4%; 95%CI= 0.4-9.4) resulted positive for SARS-COV-2. At clinical examination one cat showed sneezing and nasal discharge, the second exhibited several clinical signs: gingivitis, stomatitis, upper lip ulcer, dehydration, enlarged popliteal lymph nodes. This cat had an history of calicivirosis and resulted positive at *Leishmania* IFAT. A significant association ($p<0.05$) was found between positivity to leishmaniosis and stray cats (51.5%), co-habitation with other animals (93.9%) and subjects not treated with ectoparasiticides (84.8%). These findings confirm that the leishmaniosis is not rare in cats and occurrence might be underestimated in endemic areas (Iatta et al., 2019. PLoS Negl Trop Dis, 13: e0007594; Spada et al., 2020. Animals, 10: 817). For this reason, the cat could have an important role as reservoir host of this disease for dogs and humans, as reported also in other recent studies (Asfaram et al., 2019. J Venom Anim Toxins Incl Trop Dis, 25: 1–10; Pereira et al., 2021. Vet Parasitol, 298: 109531). Moreover, our results confirm that cat may be infected with SARS-CoV-2, although further studies are needed to establish the real prevalence in cat population. Finally, co-infection with other pathogens (FIV, FeLV) may exacerbate the intrinsic pathogenicity of *Leishmania* in cats, as previously reported (Akhtardanesh et al., 2020. Vet Parasitol Reg Stud Reports, 20: 100387).

CATALASE IMPAIRS *LEISHMANIA MEXICANA* DEVELOPMENT AND VIRULENCE

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Keywords: *Leishmania*, catalase, virulence

INTRODUCTION: *Leishmania* (Kinetoplastea: Trypanosomatidae) is a unicellular parasite causing a vector-borne disease named leishmaniasis in tropical and subtropical regions. *Leishmania* spp. are dixenous (two hosts) and their life cycle is based on two major development stages: extracellular promastigote and intracellular amastigote (Dvorák et al., 2018. The leishmaniases: old neglected tropical diseases, Bruschi F., Gradoni L, Cham, 31–77). It is commonly recognized that dixenous species have evolved from their monoxenous (= one host) relatives, and comparative analysis of their genomes showed numerous events of gene gains and losses, underlining the difference between the two groups of parasites (Lukeš et al., 2014. Mol Biochem Parasitol, 195:115–22). Catalase is one of these interesting examples. It degrades hydrogen peroxide (H2O2) producing water and oxygen, helping to protect cells from the oxidative stress (Alfonso-Prieto et al., 2009. Am Chem Soc, 131:11751–61). The common ancestor of trypanosomatids lacked the homolog of catalase-encoding gene, which have been acquired later independently at least three times by monoxenous species belonging to the subfamily Leishmaniinae, Blastocrithidiinae and genus *Vickermania* (Chmelová et al., 2022. Antioxidants, 11: 1-5). Remarkably, this gene was secondarily lost in *Leishmania* spp. These data, taken together with the fact that H2O2 is involved in regulation of *Leishmania* differentiation, suggest that the loss of the catalase-encoding gene was guided by the establishment of dixenous life cycle in *Leishmania* spp. (Kraeva et al., 2017. Infect Genet Evol, 50:121-27).

MATERIALS AND METHODS: We generated a *Leishmania mexicana* line, which expresses catalase (Lmex-CAT), using a novel bicistronic expression system, based on 2A self-cleaving peptide of Teschovirus A. These mutans were used for analysis *in vitro* and *in vivo* (in sandflies and mice infection models).

RESULTS AND CONCLUSIONS: The development of Lmex-CAT in a vector was significantly compromised, with reduced percentage of the macrophage-infective metacyclic promastigotes found in the gut of the insect compared to the control. After 72 h of macrophage infection, the number of Lmex-CAT amastigotes was significantly lower when compared to the control. In mice infection, the mutant cells showed a decrease in their virulence, with only 50% of the mice developing lesions, which also appeared later and smaller compared to the control. In summary, the stable expression of catalase in *L. mexicana* compromises parasite development in sand flies, *Leishmania* transmission potential, and their ability to infect mammal host and to produce clinical manifestations. This suggests that the loss of catalase was a necessary event for the establishment of dixenous life cycle in *Leishmania* spp.

DETECTION AND ISOLATION OF ENDOSYMBIONTS (*SPIROPLASMA* SPP.) FROM SWEDISH *IXODES RICINUS* TICKS

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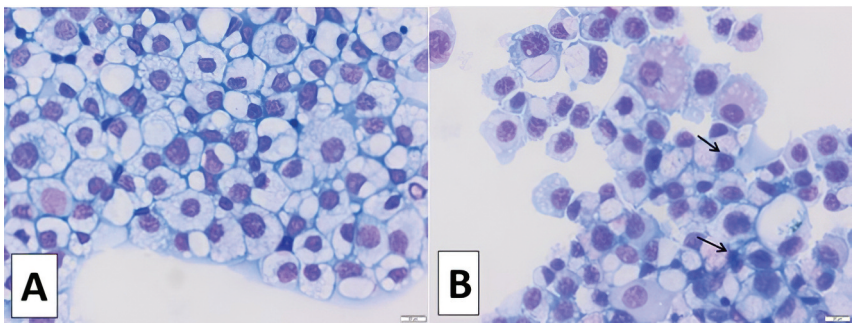
Keywords: ticks, *Spiroplasma*, endosymbionts

INTRODUCTION: *Ixodes ricinus* is the most common tick species in Sweden. It harbors many microorganisms pathogenic for humans and animals such as *Borrelia* spp., tick-borne encephalitis virus and *Babesia* spp., as well many symbiotic bacterial microorganisms (Jaenson et al., 2018. Parasit Vectors, 11: 477; Leta et al., 2021. Microbial Ecol). Spiroplasmas are vertically-transmitted endosymbionts of arthropods, including ticks, and pathogens of some arthropods and plants (Cisak et al., 2015. Ann Agric Environ Med, 22: 589–93). They are helical mycoplasmas belonging to the class Mollicutes (Trachtenberg, 2005. Curr Biol, 15: 483-84). In ticks, they can be found in the salivary glands, gut, and reproductive organs. *Spiroplasma* spp. have been detected in, and/or isolated *in vitro* from, ticks in Germany, Slovakia, Norway and several other European countries (Henning et al., 2006. Int J Med Microbiol, 296 S40: 157-61; Tveten et al., 2011. Vector Borne Zoonotic Dis, 11: 1329-34; Palomar et al., 2019. Ticks Tick Borne Dis, 10: 628-38). However, to our knowledge, there are no reports suggesting their presence in Swedish ticks. This study aimed to detect and isolate *Spiroplasma* spp. from Swedish *I. ricinus* ticks using tick cell lines as a substrate.

MATERIALS AND METHODS: Tick samples were collected from dogs and cats in different regions of Sweden (Uppsala and Skåne counties, as well as from the municipalities of Sundsvall, Örnsköldsvik and Skellefteå) and used to infect two tick cell lines derived from *Rhipicephalus microplus* (BME/CTVM23) and *I. ricinus* (IRE/CTVM19). A conventional cytocentrifugation technique combined with Giemsa staining was used to visualize the presence of bacteria. PCR was also performed on the ticks used for the isolation attempts, using *Spiroplasma* genus-specific primers targeting the 16S rRNA and rpoB genes.

RESULTS AND CONCLUSIONS: Preliminary results (see Figure 1: A. uninfected BME/CTVM23 cells. B. BME/CTVM23 cells 181 days after inoculation with internal organs from an *I. ricinus* tick (arrows: *Spiroplasma*-like inclusions). Scale bar 20um) show that some cells appear to be infected with bacteria that resemble *Spiroplasma* spp. Molecular analysis of ticks as well as of the cells that have been incubated with tick organs is ongoing and will be presented.
Isolation of *Spiroplasma* spp. will help us to study more closely the bacteria and their influence on the tick's fitness and reproduction, and their role in the persistence of pathogenic microorganisms that are hosted by ticks.



TICKS AND TICK-BORNE PATHOGENS IN SYMPATRIC POPULATIONS OF ALPINE IBEX, CHAMOIS AND DOMESTIC ANIMALS

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Keywords: *Babesia*, Anaplasmataceae, *Ixodes ricinus*

INTRODUCTION: Ticks and tick-borne pathogens (TBP) are emerging worldwide as veterinary and human pathogens (Schnittger et al., 2012). The increase in winter temperatures linked to climate change favours overwintering, latitudinal and altitudinal expansion of arthropod vectors (Semenza and Menne, 2009; Semenza and Suk, 2018). Simultaneously the fragmentation of habitats and human presence in rural environments increases contact between man and animal species boosting the onset or re-emergence of diseases. Understanding TBP dynamics in Alpine Ibex and Alpine Chamois is a unique opportunity to understand the susceptibility and adaptation of a species to tick-borne emerging pathogens.

MATERIALS AND METHODS: *Capra ibex* and *Rupicapra rupicapra* whole blood and lung tissue were sampled in two areas of Gran Paradiso National Park. In the same areas, cattle and goat grazing in shared pastures with wild ungulates were sampled and analyzed for *Babesia*/*Theileria* spp. Anaplasmataceae, *Borrelia burgdorferi s.l.* and SFG Rickettsiae. Prevalence of infection with TBP was also determined along an altitudinal gradient in questing Ixodidae ticks.

RESULTS AND CONCLUSIONS: The prevalence values of the different target pathogens are summarized in Table 1. Higher prevalence of infection were reported for *Babesia*/*Theileria* spp. and Anaplasmataceae, followed by SFG Rickettsiae and *B. burgdorferi s.l.* Sequencing showed a high variability in circulating strains with a high degree of connection between domestic and wild ungulates. The analysis of sympatric populations of ticks, livestock and wild mountain-dwelling ungulates is essential to define the degree of pathogen overlap in sympatric domestic and wild ungulates.

	Babesia/Theileria spp.			Anaplasmataceae			*Rickettsiae* SFG			*Borrelia burgdorferi* s.l.		
	neg	pos	P	neg	pos	P	neg	pos	P	neg	pos	P
wild ruminants	11	43	79.63%	11	43	79.63%	35	19	35.19%	54	0	0.00%
domestic ruminants	34	9	20.93%	17	26	60.47%	43	0	0.00%	40	3	6.98%
Ixodidae ticks	154	76	33.04%	120	112	48.26%	225	6	10.00%	219	11	4.78%

TICKS FROM WILD HOSTS IN LIGURIA: UPDATES FROM A REGIONAL MONITORING AND SURVEILLANCE PLAN

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Keywords: ectoparasites, wildlife, game

INTRODUCTION: Hard ticks (Ixodidae) are ectoparasites of domestic and wild animals, as well as humans (Madison-Antenucci et al., 2020. Clin Microbiol Rev, 33: e00083-18). Their geographical distribution and abundance may be influenced by dynamics related to wildlife (Medlock et al., 2013. Parasit Vectors, 6: 1–11). The Regional Plan of Monitoring and Surveillance of wildlife health of the Liguria Region provides for a large range of analysis on hunted game, as well as on wild carnivores and birds accidentally found dead. The Plan also includes the search for, collection and identification of ectoparasites found on these animals, which are conferred to the Istituto Zooprofilattico Sperimentale of Piedmont, Liguria and Aosta Valley (IZSPLV).

MATERIALS AND METHODS: Ticks were collected during four subsequent hunting seasons (2018-2019, 2019-2020, 2020-2021 and 2021-2022) from wild boar (*Sus scrofa*) (no.= 4363), roe deer (*Capreolus capreolus*)(no. = 337), fallow deer (*Dama dama*)(no.= 129) and chamois (*Rupicapra rupicapra*)(no.= 5) hunted in Liguria (northwest Italy). Ears, or more rarely portions of skin were delivered by local hunting associations to the IZSPLV. Each anatomical portion was univocally identified and thoroughly inspected to search for ticks. Meanwhile, ticks were also collected during necropsies of carcasses of wild animals accidentally found dead, including wolf (*Canis lupus*)(no.= 10), fox (*Vulpes vulpes*)(no.= 4), European badger (*Meles meles*)(no.= 1), and long-eared owl (*Asio otus*)(no.= 1). Ticks were removed and morphologically identified to species level according to Barker et al., 2004 (Parasitology, 129: S15–S36) and to Estrada-Peña et al., 2004 (Ticks of Domestic Animals in the Mediterranean Region, University of Zaragoza, Zaragoza, Spain, 131).

RESULTS AND CONCLUSIONS: Overall, 1421 ticks were found, belonging to five different species. *Ixodes ricinus* (no.= 930, 65.4% of the collected ticks) was the most frequent, followed by *Dermacentor marginatus* (no.= 305, 21.5%) and *Rhipicephalus sanguineus s.l.* (no.= 177, 12.5%), while *Haemaphysalis punctata* (no.= 7, 0.5%) and *Ixodes hexagonus* (no.= 2, 0.1%) were found occasionally. The prevalence rate (presence of at least one tick), calculated only for the three main hunted species, was: 3.6% for wild boar, 63.2% for roe deer and 58.1% for fallow deer. The observed difference might be influenced by the different hunting seasons: January–March for fallow deer, January–April and June for roe deer, from October to January for wild boar, or by other factors. The prevalence was not calculated on the other species, due to their low numbers or, in the case of hosts sampled at necropsy, due to their frequent bad conservation status. The tick species distribution in the different hosts varied. *I. ricinus* was found in all the examined hosts, with the only exception of the badger. It was the dominant species in roe deer and fallow deer and the only species identified out of the 88 ticks found in wolves. *Dermacentor marginatus* was associated to wild boar.

LARVICIDAL POTENTIAL OF MACROALGAL EXTRACTS OF *DICTYOTA DICHOTOMA* AND *DICTYOPTERIS POLYPODIOIDES* AGAINST LARVAE OF *AEDES ALBOPICTUS*

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Keywords: larvicidal activity, algae, mosquito control

INTRODUCTION: *Aedes albopictus* is an aggressive biting mosquito, annoying both animals and humans and it is also a competent vector of various pathogens. Among the control methods, the most used are chemical treatments against adult mosquitoes and biological or chemical insecticide against larvae. However, it is crucial to assess and develop efficient and sustainable vector control tools that take into account problems as insecticide resistance and current restriction to use of biocides in the EU. There are few studies on potential larvicidal activity of seaweeds and their compounds. The aim of this study is to investigate larvicidal properties of two algae of family Dictyotaceae (*Dictyota dichotoma* and *Dictyopteris polypodioides*), using different extraction methods.

MATERIALS AND METHODS: To evaluate the potential activity as mosquito larvicide, laboratory-reared mosquito larvae of known age (third or fourth instar) were exposed to de-chlorinated water treated with the two algal extracts at various concentration: in order to test different bioactive compounds, extraction was performed with three solvents (ethanol, acetone, water). Mortality was recorded every 24h until pupa stage and adult emergence. To exclude any possible influence of solvents used for extraction, preliminary tests were carried out using solvents without algae. To determine the activity range, mosquito larvae were first exposed to three high concentrations (100, 150 and 250 mg/l); then lower concentrations were tested to establish dose–response curves and determine LC50 and LC90 values.

RESULTS AND CONCLUSIONS: Water extract did not give appreciable effects for either of the two tested algae. *Dictyota dichotoma* was the only species that had proven larvicidal effectiveness and for which test continued at lower concentrations (50, 25, 12.5, 6.25, 3.125 mg/l), with differences in mortality due to the extraction method performed. LC50 and LC90, within 24h, were 8.81 and 24.5 mg/l with ethanol extract and 7.18 and 47.11 mg/l with acetone extract, respectively. LC50 and LC90, within 48h, were 6.72 and 12.4 mg/l with ethanol extract and 4.06 and 21.38 mg/l with acetone extract, respectively. Comparing extraction methods, both were proved to be effective. Considering LC50, data showed that *D. dichotoma* extract with acetone is more effective, reaching this value at lower concentrations in both 24 and 48 hours; however, ethanol extract had better results in terms of LC90 both in 24 and 48h. In conclusion, *D. dichotoma* proves to be a possible candidate as an alternative biological insecticide with LC50 and LC90 values lower or similar than ethanol extracts obtained from other algae species. In order to obtain the higher larval mortality by using the lower concentration of product, we can conclude that ethanol is preferable to acetone extraction.

	Ethanol extract		Acetone extract	
mg/L	24h	48h	24h	48h
LC50	8.81	6.72	7.18	4.06
LC90	24.5	12.4	47.11	21.38

VARROA DESTRUCTOR EFFECTS ON APIARIES IN HIGH AND LOW IMPACT AGRICULTURAL AREAS

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Keywords: honey bee, *Varroa destructor*, ColEval

INTRODUCTION: The development of *Apis mellifera* colonies is mostly influenced by environmental, climatic, and meteorological factors rather than by the only availability of nectariferous flora or pollinating resources. In the agricultural context, besides the many variables affecting colony development, the use and exposition of phytopharmaceuticals based on their method, period, and type of utilization could result in sub-optimal development of colonies (Evans & Chen, 2021, Colony Collapse Disorder and Honey Bee Health. 458). This decline in population caused by increased mortality, reduction in average life expectancy, and susceptibility to pathogens has consequently decreased honey production drastically.

MATERIALS AND METHODS: The comparative analysis of apiaries' performance was evaluated in two different agricultural settings: two separate apiaries located respectively in a high (vineyards and hazelnuts cultivation) and low impact (uncultured shrubs and forested areas) agricultural areas was performed between March and September 2021. During this period, considering the ColEval method instructions, data were collected monthly for different variables such as weight, relative mortality, bee population, brood extension, honey availability, and pollen quantity. In addition to the data collection, honey bee productions were sampled for subsequent multi-residue pesticide analysis. Moreover, sample collections and evaluations were performed concurrently with phytopharmaceutical treatments. We evaluated the intensity of *V. destructor* presence periodically by Varroa Easy Check.

RESULTS AND CONCLUSIONS: Both production and colony strength significantly differed between the two apiaries. *V. destructor* treatment and pesticides were associated with apiaries' productivity and strength both at the end of the active season and during overwintering.

THE MOSQUITO SPECIES OF THE CAFFARELLA VALLEY, APPIA ANTICA REGIONAL PARK, ROME, ITALY: FIRST RECORD OF *CULEX THEILERI* THEOBALD, 1903 (DIPTERA: CULICIDAE) IN ROME

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Keywords: mosquitoes, Rome, *Culex theileri*

INTRODUCTION: In this study, a mosquito monitoring activity was carried out in the Caffarella Valley Appia Antica Regional Park in Rome from September 2020 to April 2021, with the aim of updating the mosquito fauna 10 years after the last check list of the area carried out in 2012 by the Istituto Superiore di Sanità. The Caffarella Valley is a very popular urban park, completely surrounded by densely populated neighborhoods. Previous studies have confirmed the presence of competent vectors of human and animal diseases, such as the common mosquito (*Culex pipiens*), the tiger mosquito (*Aedes albopictus*) (Severini et al., 2017. JEMCA, 35: 29-32) and *Anopheles labranchiae* (Severini et al., 2021. XXVI CNIE) belonging to the *Anopheles maculipennis complex*. During this entomologica survey, 783 mosquito larvae were collected.

MATERIALS AND METHODS: All specimens were sampled at larval stage using a standard 350 ml dipper and transported to the laboratory where they were raised until adulthood. Larvae were identified as morphology. To identify the species within the *An. maculipennis complex*, and the sibling species *Anopheles claviger/petragnani*, the molecular marker ITS-2 (Ribosomal internal transcribed 2) (Profit et al., 1999. Parasitol Res, 85: 837-43) was used and after being amplified with PCR the samples were sent for sequencing.

RESULTS AND CONCLUSIONS: Out of these samples, 8 taxa have been identified morphologically or molecularly in case of sibling species, belonging to 5 genera: *Culex pipiens* (49.1%, no.= 392), *Culiseta annulata* (16%, no.= 128), *Culiseta longiareolata* (13.1%, no.= 105), *Anopheles claviger* (11.5%, no.= 92), *Aedes albopictus* (5.5%, no.= 44), *Anopheles maculipennis sl* (2%, no.= 16), *Culex theileri* (0.7%, no.= 6), *Uranotaenia unguiculata* (1.8%, no.= 15). The noteworthy datum of this survey is the finding of *Culex theileri*, a very common species in ponds and marshes in natural and rural environments but never recorded in Rome before (Romi & Sabatinelli, 1997. Diptera Culicidae. In: Zapparoli M., Gli insetti di Roma. Fratelli Palombi Editori, Roma, 246-248). This species usually feeds on mammals and birds, but often bites humans also entering houses and shelters. In general, such habitus in feeding on multiple hosts could increase the risk in spreading zoonotic diseases. Moreover, also the finding of *Ur. unguiculata*, never observed before in the park, lead us to review and update the composition of species in the whole urban area, since the last report of this species in Rome dates back to 1962. Caffarella Valley, part of the Appia Antica Regional Park, confirms its high degree of naturalness also in terms of mosquito species. On the other hand, the data collected during this monitoring activity confirmed the presence of several vectors competent for the transmission of arboviruses, throughout the year in the Caffarella Park, hence increasing the chances of human-mosquito contact and the potential transmission of pathogens.

This research was in the frame of the projects 'Residual Anophelism: distribution of potential vectors of malaria in Puglia and Basilicata, construction - updating of entomological maps and breeding tests of *Anopheles labranchiae* populations in an insectarium' (IZSPB 01/18 RC) funded by the Italian Ministry of Health.

MONITORING *AEDES INVASIVE* MOSQUITOES BY CITIZEN SCIENCE: RESULTS OF FIRST EXPERIENCES IN ITALY

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Keywords: *Aedes albopictus*, monitoring, citizen science

INTRODUCTION: Mosquito Alert Italia is a network - coordinated by the Medical Entomology (Med Ent) group of Sapienza University and including experts from ISS, IZSVenezie, MUSE and University of Bologna – with the goal to implement citizen science (CS) approaches in the monitoring invasive *Aedes mosquitoes*. The goal is to complement data from entomological monitoring with the help of citizens, while promoting awareness on mosquito biology and public health risks and on implementation of individual-based approaches to reduce mosquito reproduction. We here describe the results of two parallel studies conducted in Italy either exploiting Mosquito Alert (MA) app for mobile phones, or involving a selected group of undergraduate students.

MATERIALS AND METHODS: 1- Mosquito Alert app was launched in Italy in late 2020 and promoted with 2 press releases (Oct 2020 and May 2021) and via social media and partner's websites. Citizens participated mainly by sending mosquito records/photos, which were identified by MA Digital Entolab's experts and used to build a database.
2- Students at Sapienza and Tor Vergata Universities of Rome were asked to send photos of mosquito by MA, as well as to provide the recorded specimens to MedEnt.

RESULTS AND CONCLUSIONS: 1-From Oct 2020 to end of 2021: 4487 mosquito reports were recorded in MA; the highest number of reports came from Rome (753) and other large cities, with a prevalence in northern-eastern regions. A total of 2653 reports were associated to photos and were examined by 3 MA' Digital EntoLab experts: i) 651 photographic records were identified unambiguously as *Cx. pipiens* (24.5%) and 865 as *Ae. albopictus* (32.6%), 665 were identified as *Culicidae* (25%) and 467 were either not identifiable or other insects (17,6%); ii) 3 *Ae. koreicus*, 1 *Ae. japonicus*, and 1 *Ae. japonicus*/*koreicus* (no. = 1) were recorded; iii) despite peak of mosquito densities in Italy is in Aug-Oct, in 2021 most reports (733, of which 479 with photos) were recorded in Jun-July, reflecting need of more effective MA promotion activities in the late part of the season; iv) most *Cx. pipiens* were recorded in May-July and in Oct-Nov 2021, while most *Ae. albopictus* in Jun-Sept, reflecting the actual relative frequencies of the two species in Italy.
2- Twenty-nine students participating the project collected 460 specimens, of which 399 were identified by MedEnt as *Cx. pipiens* (43.3%) or *Ae. albopictus* (34.6%). One *An. maculipennis*, 1 *Ae. detritus* and 10 *Cs. longearolata* and two *Cs. annulata* were also identified. Molecular identification will allow identification of morphologically unidentified specimens, as well as *Cx. pipiens* and *molestus* biotypes. Results, although obtain with a low-profile communication campaign, show the potential of CS approaches in tracing novel *Aedes* invasive species, as well as in studying mosquito species distribution. More extensive and regular promotion of the app during the entire mosquito season is necessary to have more significant collections of records/specimens.

INTERLABORATORY COMPARISON FOR THE DIAGNOSIS OF ARTHROPOD OF PUBLIC HEALTH IMPORTANCE

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Keywords: proficiency tests, arthropod, entomological laboratory

INTRODUCTION: In the last decades, the laboratory community (entomological laboratories included) has used interlaboratory studies as an external quality control. In particular, during recent years, proficiency tests (PTs) have become an instrument for evaluating the laboratory performance in an objective manner. The laboratory diagnosis is crucial to identify various species of arthropods reliably and rapidly; therefore, an adequate knowledge of the entomological systematics and taxonomy is necessary. In this contest, a specific PTs was organized in 2021 for the diagnosis of arthropods of public health importance.

MATERIALS AND METHODS: Five entomological laboratories took part in the inter-laboratory comparison. The chosen format consisted in 5 insects to be identified at family, genus and species level. Moreover, sex was requested to be determined. The choice of the identification method (morphological or molecular through BOLD DNA barcode) was left to the participants. Each sample in the panel consisted of one specimen (adult) which was stored dried in an 8 mL micro-tube. During the packaging process, the integrity of each specimen was visually controlled. The samples were stored at low temperature and shipped in dry ice. Results related to the test conducted on each sample were classified as correct if conform to what expected or incorrect in the absence of an answer. The analysis aimed primarily at recording the percentages of correct or incorrect results and verified the randomness of some of the observed differences.

RESULTS AND CONCLUSIONS: The overall performance of the participants for morphological analysis was high, reaching a precision equal to 97%. Only one laboratory confirmed the identification by sequencing. The availability of diagnostic laboratories of proved competence is fundamental for the surveillance and management of arthropods of Public Health importance. Thus, this inter-laboratory comparison could be considered a first attempt to improve such competence on an experimental basis, but a step to improve it should be the dissemination of the initiative, increasing both the number of laboratories and the types of arthropods included in the interlaboratory test.

DECIPHERING THE MOLECULAR INTERACTION BETWEEN THE ASIAN TIGER MOSQUITO *AEDES ALBOPICTUS* AND THE CHIKUNGUNYA VIRUS

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Keywords: *Aedes albopictus*, chikungunya virus, transcriptomics

INTRODUCTION: The global spread of *Aedes albopictus*, from South-Est Asia to several areas of the world, represents an important health problem, considering its capacity to act as vector of various pathogens. Among transmitted arboviruses (arthropod-borne viruses), chikungunya virus (CHIKV) is currently spread worldwide, and it was responsible of two outbreaks in Italy in the last fifteen years. The virus journey in the mosquito includes the colonization of the midgut epithelial cells soon after the infectious blood meal (1-2 days post blood-feeding, dpf) followed by the diffusion into the haemolymph (3-6 dpf). Finally, the virus can enter salivary glands to be transmitted to the following vertebrate host. With the aim of investigating *Ae. albopictus*-CHIKV molecular interactions, including immune mechanisms mounted by the mosquito to fight CHIKV and strategies implemented by the virus to survive and to gain competence for transmission, a transcriptomic approach was carried out.

MATERIALS AND METHODS: Mosquito key organs and tissues (midgut and carcasses, i.e., female body without midgut) at 1 and 5 dpf, in CHIKV-infected and in control (not-infected) mosquitoes, were analysed by RNA-seq; after assembly, contigs were annotated and differential expression (DE) analysis was performed. To validate the outcome of the RNA-seq approach, we selected 5 candidates among DE groups and we performed RTqPCR in dissected tissues.

RESULTS AND CONCLUSIONS: PFAM and GO enrichment analysis highlighted the role of immune pathways (e.g., ubiquitination and RNA interference pathways) during both the early infection stage and the following diffusion throughout the haemolymph. Transcriptional analysis by RTqPCR revealed a high expression of a LRR (Leucine Rich Repeat) protein and of the antimicrobial peptide Holotricin in the haemolymph, and of a trypsin in the midgut, in agreement to RNAseq data. Also, a Phenoloxidase activating factor (PO) confirmed its transcriptional abundance in the haemolymph. In contrast, transcriptional analysis of the Vitellogenin Receptor by RTqPCR disagreed with RNAseq data, showing a specific expression in the ovaries and not in the midgut.
This work contributes to the understanding *Ae. albopictus*-CHIKV molecular interactions through a detailed RNA-seq and RTqPCR analysis of key tissues (midgut and haemolymph at 1 and 5 dpf). Different pathways (e.g., ubiquitination, RNAi) resulted modulated by *Ae. albopictus* following CHIKV infection. Moreover, activation of LRRs proteins, secretion of Holotricin and melanization (by PO) occur mainly after viral dissemination into the hemocoel. Among the strategies developed by the virus to survive, the upregulation of VitR might increase vertical transmission, and the downregulation of trypsin in the midgut could limit enzymatic degradation during blood meal digestion. Overall, we believe that further functional analyses of candidates identified in this study will provide a deeper understanding of the interaction between the mosquito and CHIKV.

MOLECULAR DETECTION OF *ANAPLASMA PHAGOCYTOPHILUM* IN A FALLOW DEER (*DAMA DAMA*) IN UMBRIA REGION

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Keywords: *Anaplasma phagocytophilum*, fallow deer, PCR

INTRODUCTION: Recently, a global interest in tick-borne diseases has grown, among the most important concerns for human and veterinary health. In Europe, *Ixodes ricinus* is the most remarkable tick species involved in the transmission of tick-borne pathogens, like *Anaplasma phagocytophilum*, the causative agent of tick-borne fever (TBF) in domestic ruminants and granulocytic anaplasmosis in various animal species, including humans. Wild ungulates population is currently expanding, also due to insufficient wildlife management programs and to the abandonment of rural areas. As a result, these animals are increasingly found in anthropized areas, in close contact with humans (Cafiso et al., 2021. Animals, 11: 3335). Wild ruminants are the maintenance host of *I. ricinus* that being an exophilic tick is also affected by environmental variables. Since no transovarial transmission of *A. phagocytophilum* has been demonstrated in ticks (Rikihisa, 2011. Clin Microbiol Rev, 24: 469–89), the infection is likely maintained in the environment by a tick-ruminant cycle (Di Domenico et al., 2016. Ticks Tick Borne Dis, 7: 782-87).
The present paper reports a case of molecular positivity to *A. phagocytophilum* in a young fallow deer found dead in the province of Terni (Umbria).

MATERIALS AND METHODS: A young male fallow deer (*Dama dama*) was subjected to necropsy to identify the causes of death. Lesions suggested a presumably predation by canids, a spleen portion was tested for tick borne pathogens detection, in the framework of a wildlife health monitoring project. The spleen sample was subjected to DNA extraction using QIAamp DNA Mini Kit (Qiagen®) according to the manufacturer's instructions, followed by a PCR for *Anaplasma* sp. (Tay et al., 2014. Trop Biomed, 31: 769–76). The sample tested positive, and it was further analyzed through a molecular typing panel specific for *A. ovis*, *A. marginale* (Torina et al., 2012. Ticks Tick Borne Dis, 3: 283-287), *A. phagocytophilum* (Alberti et al., 2005. Appl Environ Microbiol, 71: 6418–22), *A. bovis* and *A. centrale* (Park et al., 2018. Acta Vet Scand, 60: 15). The molecular investigation revealed the positivity to *A. phagocytophilum*.

RESULTS AND CONCLUSIONS: *A. phagocytophilum* can infect humans and a wide range of animal hosts: its detection in a fallow deer confirms literature data (Di Domenico et al., 2016) for Italy on the reservoir role played by wild ungulates, in the maintenance of zoonotic agents such as *A. phagocytophilum*. Furthermore, further investigations are needed to assess the pathogen's prevalence in reservoirs and vectors as well as to detect *A. phagocytophilum* genotypes linked to human infection. The circulation of *A. phagocytophilum* threats not only the human activities related to wilderness, but also city parks and green areas, given the proximity of some ungulates to the urban areas. Our finding highlights the importance of passive monitoring plans for the control and management of zoonosis linked to the presence of wildlife in Umbria region.

FIRST REPORT OF *AEDES JAPONICUS* IN THE LIGURIA REGION, NORTH WEST OF ITALY

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Keywords: *Aedes japonicus*, invasive mosquito species, entomological surveillance

INTRODUCTION: The invasive species *Aedes japonicus* is native to East Asia. From the 90's it has spread in New Zealand, US and different European countries (Kampmen et al., 2014. Parasit Vectors, 7:59).
This species was identified for the first time in Italy in 2015 at the Austrian border and then spread in the northeast of the country (Montarsi et al., 2019. Parasit Vectors, 12: 120).
In the framework of the National plan for prevention, surveillance, and response to Arboviruses, an entomological and virological surveillance of mosquitos in Liguria is performed annually. One of the aims of this plan is the detection of new invasive species.

MATERIALS AND METHODS: In 2021, surveillance was performed biweekly from June to November. Adult mosquitos were trapped by means of CO2-lure-baited BG-sentinel traps and gravid traps, in 20 sites of the Liguria region. The mosquitos were morphologically identified using entomological keys and the ECDC guidelines. After the morphological identification, for each site, females of the same species were pooled and tested for the presence of West Nile virus (WNV) and Usutu virus (USUV). After homogenization in PBS, total RNA was extracted using a commercial kit and analysed by a multiplex real-time RT-PCR for the simultaneous detection and differentiation of the two WNV Lineages (1 and 2) (Del Amo et al., 2013. J Virol Methods, 189: 321-7), and by a real-time RT-PCR specific for USUV (Cavrini et al., 2011. J Clin Virol, 50:221-3). To confirm doubtful morphological identification, genetic analyses were performed. In particular, in the reported case, genomic DNA was extracted from a pool of homogenized mosquitos and PCR reactions were performed to amplify regions of two mitochondrial genes (COI and ND4) according to methods previously described (Dawnay et al., 2007. Forensic Sci Int, 173: 1-6; Cameron et al., 2010. J Med Entomol, 47: 527-35). PCR products were sequenced on both strands and each consensus sequence was compared with those deposed in GenBank by Blastn. A similarity ≥98% was considered as a threshold for identification at the species level.

RESULTS AND CONCLUSIONS: Six mosquitoes collected in Albenga, in the province of Savona (44.067169 N, 8.158698 E) using a gravid trap, the 6th of July 2021, were morphologically identified as *A. japonicus*. The sample tested negative for WNV and USUV. The genetic analyses confirmed the morphological identification, both for COI gene and for ND4 gene.
For the first time the invasive species *A. japonicus* has been identified in the Liguria region, in the North West of Italy, six years after the species was first detected in the country and in a site hundreds of kilometers away from its area of spreading in the North East of Italy. The report could represent a new introduction in a territory characterized by intensive floriculture activities.

FINDING OF *LEISHMANIA* KDNA IN QUESTING *IXODES RICINUS* TICKS FROM THE BOLOGNA PROVINCE (EMILIA-ROMAGNA REGION, ITALY)

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Keywords: *Leishmania* sp, *Ixodes ricinus*, questing ticks

INTRODUCTION: Although sandflies are the only proven vectors of *Leishmania infantum*, recently other possible vectors have been investigated. In particular, some studies have been conducted on brown tick (*Rhipicephalus sanguineus*), due to its role as a vector of other pathogens and its close relationship with dogs (Dantas-Torres, 2011. Trends Parasitol, 27:155-59), to date recognized as the main reservoir of zoonotic visceral leishmaniasis (Travi et al., 2018. PLoS Negl Trop Dis, 12: e0006082). In this tick, transovarial transmission of L. infantum has been confirmed in laboratory conditions (Dantas-Torres et al., 2010. Exp Parasitol, 125: 184–85), as well as its ability to transmit the parasites from dogs to hamsters (Almeida et al., 2016. Rev Bras Med Vet, 38:329-33), but not to other dogs (Rakhshanpour et al., 2017. Iran J Parasitol, 12:482-89). The aim of this study was to search for *Leishmania* kDNA in questing *Ixodes ricinus* ticks from the Bologna province, where active foci of leishmaniasis are ongoing.

MATERIALS AND METHODS: Questing ticks were collected in 4 sites of the Bologna province from natural pathways and parks. Specimens were identified and processed as reported by Aureli et al. 2015 (Ann Agric Environ Med, 22:459-66). Overall, 236 DNA extracts were screened: 151 larvae pool (10 larvae/pool), 72 nymphs pool (5 nymphs/pool) and 13 adults. A real-time PCR targeting the *Leishmania* kDNA was performed (Tsakmakidis et al., 2017. Vet Parasitol Reg Stud Reports, 16: 100279).

RESULTS AND CONCLUSIONS: All the collected ticks were *I. ricinus*. From 2 of the 4 locations examined, 4 (1.7%) extracts tested positive: 1 larvae pool, 2 nymphs pool and 1 adult. Most of the previous studies have been conducted on brown ticks collected directly from mammalian hosts. This greatly increases the chance of finding a positive tick: indeed, infection rates reported in these arthropods are frequently higher than the ones found in sandflies (Dantas-Torres et al., 2010. Parasitol Res, 106: 857-60). Although the prevalence values observed in the present study are lower than those reported in literature in fed ticks (Dantas-Torres et al., 2010, l.c.; Colombo et al., 2011. Parasitol Res, 109: 267-74; Solano-Galego et al., 2012. Parasit Vectors, 5: 98; Campos and Costa, 2014. Rev Inst Med Trop Sao Paulo, 56: 297-300; Medeiros et al., 2015. BMC Vet Res, 11: 258), to the best of our knowledge this is the first report of *Leishmania* kDNA in unfed questing *I. ricinus*. These findings support the hypothesis that even in this tick species *Leishmania* can have both transstadial and transovarial transmission.

PRELIMINARY SAMPLING OF MOSQUITO VECTORS AND PATHOGENS IN DJIBOUTI CITY USING A NOVEL SURVEILLANCE METHOD BASED ON FTA CARD

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Keywords: FTA card, arbovirus, monitoring

INTRODUCTION: Several strategies are available in mosquito-borne disease surveillance, according to the objective, target species, available funding and skills of personnel. In this study, we modified a BG sentinel trap to include a sugar delivery system equipped with a nucleic acid preserving substrate (FTA card). During sugar feeding, pathogens in mosquito saliva are released on a honey-soaked FTA card, remaining detectable for several days at environmental conditions. This approach simplifies surveillance procedures in pathogen detection, decreasing time, cost and labour required, and avoiding cold chain (Hall-Mendelin et al., 2010. PNAS, 107: 11255-59).

MATERIALS AND METHODS: We deployed 12 BG sentinel traps modified with honey-baited FTA cards in Djibouti City (Djibouti) placing traps outdoors in an Italian military base where pyrethroid insecticides are used. In each site, we performed six weekly captures (from 28th January 2020 to 25th February 2020) maintaining traps active for 3-4 consecutive days. Mosquitoes and FTA cards collected from each trap were stored in silica gel at room temperature until further analysis in Italy. All mosquitoes were morphologically identified by species, and RNA extracted from FTA cards was tested for the presence of arboviruses by real time PCR and sequencing (Scaramozzino et al., 2002. J Clin Microbiol, 39(5):1922–27; Pastorino et al., 2005. J Virol Methods, 124: 65–71; Drosten et al., 2002. J Clin Microbiol, 40:2323–30). A mosquito subsample was also genotyped through DNA extraction (Rider at al., 2012. Malar J, 11: 193) and PCR to assess the presence of mutations for knockdown resistance (kdr) to pyrethroids (Martinez-Torres et al., 1999. Pestic Sci, 55:1012-20).

RESULTS AND CONCLUSIONS: Out of 13,304 collected mosquitoes, 98.7% were identified as follows: 96.8% *Culex pipiens*/*quinquefasciatus*, 2.7% *Aedes aegypti*, 0.36% *Anopheles stephensi*, 0.11% *Culex sitiens* and 0.05% *Anopheles dthali*. Despite the control efforts taken over the years against *An. stephensi*, this invasive Asian malaria vector was detected, confirming its establishment in Horn of Africa. We also show for the first time *An. dthali* in Djibouti City, a secondary malaria vector known to be present in the North of the country (UGP PNUD/Ministere de la Santé, Republique De Djibouti, PNLP 2020-2024). The high density of *Cx. pipiens*/*quinquefasciatus* in the study area can be explained by high levels of insecticide resistance observed on a mosquito subsample (no.= 133): homozygous resistant 84%; heterozygous 12.5%; homozygous susceptible 3.5%. Finally, 3 of 71 FTA cards detected West Nile (no.= 2) and Dengue (no.= 1) viruses according with the presence of vectors in the same trap. These findings suggest that the proposed sampling approach could be applied for mosquito pathogens surveillance, resulting particularly useful in remote areas and in long term storage, when laboratory and qualified personnel are not available in loco, but also to reducing efforts in pathogen detection.

AMBLYOMMA MARMOREUM (KOCH 1844) (ACARI: IXODIDAE) IN ITALY: NEW RECORDS ON MIGRATORY BIRDS FROM AFRICA

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Keywords: alien tick species, migratory birds, *Amblyomma*

INTRODUCTION: In Europe, as in other parts of the globe, migratory birds play an important role in the transportation of ixodid ticks and tick-borne pathogens (Hasle, 2013. Front Cell Infect Microbiol, 3: 48) among continents. In spring, migratory birds reach Europe, mainly from sub-Saharan Africa or from northern African countries but not much is known about the diversity and ecology of the tick species dispersed by them (Hornok et al., 2016. Parasit Vectors, 9: 101). In this study, we report the occurrence of immature ticks specimens carried by migratory birds, identified as *A. marmoreum* (Koch, 1844).

MATERIALS AND METHODS: As part of two consecutive projects focused on sampling migratory birds in spring migration from Africa to Europe, more than 2500 ticks were collected in three years (Toma et al., 2021. Exp Appl Acarol, 83: 147–64) from 2017 to 2021, during bird-ringing activities on Ventotene Island, an important stop-over site in the Mediterranean Sea. Immature *Amblyomma* specimens, analysed in the present study, were collected from each bird using a tick twister and stored in 70% ethanol. The *Amblyomma* genus is not part of the European tick fauna, hence the specimens were identified based on literature on African tick fauna (Apanaskevich et al., 2008. Folia Parasit, 55: 61-74) and in consultation with experts from the University of Pretoria. The specimens were analyzed through amplification and sequencing of the following different molecular markers: 12S rDNA (Beati & Keirans, 2001. J Parasitol, 87:32-48), 16S rDNA (Black & Piesman, 1994. Proc Natl Acad Sci U S A, 91: 10034-8), COX (Lv et al., 2014. Parasit Vectors, 7: 93), 18S rDNA and 28S rDNA (Hornok et al., 2020. Ticks Tick Borne Dis, 11: 101494). All PCR products were purified and directly sequenced at Eurofins Genomics (Ebersberg, Germany). For phylogenetic analysis, the neighbour joining method with Tajima-Nei distance (Tajima & Nei, 1984. Mol Biol Evol, 1: 269–85) was used as a tree-building model using the Accelrys DS Gene software.

RESULTS AND CONCLUSIONS: In total, 18 immature specimens of *Amblyomma* sp. (17 nymphs and 1 larva) were collected from 6 migratory bird species. In the absence of adult specimens, morphological identification was limited to assign these ticks to the *Amblyomma* genus showing from 99.94% to 95.24% similarity, depending on the molecular target, with *Amblyomma marmoreum*. In particular, the 16S sequences from our specimens showed 99.08% identity with the sequence of *A. marmoreum* isolate Kinixys from South Africa (GenBank: MW29050). Likewise, the three phylogenetic trees consistently indicated that the *Amblyomma* from Ventotene belonged to the same branch as *A. marmoreum*. In conclusion, this record confirms that during the study period, immature *A. marmoreum* reached each spring the Pontine Islands. The entry of alien tick species and their potential transmitted pathogens deserves further study also in the light of the globally ongoing climate change.
This research was in the frame of the two projects 'Risk of introduction and spread of vector-borne viruses in Italy' (IZS AM 03/14 RC), and 'Emerging and re-emerging zoonoses along the routes of migratory birds. An integrated approach to investigate the potential route of introduction and spread' (IZS AM 04/19 RC) funded by the Italian Ministry of Health.

ATTRACTION OF *AEDES KOREICUS* TO HUMANS ASSESSED BY HUMAN LANDING CATCHES

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Keywords: *Aedes koreicus*, feeding pattern, mosquito ecology

INTRODUCTION: *Aedes koreicus* is an invasive mosquito discovered in Italy in 2011 and quickly spreading in the Northern part of the country. Little information on its vector competence is available and its biology and ecology are poorly known. In particular, the host feeding behavior and the anthropophilic degree are a crucial aspect to evaluate the vector competence and human disease transmission. Herein, the anthropophilic degree was investigated by human landing catches (HLC).

MATERIALS AND METHODS: The study was carried out in five cities (three in Belluno province and two in Trento province). Samplings were carried out in 2014, 2015 and 2020, once a month from May to October. The mosquitoes were collected by aspiration using a handheld aspirator. A collection of 30' was set up hourly starting from 3.5 hours before sunset. At the same time, a BG-Sentinel trap baited with lure and CO2 was activated to evaluate the abundance of *Ae. koreicus*.

RESULTS AND CONCLUSIONS: Overall, 1,136 mosquitoes were collected by HLC (439, 235, and 462 in 2014, 2015 and 2020 respectively), belonging to seven species. The amount of *Ae. koreicus* females collected was 27, 33 and 61 in 2014, 2015 and 2020, respectively. Among mosquitoes collected, *Ae. albopictus* was the most abundant species (14.7 females/catch; 85.6%), while *Ae. koreicus* was eight times less abundant (1.8 females/catch; 10.6%) ($p<0.01$). The total mean number of *Ae. koreicus* (mosquito/catch) from 2014-2015 to 2020 increased significantly in Trentino sites (1.0 in 2014-15 vs 6.3 in 2020) but decreased in Veneto sites (1.3 in 2014-15 vs 0.4 in 2020). Our results show that *Ae. koreicus* is attracted by human even though less aggressive than *Ae. albopictus*. In Trentino province, the increase in catches probably reflects the increase in *Ae. koreicus* density. 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.

POPULATION DYNAMICS OF *AEDES INVASIVE* MOSQUITOES IN NORTHEASTERN ITALY

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Keywords: *Aedes koreicus*, *Aedes japonicus*, mosquito surveillance

INTRODUCTION: *Aedes Invasive* Mosquitoes (AIM) monitoring has been ongoing in the Veneto region since 2011 and in Friuli Venezia Giulia since 2015, after the detection of *Aedes koreicus* and *Aedes japonicus*, respectively. In 2020 and 2021, the surveillance program was implemented, according to the transboundary "*Aedes Invasive* Mosquitoes COST-Action project." This study aimed to assess changes in AIM population dynamics in three areas.

MATERIALS AND METHODS: In 2020 and 2021, five ovitraps (containers with the capacity of one liter) were placed at three locations, where AIMs were established: Belluno (BL), Montebelluna (TV) and Gemona del Friuli (UD). Oviposition supports were collected every two weeks from May to October. Eggs were counted and hatched in the IZSVe insectarium, where morphological species identification of larvae was performed. Since only part of the eggs hatched, the number of larvae identified were used to estimate the species composition of the mosquito eggs using the formula recommended by the AIM COST-Action project (total no. of eggs x no. of specimens hatched-analyzed of species X/total no. of specimens hatched-analyzed of all species).

RESULTS AND CONCLUSIONS: A total of 275 oviposition supports were collected during the monitoring seasons (145 in 2020 and 130 in 2021). The mean number of *Aedes* spp. eggs per ovitrap increased in 2021 in comparison with 2020 (192 eggs/trap in 2021, vs 100 in 2020). The egg number of *Ae. koreicus* increased in Montebelluna (49 eggs/trap in 2020, 217 in 2021) and decreased in Belluno (181 eggs/trap in 2020, 29 in 2021), while *Ae. japonicus* increased both in Belluno (70 eggs/trap in 2020, 356 in 2021) and Gemona del Friuli (1322 eggs/trap in 2020, 2363 in 2021). The mean number of *Aedes albopictus* eggs did not change significantly in the three cities. *Aedes koreicus* is increasing its density westward (areas recently colonized) but not in Belluno where it seems displaced by *Ae. japonicus*. These observations suggest a possible competition between the sibling species *Ae. koreicus* and *Ae. japonicus* with an advantage for the latter. In Gemona del Friuli *Ae. japonicus* is increasing significantly in abundance probably due to the absence of *Ae. koreicus*. Although *Ae. japonicus* and *Ae. koreicus* do not display an aggressive biting behavior towards humans, its hosts' preference has not been defined yet. However, several studies report their competence for arboviruses and nematodes of the genus *Dirofilaria*. Considering the vector competence of AIM and their ability to spread quickly, authorities dealing with these invasive species should strengthen their monitoring and control activities.

This study was carried out in the framework of AIM-COST Action.

EVIDENCE OF PYRETHROID RESISTANCE IN TWO MAIN EUROPEAN MOSQUITO VECTOR SPECIES, *AEDES ALBOPICTUS* AND *CULEX PIPIENS*

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Keywords: *Aedes albopictus*, *Culex pipiens*, insecticide resistance

INTRODUCTION: The indigenous *Culex pipiens* and the invasive *Aedes albopictus* are among the most abundant mosquito species in Europe and are an increasing public health concern due to their ability to transmit arboviruses such as West-Nile, chikungunya and dengue. Pyrethroid-based adulticides spraying are largely exploited in some European regions to reduce nuisance due to mosquito biting, despite it is recommended only in case of arbovirus outbreaks. This extensive usage (as well as the use against agricultural pests) exerts high selective pressure on the target populations, as revealed by high levels of pyrethroid resistance (PR) and high frequencies of alleles associated to PR recently reported in *Ae. albopictus* from Italy. Despite these evidence, the topic has been seldom studied so far. We here report data on phenotypic PR and on frequencies of mutations in the voltage-sensitive sodium channel (VSSC) gene associated to reduced susceptibility to pyrethroids in *Ae. albopictus* and *Cx. pipiens* across Italy and Europe.

MATERIALS AND METHODS: WHO-bioassay data were obtained for *Ae. albopictus* and *Cx. pipiens* from Italy. Data on VSSC mutations were obtained by PCR-genotyping of mutation V1016G in *Ae. albopictus* populations across Europe and of L1014/F/C/S mutations in *Cx. pipiens* populations across Italy. Further data on genotypic and phenotypic PR in European populations of both mosquito species were obtained from bibliographic research.

RESULTS AND CONCLUSIONS: In *Ae. albopictus*, literature data and results of WHO-bioassays reveal reduced pyrethroid susceptibility in 7 out of 11 analysed populations from Italy, as well as in Albania, Greece and Spain. Genotyping of over 3,400 specimens from 19 European countries shows widespread presence of the V1016G mutation in Italy (detected in 7 out of 11 regions at frequencies >50% in Emilia-Romagna and Lazio sites) and in 8 other countries (Bulgaria, France, Georgia, Malta, Romania, Spain, Switzerland and Turkey) at frequencies of 1 to 7% per site.
In *Cx. pipiens*, bioassay results reveal reduced susceptibility in all analysed Italian populations (Trentino, Emilia-Romagna, Piemonte, Liguria, Lazio, Puglia) with mortality <30% in Puglia and Emilia-Romagna. Presence of mutations L1014F/C/S was investigated only in Italy and Greece, where they reach frequencies >90%.
The data herein presented reveal widespread and elevated PR in Italian populations of the two major arbovirus vectors, and preliminary indications of potential risk also in other European countries. The situation appears particularly worrisome in the case of Italian *Cx. pipiens* populations showing PR levels so high to certainly compromise the efficacy of pyrethroid based treatments, which represent the only available weapon in case of WNV transmission. These evidence represent a wake-up call for mosquito surveillance programs across Europe and prompt extensive monitoring as well as implementation of PR management plans.

CROSS-SECTIONAL MULTICENTRIC SURVEY ON CANINE FILARIOSES IN SOUTHERN ITALY

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Keywords: *Dirofilaria immitis*, epidemiology, southern Italy

NTRODUCTION: Canine filarioses caused by *Dirofilaria repens* and *Acanthocheilonema reconditum* have been constantly reported and regarded as endemic in southern Italian regions; conversely, *Dirofilaria immitis* is considered sporadic in these regions, and negligible its risk of transmission to dogs. However, in the last decade, the number of autochthonous cases or foci of *D. immitis* in dogs form southern regions increased significantly suggesting a new distribution pattern for this parasite. To provide more evidence to the southward spread of *D. immitis* a cross-sectional multicentric survey on canine filarioses was carried out.

MATERIALS AND METHODS: Owned and sheltered dogs from six southern regions (i.e., Lazio, Campania, Apulia, Basilicata, Calabria and Sicily) were included in the survey regardless their breed, attitude and/or gender. All the dogs were elder than 1 year and had no history or were not under preventative treatment against filarioses. A blood sample was collected from each included dog and analysed with Knott's test and, if positive, further tested using a *D. immitis* specific antigen ELISA test.

RESULTS AND CONCLUSIONS: One-thousand-eighty-seven dogs were enrolled in the survey (Table). According to the Knott's test results, the overall prevalence was 17% (338/1987) being single-species infection (92.6%) more common that mixed (7.4%). *Dirofilaria immitis* was the most frequent species with an overall prevalence of 11.4% (no. 251), followed by *D. repens* (no. 98, 3.7%) and *A. reconditum* (no. 14, 0.6%). *Acanthocheilonema reconditum* was retrieved in Sicily and Calabria exclusively. Sheltered dogs were significantly more at risk for *D. immitis* (χ^2 =163.3427, $p < 0.00001$), and, in the same manner, mongrel dogs (χ^2 = 92.7365, $p< 0.00001$) and animals housed in rural areas (χ^2 = 80.7429, $p< 0.00001$) were more frequently infected by *D. immitis*.
This survey provides further evidence on the southward spread of *D. immitis* in Italy. Southern Italian regions are wrongly considered not at risk for *D. immitis* and the lack of regular chemoprophylaxis against this parasite in dogs is favouring its spread. Practitioners and dogs' owners should be aware of this risk and the adoption of efficient strategies to protect dogs and control the transmission are urgently needed.

Region	No. dogs	Positive (%)	*D. immitis* (%)	*D. repens* (%)	*A. reconditum* (%)
Sicily	607	103 (16.97)	52 (8.57)	40 (6.59)	10 (1.65)
Calabria	174	9 (5.17)	4 (2.30)	2(1.15)	2(1.15)
Basilicata	41	0 (-)	-	-	-
Apulia	417	195 (46.76)	163 (39.09)	21 (5.04)	0 (-)
Campania	598	27 (4.52)	4 (0.67)	11 (1.84)	0 (-)
Lazio	150	4 (2.67)	4 (2.67)	-	-
Total	1987	338 (11.42)	227 (11.42)	74 (3.72)	12 (0.60)

Acknowledgment: This study was partially financed by Boehringer Ingelheim Animal Health Italy.

SURVEILLANCE ACTIVITY OF *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE) IN THE MUNICIPALITY OF MESSINA

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Keywords: *Aedes albopictus*, surveillance, Messina municipality

INTRODUCTION: *Aedes albopictus* (Skuse, 1895) (Diptera: Culicidae), popularly known as "Asian tiger mosquito", is an invasive mosquito species native to Southwest Asia that has spread rapidly in many areas of Europe and the Mediterranean in recent decades. It bites humans and a wide range of mammals, birds and may act as vector for several virus and parasite species.
Tiger mosquito is considered a new threat to public health, and it is of great importance with rising number of mosquito-transmitted diseases in Europe. In this study we report surveillance data on the oviposition activity of *Ae. albopictus* in the municipality of Messina (Sicily, Italy).

MATERIALS AND METHODS: Surveillance activity on *Ae. albopictus* presence and activity in the municipality of Messina was carried out using ovitraps from June to December 2021. A total of 118 georeferenced monitoring units were selected in the study area. Ovitrap consists of black plastic container of 500 ml, filled three-quarter with water and equipped with a masonite paddle (15 × 3 cm), where *Ae. albopictus* females lay eggs. The masonite paddles and water in each ovitrap were replaced at 1-week interval. The paddles were stored in numbered plastic bags according to ovitrap id and transferred to the laboratory. The paddles were observed using a stereo microscope for detection of *Ae. albopictus* eggs and their number recorded into an electronic spreadsheet. Descriptive statistic was calculated for layed eggs and the Pearson's correlation test was used to assess correlations between egg abundance and climatic data (i.e., precipitation and/or temperatures) recorded in the 2 weeks preceding the paddle collection. Linear regression model (y=a+bx) was applied to confirms these relationships and to determine the degree of correlation.

RESULTS AND CONCLUSIONS: During the surveillance period, a total of 3.422 ovitraps were placed in the study area, and 3369 (98.4%) of them were retrieved, being 64.8% positive for eggs. Presence and activity of *Ae. albopictus* in the municipality of Messina remained high for most of the monitored weeks. Egg abundance was positively influenced by mean temperature and negatively by precipitation and relative humidity recorded in the 2 weeks before paddle sampling (Table 1). These findings show as *Ae. albopictus* is perfectly encroached in the study area posing nuisance and health issues to citizens.

Acknowledgement: This study was financed by Messina servizi Bene Comune S.p.A.

Table 1. Relationship between the mean environmental temperature (T_{Mean}), the total precipitation and the mean relative humidity (RH) of the 2 weeks before sampling and the abundance of *Ae. albopictus* egg abundance.

	Pearson r	Rainfall (mm)	T_{Mean} (°C)	RH
Egg abbundance	r	-0.4332	0.7420	-0.5868
	95% confidence interval	-0.6902 to -0.07915	0.5157 to 0.8715	-0.7846 to -0.2806
	P value	0.0189	< 0.0001	0.0008
	Linear regression model			
	R square	0.1876	0.5506	0.3444
	Equation	Y = -0.03426*X + 4.617	Y = 0.09936*X + 14.94	Y = -0.001190*X + 0.6689

ENTOMOLOGICAL AND VIROLOGICAL SURVEY ON THE INSECT VECTORS POTENTIALLY IMPLICATED IN THE TRANSMISSION OF EMERGING AND RE-EMERGING INFECTIONS IN PACOCHE NATURE RESERVE (ECUADOR): PRELIMINARY RESULTS

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Keywords: vectors, re-emerging diseases, South America

INTRODUCTION: Pacoche Reserve, located at the Ecuadorian coast, includes 5,045 hectares of terrestrial ecosystems. This area is named after the Pacoche hills, located on the San Lorenzo cape south of Manta, in the province of Manabí. In 2016, this locality presented an epizootic with the death of 44 howler monkeys (*Alouatta palliata*), probably related to the presence of yellow fever, dengue, chikungunya and Zika viruses. As a part of the activities of the Italian Cooperation in Ecuador, the Italian Fund in Ecuador for Sustainable Development (FIEDS), together with the support of the Italian Agency for Development Cooperation (AICS) have recently funded a scientific project in this protected area. Research activities, involving both Italian and Ecuadorian institutions (Istituto Superiore di Sanità, Istituto Zooprofilattico Sperimentale Abruzzo e Molise, Instituto Nacional de Investigacion en Salud Publica, Instituto Nacional de Biodiversidad) were aimed at characterizing the main arthropod vectors responsible for serious diseases in humans and animals (mosquitoes, biting midges and sand-flies) and possible pathogens in these vectors. Also, blood samples were taken from wild animals, possible amplification reservoirs, from which were analyzed possible pathogens.

MATERIALS AND METHODS: Different insect sampling methods were used: Human landing catches (HLC), CDC-light traps, GAT traps, sticky-traps, Black-light trap, ovitraps. Mosquito larvae were sought in natural and artificial breeding sites. Birds and bats were caught with mist nets activated both day and night, identified, measured, weighed and released; two feathers were plucked out and blood-spots were collected and stored on FTA cards for subsequent molecular analyses, besides, if there were any ecto parasites, those were collected. All biological samples were transported and partially analyzed at the INSPI laboratories in Quito.

RESULTS AND CONCLUSIONS: Out of 118 mosquitoes from HLC have been morphologically identified so far: 27 *Wyeomyia medioalbipes*, 5 *W. leucostigma/coenonus*, 3 *W. rooti*, 7 *W. argenteorostris*, 21 *Haemagogus equinus*, 7 *H. celeste*, 5 *Psorophora pallescens*, 5 *Aedes aegypti* and 1 *Coquillettidia fasciolata*. Sixty biting midges were collected, and among them 38 *Culicoides paraensis* group, 3 *C. debilipalpis*, and 1 *C. pusillus* were identified. 29 feathers from 18 bird species and 30 blood-spots from 15 bird and 4 bat species were sampled. RNAs were extracted from 19 FTA cards (3 from bats and 16 from birds); RT-PCRs were performed to detect dengue virus (DENV). In addition, 20 *Hg. equinus* in 4 pools were analysed for DENV. All samples were negative. Analyzes are currently underway in both countries in close collaboration and using shared methodologies and protocols. Insects will be morphologically and/or molecularly identified and some of them together with the biological samples from birds and bats will be processed to search for arboviruses potentially circulating in the study area.

ENTOMOLOGICAL MOSQUITO SURVEILLANCE FOR WEST NILE, USUTU VIRUS AND *DIROFILARIA* SPP. IN LIVESTOCK FARMS IN APULIA AND BASILICATA REGIONS (2021)

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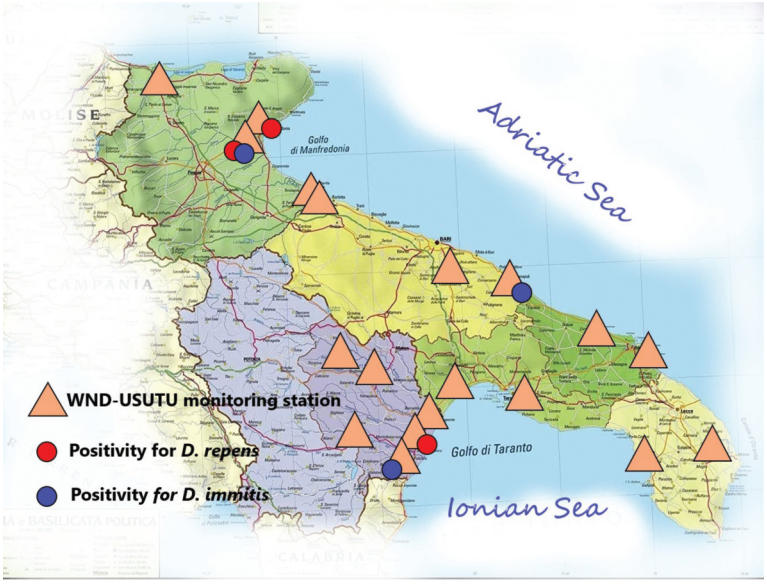
Keywords: mosquitoes, *Dirofilaria*, LAMP

INTRODUCTION: The entomological survey is a fundamental action of the national surveillance plan of arboviruses (PNA, 2020-2025) at a level region to prevent risk of pathogen transmission by arthropods, including mosquito species. During the 2021 year a mosquito monitoring was conducted in the Apulia and Basilicata regions (South Italy) as part of an integrated surveillance plan for Usutu and West Nile (WNV) infection and *Dirofilaria* spp. infestation. This work reports the results obtained.

MATERIALS AND METHODS: Mosquito traps (CDC and BG sentinel®) were positioned in selected livestock farms in each province in Apulia region and in Matera province in Basilicata region. After identification using morphological keys (Severini et al., 2009. Fragm Entomol, 41: 1-372), the culicidae were counted and pooled according to species, sex, date, type of trap. Females were molecularly tested for WNV and USUV using real-time RT-PCR (Tang et al., 2006. J Clin Virol, 36: 177-82; Del Amo et al., 2013. J Virol Methods, 189:321-27; Cavrini et al., 2011. J Clin Virol, 50: 221-23) and for *Dirofilaria repens* and *D. immitis* using both qPCR (Silbermayr et al., 2014. Parasit Vectors, 7: 226) and LAMP (Aonuma et al., 2009. Parasit Vectors, 2: 15; Raele et al., 2016. PLoS Negl Trop Dis, 10: e0004789). A leg from each *An. maculipennis s.l.* complex mosquito was used for molecular identification at species level (Folmer et al., 1994. Mol Mar Biol Biotechnol, 3: 294-99); the obtained sequences were compared with the NCBI database through BLAST runs.

RESULTS AND CONCLUSIONS: A total of 5454 (5127 F, 327 M) mosquitoes from eight genera and 17 species (*Aedes* (*Ae*) *albopictus*, *Ae. vexans*, *Anopheles* (*An*) *algeriensis*, *An. claviger*, *An. maculipennis s.l.* (= *An. labranchiae*), *An. superpictus*, *Coquillettidia richardii*, *Culex* (*Cx*) *modestus*, *Cx. pipiens*, *Cx. theileri*, *Culiseta* (*Cul.*) *annulata*, *Cul. longiareolata*, *Ochlerotatus* (*Ochl*) *caspius*, *Ochl. communis*, *Ochl. detritus*, *Orthopodomyia pulcripalpis*, *Uranotaenia unguiculata*) were collected from 19 stations (13 in Apulia and 6 in Basilicata) for a total of 572 caughts. Out of 596 examined pools, all of them were negative for both WNV and USUV viruses and 13 pools (13/572) positive for *Dirofilaria*, with seven being *D. repens* and six *D. immitis*. Specifically, *D. repens* was found in *Cx. pipiens*, *Ochl. caspius* and *An. maculipennis s.l.* (= *An. labranchiae*) and *D. immitis* was recorded in *Ae. albopictus*, *Cx. pipiens* and *Ochl. caspius*. The results obtained show the presence of a rich culicidae entomofauna in the monitored farms, confirming the variety of biotypes present in the two regions and the presence of mosquito species capable to be vectors of a variety of human pathogens. All the specimens of *An. maculipennis s.l.* identified belong to the single species *An. labranchiae*, for which positivity to *D. repens* has been recorded for the first time.



SEROPREVALENCE OF *BORRELIA BURGDORFERI S.L.* AND *MIDICHLORIA MITOCHONDRII* IN DOGS: RISK FACTORS ANALYSIS IN NORTHERN ITALY

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Keywords: *Borrelia burgdorferi s.l.*, *Ixodes ricinus*, tick bite

INTRODUCTION: Tick-borne diseases are spreading worldwide, and this trend has been widely documented for Lyme borreliosis, a bacterial disease that in western Europe is mainly transmitted by the hard tick *Ixodes ricinus*. In Italy, Lombardy was considered a low-incidence region, but recently a high-risk cluster for the resident human population has been identified (Zanzani et al., 2019).
To evaluate the risk of exposition to *Borrelia burgdorferi s.l.* (the causative agent of Lyme borreliosis) and *I. ricinus* bites for dogs living inside the described cluster, a serological survey was planned to detect antibodies anti-*B.burgdorferi* and -*Midichloria mitochondrii*, an alphaproteobacterium symbiont of *I. ricinus*.

MATERIALS AND METHODS: From May to October 2019, 149 blood samples were collected from owned dogs living in the described cluster (Sondrio and Lecco provinces). For each dog enrolled in the survey, data about age, sex and size were collected. In dogs' sera, the presence of anti-*B. burgdorferi s.l.* IgM and IgG was evaluated by a commercial indirect immunofluorescence assay (MegaScreen Fluoborrelia, MegaCor Diagnostik GmbH, Austria; cutoff titer, 1:64). To assess the possible exposition to *I. ricinus* bites in dogs in which transmission of *B. burgdorferi s.l.* did not occur, the presence of anti-*M. mitochondrii* IgG was investigated by an ELISA assay (Bazzocchi et al., 2013). Statistical analysis to determine the significant risk factors of *B. burgdorferi s.l.* seroprevalence and of *I. ricinus* bites was implemented by SPSS 20.0 (IBM, Chicago, IL).

RESULTS AND CONCLUSIONS: In the present study, 9.3% of dogs (14/149) showed detectable anti-*B. burgdorferi s.l.* IgM, and in 6.7% of dogs (10/149) anti-*B. burgdorferi s.l.* IgG were detected; overall prevalence of antibodies anti-*B. burgdorferi s.l.* was 12.1% (18/149). Out of the 149 tested dogs, 26.8% (no.= 40) were classified as positive for the presence of anti-*M. mitochondrii* IgG. Considering seropositivity to *B. burgdorferi s.l.* and/or seropositivity to *M. mitochondrii* as a consequence of at least one tick bite by *I. ricinus*, in the present study 34.9% of dogs (52/149) have been bitten. Considering seasonality, seropositivity to *B. burgdorferi s.l.* and/or *M. mitochondrii* significantly peaked in July (57.1%, 8/14) and October (83.3%, 15/18), and this result was consistent with *I. ricinus* phenology in temperate climates. Other features (province, age, sex, size) were not significantly related to *B. burgdorferi s.l.* seroprevalence and to *I. ricinus* bites risk.
Results of our study showed that in the previously described high-risk cluster of human Lyme borreliosis, the seroprevalence of *B. burgdorferi s.l.* in owned dogs was 12.1% and from spring to autumn the risk for tick bites was high. This risk coincided with *I. ricinus* seasonal dynamics in western Europe, and probably it was uniform in owned dogs population; in fact, the province in which the dogs lived, their age, sex, and size were not significant risk factors.

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ZOONOSES AND ONE HEALTH

MOLECULAR METHOD FOR DETECTION OF *TOXOPLASMA GONDII* OOCYSTS IN LEAFY-GREEN VEGETABLES: INTER-LABORATORY SOP VALIDATION AND MULTICENTRE FIELD APPLICATION

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Keywords: *Toxoplasma gondii*, molecular detection, ready-to-eat salad

INTRODUCTION: *Toxoplasma gondii* is a zoonotic pathogen with up to 60% of acquired infections associated with foodborne transmission. Consumption of raw fresh produce (FP) contaminated with *T. gondii* oocysts is one infection route. The relative importance of FP as source for human *T. gondii* infection is underestimated as standardized detection method(s) are lacking. We developed a standard operating procedure (SOP) for molecular detection of *T. gondii* oocysts in leafy green salads, validated it by a ring trial (RT) and are currently applying it in a multicentre study (MS) on ready-to-eat (RTE) salad at European level.

MATERIALS AND METHODS: Based on literature evidences, a standard operating procedure (SOP) for the molecular detection of *T. gondii* oocysts in ready-to-eat (RTE) leafy green salads was developed and validated by a comparative work between two laboratories. The key analytical steps include: i) oocysts recovery by sample washing followed by pelleting of the eluate by centrifugation; ii) DNA extraction and; iii) DNA detection by triplex real-time PCR, targeting two *T. gondii* multicopy markers and an internal amplification control. Thereafter, the SOP was implemented in seven consortium laboratories across Europe using video tutorials. The reproducibility of the SOP was further assesses in a ring trial (RT), involving nine laboratories, using three sample panels aiming to evaluate the performance of the overall procedure, the DNA extraction and the qPCR. Finally, an evidence-based sampling strategy was designed to conduct a multicentre study to assess the prevalence of *T. gondii* oocysts in RTE leafy green salads in ten countries representative of Europe.

RESULTS AND CONCLUSIONS: The SOP provided a LoD of 10 oocysts/30g salad during development and validation of the SOP. SOP implementation in the laboratories was successful and allowed identification and resolution of procedure limitations. RT analysis confirmed robustness of the procedure and comparability of results among participants. The MS sampling started in October 2021 a period of one year to potentially detect seasonal variations. Two categories of RTE salad mixes (baby leaves and cut leaves mixes) are sampled to explore potential associations between oocyst contamination and cultivation and growth conditions. In the study period, a total sample size of about 3,000 RTE salads from across Europe is expected to be tested. Positive samples will be confirmed and characterized by ITS-1 single tube nested PCR followed by Sanger sequencing. In conclusion, the application of a well-validated SOP is proving to be a useful tool to investigate the occurrence of *T. gondii* contamination in RTE salad, which is needed to assess the associated potential risk for humans.

Acknowledgments: This work was done as part of TOXOSOURCES project, EU Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

AN ORGANOTYPIC MODEL OF RETINAL *TOXOPLASMA GONDII* INFECTION: INCREASED PRODUCTION OF GSTO1 AND PNFKB

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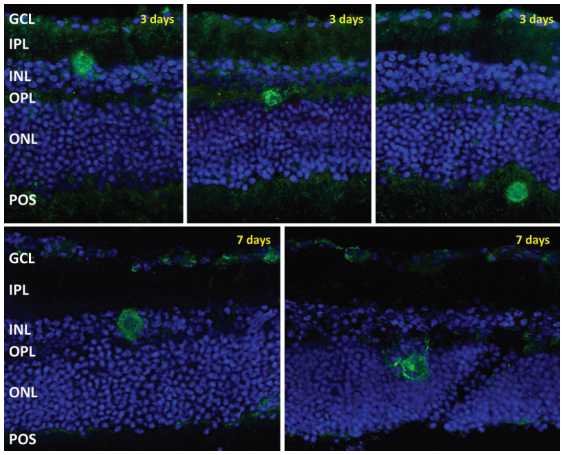
Keywords: *Toxoplasma gondii*, retina, inflammatory response

INTRODUCTION: Ocular toxoplasmosis (OT) is caused by the parasite *Toxoplasma gondii* and it represents the most common cause of eye inflammation in the world. Histopathology information of active OT lesions in humans is difficult to gather, and the majority of data about the inflammatory response and the morphological changes is based on a murine model of congenital OT. With this study we present an *ex vivo* model that facilitates the observation and manipulation of the infected retina. After stabilization of the cultured retina, we have started to analyze the inflammatory response caused by the parasite in this tissue. The first markers we have searched for are Glutathione S-transferase omega-1 (GSTO1) and Nuclear Factor kappa light chain enhancer of activated B cells (NFkB).

MATERIALS AND METHODS: Retinas dissected from 3- to 5-week-old C57BL/6J mice were cut into 4 fragments and transferred onto Millicell-CM culture inserts with ganglion cells up. Eight fragments were transferred on each insert. The inserts were placed in 6-well tissue culture plates with 1 mL of culture medium (50% MEM, 25% Hank's buffer salt solution, 25% Dulbecco's Phosphate Buffered Saline, 25 U/mL penicillin, 25 mg/mL streptomycin, 1 µg/mL amphotericin B, and 200 µM L-glutamine), which was changed every other day, and incubated for 24 hours, 3 days (D), 7D, or 10D.
Toxoplasma gondii (RH strain) were maintained in Vero cells. They were used at a concentration of 2,000/well to infect the retinal explants. Control explants were cultured in unmodified culture medium. For immunostaining, retinal fragments were fixed with 4% paraformaldehyde, embedded in cryo-gel, and cut into 10-µm-thick sections with a cryostat. The primary antibody was a polyclonal goat anti-*T. gondii* and the secondary antibody was conjugated with Alexa-Fluor-488. The sections were counterstained with DAPI. For Western blot (WB), fragments were homogenized and processed following standard protocols. Primary antibodies were directed to GSTO-1, to NFkB, or to pNFkB. Densitometric analysis of the immunoreactive bands was performed using the software ImageLab and statistical evaluation with ANOVA.

RESULTS AND CONCLUSIONS: Immunofluorescent, putative *T. gondii* cysts were observed at 3D and 7D in the inner nuclear layer (INL), outer plexiform layer (OPL), and in the photoreceptor outer segment (POS) (Figure). Results from WB showed a slight increase in GSTO1 levels at 3D, which became significant at 7D compared to controls. In addition, the ratio pNFkB/NFkB increased significantly at 3D, while at 7D it was similar to control values, suggesting a survival response in host cells by stimulating an antiapoptotic phenotype as previously shown in infected fibroblasts. Further analyses with shorter incubation times will be performed to verify this process in early phases of infection.



TARGETED CRISPR-CAS9 SCREENS *IN VIVO* IDENTIFY GRA12 AS THE MOST IMPORTANT COMMON VIRULENCE FACTOR FOR SURVIVAL OF *TOXOPLASMA GONDII*

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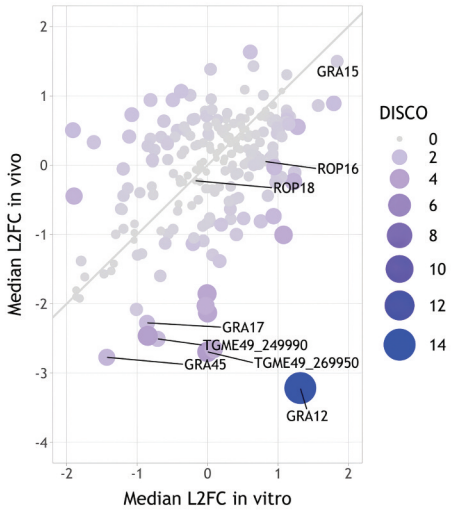
Keywords: *Toxoplasma gondii*, CRISPR, secretome

INTRODUCTION: *Toxoplasma gondii* is often considered the most successful parasite on Earth since it can infect any nucleated cell type and any warm-blooded animal, including one third of the human population. Its virulence varies widely, ranging from mildly pathogenic to virulent strains. *Toxoplasma*'s ability to survive within the infected cell and to protect its replicative niche is secured by proteins secreted into the host cell after invasion: the dense granules (GRA) and rhoptry proteins (ROP). Recent work has estimated their number to be over 200, the vast majority of which remains uncharacterised. Most effectors important for parasite survival have been shown to be either host- or parasite strain specific. However, effector proteins required to colonize multiple hosts or in common between lineages are largely unknown.

MATERIALS AND METHODS: We performed CRISPR-Cas9 genetic screens to identify factors contributing to parasite survival across different *Toxoplasma* strains and hosts. A single guide RNA (sgRNA) library targeting all putative secreted proteins was created and transfected in 4 strains with different pathogenicity, including the human lethal isolate VAND. The screens were performed in mouse strains with different susceptibility to infection to identify factors regardless of the host genetic background. Knock-out parasites were injected as a pool in mouse peritoneum, recovered 5 days after infection, and parasite's sgRNAs were sequenced to determine their relative abundance. Under-represented sgRNAs after infection indicate a role for the targeted protein in parasite survival to the host innate immune response. We then validated the role of the top hit, GRA12, as virulence factor both *in vitro* and *in vivo*, and proceeded with its functional characterisation. To identify neighbouring proteins and interactors we established a strain expressing GRA12 fused with the biotin ligase TurboID, and performed a pull-down of the endogenously tagged GRA12, followed by mass spectrometry.

RESULTS AND CONCLUSIONS: We identified GRA12 as a key protein required for parasite growth in the mouse peritoneum, regardless of the host or the parasite genetic backgrounds (Fig). Targeted deletion of gra12 in three *Toxoplasma* strains confirms its role as a virulence factor in macrophages of mice, rat and human origin, indicating a parasite strain- and species-transcendent function. Preliminary results show that GRA12 orthologs from parasites *Hammondia hammondi* and *Neospora caninum* can complement the function of TgGRA12 suggesting a likely role beyond *Toxoplasma*. Current investigation focuses on how GRA12 functions at the host-pathogen interface and ongoing experiments follow up on the results from the identified interactome. In summary, we show that GRA12 is a strain- and species-transcendent virulence factor critical for the cell-autonomous survival of *Toxoplasma*. Identification of such factors is crucial to understand the success of *Toxoplasma gondii* as generalist parasite and to device novel therapeutic strategies.



GRA12 is the most important secreted virulence factor in vivo of the hypervirulent isolate VAND. Scatter plot of median log_2-fold-change (L2FC) for each gene *in vitro* and *in vivo*. Each point is scaled according to the gene discordance-concordance (DISCO) score. Genes with the most negative *in vivo* L2FC and control genes are labelled.

IS THERE A CORRELATION BETWEEN THE ENDOSYMBIONT COMMUNITY AND DIFFERENT LINEAGES IN *IXODES FRONTALIS* TICK?

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Keywords: *Ixodes frontalis*, symbionts, molecular biology

INTRODUCTION: *Ixodes frontalis* is an ornithophilic tick species widely distributed in Europe that can vector multiple pathogens but is still understudied. Hornok et al., 2016 (Parasit Vectors, 9: 101) showed that two genetic lineages are clearly recognizable in the populations of this tick species (Haplotype A and B). Bacterial symbionts of other ticks species have been investigated and shown to have fundamental roles for ticks survival and blood metabolism, however little is known about the symbionts of *I. frontalis*. Small sized screenings showed the presence of two symbiotic species: *Midichloria mitochondrii* and *Spiroplasma ixodetis*. Thus far, there is no data on the correlation between symbiont presence and ticks' haplotypes. Here, we aim to expand this knowledge.

MATERIALS AND METHODS: *I. frontalis* specimens (larvae and nymphs) were collected from vegetation, in particular under bamboo bushes, using the flagging technique, in different areas in France and in Italy. Presence and load of *M. mitochondrii* and *S. ixodetis* were investigated with both qualitative and quantitative PCR. In addition, 16S metagenomics was performed on a subset of samples to investigate the community with an unbiased approach. COI mitochondrial gene PCRs and sequencing were also performed to discriminate between the two lineages. Furthermore, a correlation analysis was run to compare the molecular data of the symbionts with the tick haplotypes.

RESULTS AND CONCLUSIONS: Both symbionts were detected, *M. mitochondrii* with an higher prevalence (20.9%) than *S. ixodetis* (14.7%). Interestingly, a significant portion of the examined ticks (67.3%) lacked both symbionts, suggesting their role to be facultative mutualists. Until now, 16S metagenomic analysis did not reveal any other symbiont candidates. Regarding the haplotypes, the majority of the tested ticks belong to the lineage A (80%). The correlation between haplotype and presence of symbiont, showed that ticks belonging to lineage A carry both *M. mitochondrii* (26%) and *S. ixodetis* (17%), even if with different prevalence, while *M. mitochondrii* is absent in specimens of haplotype B and *S. ixodetis* is present at lower percentage (6%).

HIGH PREVALENCE OF TICK-BORNE PATHOGENS AND ASSOCIATED ENDOSYMBIONTS IN ITALIAN CITIZENS

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Keywords: tick-borne pathogens, humans, *Midichloria mitochondrii*

INTRODUCTION: The prevalence of tick-borne diseases (TBDs) is globally increasing with several zoonotic pathogens, such as *Coxiella burnetii*, *Rickettsia* spp. and *Borrelia burgdorferi sensu lato* complex, recognized as emerging or re-emerging in animals and humans (Dantas-Torres et al., 2012. Trends Parasitol, 28:437-46). The Italian peninsula is highly suitable for several outdoor activities (e.g., camping, gardening, hiking, breeding, farming, forestry work), and suburban and rural environments represent a risk for the exposure to tick bites for humans, especially in southern regions where a wide variety of ixodid ticks thrive (Otranto et al., 2014. Parasit Vectors, 7:328).
The present study aimed to assess the occurrence of zoonotic tick-borne pathogens (TBP) in people exposed to tick bites in southern Italy, as well as the potential involvement of *Candidatus Midichloria mitochondrii* (*M. mitochondrii*) endosymbiont, in TBP infection in humans.

MATERIALS AND METHODS: From February to December 2021, 135 people, exposed to tick bite in three regions of southern Italy (i.e., Apulia, Basilicata and Campania), were enrolled and their data (i.e., age, gender, outdoor activity, geographical origin and post-bite clinical findings ascribable to TBDs) recorded. Serum and blood samples were collected and screened for antibodies against *C. burnetii*, *Rickettsia* spp. and *B. burgdorferi s.l.* complex by a chemiluminescent immunoassay (CLIA, Vircell®, S.L.) and for microrganism's DNA, including also *M. mitochondrii*, by conventional PCR.
The association between TBP infection and age, gender, outdoor activity and geographical origin of patients, as well as *M. mitochondrii* infection, was evaluated by chi-square test ($p < 0.05$ was considered significant). A phylogenetic analysis on *M. mitochondrii* 16S rRNA gene sequences detected in humans was inferred using the maximum likelihood method.

RESULTS AND CONCLUSIONS: Overall, 63 people (45.9%) scored positive for TBPs, being *C. burnetii* the most frequently identified (27.4%), followed by *Rickettsia raoultii* (21.5%) and *Borrelia lusitaniae* (10.4%). The risk of TBP infection was significantly associated with the geographical region ($p<0.03$), being patients living in Basilicata more exposed to TBPs. In addition, *M. mitochondrii* DNA detected in 46 participants (34.1%) was significantly associated with TBP infections ($p<0.0001$). Phylogenetic analysis of *M. mitochondrii* sequences revealed 5 clades and 8 human sequence types correlated to vertebrates, *Ixodes* spp. and Europe. Data demonstrate a high circulation of TBPs in citizens practicing outdoor activities in southern Italy and the potential involvement of *M. mitochondrii* in TBP infections.

EARLY WARNING SIGNALS OF TICK-BORNE ENCEPHALITIS RISK

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Keywords: tick-borne encephalitis, early warning, risk assessment

INTRODUCTION: Tick-borne encephalitis is an emerging tick-borne viral infection caused by TBEv, a flavivirus affecting human and animal central nervous systems. Despite the availability of an effective vaccine, an average of 2700 confirmed human cases are reported each year in Europe, with a mean annual notification rate of 0.6 cases per 100,000 inhabitants. The virus circulates enzootically in natural foci among ticks and a number of wildlife hosts which play different eco-epidemiological roles. These include rodents, which play a major role as reservoirs and amplifiers of the infection, and deer which affect tick abundance. TBE distribution is very focal as a consequence of the complex interplay between environmental covariates and the temporal and spatial variation in the abundance of key hosts. The reduction of tick-borne diseases burden strongly depends on the level of awareness of those categories mostly exposed to tick bites including the general public. Therefore, the identification of early warning signals of changing risk can be of utility for the public health authorities for the implementation of prevention and informative campaign.

MATERIALS AND METHODS: We focused our studies on the Province of Trento (northern Italy, ~6,000km2, ~500,000 inhabitants) where TBEv cases have been recorded since 1992. By combining long term data on host abundance, tick infestation rate on rodents, seroconversion in rodents, a number of environmental variables, and TBE human cases, by the use of mathematical models we identified those predictors with higher potential to forecast changes in TBE risk.

RESULTS AND CONCLUSIONS: Between 1992 and 2020, a total of 206 TBEv human cases were recorded in the study area. We found a significant positive association between TBEv cases and the number of co-feeding ticks on rodents (Rosà et al., 2019), autumnal cooling temperature, and airborne pollen with a different time lag. In particular, we recently found a significant positive association between beech, oak, and hop hornbeam pollen abundances and TBEv cases recorded two years later. These predictors could be used by public health officers as an early warning signal for changes in TBE risk and therefore of utility to plan information and prevention actions.

INFESTATION BY *LINGUATULA SERRATA* (PENTASTOMIDA: LINGUATULIDAE) IN A GREY WOLF (*CANIS LUPUS*) IN ITALY (APULIA REGION): A NEGLECTED ZOONOSIS

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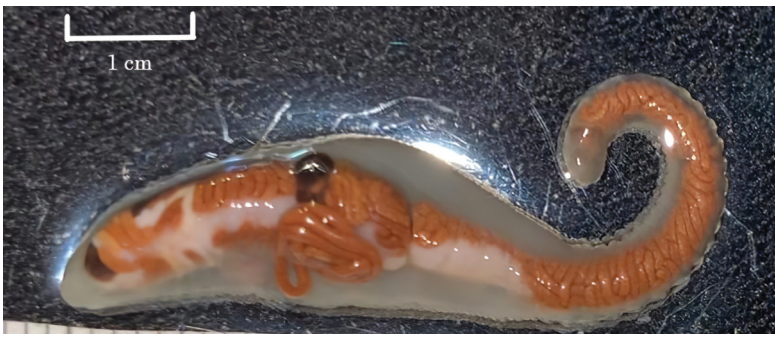
Keywords: *Linguatula serrata*, wolf, Italy

INTRODUCTION: Linguatulosis caused by the tongue-worm, *Linguatula* (L.) *serrata* is a frequent zoonosis in Middle East and Africa, rare in Europe. The adult form of the parasite typically inhabits respiratory passages of canids; herbivores and rodents represent intermediate hosts and they acquire infestation by pasture contaminated by parasite eggs. Following ingestion, the hatched larvae exit the intestine, migrate in host tissues where molt in infective nymphs (visceral linguatulosis) capable to develop into adults in final hosts (Riley, 1986. Adv Parasitol, 25: 45-128). Humans can be affected by visceral linguatulosis, with larvae encysted on viscera (Pampiglione et al., 2001. Parassitologia, 43(3):105-8) or mobile in eye (Koehsler et al., 2011. Emerg Infect Disease, 17(5): 870-72); or/and by nasopharyngeal form (Halzoun/Marrara syndrome) when they serve as aberrant final hosts by eating active nymphs in contamined poorly cooked viscera (Christoffersen and De Assis, 2013. Zool Med Leiden, 87:1-206). This report provides new information on the role of wild animals in the epidemiology of this parasitosis.

MATERIALS AND METHODS: In May 2021, a tongue-like parasite was collected from nasal cavities of a grey wolf subjected to autopsy and microscopically and molecularly examined; feces and nasal mucus were analyzed for pentastomid eggs by flotation using NaCl satured solution. DNA was extracted and amplified by conventional PCR targeting COX1 gene (Folmer et al., 1994. Mol Mar Bio Biotechnol, 3:294-99); the obtained amplicon was purified, sequenced, compared with nucleotide sequences in GenBank database using BLAST and a phylogenetic tree constructed using the MEGA6 software.

RESULTS AND CONCLUSIONS: The collected parasite was flat, annulated, 63mm long and 8mm width, with visible eggs in ovaries and four hooks surrounding mouth openings; in nasal mucus and feces were found ovoid and yellowish eggs, about 90 by 70 µm in size with inside an armed embryo. Based on morphological features, the specimen and ova were identified as *Linguatula serrata*, female and pentastomid eggs, respectively. The obtained sequence (GenBank accession number MW947492) result be grouped within a representative cluster constituted by *L. serrata* specimens collected from both Romanian and Italian dogs, respectively (accession numbers KF029447 and MZ052082).
Linguatula serrata in grey wolfs is poorly registered in Europe, with observations from Balkans'area (Pavlovic et al., 2017. J Hellenic Vet Med Soc, 68(4): 687-90) and now also documented in a wolf in Italy. This report also confirms the circulation of the parasite in Apulia region, about two decades after its detection in a stray dog in urban context (Cafiero et al., 1997. Atti SiSvet vol. LI 617-18), thus suggesting the importance to inspect for immature *L. serrata* forms both cattle and wild animals. Further studies based on molecular tools could also improve the understanding of the epidemiology of this neglected zoonosis.



SYSTEMATIC ANALYSIS OF *ACANTHAMOEBA* SP. SEQUENCE TYPE CORRELATED WITH SOURCE AND PATHOGENICITY IN ITALY

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Keywords: *Acanthamoeba*, one-health, Italy

INTRODUCTION: During the last few decades, *Acanthamoeba* have been frequently reported as a potentially pathogenic free-living protozoan, causing a sight-threatening keratitis (AK), fatal granulomatous encephalitis (GAE), and cutaneous disorders. Based on rRNA gene sequences (ASA.S1 fragment), the genus is divided into 22 genotypes (T1 – T22). Particularly, the genotype T4 has been the most associated with AK and GAE. By 2001, it had become evident that also many isolates in environmental studies were classified into sequence type T4. Clustering of AK cases or tracking of strains in the environment might be possible by examining subsequences (alleles) within the ASA.S1 fragment (Fuerst and Booton, 2020. Pathogens, 9: 1-34). The aim of the study is to analyze the distribution of *Acanthamoeba*' clinical and environmental samples in Italy by phylogenetic and statistical analyses. Sequence data retrieved from GenBank were compared with new positive' *Acanthamoeba* clinical samples obtained in the present study.

MATERIALS AND METHODS: Clinical samples: Ten samples were collected at the Laboratory of Parasitology of the Polyclinic Tor Vergata of Rome and *Acanthamoeba* specific PCR (18S) was used to identify the genotype.
Database' creation: We search all the deposited *Acanthamoeba* sequences from Italy in PubMed Nucleotide. We used a multistep strategy matching the keywords *Acanthamoeba*, Italy, Italian, FLA and free-living amoeba. Next, a GenBank "complete record" check was performed (last update March 2022) paying attention to "Country Isolation and Source".
Genotypes and Alleles characterization: 18S sequences spanning the spectrum of Italian *Acanthamoeba* diversity were downloaded and aligned with those produced here. Alleles identification was based on the Fuerst database (Fuerst and Booton, 2020. Pathogens, 9: 1-34). Phylogenetic (Maximum Likelihood) and statistical (Hierarchical Clustering on Principal Components Analysis) analyses were performed using RStudio.

RESULTS AND CONCLUSIONS: All new clinical samples characterized in the present study belonged to genotype T4, the main genotype responsible for AK, as already reported worldwide. Our multistep strategy produced a unique database with 121 sequences trimmed down by removing all input characterized in different genetic loci. Genetic analysis comprising the spectrum of Italian *Acanthamoeba* diversity (>200 sequences) confirmed the existence of different genotypes – T2, T3, T4, T11 and T15 – in our country. New allelic variations never reported so far, were identified particularly in the genotype T4 and T15. The phylogenetic analysis did not allow to solve the genetic relations among isolates from different sources. The next step will be to perform a deep statistical analysis based on *Acanthamoeba* genotypes, alleles and source to understand traits which could drive the evolution of different *Acanthamoeba*' genetic populations.

BLASTOCYSTIS IN THE "SENTINEL" ORGANISM *MYTILUS GALLOPROVINCIALIS* FROM CENTRAL TYRRHENIAN SEA COAST: AN INDICATOR OF ENVIRONMENT CONTAMINATION

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Keywords: *Blastocystis*, *Mytilus galloprovincialis*, environmental contamination

INTRODUCTION: *Blastocystis* is widespread in several terrestrial animal hosts including humans, with a large genetic heterogeneity; 32 distinct subtypes (STs) have been so far detected. In the marine realm, *Blastocystis* was detected in fish and marine mammals (Gantois et al., 2020. Microorg, 24: 8-460), as well as new STs, were specifically detected in marine turtles and cetaceans from the Mediterranean Sea (Aco et al., 2021. Abstract SoIPa Congress XXXI). However, it can be also bioaccumulated throughout the marine food web as resulting of contaminated freshwater runoff. Marine invertebrates as mussels, being filter feeders, have been previously reported contaminated by protozoan parasites (Giangaspero et al., 2009. Parasitol Int, 58:12-7; Santoro et al., 2020. Front Microbiol, 11:355) representing a potential source of infection for humans. The aim of the study is to investigate the presence of *Blastocystis* in the Mediterranean mussels (*Mytilus galloprovincialis*) from the central Tyrrhenian Sea coast.

MATERIALS AND METHODS: A molecular survey has been so far performed on a total of no.= 353 specimens of *Mytilus galloprovincialis* from 4 coastal sites in the Lazio Region (no.= 264) and Gulf of Naples (Campania region) (no.= 89), by using real-time PCR that amplifies a small fragment of the SSU rRNA gene. Then, PCR positive samples were sequenced by next-generation amplicon sequencing (NGS) (Maloney et al., 2019. Infect Genet Evol, 73:119-25) for the identification of the specific ST.

RESULTS AND CONCLUSIONS: *Blastocystis* was detected in 51 out of 353 (14.4%) Mediterranean mussels from all sampling sites. In the Lazio region, 5.3% of the samples were positive, whereas, 41.6% of individuals resulted positive in the Gulf of Naples. The analysis of full-length nucleotide sequences and phylogenetic analysis shows that the contamination in all samples was due to ST3. However, mixed infection by other STs was identified in some samples. This is the first evidence of *Blastocystis* in Mediterranean mussels. The finding seems to support that mussels bioaccumulate *Blastocystis* via contaminated freshwater runoff. Molecular surveillance of shellfish for *Blastocystis* can be used as a possible indicator of the contamination of the marine environment, taking also into account the potential risk of transmission of the protist to humans.

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INSECTICIDAL EFFICACY AGAINST *PHLEBOTOMUS PERNICIOSUS* IN DOGS TREATED ORALLY WITH FLURALANER

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Keywords: *Phlebotomus perniciosus*, fluralaner, insecticidal efficacy

INTRODUCTION: Domestic dogs are the main reservoir hosts for *Leishmania infantum*, the agent of zoonotic visceral leishmaniasis (VL), transmitted by skin inoculation of metacyclic promastigotes of *Leishmania* by infected phlebotomine vectors during blood meal. Highlighting how dog's skin represents the ultimate boundary to implement protection. The sand fly *Phlebotomus perniciosus* is the main vector of zoonotic VL in the western Mediterranean region, including southern Europe (Portugal, Spain, southern France, Italy, and northwest Croatia) and Maghreb countries (Morocco, Algeria, and Tunisia), and also experimentally shown as potential vector in *Leishmania tropica* transmission, agent of Canine leishmaniosis in north Africa and the Middle East. Traditionally topical treatments i.e. collars, spot-ons or sprays, containing synthetic pyrethroids give dog's protection from sand fly bites. Isoxazolines are a novel class of systemic insecticides with lethal effects against arthropod vectors, approved as veterinary drugs for dogs and cats protection against ectoparasites. Fluralaner has been shown as an active insecticidal compound based on high level of efficacy and persistence. The aim of this study was to evaluate the insecticidal efficacy of oral fluralaner in dogs bitten by *P. perniciosus*.

MATERIALS AND METHODS: In two different locations, two parallel-group, negative-controlled, randomized, masked laboratory trials with equivalent designs were performed using two different pathogen-free laboratory-bred *P. perniciosus* strains for the challenge. In each trial, initially ranked on natural attractiveness to sand flies, 12 purpose-bred beagles, were randomly allocated to two groups (6 animals/group). In the control group dogs were not treated, while received fluralaner orally at the approved dose on day 0, in the other group. Each dog was subsequently exposed to an average of 70 unfed live sand fly females on days 1, 28, 56 and 84. Viability of blood-fed females was then evaluated for up to 96 h after exposure, and insecticidal efficacy was measured as the survival rate of flies fed on the fluralaner-treated dogs versus that of dogs in the control group. Significance was calculated for the proportion of live fed sand fly counts from treated versus control group dogs.

RESULTS AND CONCLUSIONS: Comparison of the survival proportions between treated and control groups, showed that fluralaner insecticidal efficacy was highly significant in both trials ($p < 0.001$ or $p < 0.01$ in different assessments) through day 56. In the first trial, efficacy reached 100% on days 1 and 28, and 99.1% on days 56. In the second trial, the insecticidal efficacy was 98.5, 100 and 85.9%, respectively on the same days. On day 84, efficacy was in the range of 53-57% ($p < 0.05$) in the first trial and 0% in the second trial. A single oral fluralaner administration to dogs under laboratory conditions results in strong and reproducible insecticidal efficacy against *P. perniciosus* for at least 8 weeks.

INCREASE OF GLUTATHIONE-S-TRANSFERASE OMEGA 1-1 AUTOANTIBODIES IN SERA FROM TRICHINELLOSIS PATIENTS (*TRICHINELLA SPIRALIS* AND *TRICHINELLA BRITOVI*)

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Keywords: glutathione-S-transferase omega, autoantibody, *Trichinella*

INTRODUCTION: The glutathione-S-transferases omega (GSTOs) are multifunctional enzymes involved in cellular defense. GSTOs exhibit a series of unique properties compared to other GSTs (Board et al., 2000. J Biol Chem, 275:24798-806). GSTO1-1, the most studied GSTO, has also been shown to possess antiapoptotic activity (Piaggi et al., 2010. Carcinogenesis, 31:804-11), regulates the TLR4 pro-inflammatory signalling through the regulation of NF-κB and is required for the lipopolysaccharide-stimulated induction of NADPH oxidase (Menon et al., 2015. Cell Sci, 128:1982-90). Auto-antibodies against GSTO1-1 were detected in serum of patients with esophageal squamous cell carcinoma (Li et al., 2014. Tumour Biol, 35:10871-7).
Autoimmune reactions have been described in trichinellosis patients (Macura-Biegun et al., 1998. Comp Immunol Microbiol Infect Dis, 21: 101-6; Pratesi et al., 2006. Parasite Immunol, 28: 447–451). Since GSTO1 expression progressively increases in the nurse cell during mouse experimental infection with *T. spiralis* (Piaggi et al., 2021. Vet Parasitol, 297: 109114) we investigated the possible presence of GSTO1-1 autoantibodies during *Trichinella* infection in humans.

MATERIALS AND METHODS: GSTO1-1 autoantibody were analyzed in sera from 15 *Trichinella spiralis* infected individuals (several years after infection), 32 *Trichinella britovi* infected subjects and 19 healthy subjects by an indirect ELISA procedure set up in our laboratory.
The presence of anti-GSTO1-1 antibodies was evaluated also by immunofluorescence, in fact after sera from trichinellosis patients were pre-incubated with purified recombinant GSTO1-1 protein, they were added to wild type HeLa or GSTO1-1 CRISPR-cas 9 knock-out HeLa or HepG2 cells and the imaging results were compared with those obtained by incubating the same cell types with untreated sera.

RESULTS AND CONCLUSIONS: Immunofluorescence: sera from *T. spiralis* and *T. britovi* infected patients produced fluorescence staining in the cytoplasm of HepG2 and HeLa cells which was significantly reduced when sera were pre-adsorbed with purified GSTO1-1 protein; almost no staining was present when GSTO1-1 knockout cells were incubated with sera patients, either pre-adsorbed or untreated.
ELISA: antibodies against GSTO1-1 were detectable in all evaluated samples (either from *T. spiralis* or *T. britovi* infected patients), progressively increasing during infection.
It is arguable that this increase of GSTO1-1 autoantibodies might reflect that in the expression and production of the protein in the inflamed skeletal muscles upon *Trichinella* infection and its release in the extracellular compartment.
Our data demonstrate that anti-GSTO1-1 antibodies can be induced by different *Trichinella* species, both either in acute or in late-stage patients.

EPIDEMIOLOGICAL INVESTIGATION ON TOXOPLASMOSIS IN WATER BUFFALO FARMS IN SOUTHERN ITALY

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Keywords: *Toxoplasma gondii*, water buffalo, serological survey

INTRODUCTION: Toxoplasmosis is one of the most common parasitic zoonoses worldwide caused by the protozoa *Toxoplasma gondii*. Beyond its impact on public health, toxoplasmosis has also important veterinary implications, because it causes in livestock abortions, fetal mummifications, stillbirths and the birth of weak offspring with negative economic impacts (Hill et al., 2015. Anim Health Res Rev, 6: 41-61). Water buffaloes are considered resistant to clinical disease due to *T. gondii*, so there are studies reporting only serological evidence of natural infection in these animals (Dubey, 2010. General Biology, 1-72). Due to the increasing of the use of buffalo meat and milk for human consumption, concern about sanitary conditions on buffalo farms has increased over the past 10 years. In order to reduce the risk of human infection with *T. gondii*, the knowledge of the epidemiological data and the identification of the potential risk factors associated with the infection in farm animals is of fundamental importance. Therefore, the aims of this study are to: i) determine the seroprevalence of *T. gondii* infection in water buffaloes and ii) evaluate the risk factors associated with the infection in water buffalo farms located in Salerno province (Southern Italy).

MATERIALS AND METHODS: This preliminary study was performed between January and March 2022 on 2,368 adult lactating buffaloes, representing 30% of the total number of animals on 20 farms in the Salerno province. Farms were selected according to the type of production orientation, type of housing and number of animals milked. Serological screening was carried out using blood samples collected during the prophylaxis for Brucellosis and analysed by an indirect ELISA kit (ID Screen®, Indirect Toxoplasmosis Multi-Species, IDVET, France), according to the manufacturer's instructions. In addition, in each farm a formulated questionnaire was given to assess the risk factors. The questionnaire included questions on several management variables (type of production, number of animals, presence of cats and any rodent control measures) and on the presence of abortion in water buffalo farms.

RESULTS AND CONCLUSIONS: Overall out of 2,368 water buffalo animals tested, 381 (16.1%; 95% Confidence Interval [CI] = 14.6–17.6) were seropositive for *T. gondii*. Furthermore, of a total of 20 water buffalo farms analysed, 19 resulted positive for *T. gondii* (95.0%; 95%CI = 73.1-99.7). Finally, a strong association was found between the seropositivity to *T. gondii* and the presence of cats and abortion in the water buffalo farms tested ($p < 0.05$).
The results of this study highlight the current epidemiological situation of *T. gondii* in the Salerno province, confirming the presence of a very high seroprevalence in buffalo farms. Further investigations and studies to prevent and control food-borne transmission of *T. gondii* in humans will be crucial.

MITOCHONDRIAL DATA ON *TRICHURIS* FROM *MACACA FASCICULARIS* SUPPORT EVIDENCE OF *TRICHURIS TRICHIURA* COMPLEX OF SPECIES

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Keywords: *Trichuris*, mtDNA, neglected diseases

INTRODUCTION: Whipworms are soil-transmitted parasitic intestinal nematodes infecting mammals, and *Trichuris trichiura* usually infects humans and other primates. Recent molecular studies report a more complex scenario suggesting the presence of a *T. trichiura* complex of species. Several taxa specifically infect only one primate species, while other taxa are able to infect a range of primate species (Callejón et al., 2013. Parasitol Res, 112: 3933; Cavallero et al., 2015. Infect Genet Evol, 34: 450; Hawash et al., 2016. Parasit Vectors, 9:37). The aim of the present study was to identify adult *Trichuris* spp. specimens recovered from a dead long tailed macaque (*Macaca fascicularis*) living in captivity at the Wild animals Recovery Center of Maremma (central Italy).

MATERIALS AND METHODS: Ten adult nematodes (3 males and 7 females) were collected from the intestinal caeca of *M. fascicularis* during necropsy, washed with saline solution and then used for morphological and molecular characterization, using sequencing of two mitochondrial genes (cox1, rrnL). (Cavallero et al., 2019. Vet Parasitol, 272: 23; Rivero et al., 2021. Front Vet Sci, 7: 626120). The obtained sequences were compared to GenBank retrieved data, and used for phylogenetic inferences with the Maximum Likelihood method (Kumar et al., 2016. Mol Biol Evol, 33:1870).

RESULTS AND CONCLUSIONS: Morphological features and measurements confirmed the similarity of the collected specimens to members of the *T. trichiura* complex. Nine good quality rrnL sequences and four cox1 sequences were obtained and used for phylogeny inferences, with final datasets of 43 input and 460bp and of 32 input and 341bp, respectively. Both phylogenetic trees placed *Trichuris* from *M. fascicularis* here analysed into the so-called "Clade 2" branch (according to cited literature), with high statistical support (99%-100%). This branch includes *T. trichiura* with a broad host range, shared by several primate species as the Japanese macaque, the Barbary macaque, the green monkey, the baboon, and humans. Such evidence confirms that the *T. trichiura* complex includes taxa with different degree of host affiliation. Moreover, captive primates have more opportunities to reach humans that wild primates have, highlighting a higher potential for zoonotic infections in confined environments.

PROVIDING BETTER UNDERSTANDING OF CLIMATE AND ENVIRONMENTAL DRIVERS OF SAND FLY BORNE DISEASES – THE CLIMOS PROJECT

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Keywords: big data, phlebotomine sand flies, *Leishmania*

INTRODUCTION: Over the last two decades, three successive research consortia (EDEN, EDENext and VectorNet) aimed at improving knowledge, surveillance, and control of vector-borne diseases in Europe and neighboring countries. Among these, sand fly-borne diseases including leishmaniasis and phlebovirosis represent an important public health and veterinary concern.

MATERIALS AND METHODS: We here present the main ideas of a novel effort to tackle sand fly borne diseases (SFBDs) – the CLIMOS project.

RESULTS AND CONCLUSIONS: CLIMOS - Climate Monitoring and Decision Support Framework for Sand Fly-borne Diseases Detection and Mitigation with Cost-benefit and Climate-policy Measures - aims to complement and build on previous efforts, bringing together researchers, health-care and veterinary practitioners, technology platform designers and at-risk communities, to conduct innovative and applied research seeking to better prepare for current and future impacts of climate and environmental changes on human and animal health, using sand flies and the diseases they transmit as a model system.
CLIMOS will:

- develop a general public health risk assessment method for SFBDs through integration of climate, environmental and One Health disciplines and data sciences;
- utilize big data from Earth-observing satellites and ground-level surveillance records, to map the locations of disease-carrying insects and provide health, climate and environmental services to keep communities safe, and
- integrate economic and social sciences, to enable socio-economic assessments of impacts of the incidence and spread of SFBDs on individuals and societies.

MOLECULAR INVESTIGATION ON THE PREVALENCE OF ZOONOTIC ENTERIC PROTISTS IN CAPTIVE WILD ANIMALS IN NORTH-EASTERN ITALY

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Keywords: protists, captive wild animals, zoonoses

INTRODUCTION: In recent years, several important intestinal zoonotic pathogens have been reported in captive wildlife all over the world, highlighting captive wild animals may be involved in the transmission of these pathogens (Zhang et al., 2021. BMC Vet Res, 17: 332). The aim of the present study is to evaluate the presence of the intestinal parasites *Cryptosporidium* spp., *Giardia intestinalis* and *Blastocystis* in animals from two zoological gardens of northern Italy.

MATERIALS AND METHODS: In 2021 an overall number of 363 faecal samples, collected seasonally from 101 animals including carnivores, herbivores, non-human primates (NHP) and reptiles living in two zoological gardens in Northern Italy, were analysed for enteric protists infection. Detection of *Cryptosporidium* spp. and *Giardia intestinalis* was performed targeting SSU rRNA through nested PCR (Caffara et al., 2013. Vet J, 19: 531-33) and qPCR (Verweij et al., 2003. Mol Cell Probes, 17: 223-25), respectively. *Giardia intestinalis* assemblage was studied by sequencing β giardine or TPI gene (Cacciò et al., 2002. Int J Parasitol, 32:1023-30; Zhao et al., 2015. Parasitology, 142: 800-6). Molecular detection of *Blastocystis* was performed using qPCR as described by Stensvold et al., 2012 (J Clin Microbiol, 50:1847-51) targeting SSU rRNA gene and the typing of the isolates was attempted according to the protocol proposed by Gabrielli et al., 2021 (Microorganisms, 9: 1656) on the same gene.

RESULTS AND CONCLUSIONS: All samples analysed were negative for *Cryptosporidium* spp., while 23.7% (86/363) were positive for *G. intestinalis* at qPCR, including species from all groups, with the highest prevalence in NHP (61.5%; 16/26), in which assemblage B was isolated. Assemblage E and F were also found in an oryx and a civet, respectively. *Blastocystis* spp. was detected in 40.5% (147/363) of the faecal samples, with highest prevalence in herbivores (86.4%; 22/26) and NHP (84.6%; 33/38). One and 16 animals resulted positive in all four seasonal samplings for *Giardia duodenalis* and *Blastocystis* spp. respectively. The presence of *Giardia* assemblage B confirms that NHP are a potential reservoir for zoonotic transmission. Typing of the *Blastocystis*' isolates is ongoing.

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SANDFLIES SURVEILLANCE CONFIRMED TOSCANA VIRUS AND *LEISHMANIA INFANTUM* CIRCULATION IN NORTHEASTERN ITALY

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Keywords: *Phlebotomus perniciosus*, phlebovirus, leishmaniosis

INTRODUCTION: In August 2021, the Veneto Region Reference Laboratory confirmed three human cases of Toscana virus (TOSV) infection (two with flu-like symptoms and one with meningitis). Epidemiological investigation revealed that two patients had not travelled outside the Veneto region during the previous four weeks, while the third case was imported from Tuscany. An entomological investigation was carried out to assess TOSV circulation in the phlebotomine vector.

MATERIALS AND METHODS: Two collections were performed in two sites (Este and Baone in Padua province), where it was suspected that patients had been infected. In total 2 CDC-CO2 light traps and 35 sticky traps worked for one night from sunset to sunrise. Collected sandflies were identified and pooled by site, date, species and gender. TOSV and *Leishmania* sp. detection was performed by molecular analysis following established experimental procedures.

RESULTS AND CONCLUSIONS: A total of 245 sandflies (104 females, 129 males and 12 not det.) were collected mostly by CDC-CO2 light traps (98%). Sandflies were identified as *Phlebotomus perniciosus* (227/245; 92.7%), *Sergentomyia minuta* (3/245; 1.2%) followed by *P. mascittii*, *P. neglectus* and *P. perfiliewi* (1/245; 0.4%). One pool of 17 females and one of 28 females of *P. perniciosus* were positive to TOSV and *L. infantum*, respectively; prevalence was 0.6% for TOSV and 1.4% for *L. infantum*. Despite the limited number of samplings, the entomological surveillance has been useful to detect TOSV circulation in the city of Este. Moreover, it revealed the presence of *L. infantum* in *P. perniciosus*. Although autochthonous cases of canine leishmaniosis have been observed in the surrounding area (Colli Euganei), the parasite has never been detected in the vector. Recently, several scientists have observed that phlebotomine sandflies are able to colonize areas characterized by environmental conditions considered unsuitable for their development, such as plain, urban or peri-urban areas. Our study confirms this trend, since we collected a higher number of sandflies specimens than previous collections. The increased vector density and the evidence of TOSV and *L. infantum* activity in the Veneto Region, along with animal and human movements from endemic areas, requires strengthening the surveillance of diseases transmitted by phlebotomine sandflies.

COMPARISON OF CITIZENS' PERCEPTION AND ENTOMOLOGICAL SURVEILLANCE DATA FOR MOSQUITO-BORNE DISEASES PREVENTION IN VENETO AND FRIULI VENEZIA GIULIA

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Keywords: Vector-borne disease, mosquitoes, questionnaire

INTRODUCTION: Mosquitoes are known to be a source of nuisance, but they are also vectors of pathogens for animals and humans. To investigate the knowledge and perception of citizen about mosquitoes, a survey was conducted in Veneto and Friuli Venezia Giulia (FVG) regions. Information obtained has been compared with data provided by entomological surveillance and human cases of arbovirosis to assess the correspondence between the people's opinion and scientific knowledge.

MATERIALS AND METHODS: Citizen opinions were retrieved by structured questionnaire between 11 and 22 September 2020 using a mixed-mode method, that combined two techniques: CAWI (Computer Assisted Web Interviewing) and CATI (Computer Assisted Telephone Interviewing). Data collected from the questionnaire were compared with entomological data, especially regarding: 1) perception of the presence of mosquitos in the area where respondents live; 2) knowledge of mosquitos as vectors of diseases; 3) knowledge about the presence of mosquito-borne diseases in the area where respondents live.

RESULTS AND CONCLUSIONS: Responders to the questionnaire were 1,001 (600 in Veneto and 401 in FVG). 85.3% of residents from Veneto and 78.8% from FVG claimed there are many mosquitoes in the area where they live. This answer fits with data from entomological surveillance, in particular in Veneto region where the number of mosquitoes captured is very high. 82.5% of residents in Veneto and 85.5% in FVG knew that mosquitoes can transmit diseases; in addition, 74.5% of respondents in Veneto and 76.8% in FVG were aware that mosquitoes of different species can transmit different diseases. Regarding the mosquito-borne diseases, respondents knew about West Nile disease circulation in recent years. West Nile disease is now endemic in these two regions, which is why it is the best known vector-borne disease. In fact, 31.7% of respondents in Veneto and 17.5% in FVG recognized the presence of this disease in the area where they live. In conclusion, the citizens of the Northeast of Italy have shown a good knowledge of mosquitoes and the diseases transmitted by them. The data collected provide useful insights for the design of communication strategies oriented to promote public awareness of mosquito-borne diseases.

This work was supported by the Public Health Department of Veneto and Friuli Venezia Giulia regions.

ANALYSIS BY ECOLOGICAL NICHE MODELLING OF THE CURRENT RISK OF DIROFILARIASIS TRANSMISSION IN SPAIN AND PORTUGAL, AND ITS FUTURE PROJECTION UNDER CLIMATE CHANGE SCENARIOS

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Keywords: *Dirofilaria* spp., ecological niche modelling, Spain and Portugal

INTRODUCTION: Dirofilariasis is a vector-borne zoonotic disease caused by several species of the genus *Dirofilaria* spp., being *D. immitis* and *D. repens* the most important ones. Canids and felids, both domestic and wild, are the main reservoirs. Their presence depends on environmental and bioclimatic factors that condition the presence of these vectors. In Spain there is a previous work where a simple model was developed using Geographic Information Systems, using three variables: temperature, rainfall and distribution of irrigated crops. Our objective is to analyse by means of Ecological Niche Modelling (ENM) the current risk of *Dirofilaria* transmission in Spain and Portugal, taking into account new factors and making a projection into the future.

MATERIALS AND METHODS: ArcGIS was used to process predictor variables (bioclimatic, distribution of surface and groundwater bodies, *Cx. pipiens* habitat, land use, biogeographical regions, vegetation layers, etc.) and presence of infected animals. The MaxEnt algorithm was used to develop NEMs for *Dirofilaria* spp. and *Cx. pipiens*.

RESULTS AND CONCLUSIONS: The highest risk of infection is in the southern and eastern provinces of the peninsular territory, with new areas appearing in southern Portugal and its coastal areas, as well as in the north of the peninsular. Furthermore, the risk of infection also increases in those inland areas with higher rainfall and higher soil humidity. The presence of irrigated land is also positively correlated with the presence of the disease. Finally, the 20- and 40-year projection according to climate change scenarios shows a clear potential increase in the risk of infection in Spain and Portugal. This methodological proposal is interesting from the One Health point of view, as it offers clear guidelines to carry out control measures to avoid the risk of infection in animals and humans.

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FIRST COMPREHENSIVE MAP OF CANINE ANGIOSTRONGYLOSIS IN DOMESTIC DOGS IN SPAIN

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Keywords: *Angiostrongylus vasorum*, Spain, domestic dogs

INTRODUCTION: Canine angiostrongylosis is a disease caused by the parasitic nematode *Angiostrongylus vasorum* that mainly affects canids, both domestic and wild, with foxes, jackals, wolves and domestic dogs being the ones for which most data are known. In Spain, studies and cases of infected domestic dogs have been reported only in the Iberian Peninsula. The aim of this study was to complete and update the map of the presence of *A. vasorum* in domestic dogs in Spain, considering all the regions.

MATERIALS AND METHODS: From January 2020 to March 2022, a total of 5544 blood samples from domestic dogs coming from all autonomous cities and communities of Spain were collected for the study. For this, 62 veterinary clinics voluntarily collaborated by providing the samples; these were randomly collected from the canine patients who attended their clinics, provided they met the inclusion criteria. Age at presentation to the clinics, breed, sex and habitat were recorded for each dog. Serum samples were tested for the presence of circulating antigens of *A. vasorum* using Angio DetectTM (IDEXX Laboratories Inc., USA) following the manufacturer's instructions. Descriptive analysis of the variables considered was carried out considering the proportions of the qualitative variables. Chi-square and Fisher's exact tests to compare the proportions were performed. In all cases, the significance level was established at $p < 0.005$.

RESULTS AND CONCLUSIONS: The overall prevalence on canine angiostrongylosis in Spain was 1.41%. The autonomous community with the highest prevalence was Murcia (4.12%) followed by the Basque Country (3.25%), Asturias (2.50%) and Cantabria (2.40%). The remaining autonomous communities did not exceed 2% being less than 1 % in with no positive dogs in Balearic Islands and in the autonomous cities of Ceuta and Melilla (0%). Taking into account the climatic characteristics of each Spanish region, the highest prevalence were obtained in the Basque Country (3.25%) with CfB climatology and Murcia (4.12%) with BsK and BSh. In Asturias (2.50%) and Cantabria (2.40%), the climatology was of Cfb. In the Balearic Islands and in the autonomous cities of Ceuta and Melilla where the climatology is of Csa, no infected animals were reported. In the rest of the autonomous communities, where prevalence were lower than 2%, the predominant climatologies were Csa, Csb and Cfb. The data shown here demonstrate the lack of control measures in domestic dogs, which is why it is necessary to carry out preventive campaigns by veterinary staff in collaboration with pet owners. At the same time, more studies are needed to address the study of these diseases in both wild and domestic animals and thus study their evolution.

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COMPARATIVE TRANSCRIPTOME OF *A. PEGREFFII* THIRD-STAGE LARVAE PROVIDES NEW INSIGHT INTO THE PARASITE-HUMAN DENDRITIC CELLS INTERACTION

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Keywords: *Anisakis pegreffii*, transcriptome, dendritic cells

INTRODUCTION: Anisakiasis is a zoonotic disease provoked by the accidental ingestion of the third larval stage (L3) of the species *A. pegreffi* and *A. simplex* (*s.s.*), infecting edible parts of fish or squids, which are consumed raw and/or undercooked (Mattiucci et al., 2018. Adv Parasitol, 99: 93-263). In humans, larvae induce a TH2 immune response, starting with the recognition of the pathogen by pattern recognition receptors expressed on the dendritic cells (DCs) that control the responses required to eliminate these pathogens while maintaining host homeostasis (Terrazas et al., 2010. J Biomed Biotechnol, 2010: 357106). In experimental conditions, Napoletano et al., 2008 (Parasite Immunol, 40: e12527) highlighted the impact of *A. pegreffii* on DC viability, DC maturation, phagosomal radical oxygen and phosphorylation of ERK1,2 pathway (Napoletano et al., 2018. Parasite Immunol, 40: e12527). The aim of the present study was to carry out a comparative transcriptomic analysis of L3 stage of *A. pegreffii* maintained in vitro with and without DCs, in order to reveal the differentially expressed genes in *A. pegreffii* larvae involved in the immunomodulatory dynamic.

MATERIALS AND METHODS: Alive L3 of *A. pegreffii* were cultured *in vitro* with DCs, at 37°C and 5% CO2. In parallel, alive L3 were seeded in the culture medium in absence of DCs. At 24h post-incubation L3 were collected and stored in RNA later. RNA was extracted using TRIzol reagent. cDNA library was prepared using the TruSeq Stranded mRNA kit (Illumina). Ligated products of 200 bp were excised from agarose gels and PCR amplified. Products were single-end sequenced on an Illumina TruSeq platform. The obtained row data was subjected to bioinformatic analyses by dedicated programs and validation tools.

RESULTS AND CONCLUSIONS: In total, 1203 differential expressed genes were identified: 573 and 628 genes as significantly up-or down-regulated, respectively. Functional analysis based on gene ontology (GO) classification system and the Kyoto encyclopaedia of genes and genomes (KEGG) database revealed a repository of *A. pegreffii* genes encoding a variety of proteases with the potential both to promote TH2 polarization through DC modulation, as well as a more permissive environment for their survival. Understanding the candidate genes in DCs–L3 interaction will not only increase our knowledge on the interaction between the zoonotic parasite with the accidental human host, but it will also help to provide targets in further intervention in both diagnosis of the infection and therapy treatments.

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INTESTINAL PARASITES INFECTING SQUIRREL MONKEYS (*SAIMIRI CASSIQUIARENSIS*) IN A HUMAN - NON HUMAN PRIMATE INTERFACE IN COLOMBIA

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Keywords: intestinal parasites, non-human primates, zoonotic transmission

INTRODUCTION: Zoonotic diseases are considered a major threat to public health and wildlife conservation. The risk of zoonotic transmissions is high between human and non-human primates (NHP) living in spatial proximity, especially as a consequence of forest loss and fragmentation (Sirima et al., 2021. Parasit Vectors, 14: 354). Under this scenario, the screening of parasites in NHP is important to inform public health policies from the One-Health perspective. This study aimed to identify and molecularly characterize intestinal parasites of free-ranging squirrel monkeys living in three forest fragments in Colombia where NHP are in close proximity to humans, and often subjected to food provisioning.

MATERIALS AND METHODS: Ninety-seven faecal samples from squirrel monkeys (*Saimiri cassiquiarensis*) were collected immediately after defecation and stored in 96% ethanol and 10% formalin solution. Faecal smears and flotation were performed (Botero & Restrepo, 2012. Parasitosis humanas, Corporación para Investigaciones Biológicas, Medellín), and samples microscopically classified as positive for Ascarididae were processed for molecular characterization (Cavallero et al., 2013. PLoS Negl Trop, 7(4): e2170).

RESULTS AND CONCLUSIONS: 98% of the samples were positive for intestinal parasites. Protozoans (*Blastocystis* sp., *Dientamoeba* sp., Entamoebidae, *Giardia* sp.), Nematodes (Ascarididae, *Strongyloides* sp., *Trypanoxyuris* sp.), Cestodes (*Hymenolepis* sp.), Trematodes (*Controrchis* sp.), and Acanthocephalans were identified based on morphology, while *Ascaris lumbricoides* was confirmed by molecular techniques.
The finding of intestinal parasites with zoonotic potential suggests epidemiological implications. We recommend conducting regular parasite surveys in NHP in order to monitor the potential zoonotic transmission risk. Additionally, educational activities with the exposed local communities should be encouraged in order to increase the awareness regarding the potential risk of zoonotic transmissions, and the importance of avoiding food provisioning and physical contact with NHP.

MOLECULAR CHARACTERIZATION OF *ECHINOCOCCUS GRANULOSUS SENSU LATO* IN VENETO REGION (NORTHEASTERN ITALY)

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Keywords: *Echinococcus granulosus*, genotypes, domestic animals

INTRODUCTION: Cystic echinococcosis (CE) is a zoonotic disease caused by tapeworms of the species complex *Echinococcus granulosus sensu lato* (*E. granulosus*) affecting animals and humans worldwide. To date, the Italian prevalence of CE is heterogeneous with higher prevalence in Sardinia and southern areas. The aim of this study was to fill the knowledge gap of *E. granulosus* distribution and genotype diversity in Veneto region.

MATERIALS AND METHODS: A total of 38 hydatid cyst specimens (liver and/or lung) were collected from sheep (no.= 24), cattle (no.= 6), pigs (no.= 3) and wild boar (no. = 1) during routine meat inspection in different municipalities of Veneto from 2019 to 2022. DNA was extracted from either germinal layer or protoscoleces and amplified with two set of primers targeting part of mithocondrial cox1 (38 cysts) and nad5 (22 cysts) gene regions (Nakao et al., 2000. Mol Biochem Parasitol, 111: 415-24; Kinkar et al., 2018. Infect Genet Evol, 64:178-84). Sequences obtained from PCR products were used for taxonomic confirmation and phylogenetic analysis.

RESULTS AND CONCLUSIONS: All cysts were identified as *E. granulosus*. Genotype G1-G3 was confirmed in the majority of cysts (92.1%; 35/38) from sheep, cattle and wild boars, whereas the remaining 3 cysts from pigs (7.9%; 3/38) were identified as genotype G6/G7. The nad5 sequences identified genotype G1 in 11 (50.0 %; 11/22) cysts and genotype G3 in 11 (50.0 %; 11/22), both in liver and lungs of either cattle, sheep or wild boar. Of note, a sheep was G1 and G3 genotype coinfected with G1 detected in liver and G3 in lung. The novelty of this study is:

- the presence of genotype G6/G7 in Veneto region (northeastern Italy) in a domestic sow. Although this genotype is already present in southern Italy (mainland and Sardinia) in wild boars, suggesting they are likely serving as reservoir of the G7 genotype, the only report in domestic pigs is from Emilia Romagna (northern Italy) in 2021;
- the coinfection of G1 and G3 in the same animal (sheep) strengthens the indication of a coexistence of the two genotypes in the same area. It is not possible to discriminate if the animal got re-infected with a different genotype or if G1 and G3 were associated to the same infection;
- the results of the study do not represent the prevalence of CE infection in that area, as the data are biased by the convenient sample. Indeed, we characterized cysts submitted to the laboratory for diagnostic purpose. Anyway, our observations support the local and global trend of G1-G3 genotypes being the most frequently identified genotypes from intermediate hosts.

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TOXOPLASMOSIS IN ITALY: WHERE ARE WE? A PICTURE FROM AN ONLINE QUESTIONNAIRE

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Keywords: Toxoplasmosis, online survey, Italy

INTRODUCTION: Toxoplasmosis is a parasitic disease that fits perfectly within the One Health perspective and the data presented here comes from an online questionnaire we developed aimed at obtaining a "picture" of the distribution of the parasite throughout Italy, focusing on the number of seroconversions recorded in pregnant women, together with questions aimed at understanding if there is uniformity in diagnostic methods and pharmacological protocols applied both in women and in newborns.
Currently, in Italy cases of congenital toxoplasmosis must be signaled to the health minister but the diffusion of these data is lacking and very fragmented.

MATERIALS AND METHODS: Twenty-six questions were posed to clinicians (gynecologists, clinical microbiologist, neonatologists) from 2020 and 2021. The referred period for the data collection was January 2018-December 2020.

RESULTS AND CONCLUSIONS: The data presented here are preliminary and derive from 42 responders, 19 coming from clinicians from North of Italy, 11 from Central and 12 from the South. The majority of practitioners work in a public hospital (29/42). Preliminary diagnosis in pregnant women is based on serology (IgG-IgM), followed by more specific exams as avidity, PCR, amniocentesis. However, diagnosis of toxoplasmosis in confirmed cases between January was made with matched serology IgG+IgM, IgG avidity and PCR on amniotic fluid.
No cases of seroconversion in pregnancy were recorded by seven clinicians. Seventeen reported less than 5 cases, nine 6-10 cases and nine more than 10 with peaks of more than 40 cases. The majority of seroconversions occurred in the first quarter of gestation (16/42), eight in the second and two in the third. The consumption of raw or undercooked meat, of contaminated fruits and vegetable, of short-aged cured meat and the contact with faeces of young cats were certainly the main sources identified, while 15 clinicians answered that it was not possible to identify it/them.
In the case of seroconversion before the 18th week of pregnancy, the majority of clinicians apply the protocol with Spiramycin (1g/every 8 hours). If seroconversion occurs after the 18th week, 13 apply treatment with Spiramycin and 15 prefer the protocol with Pyrimethamine/Sulfadiazine/Folinic Acid. In 15 cases clinicians answered that the protocol used in case of maternal toxoplasmosis did not, or not always, prevent the occurrence of congenital toxoplasmosis and this could be due to incorrect administration of therapy or to late administration.
Looking at newborns with congenital toxoplasmosis the results of the survey indicated that in the majority of cases physicians used Pyrimethamine + Sulfadiazine + Folinic Acid but with different time/concentration protocols depending if the toxoplasmosis is clinically manifested or subclinical.
The preliminary data collected with the present survey indicates that toxoplasmosis is diffused along all the Italian territory and the establishment of a functional, easy-to-use national database would be desirable.

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EXPRESSION ANALYSIS OF *PLASMODIUM FALCIPARUM* OOKINETE-SPECIFIC GENES IN *ANOPHELES COLUZZII* MOSQUITOES

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Keywords: *An. coluzzii*, *P. falciparum* ookinete, Malaria transmission markers

INTRODUCTION: The possible role of the human and mosquito genetic backgrounds in the efficiency of *Plasmodium falciparum* transmission from the human host to the *Anopheles* vector has been so far investigated through demanding in vivo and ex vivo experiments with significant logistical, technical and ethical obstacles. More efficient experimental approaches are needed to probe this important aspect of malaria epidemiology.
The aim of the present study is to develop molecular methods to detect and quantify the ookinete stage of the malaria parasite. Nucleic acid extraction (DNA and RNA) from mosquitoes collected in the first 24 hours after infectious blood meal (i.e., during the ookinete maturation) might allow the simultaneous molecular characterization of the human and mosquito hosts and of the possible parasite infection originating from the last blood meal before capture. This tool would allow to study, using biological samples obtainable from single mosquitoes, the possible influence of the human and mosquito genetic background on the transmission of the parasite from the human host to the mosquito vector.
The expression profiles of ookinete markers were therefore investigated in *Plasmodium falciparum*-infected mosquitoes collected at different time-points (TP) after an infectious blood-meal: the Circumspozoite and TRAP-Related Protein (CTRP), Secreted Ookinete Adhesive Protein (SOAP), von Willebrand factor A domain-related Protein (WARP) and Chitinase 1 (CHT1) transcripts.

MATERIALS AND METHODS: *An. coluzzii* mosquitoes were experimentally infected with *P. falciparum* NF54 strain and collected at different TPs post-infection (TP12, TP18, TP24 and TP36 hpi, hours post-infection) spanning the formation and maturation of the ookinete stage inside mosquito midgut. Total RNA and genomic DNA were obtained from ~ 50 females/TP through both phenol-chloroform and spin-column chromatography extraction methods. A SYBR green qPCR assay was developed to analyze the transcriptional profile of selected ookinete genes (ctrp, soap, warp and cht1) across the four TPs, applying the relative quantification procedure. Furthermore, external calibration standard curves were designed to perform absolute quantifications of the markers inside single infected mosquitoes.

RESULTS AND CONCLUSIONS: Our study provides a first transcriptional analysis and molecular quantification of *P. falciparum* ookinete marker genes inside single *An. coluzzii* mosquitoes. RTqPCR results confirmed that ctrp, soap, warp and cht1 show a progressive increase in their expression during ookinete maturation, with a peak in transcript abundance at 24 hpi during midgut invasion. Moreover, absolute quantification assays showed that the four markers were transcribed with comparable intensities, ranging between 100-10000 mRNA copies/mosquito.
This pilot study suggests the possibility to investigate the role on malaria transmission of human and mosquito genetic variability through large-scale field studies based on the collection of freshly-fed mosquitoes.

INTEGRATED EVALUATION OF VECTOR BITING RHYTHMS AND HUMAN HABITS IN BURKINA FASO REVEALS HIGH RESIDUAL MALARIA TRANSMISSION DESPITE THE EXTENSIVE COVERAGE OF INSECTICIDE TREATED NET (LLIN)

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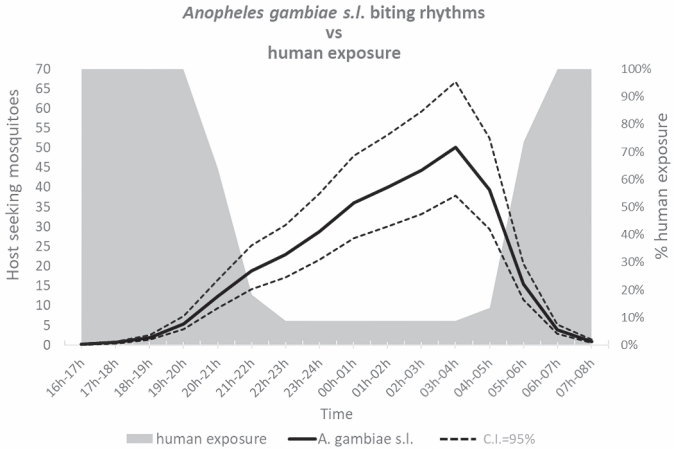
Keywords: *Anopheles*, malaria transmission, vector biting rhythms

INTRODUCTION: Burkina Faso is among the 10 high sub-Saharan countries where a stalling in the fight against malaria has been registered (World Malaria Report, 2020). In this area the incidence remains still very high despite LLIN distribution campaigns are occurring since 2010 as main malaria vector control strategy recommended by WHO. Our overarching goal is to understand how vector behaviours in response to LLINs (i.e. increased zoophagy, outdoor biting and/or altered biting rhythms) and their interactions with human activities and net usage can contribute to this epidemiological scenario. In previous studies, conducted within 5 years after LLIN introduction in Goden village in Burkina Faso, we defined a scenario of marked zoophily in the dominant vector species (*Anopheles coluzzii* and *Anopheles arabiensis*) despite sporozoite (SR=6.1%) and entomological inoculation (EIR=1.4 infective bites/person/hour) rates in the range observed in pre-intervention settings. (Pombi et al., 2018. Sci Rep, 8:12806; Perugini et al., 2020. Parasit Vectors, 13:277). We here present an analysis of malaria transmission risk in Goden on the basis of Human Landing Catches (HLC) conducted during the whole mosquito biting period, alongside a survey on human habits and net usage.

MATERIALS AND METHODS: Human host-seeking mosquitoes were collected by HLC inside and outside three houses, from 16:00 to 08:00, for a total of 8 nights in September 2020. A questionnaire about timing of human activities and net usage was submitted to the head of households from half of village compounds (i.e. 80 compounds). A subsample of collected mosquitoes was tested for *Plasmodium* sp. infectivity (Bass et al., 2008. Mal J, 7:177) while mosquito biting rhythms were analysed by Generalized Additive Models.

RESULTS AND CONCLUSIONS: A subsample of 1070 specimens - out of the 15201 *A. gambiae s.l.* females collected- showed 2.7% of SR. Vector population exhibits an equal human biting rate both indoors and outdoors, with a roughly homogeneous plateau of intense biting activity of 37.4 mosquitoes/person/hour from 22:00 to 5:00 and a non-negligible biting pressure (mean of 6.6 mosquitoes/person/hour) before and after this large time window (Figure). Under a hardly realistic scenario of full human exposure, the EIR during the 16 hours of HLC is 7.2 infective bites/person (ibp). However, adjusting human exposure according to LLIN usage as estimated based on questionnaires, the EIR drops to 1.5 ibp. Interestingly, 0.51 infective ibp occurred before 20:00 and after 6:00 when 100% of inhabitants are awake and thus fully exposed to bites (corresponding to 25 ibp/month). This result brings out a non-negligible gap in LLIN protection sustaining the hard core of residual malaria transmission in the village. We expect that the interplay between human and vector behaviours may represent one of the main factor accounting for the persistent high malaria transmission in other epidemiologically similar settings characterized by high vector density and insecticide resistance.



LABORATORY BREEDING OF *PHORTICA* SPP. (DIPTERA: DROSOPHILIDAE), VECTORS OF THE ZOONOTIC EYEWORM *THELAZIA CALLIPAEDA*

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Keywords: *Phortica* spp., laboratory rearing, vector-borne disease

INTRODUCTION: The establishment of arthropod colonies is an important tool to manipulate disease vectors, investigate their life history traits, and their vector competence, prevention of vector borne pathogen transmission, or to assess insecticidal/repellent activities. Some species of drosophilid flies belonging to the genus *Phortica* (*Phortica variegata* and *Phortica okadai*) feed on ocular secretions of mammals, acting as biological vectors of the zoonotic eyeworm *Thelazia callipaeda* (Otranto et al., 2005. Parasitology, 131: 847). Recently, a third species, *Phortica oldenbergi*, has been experimentally demonstrated as intermediate host of *T. callipaeda*. This study describes an effective breeding protocol of *Phortica variegata* and *Phortica oldenbergi* in insectary conditions.

MATERIALS AND METHODS: Gravid flies of *P. oldenbergi*, *P. variegata* and *Phortica semivirgo* were field collected in wooded areas of Lazio region (Italy) (Pombi et al., 2020. Parasit Vectors, 13: 1-9) and allowed to oviposit singularly to obtain isofamilies. Flies were maintained in ovipots (200ml) with a plaster-covered bottom to maintain high humidity level inside. Adult feeding was guaranteed by freshly apple and a liquid dietary supplement (80% distilled water, 20% snail extract–based syrup and 0.009% sodium chloride), while larval development was obtained by two *Drosophila*-like agar feeding media: a Standard one (86% water, 5.6% yeast, 3.9% sucrose, 0.5% agar, 3% cornmeal flour and propionic acid) and another Chestnut flour-based (84.8% water, 6.69% yeast, 4.46% sucrose, 0.66% agar, 2.67% chestnut flour, 0.66% banana, >0.001% propionic acid). All conditions were kept in a climatic chamber with a photoperiod of 14:10h light: dark, 26±2°C and 80±10% RH.

RESULTS AND CONCLUSIONS: From 130 field collected *Phortica* spp. three generations (i.e., 783 F1, 109 F2, 6 F3) were obtained. *Phortica oldenbergi* was the species with higher breeding performance, being the only species reaching F3, followed by *P. variegata*. Chestnut-based feeding medium allowed higher adult production and survival probability in both *P. oldenbergi* and *P. variegata*. Adult production/female was promising in both species (*P. oldenbergi*: 13.5 F1/f; *P. variegata*: 4.5 F1/f; Table), indicating the possibility to obtain stable colonies. This standardized breeding protocol, based on controlled climatic parameters and fly densities, together with the introduction of an enriched feeding medium, allowed to investigate aspects of life history traits of *Phortica* spp. involved in the transmission of *T. callipaeda*. Obtaining F3 generation of these species for the first time paved the road for the establishment of stable colonies, an essential requirement for future studies on these vectors in controlled conditions.

Species	Medium	Parental generation		F1 generation		F2 generation	
		Wild Females	Adults/WF	F1 females	Adults/F1	F2 females	Adults/F2
Phortica oldenbergi	Chestnut	4	13.5 (min: 1, max: 79, SD: 13.2)	61	0.1 (min: 3, max: 13, SD: 2.4)	13	0
	Standard	67	2.9 (min: 1, max: 31, SD: 4.1)	189	0.3 (min: 1, max: 25, SD: 2.1)	43	0.1 (min: 1, max: 2, SD: 0.7)
Phortica semivirgo	Standard	3	7.0 (min: 4, max: 23, SD: 4.6)	21	0	0	0
Phortica variegata	Chestnut	6	4.5 (min: 1, max: 17, SD: 4.3)	19	0	0	0
	Standard	50	1.8 (min: 1, max: 29, SD: 2.6)	85	0.01 (min-max: 1, SD: 0.05)	1	0

PHORTICA OLDENBERGI (DIPTERA: DROSOPHILIDAE): A NEW VECTOR OF *THELAZIA CALLIPAEDA* EYEWORM IN EUROPE

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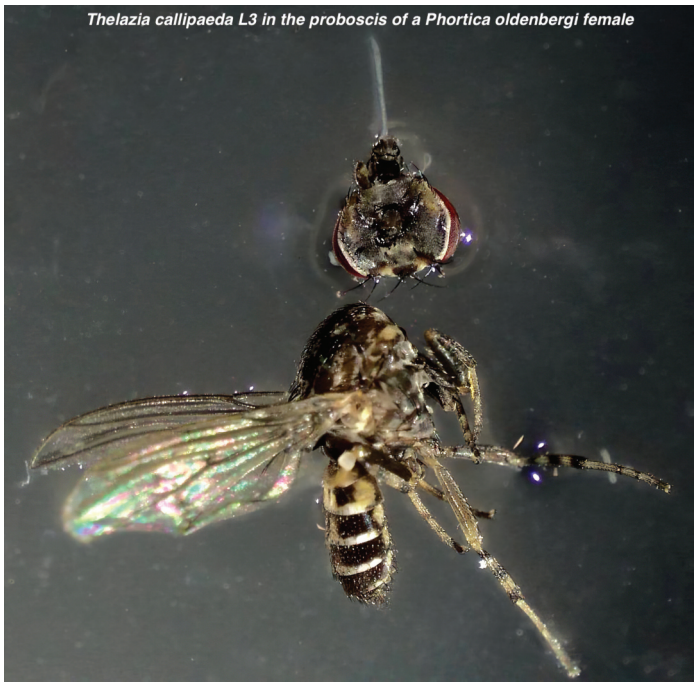
Keywords: *Phortica oldenbergi*, *Thelazia callipaeda*, vector

INTRODUCTION: *Thelazia callipaeda* is a zoonotic nematode parasitizing the eyes of a wide range of vertebrate hosts, primarily dogs. This parasite, also known as oriental eyeworm, is transmitted by *Phortica variegata* and *Phortica okadai* in Europe and Asia, respectively (Otranto et al., 2020. Trends Parasitol, 37:263-64). To date, around 130 *Phortica* spp. are described worldwide, of which many present the lachryphagy as feeding behavior. This peculiar feeding habit makes these flies vectors of eyeworms (Máca and Otranto, 2014. Parasit Vectors, 7:516). Therefore, in this study we investigated the role played by a third species, *Phortica oldenbergi*, as vector of *T. callipaeda* in Europe.

MATERIALS AND METHODS: A total of 140 *P. oldenbergi* fruit flies were experimentally infected with *T. callipaeda* L1 recovered from gravid females collected from the eyes of naturally infected dogs. Conventional PCR was performed on the dissected flies using primers that amplify a portion of the partial mitochondrial cytochrome c oxidase subunit 1 gene (cox1).

RESULTS AND CONCLUSIONS: Seventy-four (i.e., 60 females and 14 males) *P. oldenbergi* specimens died at 5 days post infection (d.p.i.) (±1) and scored negative for *T. callipaeda* larvae at the dissection. The 66 *P. oldenbergi* that survived (i.e., 20 males and 46 females) were dissected at 21 d.p.i. From those, *T. callipaeda* L3 were detected in the proboscis of two females (3.0%; Figure 1). Overall, at the molecular analysis, 11.4% (no. = 16/140; 3 males and 13 females) drosophilids scored positive for the presence of *T. callipaeda* DNA, all belonging to haplotype 1. Data herein reported bring further insights in the biology of *T. callipaeda* by adding *P. oldenbergi* as a new potential intermediate host/vector under experimental conditions. However, the role of this drosophilid species in the transmission cycle of *T. callipaeda* should be further confirmed under natural conditions. This new finding is of interest to science also considering the possible spreading of *P. oldenbergi* southwards Europe. Studies on the biology (e.g., feeding behavior, host preferences) and current distribution of this fly species are advocated to assess its impact on the epidemiology of thelaziosis caused by *T. callipaeda* in Europe.



Thelazia callipaeda L3 in the proboscis of a Phortica oldenbergi female

HEPATOZOON SPECIES INFECTING DOMESTIC CATS FROM REGIONS OF THE MEDITERRANEAN BASIN

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Keywords: cats, *Hepatozoon* spp., epidemiology

INTRODUCTION: Domestic cats with outdoor lifestyle may be exposed to arthropod vectors such as ticks, sand flies, fleas and mosquitos as well as to pathogens they transmit. The epidemiology of tick-borne diseases in cats is less investigated than in dogs (Otranto & Dantas-Torres, 2010. Parasit Vectors, 3:2). Moreover, the vectors of feline *Hepatozoon* species are currently unknown. The aim of this study was to assess the prevalence of apicomplexan parasite infections and associated risk factors in cats from different countries of the Mediterranean basin.

MATERIALS AND METHODS: From September 2019 to August 2021, blood samples were collected from 600 cats living in France, Greece, Israel, Italy, Portugal and Spain, including 100 for each region. Data on animals (i.e., age, sex, breed, housing conditions and provenience), clinical signs and laboratory parameters were recorded. Cats were grouped according to age (Quimby et al., 2021. J Feline Med Surg, 23:211-33) in kittens (up to 1-year), young (between 1- 6 years), mature (between 7-10 years) and senior (older than 10 years). Blood samples were tested for *Hepatozoon* spp. and piroplasmids by conventional PCR targeting partial 18S rRNA gene. The association between infected cats and risk factors (signalment records), clinical signs and laboratory abnormalities was evaluated by chi-square test ($p < 0.05$ was considered significant).

RESULTS AND CONCLUSIONS: The cat population was composed of 52.2% females and 47.8% males, of which 20.4% kittens, 54.8% young adults, 11% mature adults and 13.8% seniors. Cats were sheltered in outside environment / free roaming (55.8%) or owned (44.2%), being the majority common European breed (89%). The overall prevalence of *Hepatozoon* spp. infection was 14.5% (no. = 87), being significantly higher in cats from Greece (30%) and Portugal (23%), than Spain (15%), Israel (15%) and France (4%). Cats from Italy scored all negative. Out of 87 positive cats, 86 (98.8%) were infected by *Hepatozoon felis* and 1 (1.2%) by *Hepatozoon silvestris* from Portugal. No piroplasmid's DNA was amplified. The risk of *Hepatozoon* spp. infection was significantly associated with the geographical area (i.e., Greece and Portugal; $p=0.01$) and housing conditions (sheltered/ free roaming vs owned; $p=0.03$). Among infected cats, 27.6% (24/87) presented at least one clinical sign with vomiting (29.2%, $p=0.2$) and malnutrition (25%, $p=0.6$) as the most common. The laboratory parameters were available in 53 animals, being increased ALT values (47.2%, $p<0.05$), leucocytosis (47.2%, $p<0.05$) and proteinemia (45.3%, $p= 0.7$) the most frequent. Data suggest that *H. felis* largely circulates within feline populations in the Mediterranean basin, mainly in sheltered or free roaming cats, with subclinical manifestations or only occasionally non-specific clinical signs. The finding of *H. silvestris* indicates the circulation of this recently described species in domestic cats of Europe (Giannelli et al., 2017. Ticks Tick Borne Dis, 8:721-724; Hodžić et al., 2017. Parasitology, 144:650-61).

VECTOR-BORNE PATHOGENS IN HUNTING DOGS OF SOUTHERN ITALY

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Keywords: hunting dog, Italy, vector-borne pathogen

INTRODUCTION: Vector-borne diseases are caused by a wide range of infectious and parasitic agents transmitted by blood-feeding arthropods, such as ticks, fleas, lice, mosquitoes and phlebotomine sand flies (Otranto et al., 2009. Parasit Vectors, 26: S1-S2). Due to their lifestyle, hunting dogs may be exposed to a plethora of vector-borne pathogens (VBPs), acting as potential reservoirs of infection for humans in rural areas (Sgroi et al., 2021. Tranbound Emerg Dis, 00: 1-8). Thus, this study aimed to assess the occurrence of VBPs in a hunting dog population of southern Italy and to evaluate the relative performance of different diagnostic tools.

MATERIALS AND METHODS: Between 2014 and 2017, blood and serum samples from hunting dogs (no. = 1,433) were collected in rural areas of Campania region, Southern Italy. All samples were tested by Knott's technique for filarioids, serologically (SNAP® 4Dx® Plus) for *Anaplasma* spp., *Borrelia burgdorferi sensu lato*, *Dirofilaria immitis* and *Ehrlichia* spp. and by qPCR for all except *B. burgdorferi s.l.* of the above pathogens plus *Babesia* spp. and *Leishmania infantum*. Logistic regression was run to evaluate the statistical associations between the risk of VBP infection and independent variables and K-Cohen formula for assessing the concordance among diagnostic methods.

RESULTS AND CONCLUSIONS: Out of 1,433 dogs, 321 (i.e., 22.4%, 95% CI: 20.3 - 24.6) tested positive for VBPs by using at least one diagnostic tool. Of the animals sampled, 28 (1.9%) were positive for filarial species, being *Acanthocheilonema reconditum* the most prevalent species (1.6%), followed by *D. immitis* (0.2%) and *Dirofilaria repens* (0.1%), with one co-infested dog (0.07%, *A. reconditum* - *D. repens*). Overall, 140 (9.8%) and 231 (16.1%) dogs scored positive for at least one VBP by serological and molecular methods, respectively. The most prevalent pathogens detected were *Ehrlichia* spp. (7.3%) with SNAP® 4Dx® Plus, and *A. reconditum* (7.7%) by qPCR. Most of the co-infections were by *Anaplasma* spp. - *Ehrlichia* spp. (1.9%) serologically and by *A. reconditum* - *Ehrlichia canis* (0.4%) by PCR. Statistical analyses reported a significant association ($p < 0.001$) between *A. reconditum* infection and both, *Ehrlichia* spp. seropositivity and geographical origin of dogs. A high agreement among diagnostic methods was found, with 99.9%, 94.0% and 95.7% agreement observed between Knott vs SNAP® 4Dx® Plus, Knott vs qPCR, and SNAP® 4Dx® Plus vs *D. immitis* qPCR, respectively. The data demonstrates a high prevalence of VBPs in hunting dogs, indicating that this group of animals is largely exposed to several arthropod vector species and suggesting the transmission risk of zoonotic agents to humans in rural areas of Southern Italy. A multi-diagnostic approach and a deeper cooperation among healthcare and other stakeholders are required to prevent VBP infections to animals and humans.

IMPLEMENTING THE EVALUATION OF WILDLIFE IMPACT ON TICK-BORNE ZOONOSES WITH NON-INVASIVE REMOTE CONTROL

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Keywords: Tick-borne diseases, emerging infectious diseases, random encounter method

INTRODUCTION: With their increasing relevance for human and domestic animal health, as well as the economy, the necessity of a comprehensive approach to study and control Tick-Borne Zoonoses (TBZ) has become clear (Madison-Antenucci et al., 2020. Clin Microbiol Rev, 33: e00083-18). Wildlife acts both as a reservoir for such diseases and as a vehicle to spread the vector, thus having a crucial role in their transmission (Gortázar et al., 2007. Eur J Wildl Res, 53:241–56). The possibility of applying innovative methods for wildlife monitoring to the study of TBZ epidemiology is an appealing way to gather better insights into these pathogens' epidemiology.

MATERIALS AND METHODS: In La Mandria Natural Park (Piedmont, IT) we sampled 14 stations with camera trapping, tick dragging and environmental sensors. To consider seasonality, the study was performed with 8 bi-weekly repetitions, 4 in summer and 4 in spring. We detected Piroplasms (*Babesia* spp./*Theileria* spp.) and Anaplasmataceae by PCR in both collected pooled ticks and spleen samples of culled wild ungulates. Eventually, we linked the prevalence of TBZ in questing ticks to wild ungulates quantitative temporal occurrence, calculated from camera trap data. When analysing relationships, we also considered environmental factors, ticks' abundance and species and wild ungulates abundance. The overall prevalence of TBZ in spleen samples was related to TBZ prevalence in questing ticks and to wild ungulates densities calculated through the REM/REST (Nakashima et al., 2018. J Appl Ecol, 55: 735–44).

RESULTS AND CONCLUSIONS: 681 questing ticks have been collected, most of the species *Ixodes ricinus*, presenting a high prevalence for Piroplasms (ranging from 6.7% to 75.3%) and no *Anaplasma phagocytophilum*. A similar pattern was found in red deer's and wild boar's spleen, with a prevalence of 75.2% for Piroplasms and only 2 positivity to Anaplasmataceae. Wild boar, red deer, roe deer and fallow deer quantitative temporal occurrence was calculated thanks to the REM analysis. The highest densities were obtained for wild boar (15.3 individuals/km^2) and red deer (11.3 individuals/km^2), while roe deer presented the lowest density (1.96 individuals/km^2). From statistical analysis, we observed an additive effect of the time spent by wild ungulates in front of a specific site and questing ticks (and thus the prevalence of TBZ in vectors) in summer and a detractive effect in early spring.
The impact of wild ungulates temporal occurrence in a precise site over TBZ is a significant risk factor that should be considered when planning the prevention and control of such diseases and defining actions for wildlife management. Camera trapping has shown to be not only an effective way to monitor wildlife population, but also a useful tool to improve the epidemiological study of TBZ in their wildlife interface, gathering detailed information otherwise impossible to obtain.

GENETIC AND MORPHOLOGICAL CHARACTERIZATION OF A SIBLING SPECIES OF THE *CONTRACAECUM MULTIPAPILLATUM* (*S.L.*) COMPLEX, PARASITE OF PELICANS AND FISH FROM PERÙ AND COLOMBIA

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Keywords: *Contracaecum multipapillatum* (*s.l.*), sibling species, *Pelecanus* spp.

INTRODUCTION: The genus *Contracaecum* (Railliet & Henry, 1912) includes, among the avian species, nematode parasites of piscivorous birds associated with freshwater, brackish, and marine ecosystems throughout the world. Among them, *C. multipapillatum* (von Drasche, 1882), was first described as *Ascaris multipapillata* (von Drasche, 1882) from the American wood ibis *Tantalus loculator*. In the western hemisphere it was reported by Courtney & Forrester, 1974 (J Helm Soc Wash, 41:89-93), Deardorff & Overstreet, 1980 (Proc Biol Sci Wash, 93:1035-79), Navone et al., 2000 (J Parasit, 86:807-810), Dronen et al., 2003 (Comp Parasit, 70:140-54) and Dyer et al., 2022 (Avian Path, 31:441-448). Molecular systematics approach has so far allowed to characterise *Contracaecum multipapillatum* (*s.l.*) worldwide: *C. multipapillatum* (*s.l.*) (D'Amelio et al., 2007. Parasitology, 134: 1041-51), *C. overstreeti* and *C. gibsoni* (Mattiucci et al., 2010. Syst Parasit, 75: 207-24), *C. pyripapillatum* and *C. multipapillatum* (Shamsi et al., 2008. Parasit Res, 103: 1031-39); *C. multipapillatum* (Davidovich et al., 2022. FWP, 26: e00147). Aim of this study was to genetically characterise adults and L3 stage larvae of *Contracaecum* spp. collected respectively from pelicans and fish of Perù and Colombia.

MATERIALS AND METHODS: A total of 10 and 12 adult specimens of *Contracaecum* spp. were collected, respectively in *Pelecanus thagus* of Peru' and *P. occidentalis* from Colombia. In addition, 40 and 20 L3 stage larval specimens were collected, respectively, from the fish species *Mugilis cephalus* of Peru and *Hoplias malabaricus* of Colombia. Cephalic and caudal ends of the worms were used for morphology, while, the central ones for genetic analysis of the mitochondrial mtDNA cox2, (Mattiucci et al., 2008. Syst Parasit, 69:101-21), ITS region of rDNA (Zhu et al., 1998. Int J Parasitol, 28:1911–21) and ribosomal RNA (rrnS) (D'Amelio et al., 2007. Parasitology, 134: 1041-51) genes. Phylogenetic analysis of the obtained sequences at those gene loci was performed by Bayesian inference (BI) and Maximum Likelihood (ML) in comparison with the sequences of *Contracaecum* spp. from aquatic birds so far deposited in GenBank.

RESULTS AND CONCLUSIONS: Adult males were morphologically referred to the species *C. multipapillatum* (*s.l.*) according to the main features of the morphospecies; the larvae were morphologically referred to *Contracaecum* Type 2 (Moravec, 1994. Acad Publishers, London: 473pp). The phylogenetic tree topology of BI and ML analysis depicted all the sequences at those gene loci from the adults worms from pelecans as well as the larvae from fish, are clustering in the same highly supported clade. This represents a clearly distinct phylogenetic lineage with respect to all the previously reported avian parasites of the genus *Contracaecum*. The taxon is included in the major subclade formed by previously detected species of the *C. multipapillatum* (*s.l.*) complex, parasites of Pelicanidae. Morphological characters of adults for the nomenclature designation of the taxon were also studied.

MIND THE GAP! CLINICAL RECOGNITION AND DEVELOPMENT OF DIAGNOSTIC TOOLS FOR THE ZOONOTIC *DERMANYSSUS GALLINAE* INFESTATION IN HUMANS

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Keywords: skin prick test, IgE, guidelines

INTRODUCTION: *Dermanyssus gallinae* is a haematophagous ectoparasite primarily known as a pest of birds, mainly poultry. It occasionally feeds on a range of mammals, including humans. Although *D. gallinae* is becoming of growing concern in human medicine as the risk of the infestation is increasing in the urban environment, the number of cases is underestimated, since most of them go underreported or are misdiagnosed, due to the unawareness of physicians and the lack of tools for making a correct diagnosis (Cafiero et al., 2019. Avian Pathol, 48: S22-S34).
The objectives of this research are: a) achieving a reliable appreciation of the magnitude of the *D. gallinae* infestation in humans; b) developing a quantitative skin prick test (SPT) and evaluating the presence of circulating *Dermanyssus*–specific IgE; c) drawing up and disseminate guidelines for recognizing and managing dermanyssosis in humans.

MATERIALS AND METHODS: One thousand (100 mg) laboratory-reared specimens of *D. gallinae* were purchased and, at the Allergology Unit, University of Bari, the specimens were placed in PBS without Ca and Mg and EDTA plus protease inhibitor and disrupted by sonication. The final ultracentrifugation step provided a semi-purified extract (Albanesi et al., 2019. Postepy Dermatol Alergol, 36: 98-103). The protein content of the extract was assessed by Bradford technique and a polyacrylamide gel electrophoresis (SDS-PAGE) was performed by a Mini-Protean II apparatus.
Between July 2021 and March 2022, eight poultry farms, localized in the province of Foggia, were visited and nine farmers were recruited based on their answers to a questionnaire. In addition, two patients, routinely examined at the Allergology Unit of the Ospedali Riuniti, Foggia, presented dermatological lesions overlapping those caused by *D. gallinae* and were included in the study. All the recruited subjects were subjected to skin prick tests for *D. gallinae* and for the house dust allergy caused by *Dermatophagoides pteronyssinus* and *Dermatophagoides farina*, with their specific extract, in order to evaluate patients' cross-reactivity.

RESULTS AND CONCLUSIONS: The protein content of the extract was 2.7 mg/ml and the analysis by SDS-PAGE detected 15 protein bands. Four out of the 11 patients (36.4%) exhibited positive skin prick test reactions for *D. gallinae* and, out of them, three showed a clinical picture compatible with dermanyssosis, whereas three (27.3%) of the recruited subjects had positive skin tests for *Dermatophagoides* and out of them two showed symptoms (Table 1). Two patients were positive for both and six did not have any reactions. These preliminary results are very encouraging, and this ongoing study represents the first attempt to develop quantitative skin prick testing capable of revealing possible mast cell-bound *Dermanyssus*–specific IgE in exposed individuals and suspected patients. In addition, the dissemination of accurate guidelines for physicians will represent a meaningful contribution for tackling the *D. gallinae* infestation in humans.

Table 1. Skin prick testing results

Patient	SPT *Dermanyssus gallinae*	*Dermanyssus gallinae* symptoms	SPT *Dermatophagoides*	*Dermatophagoides* symptoms	Conclusions
1	Pos.	Yes	Neg.	No	Allergy to *Dermanyssus gallinae*
2	Pos.	No	Pos.	No	No Allergy: IgE specific for *Dermanyssus gallinae* and *Dermatophagoides*
3	Pos.	Yes	Neg.	No	Allergy to *Dermanyssus gallinae*
4	Neg.	No	Neg.	No	No Allergy
5	Neg.	No	Neg.	No	No Allergy
6	Neg.	No	Neg.	No	No Allergy
7	Neg.	No	Neg.	No	No Allergy
8	Neg.	No	Neg.	No	No Allergy
9	Neg.	No	Neg.	No	No Allergy
10	Pos.	Yes	Pos.	Yes	Allergy to *Dermanyssus gallinae* Allergy to *Dermatophagoides*
11	Neg.	Yes	Pos.	Yes	Allergy to *Dermatophagoides*

SPT= skin prick test; pos.=positive; neg.=negative.

ANISAKIASIS: EXPANDING THE REPERTOIRE OF POTENTIAL *ANISAKIS* INFLAMMATORY MODULATION STRATEGIES ON HUMAN FIRST LINE OF DEFENSE

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Keywords: *Anisakis*, Caco-2, Inflammation

INTRODUCTION: The co-evolution between humans and helminths stimulated parasites to develop evasion and immunosuppression mechanisms enabling the establishment of chronic infection and tumorigenesis (Maizels et al., 2018. Immunity, 49:801-18). Although the marine nematode *Anisakis* is an example of accidental parasite of humans, it is able to elicit an acute or chronic inflammatory and immune response, leading to a fish-borne zoonosis called gastric, intestinal, ectopic or allergic anisakiasis (Nieuwenhuizen et al., 2016. Parasite Immunol, 38:548-57). To date, few studies investigated the potential immunomodulation strategies exerted from Anisakis in humans (reviewed by Cavallero et al., 2022. Pathogens, 11: 285). The aim of this study was to analyze the gene expression of two pro-inflammatory cytokines (IL-6, IL-8) at three timepoints, in *in-vitro* human epithelial colorectal adenocarcinoma cells (Caco-2), mimicking the human epithelial barrier, exposed to *Anisakis* agents of pathogenicity represented by live larvae (L3), crude extract (CE) and exosomes (EVs).

MATERIALS AND METHODS: A total of 216 L3 were collected and used for L3 incubation (9), CE preparation (27) and EV isolation and quantification (180). The EVs size distribution and concentration were measured using Nanoparticle Tracking Analysis (NTA). Incubation with Caco-2 cells was set at 1h, 6h and 24h, at 37°C in the presence of 5% CO2. Cells total RNA was used for quantitative Real-Time PCR analysis.

RESULTS AND CONCLUSIONS: NTA confirmed the presence of EVs with a mean size of 141,7 nm and a concentration of 1,32 x 1010 particles/ml. qRT-PCR of Caco-2+L3 showed a significant early downregulation of IL-6 (1h, $P<0.01$) and IL-8 ($P= 0.054$), compared to controls, followed by a moderate not statistically relevant upregulation at 6h and 24h. On the contrary, Caco-2+CE showed a strong upregulation of IL-6 at 1h and 6h ($P<0.05$) that seemed to switch off at 24h ($P< 0.05$), while IL-8 expression was not affected compare to controls. Finally, Caco-2+EVs showed a relevant early (1h) upregulation in IL-8 and IL-6 gene expression ($P<0.05$), followed by a slight downregulation (not significant) in the other checkpoints (6h, 24h) compare to not treated samples. These results, in agreement with our previous data obtained from ELISA tests on IL-6 and IL-8 at 24h, showed an evident modulation of host's first line of defense. Caco-2 cells seemed not completely alarmed by the presence of L3 and its exosomes, suggesting an early mild modulation strategy implemented by the parasite to survive in an inhospitable environment. On the other hand CE, which represents a cocktail of L3 antigens mimicking its senescence, clearly stimulated inflammation. To investigate these mechanisms, we are validating a multiplatform approach accounting for the new suitable 3D model based on intestinal organoids, comparative transcriptomics and EVs.

PREVALENCE AND MOLECULAR CHARACTERIZATION OF GASTROINTESTINAL PROTOZOANS FROM POLICLINICO TOR VERGATA, ROME: A ONE-YEAR SURVEY

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Keywords: multiplex Real-Time PCR, genotyping, zoonoses

INTRODUCTION: Detection of intestinal parasites by molecular methods is becoming an increasing common practice in Italy. Indeed, comprehensive molecular assays have been used for gastroenterology diagnostics in recent years as an efficient approach for protozoans' detection in fecal samples. In this study a one-year survey within the second Covid-19 pandemic period was carried out to assess prevalence and molecular genotyping of different gastrointestinal protozoans by means of a multiplex Real-Time PCR. The aim was to display a more comprehensive picture of parasites presence and diversity in the considered patients.

MATERIALS AND METHODS: A one-year study (March 2021-March 2022) was conducted over 377 clinical fecal samples from symptomatic patients (with and without a formal request for a parasitological examination) in the Laboratory of Parasitology of Policlinco Tor Vergata, Rome. *D. fragilis*, *Blastocystis* spp., *G. duodenalis*, *Cryptosporidium* spp., *E. histolytica*, and *C. cayetanensis* were investigated by multiplex Real-Time PCR (AllplexTM Gastrointestinal Panel-Parasite Assay) and molecularly characterized by specific end-point PCR. Genotypes and subtypes allocation was performed, wherever possible, by comparison of the obtained sequences with those retrieved from NCBI GenBank by MEGAX.

RESULTS AND CONCLUSIONS: Overall, 87/377 (23%) positive samples were observed. Twenty-five (6.6%), 45 (11.9%), 5 (1.3%), 8 (2.1%) samples were positive for *D. fragilis*, *Blastocystis* spp., *G. duodenalis*, *Cryptosporidium* spp., respectively. All samples resulted negative for *E. histolytica* and *C. cayetanensis*. Co-infections were detected in 9 samples (2.4%): 7 with the association *Blastocystis* spp. + *D. fragilis* (1.8%), one with the association *Blastocystis* spp. + *G. duodenalis* (0.3%) and one with the association *Blastocystis* spp. + *Cryptosporidium* spp. (0.3%).
With regard to the molecular characterization: the obtained *D. fragilis* sequences (no. = 6) belonged to Genotype 1, as commonly reported in human. *Blastocystis* spp. ST1 (2/16), ST2 (2/16), ST3 (6/16), ST4 (6/16) subtypes were identified, as expected. Finally, the *Cryptosporidium* isolate was identified as *C. parvum*.
Whereas not all patients presented a specific request for a parasitological screening, the overall positivity value of 23% appears consistent: almost one out of four patients resulted as infected at least with one gastrointestinal protozoan. Whether the technique switching from microscopy to molecular assays, or the patient population's characteristics, affected by Covid-19 pandemic, could have played a role in the observed results. However, it is necessary to evaluate and monitoring these prevalence values routinely. The availability of multiplex Real-Time PCR tool enables to pursue this aim and also to obtain more targeted samples for the molecular characterization, the use of which remains crucial for diversity analysis and to identify potential zoonotic isolates.

SURVEY ON THE PRESENCE OF *LEISHMANIA INFANTUM* IN WILD ANIMALS FROM THE PROVINCE OF BOLOGNA (NORTH-EASTERN ITALY)

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Keywords: leishmaniasis, wildlife, Italy

INTRODUCTION: In Italy, *Leishmania infantum* is the only species responsible for zoonotic visceral leishmaniasis (ZVL), having dog as traditionally recognized reservoir in central and southern regions. In the Emilia-Romagna (ER) region, recurrent outbreaks of human visceral leishmaniasis (HVL) have been reported starting from the '70s (Pampiglione et al., 1982. G Mal Infett Parassit, 11:1475-80), and recent studies have characterized human strains as genetically different from those found in dogs within the same area (Rugna et al., 2017. Vector Borne Zoonotic Dis, 17:409-415). To identify any animal reservoirs other than dogs, a survey has been conducted from 2019 to 2021 on wild animals in the province of Bologna (ER), where foci of HVL are currently active.

MATERIALS AND METHODS: Earlobe skin and spleen were collected from 33 hunted roe deer (*Capreolus capreolus*) and 5 damaged hedgehogs (*Erinaceus europaeus*) from the province of Bologna (ER). The presence of *L. infantum* was tested with a real-time PCR (Tsakmakidis et al., 2017. Vet Parasitol Reg Stud Reports, 16:100279) and sequencing of the ITS1. For strains discrimination, a new nested PCR protocol targeting the cysteine protease B (CPB) was developed and optimized.

RESULTS AND CONCLUSIONS: The presence of *L. infantum* was detected in 11 roe deer (33.3%) and 4 hedgehogs (80%). The analysis of the CPB revealed the presence of a strain previously isolated in human patients of the same area but not in dogs (Rugna et al., 2017. Vector Borne Zoonotic Dis, 17:409-415), in all specimens tested but one. In Europe, the finding of *L. infantum* in hedgehogs was limited to Spain, where it was firstly reported in one specimen by Muñoz-Madrid et al., 2013 (Acta Trop, 128:706–709) and later in Catalonia with a prevalence of 34.4% in the specimens examined (Alcover et al., 2020. Prev Vet Med, 175: 104874). Concerning roe deer, to the best of our knowledge, this is a first report of *Leishmania* infection in this species. Parasites were found in all earlobe skin samples and (only in one sample) also in spleen. These preliminary data, along with the results of a recent study on blood meal preferences of sandflies in the same area, showing the roe deer as the most bitten animal (Calzolari et al., 2022. Acta Trop, 226:106246), highlight the possible involvement of these wild animals in the epidemiology of leishmaniasis in the area under study.

BLASTOCYSTIS IN ITALY: GENETIC CHARACTERIZATION AND META-ANALYSIS FROM 1989 TO 2022

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Keywords: *Blastocystis*, one-Health, Italy

INTRODUCTION: *Blastocystis* sp. is one of the most common gastro-intestinal parasites in humans and displays a global distribution. It is observed also in animal hosts, and there is evidence to support *Blastocystis'* zoonotic transmission. One factor thought to be key in explaining *Blastocystis*' pathogenicity and zoonotic potential is the existence of a remarkable degree of genetic variability, as revealed by the 18S, which is thus used to divide the genus into subtypes (Maloney and Santin, 2021. Microorganisms, 9: 1-21). The aims of the study are i) to conduct a systematic review and a meta-analysis on diverse studies carried out in Italy, which reported *Blastocystis*-positive samples from humans, animals and environmental sources; ii) to characterize *Blastocystis* subtypes and 18S alleles in new positive' *Blastocystis* from symptomatic patients comparing them with genetic data retrieved from the meta-analysis step.

MATERIALS AND METHODS: Systematic Analysis: peer-reviewed published papers on *Blastocystis*' detection in Italy were retrieved via systematic exploration in four international electronic databases. The searching procedure was fulfilled using Medical Subject Heading terms alone or in combination. Eligibility criteria for articles inclusion were determined. Next, necessary information for meta-analysis steps were extracted to tabulate the information in an efficient way.
Human *Blastocystis* samples: 40 samples resulted *Blastocystis*' positive by microscopy or qPCR (Allplex). The samples were collected at the Laboratory of Parasitology of the Polyclinic Tor Vergata of Rome, from 2014 to 2022 and then sequenced (18S).
STs and Alleles characterization: 18S sequences covering the spectrum of Italian *Blastocystis* diversity, retrieved from the systematic analysis step, were downloaded and aligned with those produced in-house. Maximum likelihood analysis was performed using RStudio. Next, all sequences were used to query the *Blastocystis* 18S database for alleles identification.

RESULTS AND CONCLUSIONS: All *Blastocystis* positives' samples from patients were genetically characterized. Six subtypes (STs) were detected, with different prevalence: ST4 (47.5%), ST3 (25%), ST1 (17.5%), ST2 (5%), ST6 (2.5%) and ST7 (2.5%).
Thirty-six articles relating to our country, from 1989 to 2021 were considered in the analysis: ten of them molecularly characterized *Blastocystis* STs, but only seven deposited the DNA sequences (>40 sequences). Phylogenetic analysis comprising the spectrum of Italian *Blastocystis* diversity (>100 sequences) confirmed the existence of several STs (ST1, ST2, ST3, ST4, ST5, ST6, ST7, ST8, ST15). In contrast new allelic variations never reported so far, were identified in each STs except for ST1, updating the current knowledge on the prevalence and genetic diversity of this protozoan.

ZOONOTIC EPISODES OF SCABIES: WHAT DO WE KNOW? A GLOBAL OVERVIEW

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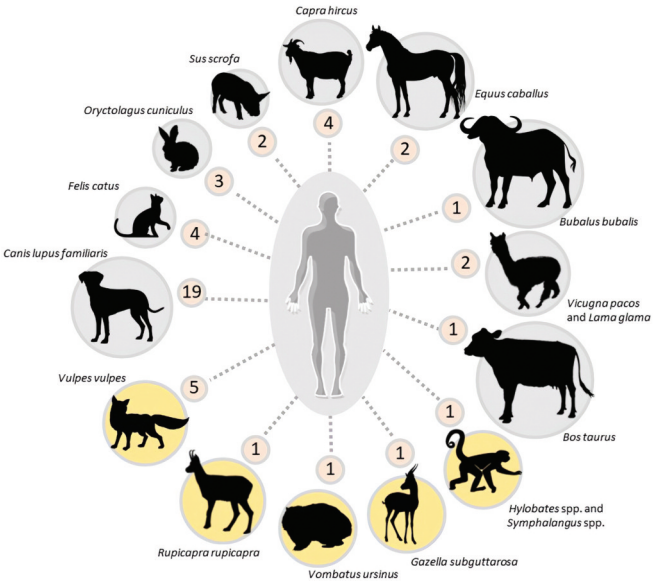
Keywords: pseudoscabies, zoonotic scabies, *Sarcoptes scabiei*

INTRODUCTION: Zoonotic scabies (ZS), also referred to as "pseudoscabies", is considered a self-limiting disease with a short incubation period and transient clinical skin signs. It is commonly thought that *Sarcoptes scabiei* mites from animals are unable to reproduce and persist on human skin, however, several ZS case reports have mentioned the persistence of symptoms and occasionally mites for weeks (Estes et al., 1983. J Am Acad Dermatol, 9: 397-401).
Furthermore, *S. scabiei* cross-transmission between different animal species has been reported in more than 50 species under natural or human-driven conditions (Escobar et al., 2021. Transbound Emerg Dis, 1-16) highlighting the pronounced epidemiological plasticity of this ectoparasite.
Nonetheless, online bibliographic enquiries using terms such as "pseudoscabies" or "ZS" point out that literature on ZS is not as abundant as expected and is partially outdated and difficult to retrieve. In addition, reviews on ZS episodes are lacking, except for sporadic contributions focusing on zoonotic canine scabies. The aim of this review was to collect and organize the sparse literature referring to *S. scabiei* zoonotic transmission, focusing on the source of the outbreak, the circumstances leading to the transmission of the parasite, the diagnosis including the identification of the *Sarcoptes* "strain" involved, and the applied treatments.

MATERIALS AND METHODS: We searched and selected relevant papers through three electronic databases (Scopus, Web of Science and Google Scholar) published until November 2021 with no time or language limits. The search strategy included the key words: "*Sarcoptes scabiei*", "scabies", "human", "animals", "sarcoptic mange", "pseudoscabies", "zoonosis", "zoonotic disease".

RESULTS AND CONCLUSIONS: A total of 46 articles, one conference abstract and a book were collected describing ZS cases associated with twenty animal hosts in five continents. Dogs were the most common source among pet owners, while diverse livestock and wildlife contributed to the caseload as an occupational disease.
Descriptive data on ZS episodes are still limited. The number of events may be greatly underestimated considering that the literature collected in this review includes outdated case reports, anecdotal reports and grey literature.
Genetic epidemiological studies of ZS outbreaks are still limited in number, but tools are available to fill this knowledge gap in the near future (Mofiz et al., 2016. Gigascience, 5:1). Further research is also needed to understand the apparent heterogeneity in the morbidity, disease severity and timing of the response to treatment among people infected with different animal-derived strains.
More first-hand information by dermatologists is also warranted to understand the variability in the morbidity, disease severity and timing of the response to treatment among people infected with different animal-derived strains.



OCCURRENCE OF *ECHINOCOCCUS MULTILOCULARIS* AND OTHER TAENIDS IN RODENT INTERMEDIATE HOST IN THE BOLZANO PROVINCE (ITALY)

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Keywords: *Echinococcus multilocularis*, intermediate host, small rodents

INTRODUCTION: *Echinococcus multilocularis* (*Em*) is a small tapeworm affecting wild and domestic carnivores and voles in a typical prey-predator life cycle. It can cause Alveolar echinococcosis in intermediate hosts, including small mammals and accidentally humans. In Italy, *Em* is documented in the northern Italian Alps since 1997. Two studies of cestod-eggs- foxes monitoring (2001/2005 and 2012/2018) confirmed the distribution of *Em* limited to Bolzano province. This study aimed to investigate the occurrence of tapeworms, primarily *Em*, in small rodents from Bolzano province since there is little to no data about *Em* prevalence in intermediate hosts.

MATERIALS AND METHODS: A total of 75 livers from small rodents (Cricetidae and Muridae) were collected in different municipalities of Bolzano province from 2019 to 2021. All livers were screened for parasitic lesions (presence of cysts mostly). From all specimens, DNA was extracted and amplified with a multiplex PCR for *Em*, *E. granulosus* and Taenidae (Trachsel et al., 2007. Parasitology, 134:911–20). Sequences obtained from PCR products were used for taxonomic confirmation and phylogenetic analysis.

RESULTS AND CONCLUSIONS: Out of 75 liver samples, 10 animals (13.33%) tested positive for *Em*. Those results confirmed the presence of *Em* in the northeastern Alps of Italy (province of Bolzano) previously reported in foxes, and reported the presence *Em* in Val Passiria for the first time, highlighting the spread of *Em* toward new areas.
Presence of *Taenia* spp. was confirmed in 21 livers (28%), including *Taenia taeniaeformis* (*Tt*) in 15 specimens (20%), *Taenia crassiceps* in 5 animals (6.6%) and *Taenia martis* (1.3%) in a single vole. To note, 3 animals were *Em* and *Tt* co-infected.
In agreement with previous studies, *Tt* was the most abundant cestode in the small rodents tested followed by *T. crassiceps* both designated zoonotic as well, causing cysticercosis and strobilocercosis, respectively. *Taenia martis*, which is known to be circulating in Europe with a life cycle affecting mustelids, was described as cause of human cerebral cysticercosis in immunocompetent hosts in Europe. However, cases in humans are sporadic worldwide and do not represent a major public health concern.
To our knowledge this is the first description of *Em* prevalence in intermediate host in Italy and it contributes to address the role of density and dispersal pathways of small mammal community composition in the maintenance and spreading of this severe zoonosis.

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A COMPLETE CLEARANCE OF CIRCULATING *WOLBACHIA* DNA IN *DIROFILARIA IMMITIS*-NATURALLY INFECTED DOGS AFTER TREATMENT WITH DOXYCYCLINE

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Keywords: *Wolbachia*, *Dirofilaria immitis*, doxycycline

INTRODUCTION: Bacteria of the genus *Wolbachia* are endosymbionts of parasitic filarial nematodes, including *Dirofilaria immitis*, and represent a target for the treatment of canine heartworm disease (HWD) (Kramer et al., 2018. Vet Parasitol, 254: 95–7). Dogs infected with *D. immitis* can develop inflammatory reactions also due to the release of *Wolbachia* that partially contributes to the severity of the clinical signs of HWD (Bazzochi et al., 2003. Vet Parasitol, 117: 73–83). The treatment of doxycycline in combination with Imidacloprid + Moxidectin (Advocate®) showed efficacy by reducing the circulating microfilariae (mfs) as well as eliminating *Wolbachia* (Savadelis et al., 2017. Parasit Vectors, 10: 245). Therefore, the time course of *Wolbachia* DNA was monitored in a canine population treated with doxycycline in combination with a long-term application of Advocate® and living in an hyperendemic area for HWD (Linosa island, Southern Italy, Sicily).

MATERIALS AND METHODS: The study was conducted from October 2020 (T0) to October 2021 (T6). At T0, dogs were divided in three groups accordingly to their positivity to *D. immitis* by antigen and Knott's tests (Knott, 1939. Trans R Soc Trop Med Hyg, 33: 191-6). Dogs that scored positive to both tests (G1) or to antigen test only (G2) were submitted to doxycycline (10mg/kg BID PO) treatment and 10% Imidacloprid + 2.5% Moxidectin (Advocate®), while those negative to both tests (G3) received only 10% Imidacloprid + 2.5% Moxidectin (Advocate®). All dogs followed-up for one year were monthly treated with Advocate® and monitored by antigen (T1-T6) and Knott's tests (T1-T2). During the whole period, all blood samples were screened for *Wolbachia-D. immitis* DNA load by quantitative real-time PCR (qPCR) and positivity was established based on the threshold cycle (Ct value up to 38.5). The statistical analysis for the prevalence of *Wolbachia* were analyzed by GraphPad Prism v. 8.0.0, where p values <0.05 were considered significant.

RESULTS AND CONCLUSIONS: At T0, 88.2% of the microfilariemic dogs from G1 were positive for *Wolbachia* DNA (Ct from 29.79 to 38.55), and none of animals from G2. *Wolbachia* was no longer detectable in dogs from G1 following 1 month of doxycycline until the end of the study, and mfs were cleared at T2. No correlation was found between mfs load and Ct value for *Wolbachia* DNA. Beyond that, dogs from the groups G2 and G3 did not present amplification for *Wolbachia* from timepoint T0 to T6. All dogs from the G1 and G2 were negative for *D. immitis* antigen at the 12th month (T6).

Results of this study suggest that the successful elimination of mfs by doxycycline is associated with complete clearance of *Wolbachia* DNA in *D. immitis*-naturally infected dogs.

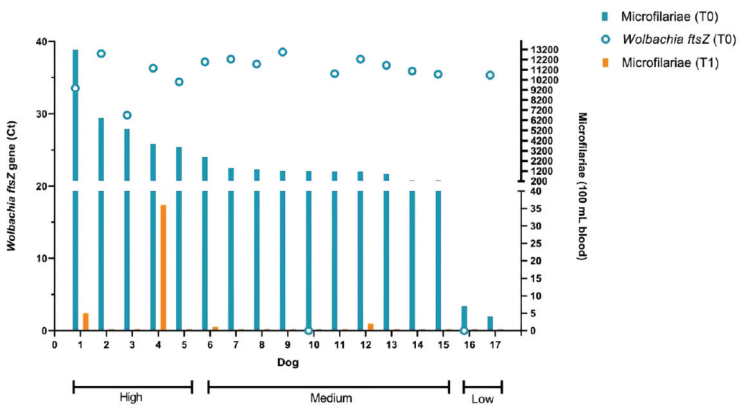

Figure 1 *Dirofilaria immitis* microfilariae (mfs) counting and *Wolbachia* load from G1 (Ag^+/Mfs^+) at T0 (before the treatment) and T1 (1 month after the treatment) related to each dog (n = 17) mfs load score (high >3000; medium >200 and <2999; and low >199). *Wolbachia* load at T1 were not present.

CROSS-PROTECTIVE IMMUNE-MODULATION IN PARASITIC INFECTIONS: THE *WOLBACHIA* SURFACE PROTEIN FROM FILARIAL NEMATODES DETERMINES MACROPHAGE ACTIVATION AND KILLING OF *LEISHMANIA* PARASITES

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Keywords: symbionts, *Wolbachia*, leishmaniases

INTRODUCTION: Filariasis and canine leishmaniasis are vector borne-diseases whose natural history is strongly interconnected. Experimental evidence indicates that heartworm infection could be one of the factors that determine protective immune-modulation against canine leishmaniasis (Maia et al., 2016. Parasit Vectors, 9: 170). Indeed, in parallel with the progressive disappearance of heartworm disease in dogs of the Po river Valley, a diffusion of leishmaniasis was observed, with widespread outbreaks. The filarial symbiont *Wolbachia* and its associated molecules have been implicated in classic, M1 activation, of macrophages, which is a condition that favours the killing of *Leishmania* parasite and the resolution of the diseases (Burza et al., 2018. Lancet, 392: 951-70). Therefore, we have examined the potential of AsaiaWSP, a bacterium engineered for the expression of the *Wolbachia* surface protein, as an inductor of macrophage activation, which is expected to determine the killing of *Leishmania* parasites (Varotto-Boccazzi et al., 2020. Pharmacol Res, 161: 105288).

MATERIALS AND METHODS: *In vitro* experiments on a murine immortalized macrophage cell line were carried out, with the aim of determing the immune activation induced by the engineered bacterium. Particularly, M1/Th1-cytokines, nitrites and costimulatory molecules were analyzed through ELISA and flow cytometry analyses. In addition, the effect of immune activation on the killing of *Leishmania infantum*, after stimulation of macrophages with AsaiaWSP, was evaluated by microscopic observation.

RESULTS AND CONCLUSIONS: The incubation of macrophages with the bacterium AsaiaWSP induced M1 activation with the release of M1/Th1 cytokines and co-stimulatory markers. In addition, a reduction of the number of amastigotes of *L. infantum* in challenged macrophages was observed, with an effect comparable to that determined by the drug amphotericin B. Considering the potential of *Wolbachia* to train the immune response on the M1 side, filarial nematodes and their symbionts are worth of further investigations, in order to uncover novel immune-modulating molecules that could be used to control leishmaniases and other M1-impaired infections.

VARROA DESTRUCTOR AND *VARROA*-TRANSMITTED VIRUSES: IMPACT ON ENVIRONMENTALLY MONITORED APIARIES

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Keywords: *Varroa destructor*, Colony collapse, honey bees

INTRODUCTION: The Worldwide population of *Apis mellifera* is declining. Emerging diseases and climate change serve as the most important factors for Colony Collapse (Goulson et al., 2015. Science, 347: 1255957). Acute Bee Paralysis Virus (ABPV), Deformed Wing Virus A and B (DWV-A and DWV-B) are among the six RNA viruses with the highest adverse effects on European honey bee health (Schurr et al., 2019. J Virol Methods, 270: 70–8). While persistence of these pathogens within colonies is primarily maintained through vertical transmission in honey bees, it is furthermore enhanced via horizontal transmission mainly by ubiquitous *A. mellifera* ectoparasite *Varroa destructor* (Chen et al., 2006. Appl Environ Microbiol, 72: 606-11). Since such viruses are able to develop infections with no visible clinical symptoms, monitoring their viral load titer by accurate (precise and specific) RT-qPCR is critical in the control and prevention of honey bees' decline (Amiri et al., 2015. PLOS ONE, 10: e0140272). In this study, we conducted a molecular survey by investigating the prevalence of *V. destructor*-borne viral diseases on a chronological basis to find possible associations between clinical signs', *V. destructor* prevalence, and phytosanitary treatments.

MATERIALS AND METHODS: Honey bees and *V. destructor* mites were collected from 40 colonies of two separate apiaries in the province of Cuneo (Piedmont region, Italy) between March and October 2021. A total of 140 honey bee (~200 µg of homogenate per each hive) and 104 *V. destructor* samples (pools varying in mite number per each hive) were used for total RNA extraction by Tri Reagent® (Sigma-Aldrich; Merck KGaA) and blackPREP Tick DNA/RNA Kit (Analytik Jena, Germany) respectively. cDNA synthesis was performed using QuantiTect® Reverse Transcription Kit (Qiagen, Hilden, Germany) in a final volume of 25 µl from 1 µg and 15ng of every honey bee and *V. destructor* mite RNA samples respectively. In order to investigate the presence and prevalence of ABPV, DWV-A and DWV-B viruses, qualitative PCR and Two-Step quantitative PCR were performed using specific primer and probes on both bee and mite samples.

RESULTS AND CONCLUSIONS: Among 140 honey bee samples investigated for DWV-A and ABPV viruses; 11 samples from different sampling periods were tested positive for DWV-A while only one sample was positive for ABPV suggesting a prevalence in line with results reported in other studies. Our results while demonstrating the presence of both DWV-A and ABPV viruses are also suggestive of an association between viral prevalence and anti-parasitic treatments efficacy aiming at *V. destructor* elimination. Moreover, increased positive results in samples collected following anti-parasitic treatment may be informative of probable resistance to such agents in *V. destructor* mites.

MOLECULAR PREVALENCE OF *BABESIA CANIS* INFECTION IN *DERMACENTOR RETICULATUS* TICKS COLLECTED IN A NATURAL PARK IN ITALY

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Keywords: piroplasmida, babesiosis, tick-borne pathogens

INTRODUCTION: *Dermacentor reticulatus* is one of the most important vectors of tick-borne pathogens (TBPs) in Europe causing diseases in animals and humans. Indeed, it is the proved vector of *Babesia canis*, but it can also transmit other pathogens of veterinary importance, such as *B. caballi*, *Theileria equi*, and *Anaplasma marginale*. The tick could transmit TBPs of public health relevance as *Rickettsia* spp. and some tick-borne encephalitis viruses (Foldvari et al., 2016). *B. canis* the protozoan agent of canine babesiosis (CB), a significant hemoparasitic disease of dogs, causing hemolytic anemia, splenomegaly, thrombocytopenia, and fever.
The circulation of *D. reticulatus* and the association of the tick with *B. canis* infection was recently reported in northern Italy (Olivieri et al., 2016). Therefore, a longitudinal study was planned mainly aimed to detect the molecular prevalence of *Babesia* spp. and its seasonal variation in *D. reticulatus* questing ticks collected in a natural park in Italy to define the temporal infection risk for dogs. An ancillary aim was the detection of *Rickettsia* spp.

MATERIALS AND METHODS: The study was carried out in the Groane Regional Park, located in the peri-urban area of Milan and Monza Brianza provinces (Lombardy). Ticks were collected from April 2015 to June 2016 in five permanent transects using both dragging and flagging techniques on the leaf litter and the vegetation. Collected ticks were morphologically identified by taxonomic keys (Arthur, 1962; Pomerantzev, 1950). Overall, 488 adult questing ticks, including 241 female and 247 male exemplars, were processed for DNA extraction. A screening real-time PCR on 18S rRNA was performed for the detection of piroplasmid DNA. On positive samples, a conventional PCR for Babesia spp. and subsequent sequencing was carried out. Besides, a real-time PCR on ITS-2 for *Rickettsia* spp. was also performed.

RESULTS AND CONCLUSIONS: Out of 488, 58 ticks (24 females and 34 males) were positive for *Babesia*/*Theileria*, resulting a molecular prevalence of 11.9%. Positive ticks were mostly collected in March (no. = 30) and April (no. = 21), when in early spring the peak of tick activity occurred (Olivieri et al., 2017). Positive ticks also occurred in February (2) and May (5). Obtained sequences from 408-bp PCR fragments confirmed a homology of 100% with *B. canis* sequences deposited in GenBank. No tick resulted positive for *Rickettsia* spp. This study evidenced a conspicuous circulation of *B. canis* infection in *D. reticulatus* adult questing ticks and confirms their role in the epidemiology of CB. The risk of acquiring the CB for owned dogs using the peri-urban park is significant in spring months. Considering the efficient transovarial transmission of *Babesia* in the ticks, tick prophylaxis is strictly required and should cover the entire period during which *D. reticulatus* is active to prevent CB. Even if no tick was found positive to *Rickettsia* spp. and *D. reticulatus* only seldom bites humans, its capacity as a vector of zoonotic pathogens should not be neglected.

DEVELOPMENT OF AN INTEGRATED GEO-EPIDEMIOLOGICAL SYSTEM FOR THE ASSESSMENT OF OCCUPATIONAL RISK OF TICK-BORNE PATHOGENS

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Keywords: software GIS, occupational exposure, northeast Italy

INTRODUCTION: In Europe, ticks are one of the most important vectors of human and animal infectious diseases. Certain occupational categories are at increased risk of exposure to potentially infected ticks. The aim of this project was to perform a risk assessment of tick-borne pathogens (TBPs) among those workers most exposed to tick bites in the “Triveneto” area.

MATERIALS AND METHODS: Three categories of data were collected: i) presence and abundance of tick species in past and recent times; ii) occurrence of TBPs in ticks; iii) environmental and meteorological data. These data were used to model the ecological niche of ticks as to predict their habitat suitability range and to build risk maps for TBP exposure. Ticks were collected by dragging in 2020-2021 in Veneto and Friuli Venezia Giulia regions; these data were integrated with historical tick collections (samplings of 2006-2017 period). After morphological tick identification, molecular tests were performed for *Borrelia*, *Rickettsia*, and *Anaplasma*, *Babesia*/*Theileria* species, TBE and CCHF viruses in pooled (larvae and nymphs) or adult ticks. Estimates of pooled prevalence (EPP) and confidence limits 95% (CL) for variable pool size were calculated. Blood samples from transhumant sheep herds located in historical TBP detection areas were serologically screened. TBPs data in ticks were modeled with the environmental and meteorological data using Maxent and GLMs to define infected ticks’ ecological niche, which was subsequently studied in relation to pathogen detection in animal samples. A dedicated website to report information on the risk of infections and prevention measures is in progress.

RESULTS AND CONCLUSIONS: In 2020-2021, 43 tick samplings were conducted and 2403 ticks (all stages) were collected; of these, 1388 were pooled (175 total pools) and screened for TBPs. A first screening for TBPs detected 5 positive samples for *Borrelia* spp. (EPP=0.36; CL=0.13-0.78), 48 for *Rickettsia* spp. (EPP=4.03; CL=3.01-5.26), 20 for *A. phagocitophylum* (EPP=1.53; CL=0.96-2.30) and 30 for *Babesia*/*Theileria* spp. (EPP=2.27; CL=1.56-3.18). Fourteen samples presented co-infections or multiple TBPs detections. Further tests are ongoing to confirm the TBPs screening results. No positive samples were detected for CCHFV and TBEV. To date, 463 sera from domestic and wild ruminants have been collected but not tested for TBPs yet. A complete environmental/meteorological and TBP database was created and preliminary ecological niche analyses showed a relatively broad habitat suitability range for *Anaplasma* (mostly defined by autumnal vegetation index), whereas the narrowest range was observed for *Borrelia* (mostly defined by coldest temperature and altitude). Website-building activities and information development have started. Further spatial analyses are ongoing and the last step will be to develop a risk map of TBP exposure for workers.

This work was supported by INAIL (Istituto nazionale Assicurazione Infortuni sul Lavoro) (founding source: CUP C94I20000220001).

MALARIA EPIDEMIOLOGY AMONG CHILDREN AND PREGNANT WOMEN, WESTERN EQUATORIA STATE, SOUTH SUDAN

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Keywords: malaria, epidemiology, South Sudan

INTRODUCTION: The study “Malaria epidemiology among children and pregnant women, Western Equatoria State, South Sudan” is the operational research component of the project “Enhancement of Malaria response in South Sudan through the improvement in access, utilization and quality of preventive/diagnostic/curative services and their integration to the three levels of care of the health system of Amadi State” implemented by Doctors With Africa CUAMM and funded by the Italian Agency for Development Cooperation in the framework of the technical support spending to the Global Fund for AIDS, Tuberculosis and Malaria. The study has been approved by South Sudan Ministry of Health and aims at narrowing gaps in malaria epidemiology knowledge and diagnosis skills through large-scale molecular investigations and capacity building at primary health care centers (PHCC) in 3 Counties.

MATERIALS AND METHODS: The study is conducted at 3 PHCCs (Mundri, Lakamadi, Mvolo), targeting children under 5 years and pregnant women, who are recruited on a volunteer basis during routine outpatient and ante-natal care visits. Recruitment started in December 2021 and will last for 6 months, reaching 2000 participants. For each participant, malaria diagnosis is performed by Rapid Diagnostic Test (RDT), microscopy observation of thick and thin blood films and Next Generation Sequencing (NGS) of DNA extracted from Dried Blood Spot.

RESULTS AND CONCLUSIONS: Prior the start of the study, laboratories at the 3 PHCC have been equipped by CUAMM with instruments, consumables and reagents needed for malaria diagnosis. UNIPI has conducted intensive training at each PHCC on malaria clinical, epidemiology and diagnostic aspects as well as on study Standard Operating Procedures, followed by on-job supervision during recruitment. Despite staff strong motivation, malaria microscopy could not be implemented at PHCC level as initially planned, due to excessive workload, and this activity has been centralized at Lui Hospital, referral site for the 3 PHCC. Results of RDT showed a 39% overall positivity in children and 13% in pregnant women in the December 2021 - March 2022 period, with important differences between months and sites. Notably, 9% of RDT showed positivity for non-*Plasmodium falciparum* species, a previously unreported observation.
At the end of the study, results from different methods will be compared to investigate causes of discordance and improve the quality of malaria diagnosis. Malaria prevalence will be compared among seasons, PHCC and population groups. NGS will not only be used for parasite DNA detection and speciation but also P. falciparum genotyping of HRP2/3 deletion associated with RDT false negative results as well as of mutations associated with resistance to antimalarials. The generated data are expected to inform National Malaria Control Program planning and monitoring of malaria control interventions.

COMPARISON OF THREE COPROLOGICAL METHODS IN DETECTING *OXYURIS EQUI* INFECTION IN HORSES AND SEASONAL TREND IN ITALY

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Keywords: pinworm, scotch test, coprology

INTRODUCTION: *Oxyuris equi*, the equine pinworm, is a fairly large nematode that resides as adult in the small and dorsal colon of equids. Gravid female parasites migrate out through the anus to deposit eggs in a sticky fluid on the perianal region, causing itching, irritation, tail rubbing, broking hairs and excoriated skin. Due to the egg-laying behavior of female, pinworm diagnosis is based in equine practice on "Scotch Test", a piece of cellophane tape applied to the perianal area to collect eggs from the skin of the anus. However, occasionally, pinworm eggs may be observed during coprological examination (Reinemeyer and Nielsen, 2014. Equine Vet Educ, 26: 584-91). The aim of this study was to compare the sensitivity of three diagnostic techniques: Mini-FLOTAC (MF), Proudman Test (PT) and Scotch Test (ST) for detecting *O. equi* eggs and to evaluate the seasonal pinworm egg laying.

MATERIALS AND METHODS: The study was performed from January 2019 to March 2022 on 3532 horses randomly distributed on Italian territory. For each animal two ST were performed on the perianal area and then a fecal sample was collected directly from the rectum. Each sample collection time point was assigned to a corresponding season: winter (21 Dec–20 Mar), spring (21 Mar–20 June), summer (21 June–21 Sep) and autumn (22 Sep-20 Dec). The fecal samples were analyzed in duplicate using both MF and PT using a Sheather's saturated sugar solution (specific gravity = 1.25). Statistical analysis was performed using Multiple Correspondence Analysis (MCA).

RESULTS AND CONCLUSIONS: Considering all three methods tested, a total of 21,192 assays was performed. On 3532 horses, 133 were *O. equi* positive, for at least one coprological method, with overall prevalence of 3.8%. The detection of *O. equi* infection varied between applied coprological techniques. Of positive horses, 95/133 (71.4%) were positive only to ST; 22/133 (16.5%) to ST and MF; 6/133 (4.5%) to ST and PT; 7/133 (5.3%) only to MF and 3/133 (2.3%) only to PT suggesting a significant different sensitivity between techniques. Seasonal prevalence of *O. equi* was 5.7% in winter, 4.3% in summer, 2.8% in autumn, 2.3% in spring showing significant differences ($p<0.01$) between seasons. The reasons of this variability are still unknown but considering the optimal temperature (25 °C) for embryogenesis and development of *O. equi* eggs (Yevstafieva et al., 2020. Biosyst Divers, 28: 125-30) and the prepatent period of the pinworms (approximately 5 months), probably winter represents the patent period of the infection acquired during the summer, but further studies are needed. In conclusion the results of this study show that almost all the positive horses (92.5%) were detected using the ST, that can be considered the gold standard method for the diagnosis of pinworms infection in equids. Therefore, it is always advisable to associate the ST to the fecal egg count performed using the Mini-FLOTAC for this nematode infection.

SURVEY ON ENDOPARASITES IN TWO LOCAL BREEDS OF SHEEP IN NORTH-EASTERN ITALY

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Keywords: endoparasites, sheep, autochthonous breed

INTRODUCTION: Endoparasite infections considerably affect the health and productivity of sheep worldwide. The conservation of local breeds cannot overlook this major threat. This study aimed to assess the presence of gastrointestinal parasites and bronchopulmonary nematodes (BPN) in sheep farms of two autochthonous breeds with limited diffusion, Alpagota and Lamon, within the framework of the SHEEP AL.L. CHAIN project, Veneto Region (Italy).

MATERIALS AND METHODS: The study was performed during summer 2020 in 9 Alpagota and 6 Lamon farms. Data on parasite control practices were collected. For each farm, the sample size was calculated according to Maurizio et al., 2021 (Vet Sci 8: 69), and 197 faecal samples (134 Alpagota and 63 Lamon) were collected from the rectum and individually analyzed by McMaster technique. Coccidia were only counted in sheep < 1 year old. Subsequently, 20 pools were composed thoroughly mixing 5 grams of faeces from 6-12 animals and processed by modified-Baermann technique to isolate first-stage larvae of BPN. Coproculture was also performed on 11 pools (from 10 farms) and 1087 third-stage larvae of gastrointestinal strongyles (GIS) were subsequently identified. The influence of risk factors (number of treatments/year, use of pasture, farm size, age, sex and breed) on the GIS burden was investigated through a negative binomial regression model, using the software STATA® 12.1.

RESULTS AND CONCLUSIONS: Only one farmer occasionally performed coprological examinations, while all except one treated their animals at least once a year. All farms were positive for parasites. Coccidia and GIS were present in all farms and overall reached a considerable prevalence (68.5% and 74.1% respectively) and abundance (8022 OPG, calculated only on sheep <1year old, and 291 EPG respectively). *Strongyloides*, *Nematodirus*/*Marshallagia*, *Trichuris*, *Capillaria* and cestoda showed limited values for both indexes and were recovered in a minority of farms. *Skrjabinema* was never found. BPN were found in 10 pools from 8 farms and were identified as *Muellerius*, *Protostrongylus* and *Neostrongylus*. Farm mean FEC and 95%CI for GIS are displayed in Figure 1, showing a generally high burden in Lamon farms, and a high variability in burden among Alpagota farms. Based on the model results, Lamon sheep were significantly more parasitized than Alpagota sheep ($p<0.001$). Other significant risk factors for GIS abundance were the lack of anthelmintic treatments ($p<0.001$), sex (males were more affected, $p<0.01$) and access to pasture ($p<0.05$). Coprocultures were dominated by the *Trichostrongylus*/*Ostertagia*/*Teladorsagia* morphotype (49%), followed by *Haemonchus* (37%), *Oesophagostomum*/*Chabertia* (9%) and *Bunostomum*/*Gaigeria* (6%). This study confirms that endoparasites are widespread among Alpagota and Lamon sheep, the latter being consistently more affected. Treatments are common despite the lack of prior diagnosis, suggesting that health management of all farms must be improved to ensure a better targeting of treatments.

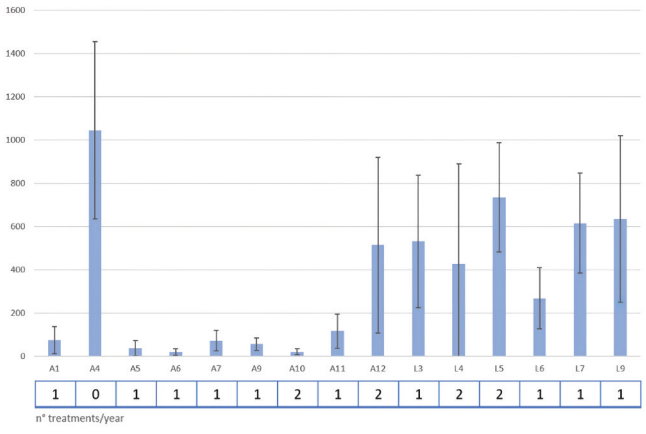

Figure 1 Mean EPG and 95%CI of GIS in Alpagota (A) and Lamon (L) farms. The confidence interval is calculated according to Maurizio et al., 2021 Vet Sci. 8: 69. In the grid, the number of treatments per year of each farm is reported.

UPDATED MORPHOLOGICAL KEYS FOR THE IDENTIFICATION OF GASTROINTESTINAL NEMATODE LARVAE IN SHEEP FROM SARDINIA, ITALY

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Keywords: gastrointestinal nematodes, larvae, microscopical analysis

INTRODUCTION: Gastrointestinal nematodes (GIN) are ubiquitous and represent a major health and production concern in sheep farming (Roeber et al., 2014. Vet Parasitol, 205:619-28; Mavrot et al., 2015. Parasit Vectors, 8:557). Given the differences in pathogenicity among these parasites, and current problems regarding anthelmintic resistance, specific diagnosis of GIN is of significant importance (Redman et al., 2019. Vet Parasitol, 275:108933). The identification of third stage larvae (L3) at least to the genus level is currently performed through microscopical analysis (Van Wyk et al., 2013. Onderstepoort J Vet Res, 80: 539; Roeber et al., 2014. Vet Parasitol, 205:619-28). However, substantial variations exist between protocols and morphological keys published in the scientific literature (MAFF, 1986. Manual of veterinary parasitological laboratory techniques. HM Stationery Office, Ministry of Agriculture, Fisheries and Food, London; McMurtry et al., 2000. Vet Parasitol, 90:73-81; Van Wyk et al., 2013. Onderstepoort J Vet Res, 80: 539). Hence, this study aimed to produce a practical and updated guide for the identification of infective ovine GIN larvae.

MATERIALS AND METHODS: A total of 173 GIN L3 from pooled sheep faecal samples (Sardinia, Italy) was morphological identified using existing keys and protocols. DNA was extracted from each L3 and used for molecular identification. All morphological and molecular data were combined to produce the final guide.

RESULTS AND CONCLUSIONS: GIN L3 *Trichostrongylus* spp., *Teladorsagia circumcincta*, *Haemonchus contortus*, *Cooperia curticei*, and *Chabertia ovina* were microscopically and molecularly identified in this study. Overall, 73.5% of larvae were correctly identified based on microscopical analysis and 91.8% were correctly classified into their respective preliminary sheathed tail groups (A: short, B: medium, C: long). A significant difference in sheathed tail length was found between these groups ($P < 0.001$), as well as between *H. contortus* and *C. curticei* ($P < 0.001$) L3. Further differentiation within Group A can be achieved based on the presence of a cranial inflexion, caudal tubercles and full body length measurements (*Trichostrongylus* spp. <720µm, *T. circumcincta* ≥720µm). Larvae in Group B can be differentiated based on sheathed tail morphometry (*H. contortus* >65µm, *C. curticei* ≤65µm), the presence of refractile bodies, full body length measurements (*H. contortus* ≤790µm, *C. curticei* >790µm) and shape of the cranial extremity. Lastly, all traits proposed for the differentiation of *Oesophagostomum* spp. and *C. ovina* larvae (Group C) were found to have considerable restrictions. Moreover, a significant difference in full body length was found between *Trichostrongylus* spp. vs. *T. circumcincta* ($P < 0.001$) and *H. contortus* vs. *C. curticei* ($P < 0.001$) L3. In conclusion, for an accurate microscopical diagnosis is important to examine each larva in its entirety (considering multiple characteristics).

A NEW QPCR APPROACH FOR THE SIMULTANEOUS DETECTION OF *CYTAUXZOON* SPP. AND *HEPATOZOON* SPP. IN FELIDS

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Keywords: *Cytauxzoon*, *Hepatozoon*, qPCR

INTRODUCTION: *Cytauxzoon* spp. and *Hepatozoon* spp. are protozoa responsible of cytauxzoonosis and hepatozoonosis in a wide range of mammals worldwide. Nevertheless, they are still little studied in felids. Molecular assays reported in literature (usually conventional PCR protocols and among them nested-PCRs) are often time- and cost-consuming with different sensitivity/specificity. Real-time quantitative polymerase chain reactions (qPCRs) to detect some piroplasms' species such as *Theileria anulata* in cattle and buffalo (Ros-García et al., 2012. Parasit Vectors, 5:171; Kundave et al., 2014. Trop Biomed, 31:728-35), *Theileria equii* and *Babesia caballi* in horses (Lobanov et al., 2018. Parasit Vectors, 11:125) are reported. Since qPCR protocols targeting simultaneously *Hepatozoon* and *Cytauxzoon* have never been set up, the aim of this study was to develop a new qPCR assay to quickly screen a large number of samples.

MATERIALS AND METHODS: Primers designed by Tabar et al., 2008 (Vet Parasitol, 151: 332-6) were used to amplify a 373 bp region of 18S-rRNA gene of the order Piroplasmida by SYBR green qPCR. Standard curves and limit of detection of the assay were determined by using 5 (i.e. 1, 10, 10^2, 10^3, 10^4 copies/µl) fold dilution series of DNA of *Babesia microti* ATCC isolate, and the specificity tested on a panel of different species of protozoa (ATCC isolates of *B. microti* and *Toxoplasma gondii*, sequenced field samples of *Cytauxzoon europaeus*, *Hepatozoon felis*, *Hepatozoon silvestris*, *Babesia venatorum*, *Babesia caballi*, *Babesia bigemina*, *Leishmania infantum*).
The assay was tested on experimental samples, i.e. whole blood from 206 owned/stray cats and 12 captive exotic felids (i.e. tiger, lion, leopard, caracal), and organs and blood clots of 19 wild cats. Each assay was performed in duplicate. Results were achieved through the melting curve temperature (Tm) analysis.

RESULTS AND CONCLUSIONS: This assay showed high specificity for piroplasms and high sensitivity (limit < than 10 copies/µl). Based on Tm is possible to quickly distinguish *Cytauxzoon* spp. infection from *Hepatozoon* spp. as the results of species-specific temperature peak (i.e. 81°C *C. europaeus*, 78°C *H. felis*, 78.5°C *H. silvestris*).
In addition, the qPCR was able to detect and differentiate some other piroplasms such as *T. gondii* (75°C), *B. venatorum* (79°C), *B. caballi* (80°C), *B. bigemina* (80.5°C), and *B. microti* (81°C). The limit of the study is represented by the same Tm of *C. europaeus* and *B. microti*. This case unavoidably requires a further step of sequencing for the distinction.
Overall, 12 cats were positive to *H. felis*, 19 to *H. silvestris* and 6 to *C. europaeus*, 1 tiger to *H. felis* and 1 to *H. silvestris*, 6 wild cats to *H. felis*, 2 to *H. silvestris* and 3 to *C. europaeus*. All confirmed by conventional PCR and subsequent sequencing. This procedure could represent a useful method to confirm *Cytauxzoon* spp. and *Hepatozoon* spp. infection in felids, to evaluate other potential piroplasms infection, and to quickly screen a large number of samples.

MICROSCOPIC AND MOLECULAR DETECTION OF *AELUROSTRONGYLUS ABSTRUSUS* IN NATURALLY INFECTED CATS

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Keywords: *Aelurostrongylus abstrusus*, PCR, copromicroscopy

INTRODUCTION: *Aelurostrongylus abstrusus*, the feline lungworm, is distributed worldwide and infects bronchioles, alveolar ducts, and alveoli of domestic cats. Feline aelurostrongylosis can vary from subclinical to life-threatening disease. The detection of L1 using the Baermann method is the gold standard for diagnosis, however some molecular assays have demonstrated better diagnostic performance than copromicroscopy. This study evaluated the diagnostic utility of a species-specific, PCR protocol for *A. abstrusus* using different biological samples collected from cats living in various endemic regions.

MATERIALS AND METHODS: One hundred privately-owned cats from Italy (no. 60) and Greece (no. 40) were included in the study. Individual fecal samples were obtained and, for cats from Italy, a pharyngeal swab as well. Fecal samples were examined using flotation and Baermann. Based on the copromicroscopic results, cats were enrolled to create three groups, i.e. Group A (no. 50 cats with *A. abstrusus* infection regardless of positivity for other helminths), Group B (no. 25 cats negative for *A. abstrusus* but positive for at least any other helminth, including other lungworms), Group C (no. 25 cats negative for any helminth). DNA extracts from individual aliquots of feces, floatation supernatant, Baermann's sediment and pharyngeal swabs were subjected to an *A. abstrusus*-specific PCR (Traversa et al., 2008. Parasitol Res, 103: 1191-16). The PCR results were compared with those obtained using Baermann method to determine the sensitivity and specificity, as follows:
Sensitivity: no. cats positive by PCR for *A. abstrusus* / no. cats positive by copromicroscopy for *A. abstrusus*.
Specificity: no. cats negative by PCR for *A. abstrusus* / no. cats negative by copromicroscopy for *A. abstrusus*.

RESULTS AND CONCLUSIONS: Cats infected with *A. abstrusus* in Group A scored either negative (no. 30) or positive (no. 20), with varying percentages, for other helminthes. Cats in Group B were positive for different species of intestinal and and respiratory helminths. At least one fecal aliquot or the pharyngeal swab scored positive by PCR in 48/50 (96%) cats enrolled in Group A. All samples from Groups B and C were PCR-negative, except for 6. The overall sensitivity and specificity of the PCR assay here used were 96% (48/50) and 94% (44/50), respectively. Detailed PCR results are shown in Table 1. These results confirm the high sensitivity and specificity of the PCR and demonstrate that the pharyngeal swab is the most suitable and practical sample for PCR in clinical settings. The use of the species-specific PCR herein investigated may have crucial clinical implications, as it allows detection of *A. abstrusus* DNA in occult/pre-patent infections, as well as in the presence of mixed infections with other endoparasites.

TABLE 1. Overall positivity in a nested PCR species-specific for *Aelurostrongylus abstrusus* of different fecal aliquots and pharyngeal swab collected from cats of the present study.

Group	PCR feces n/tot (%; 95% CI)	PCR flotation n/tot (%; 95% CI)	PCR Baermann n/tot (%; 95% CI)	PCR pharyngeal swab n/tot (%; 95% CI)	Total PCR n/tot (%; 95% CI)
		Italy			
A	16/25 (64; 42.5-82)	15/25 (60; 38.7-78.9)	21/25 (84; 63.9-95.5)	21/25 (84; 63.9-95.5)	24/25 (96; 79.7-99.9)
B	0/15 (0)	1/15 (6.7; 0.2-32)	2/15 (13.3; 1.7-40.5)	0/15 (0)	2/15 (13.3; 1.7-40.5)
C	0/20 (0)	0/20 (0)	0/20 (0)	2/20 (10; 1.2-31.7)	2/20 (10; 1.2-31.7)
		Greece			
A	22/25 (88; 68.8-97.5)	18/25 (72; 50.6-87.9)	20/25 (80; 59.3-93.2)	NA	24/25 (96; 79.7-99.9)
B	1/10 (10; 0.3-44.5)	1/10 (10; 0.3-44.5)	2/10 (20;2.5-55.6)	NA	2/10 (20;2.5-55.6)
C	0/5 (0)	0/5 (0)	0/5 (0)	NA	0/5 (0)
		Total			
A	38/50 (76; 61.8-86.94)	35/50 (70; 55.4-82.1)	41/50 (82; 68.6-91.4)	21/25 (84; 63.9-95.5)	48/50 (96; 86.3-99.5)
B	1/25 (4; 0.1-20.4)	2/25 (8; 1-26)	4/25 (16; 4.5-36.1)	0/15 (0)	4/25 (16; 4.5-36.1)
C	0/25 (0)	0/25 (0)	0/25 (0)	2/20 (10; 1.2-31.7)	2/25 (8; 1-26)

n: number of positive cats; tot: number of examined cats; CI: confidence interval; NA: not applicable, pharyngeal swabs collected only for cats enrolled in Italy.

MOLECULAR CHARACTERIZATION OF *DICTYOCAULUS CERVI* IN RED DEER (*CERVUS ELAPHUS*) IN TWO STUDY AREAS IN THE ITALIAN ALPS

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Keywords: wild ungulate, lungworms, dictyocaulosis

INTRODUCTION: *Dictyocaulus* is a worldwide distributed roundworm genus causing respiratory disease in wild and domestic ruminants. In the latter, infection with fatal bronchopneumonia often occurs, leading to reduced production yields and major economic losses; in cervids, available data on the infection is poor. Species identification is crucial for epidemiological studies and risk assessment, since domestic/wild ruminants cross-infections may occur. Morphological identification of *Dictyocaulus* spp. is challenging, and taxonomy, especially in cervids, is unresolved. Molecular analyses have improved species identification, as for *Dictyocaulus eckerti* (DE) complex. DE was primarily described as a group infecting several cervid species, including red deer *Cervus elaphus*. Separate lineages have been described for DE over the years: e.g., the recently defined species *Dictyocaulus cervi* (DC) in red deer (based on both genetic and morphological features) (Pyziel et al., 2017. J Parasitol, 103:506-18). In cervids, exact species identification could be useful for wildlife management and conservation, as well as for the potential interactions with domestic ruminants during the grazing season. Although dictyocaulosis has been already described in the Italian Alps, information is poor and outdated. The aim of this work was to expand the knowledge on *Dictyocaulus* spp. in red deer from two Italian study areas (Valle d'Aosta – VdA; Lombardy sector of the Stelvio National Park – SNP).

MATERIALS AND METHODS: The dissection of respiratory tracts of 250 individuals (VdA=104; SNP=146) was performed in the culling seasons 2017-19. The collected adult lungworms were morphologically characterized and measured using optical microscopy; PCRs were performed on partial 18S rDNA, ITS2 and cox1 genes for a randomly selected subgroup of specimens (17 from VdA, 21 from SNP). Phylogenetic analyses were inferred based on the obtained gene sequences.

RESULTS AND CONCLUSIONS: Lungworms prevalence was 22% in VdA and 21.9% in SNP; mean abundance of lungworms (±SD) in all the examined animals was 1.2 ± 3.6 and 1.6 ± 5.6, mean intensity of infection (±SD) was 5.6 ± 5.7 and 7.3 ± 8.9 lungworms per infected individual in VdA and SNP respectively. Worms were morphologically compatible with DC. The amplified gene sequences identified 15/17 lungworms from VdA and 20/21 from SNP as DC. The remaining specimens were classified as *Dictyocaulus* spp. and defined as a separate lineage from DC/DE. DC isolates were grouped in two well defined clusters based on cox1 sequences, with SNP isolates closely related to DC, while VdA isolates clustered with putative DE sequences.
These results show the presence of DC in red deer in two distinct areas in the Italian Alps and increases knowledge on dictyocaulosis in red deer in Italy. Further studies should be performed on the detected *Dictyocaulus* spp. isolates to assess possible cross-infections with domestic ruminants and better understand the epidemiology of dictyocaulosis in red deer.

GASTROINTESTINAL HELMINTH FAUNA OF SYNANTHROPIC RODENTS IN EMILIA-ROMAGNA REGION, ITALY

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Keywords: *Rattus* sp., *Mus musculus*, intestinal helminths

INTRODUCTION: Many rodents species have become synanthropic and live in close association with humans, leading to various forms of adverse interactions and playing a significant role in human health, welfare and economy. They play an important role as vectors or reservoirs of various pathogens, including many zoonotic agents. There are limited surveys on helmintofauna of such rodents in Italy (Macchioni, 1966. Ann Fac Med Vet Pisa, 19: 340-51) and recent data are lacking. The aim of this study was to conduct an epidemiological survey of the gastrointestinal helminth fauna of synanthropic rodents present in Emilia-Romagna region.

MATERIALS AND METHODS: In total, the carcasses of 111 synanthropic rodents (43 *Rattus norvegicus*, 27 *R. rattus* and 41 *Mus musculus*) were collected by professional rodent control service from the provinces of Forlì-Cesena and Ravenna. Each gastrointestinal tract was opened lengthwise, and the content was observed under a stereomicroscope after sedimentation and washing steps. Further steps of sedimentation and flotation of the intestinal content were performed for microscopic egg investigation. Collected parasites were fixed in ethanol and morphologically identified. Descriptive statistics was performed on all the different variables considered, and Chi Square test was used to evaluate possible correlations between parasitological results and the origin, host species and sex.

RESULTS AND CONCLUSIONS: On a total of 111 gastrointestinal tracts examined, 72.1% of rodents tested positive for helminths. Concerning *R. rattus*, *Syphacia muris* (59.3%) and *Aspiculuris tetraptera* (55.6%) were the most prevalent nematodes, while *Rodentolepis nana* (11.2%) and *Hymenolepis diminuta* (7.4%) were the most common cestodes. *Brachylaema recurva* was the only trematode, recovered in one animal. *Heterakis spumosa* (60.5%) and *Nippostrongylus braziliensis* (55.8%) were the two prevalent species of nematodes found in *R. norvegicus*, *H. diminuta* (14%) the only one cestode found in this species. The most frequently recovered species in *M. musculus* was *Syphacia obvelata* (39%), while other species such as *Heligmosomoides polygrus* (4.9%), and *Trichuris muris* (2.4%) were found in a limited number of individuals. Helminth eggs were detected in 42.7% of the examined samples, with contrasting results when compared to necropsy findings. Our results confirm the widespread occurrence of parasites such as *Syphacia* sp., *N. braziliensis* and *H. spumosa*, species with a simple and direct life cycle, already reported in our country. To be noted the presence of some parasites with zoonotic concern, such as *S. obvelata* (Deok-Gyu et al., 2015. Korean J Parasitol, 53: 135-39), *R. nana* (Panti-May et al., 2020. Parasitol Int, 75: 102042), *H. diminuta* (Panti-May et al., 2020. Parasitol Res, 119: 1997-2004), which in hygienically degraded environments may represent a risk of human transmission.

SARCOCYSTIS SPP. IN WILD BOAR (*SUS SCROFA*) FROM SOUTHERN ITALY: AN EPIDEMIOLOGICAL AND MOLECULAR SURVEY

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Keywords: sarcocystosis, wildlife, public health

INTRODUCTION: Domestic and wild swine are intermediate hosts of two *Sarcocystis* species: *Sarcocystis miescheriana* (syn. *S. suicanis*) and *S. suihominis*. A third species, named *S. porcifelis*, has been reported, but its presence in pigs has not been confirmed. *S. suihominis* is the only zoonotic species with public health concern (Rosental, 2021. Res Vet Sci, 136: 483-90) and few data are available on human risk of infection in Italy. However, considering the high consumption of wild boar meat (Guardone et al., 2022. Animal, 12: 263), this study aims to carry out an epidemiological survey on *Sarcocystis* spp. in wild boar and to develop a Multiplex PCR assay to identify both *Sarcocystis* spp. (*S. miescheriana* and *S. suihominis*) previously detected in Italy in wild boar (Gazzonis et al., 2019. Parasitol Res, 118:1271–87).

MATERIALS AND METHODS: The study was performed in Campania region on 311 wild boars culled during two hunting seasons (2019–2020). Animals were classified according to the hunting area, sex and age considered predictor factors for positive status. Carcasses were examined by veterinarians involved in the regional project "Piano Emergenza Cinghiali in Campania". Histological examination was performed on 997 tissues (269 oesophagus, 277 diaphragms, 298 hearts and 153 tongues) that were 10% neutral buffered formalin fixed and paraffin wax embedded. Thick sections (4 µm) were stained with haematoxylin and eosin and observed by light microscopy. Positive tissues were confirmed molecularly at the Department of Veterinary Medicine of Turin University. DNA was extracted from a pool of positive tissues for each animal. Specific primers targeting the mtDNA cox1 gene were designed aligning the partial sequences of *S. miescheriana* and *S. suihominis* available in GenBank. The newly designed primers were first tested individually and then combined, until a single common forward primer and two specific reverse primers were obtained.

RESULTS AND CONCLUSIONS: At histological examination, *Sarcocystis* spp. were detected in 251/311 wild boars (80.7%). The sarcocysts measured 90x58 µm, the wall was 2.9±0.86 µm thick with finger-like protrusions on the surface, filled with banana shaped bradyzoites, attributable to *S. miescheriana*. The mean number of sarcocysts was 3.4 (standard deviation = 5.8, variation = 1–55). Statistically significant differences ($p<0.05$) were reported for age and sampled muscle. Adult wild boars were at higher risk of infection; moreover, heart and oesophagus were the most infected tissues.
Multiplex PCR showed that the primers were species specific and able to amplify the target species. One hundred four samples were tested molecularly confirming the positivity to *S. miescheriana*. The high prevalence of *S. miescheriana* reported in this study associated to the low prevalence of *S. suihominis* in wild boars in Italy (Gazzonis et al., 2019. Parasitol Res, 118: 1271–87) suggests the low zoonotic potential of wild boar meat consumption.

Table1. Result of statistical analysis to assess association between *Sarcocystis* spp. infection and exposure variables collected through the study

Variable	Category	N° positive/N° examined	Prevalence % (95% CI)	P value	OR
Hunting area	Avellino	46/62	74.2 (63.3-85.1)	0,37	-
	Benevento	29/38	76.3 (62.8-89.9)		
	Caserta	21/25	84.0 (69.6-98.4)		
	Salerno	155/186	83.3 (78.9-88.7)		
Sex	Male	131/156	84.0 (78.2-89.7)	0,14	-
	Female	120/155	77.4 (70.8-84.0)		
Age Classes	Piglet (<1 year)	19/31	61.3 (44.1-38.8)	**0,01**	REF
	Subadult (1-2 years)	105/129	81.4 (74.7-88.1)		2.76
	Adult (>2 years)	127/151	84.1 (78.3-89.9)		3.34
Muscle	Heart	152/298	51.0 (45.3-56.7)	**<0.05**	1.44
	Oesophagus	119/269	44.2 (38.3-50.2)		1.10
	Diaphragm	116/277	41.9 (36.1-47.7)		REF
	Tongue	47/153	30.7 (23.4-38.0)		0.62

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