# XXXIII

## Congresso Nazionale
## della Società Italiana di Parassitologia
## Padova, 18-21 giugno 2024

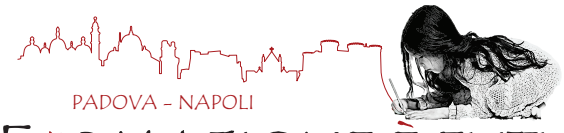
PADOVA - NAPOLI

# FORMAZIONE È FUTURO
## IN PARASSITOLOGIA
## SOIPA 2024

SOIPA
Società Italiana di Parassitologia

# XXXIII

## Congresso Nazionale
## della Società Italiana di Parassitologia



PADOVA - NAPOLI

FORMAZIONE È FUTURO
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Padova, 18-21 giugno 2024
Palazzo del Bo
Best Western Plus Hotel Galileo

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SOIPA 2024

## XXXIII Congresso della Società Italiana di Parassitologia

Padova 18-21 giugno 2024

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Kassiopea group

Via S. Tommaso d'Aquino, 20 - 09134 Cagliari
Tel +39 070 651242
soipa2024@kassiopeagroup.com
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### PROGETTAZIONE GRAFICA

Goldgraphic

Via Gen. F. Pignatelli, 33C - 80141 Napoli
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# INDICE GENERALE



## PLENARY LECTURES



## KEY NOTES



## SYMPOSIA

## ORAL COMMUNICATIONS AND POSTERS

# PRESENTAZIONE

A nome di tutto il Comitato Organizzatore siamo lieti di darvi il benvenuto al **XXXIII Congresso Nazionale della Società Italiana di Parassitologia** (SoIPa) che si terrà a **Padova**, dal **18** al **21 giugno 2024**, tra il **Palazzo del Bo**, sede storica dell'Ateneo patavino situata nel cuore della bellissima città veneta, e il **Best Western Hotel Galileo**, ubicato in prossimità del centro cittadino.

Dopo la positiva esperienza vissuta a Napoli con SoIPa 2022, anche questa edizione vedrà un Comitato organizzatore "multi-universitario", con una squadra giovane, affiatata ed entusiasta di Colleghe/i delle sedi di Padova, Napoli e Udine.

La tematica del Congresso "**Formazione e/è Futuro in Parassitologia**" vuole sottolineare come la formazione delle nuove generazioni rappresenti un immenso valore, dal quale non si può prescindere per promuovere e garantire la trasmissione delle conoscenze, aprendo le porte a nuove scoperte e frontiere nel panorama parassitologico in termini di competenze biologiche, naturalistiche, mediche e veterinarie. Seguendo lo spirito inclusivo della nostra società questo tema conduttore sarà affrontato in modo multi- e inter-disciplinare coinvolgendo esperti nelle diverse branche della parassitologia.

Il programma è molto ricco. Oltre alle **Comunicazioni in sessioni parallele** ci saranno **18 Simposi**, sia accademici sia sponsorizzati da Aziende, su argomenti di grande attualità nell'ambito della parassitologia. Numerosi e prestigiosi saranno gli **invited speakers** provenienti da diverse parti del mondo. Anche per questa edizione sono previsti i **SoIPa Awards**, destinati ai soci giovani iscritti alla SoIPa e messi a disposizione dal Consiglio Direttivo.

Non mancheranno i momenti di confronto sociale e di convivialità, con lo spirito di allegria e amicizia che da sempre caratterizzano i Congressi SoIPa!

Sarà un piacere rivederci tutti a Padova e siamo certi che la vostra attiva partecipazione renderà SoIPa 2024 un'esperienza straordinaria!

A prestissimo

I co-Presidenti del Comitato organizzatore
Antonio Frangipane di Regalbono e Laura Rinaldi

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# MARTEDÌ 18 GIUGNO 2024

*Palazzo del Bo, Aula Magna*

## 16:00 REGISTRAZIONE DEI PARTECIPANTI

## 17:30 CERIMONIA INAUGURALE

### SALUTI ISTITUZIONALI

### PLENARY LECTURES

#### COMMUNICATION STRATEGIES IN PARASITOLOGY

Gioia Capelli[1], Barbara Tiozzo Pezzoli[2]

*[1]Past Health Director at Istituto Zooprofilattico Sperimentale delle Venezie; [2]Communication expert at Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Italy*

#### TRAINING NEEDS FOR THE CONTROL OF NTDs IN ENDEMIC COUNTRIES

Antonio Montresor

*Past Responsible Officer for intestinal parasites at the WHO Department of Neglected Tropical Diseases (Geneva, Switzerland); Adviser at the Ivo de Carneri Foundation (Milan, Italy)*

## 19:00 COCKTAIL DI BENVENUTO

## *Best Western Plus Hotel Galileo*

| Mercoledì 19 giugno 2024 | | |
|---|---|---|
| **Sala Luna** | **Sala Europa** | **Sala Ganimede** |
| **8:30 - 10:30** | | |
| **SIMPOSIO**<br>Studi di valutazione della competenza vettoriale | **SESSIONE COMUNICAZIONI 1**<br>Parasitic infections in farm animals and drug resistance | **SESSIONE COMUNICAZIONI**<br>SoIPa Awards 1 |
| **Coffee Break** | | |
| **11:00 - 13:00** | | |
| **SIMPOSIO**<br>Advances in leishmaniases research: from vector/parasite biology to the disease control | **SIMPOSIO**<br>Extracellular vesicles and circulating RNAs in parasite-host interplay | **SESSIONE COMUNICAZIONI 2**<br>Eco-parasitology and wildlife |
| **Lunch** | | |
| **14:00 - 16:00** | | |
| **SIMPOSIO**<br>Cystic and alveolar echinococcosis: large-scale studies and their impact on public health | **SIMPOSIO VETOQUINOL**<br>Gatti - Parassiti - One Health | **SESSIONE COMUNICAZIONI 3**<br>Parasitic and vector-borne diseases in companion animals |
| **Coffee Break** | | |
| **16:30 - 18:30** | | |
| **SIMPOSIO**<br>Microbial symbiosis in arthropod vectors | **SIMPOSIO**<br>L'invasore non arriva mai da solo: aspetti parassitologici delle invasioni biologiche | **SESSIONE COMUNICAZIONI 4**<br>Vectors and vector-borne diseases 1<br>Key notes Boehringer Ingelheim Animal Health 18:00-18:30 |
| **18:30 Assemblea dei Soci - Sala Luna** | | |

| **20:30 - 22:00** | | |
|---|---|---|
| | **SIMPOSIO BOEHRINGER INGELHEIM ANIMAL HEALTH**<br>A cena con i parassiti: le nuove sfide diagnostiche per il clinico | |

## *Best Western Plus Hotel Galileo*

| Giovedì 20 giugno 2024 | | |
|---|---|---|
| **Sala Luna** | **Sala Europa** | **Sala Ganimede** |
| **8:30 - 10:30** | | |
| **SIMPOSIO**<br>Inside your brain: cosa succede quando i parassiti usano il cervello | **SIMPOSIO**<br>Apicomplexa negletti ed emergenti negli animali d'affezione, da reddito e selvatici | **SESSIONE COMUNICAZIONI**<br>SoIPa Awards 2 |
| **Coffee Break** | | |
| **11:00 - 13:00** | | |
| **SIMPOSIO**<br>La parassitologia umana in Italia: esperienze pluriennali in ambito diagnostico | **SIMPOSIO**<br>Donne in Parassitologia: dalle parassitosi "gender-biased" al soffitto di cristallo | **SESSIONE COMUNICAZIONI 5**<br>Parasites in fish and other aquatic animals |
| **Lunch** | | |
| **14:00 - 16:00** | | |
| **SIMPOSIO**<br>Micologia veterinaria: non solo dermatofiti | **SIMPOSIO MSD**<br>One Health: una salute unica e una sola scienza. L'esperienza della Regione Veneto per la prevenzione e il contrasto delle zoonosi da vettore | **SESSIONE COMUNICAZIONI 6**<br>Zoonosis, medical tropical parasitic diseases and One Health |
| **Coffee Break** | | |
| **16:30 - 18:30** | | |
| **SIMPOSIO**<br>La didattica parassitologica in Italia | **SESSIONE COMUNICAZIONI 7**<br>Zoonoses and One Health | **SESSIONE COMUNICAZIONI 8**<br>Diagnostics, phylogeny and genomics |
| **18:30 Collegio docenti SoIPa - Sala Luna** | | |
| **20:30 Cena sociale** | | |

## *Best Western Plus Hotel Galileo*

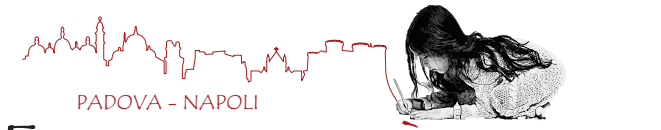
| Venerdì 21 giugno 2024 | | |
|---|---|---|
| **Sala Luna** | **Sala Europa** | **Sala Ganimede** |
| **8:30 - 10:30** | | |
| **SIMPOSIO**<br><br>Artropodi vettori e patogeni trasmessi: l'approccio integrato del progetto PNRR INF-ACT | **SIMPOSIO**<br><br>Parassiti, zoonosi e spillover, nella storia antica e recente di *Homo sapiens* | **SESSIONE COMUNICAZIONI**<br><br>SoIPa Awards 3 |
| **Coffee Break** | | |
| **11:00 - 13:00** | | |
| Presentazione CEVA 11:00-11:10<br><br>**SESSIONE COMUNICAZIONI 9**<br><br>Vectors and vector-borne diseases 2 | **SIMPOSIO**<br><br>Passato, presente e futuro della formazione e della ricerca sulle malattie parassitarie delle api da miele | **SESSIONE COMUNICAZIONI 10**<br><br>Mycotic diseases and alternative methods for parasite control |
| **13:00 Cerimonia di chiusura - Sala Luna** | | |

Simposio in lingua inglese / Symposium held in English

# XXXIII

CONGRESSO NAZIONALE
DELLA SOCIETÀ ITALIANA DI PARASSITOLOGIA
PADOVA, 18-21 GIUGNO 2024

# PLENARY LECTURES



## COMMUNICATION STRATEGIES IN PARASITOLOGY

Capelli G., Tiozzo Pezzoli B.

*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy*

In the last decades, the relationship between science and society has been undermined by risky events that seriously affected man, animals, and the environment compromising people's trust in science. Communicating risk remains a major challenge for institutions and experience has shown there are no proven recipes for its implementation. Nonetheless, research and practice demonstrate that there are some hard and fast principles to follow in order to ensure the good outcome of the communication and build trust. In this presentation, I will briefly introduce the discipline of risk communication and then discuss its pivotal role as a tool for risk prevention and management during emergencies. Examples of communication strategies applied to parasitology will lead us to understand how professionals can be taught and importantly contribute to develop interventions to successfully promote full risk awareness and healthy behaviours in daily life and ensure public health.

# TRAINING NEEDS FOR THE CONTROL OF NTDs IN ENDEMIC COUNTRIES

Montresor A.

*Department of Neglected Tropical Diseases (WHO, Geneva, Switzerland)*

Twenty one parasitic, viral and bacterial diseases are considered neglected tropical diseases by WHO. The common characteristic is that all NTDs are linked to poverty.
All together NTDs are causing morbidity that is in the order of the ones caused by malaria, TB or HIV and this fact enabled to attract interest from ministries of health, researchers and funding agencies, especially considering that NTD control is much more easy and low cost than malaria, TB or HIV.
In the last 10 years, control activities for NTD control rapidly scaled up, but to reach the target identified by WHO for 2030 a number of professional roles should be filled to cover all the aspects of the NTD control.
For the assessment of the epidemiological situation, efficient diagnostic tools are in need but also staff able to organize, conduct surveys and analyse the results. For the design of the control intervention the capacity to manage large quantities of medicines from producer stores until the administration points is needed. For implementation of the interventions personnel able to train volunteers on drug administration, able to manage side effects and organize accompanying measures is needed.
WHO is organizing several training courses for MoH personnel, but the help of collaborating centers and academia is needed to fill the gap.

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# KEY NOTES

PADOVA - NAPOLI

FORMAZIONE È FUTURO
IN PARASSITOLOGIA
SOIPA 2024

# *DIROFILARIA IMMITIS* AND *ANGIOSTRONGYLUS VASORUM* IN CENTRAL ITALY

Traversa D.*[1], Morelli S.[1], Colombo M.[1], Di Cesare A.[1], Astuti C.[1], Diaferia M.[2], Barlaam A.[3], Paoletti B.[1], Maggi R.[4], Totaro G.[5], Falcone A.[6], Veronesi F.[2]

*[1]Department of Veterinary Medicine, University of Teramo, Teramo, Italy; [2]Department of Veterinary Medicine, University of Perugia, Perugia, Italy; [3]Department of Agricultural Sciences, University of Foggia, Foggia, Italy; [4]Vet Practitioner, Roma, Italy; [5]Vet Practitioner, Manfredonia, Italy; [6]Vet Practitioner, Chieti, Italy*

**Keywords:** Epidemiology, Dirofilariosis, Angiostrongylosis.

**INTRODUCTION:** *Dirofilaria immitis* and *Angiostrongylus vasorum* are major nematodes infecting domestic dogs, transmitted by mosquitoes and terrestrial gastropods, respectively. Paratenic hosts may also be source of infections by *A. vasorum*. Adult stages reside in the pulmonary arteries of definitive hosts and cause potentially life-threatening diseases. Several factors (*e.g.* climate changes, conurbation, movements of animals) have recently changed their distribution patterns in European countries, including Italy. A continuous surveillance is crucial for planning proper treatment and prevention measures. The present study has updated current data on the distribution of dirofilariosis and angiostrongylosis in dog populations of central Italy.

**MATERIALS AND METHODS:** A total of 1832 dogs living in central Italy were selected according to purposed inclusion and exclusion criteria: 380, 379, 347, 366 and 360 dogs in Umbria (site A), Marche (site B), Abruzzo (site C), Molise/Northern Puglia (site D) and Lazio (site E), respectively. Data on signalment, anamnesis and clinical signs were recorded from each dog. Individual blood and faecal samples collected with the consent of the owner/responsible were subjected to Knott's and copromicroscopy (Baermann's test and floatation).

**RESULTS AND CONCLUSIONS:** At Knott's test *D. immitis* and/or *Dirofilaria repens* were detected in 30 (1.6%) and 129 dogs (7%): 15 (3.9%) and 68 (17.9%), 4 (1%) and 38 (10%), 2 (0.6%) and 8 (2.3%), 3 (0.8%) and 3 (0.8%), and 6 (1.7%) and 12 (3.3%) in sites A to E, respectively. Sixty-two dogs (3.4%) were positive for *A. vasorum*, *i.e.* 39 (10.3%) in site A, 12 (3.2%) in site B, 6 (1.7%) in site C, 2 (0.5%) in site D and 3 (0.8%) in site E. Larvae of *Strongyloides stercoralis* were found at the Baermann's test of 11 (0.6%) dogs. At floatation, Ancylostomatidae, *Trichuris vulpis* and roundworms were found in 291 (15.9%), 232 (12.7%), and 169 (9.22%) dogs. Taeniid eggs and *Cystoisospora canis* oocysts were found in 4 (0.2%) and 12 (0.65%) dogs. Eggs of the respiratory nematodes *Capillaria aerophila* and *Capillaria boehmi* were detected in 131 (7.1%) and 44 (2.4%) dogs. This study confirms that *D. immitis* is enzootic in regions of central Italy even where until a few years ago it was unexpected or undetected. At the same time, *A. vasorum* occurs often in dog populations of central Italy, as other extra-intestinal nematodes (*i.e. D. repens* and *Capillaria* spp.) that are more frequently detected than in the past. Intestinal nematodes are confirmed to be highly prevalent in dog populations. The present data underlines the importance of routinary diagnostic examinations in dogs living in enzootic areas to early diagnose and treat these potentially fatal parasites. These findings show the importance of a high level of vigilance and indicate the need for appropriate preventive measures both in enzootic areas and in non-enzootic regions or where *D. immitis* and *A. vasorum* are considered unexpected.

# *ANGIOSTRONGYLUS VASORUM* IN THE CLINICAL SETTING

Furlanello T.*

*San Marco Veterinary Clinic and Laboratory, Veggiano, Italy*

**Keywords:** Angiostrongylosis, Coagulation abnormalities, Serum electrophoresis.

Angiostrongylosis poses a significant challenge for clinicians due to its wide array of clinical manifestations. Dogs affected by angiostrongylosis may exhibit various symptoms, spanning from cardiopulmonary issues such as coughing, dyspnea, and syncope to neurological complications like seizures, obtundation, and para- and tetra-paresis, primarily linked to central nervous system hemorrhage. Moreover, overt hemorrhage, such as epistaxis and prolonged post-surgical bleeding, can commonly occur, while some cases may remain subclinical. Uncommon presentations may arise from larval migration, notably in organs like the kidneys or myocardium, or from adult worm development, such as in ocular tissues. Respiratory disease is a predominant feature of angiostrongylosis in dogs, often characterized by verminous pneumonia due to inflammation around L1 larvae penetrating the alveoli. Clinical manifestations range from coughing and dyspnea to chronic fatigue and syncope. Indirect effects, like pulmonary hypertension secondary to vascular changes, further complicate the condition. With the increasing use of ultrasonography in pulmonary diseases, clinicians can detect subpleural pulmonary nodules, which serve as markers for angiostrongylosis, proving more diagnostically significant than the conventional POC test (Venco et al., 2021. Vet J, 271:105649). In clinical practice, coagulative abnormalities are frequently observed, often previously mistaken for rodenticide intoxication. Our experience suggests that these abnormalities stem from hyperfibrinolysis and hyperfibrinogenolysis, leading to severe hypofibrinogenemia (Zoia & Caldin, 2004. J Small Anim Pract, 56:78). Notably, measurement of fibrinogenemia is often overlooked in routine clinical assessments, missing a crucial diagnostic indicator. Serum electrophoresis reveals a notable peak in the beta-3 fraction. This finding slightly contrasts with a previous report of a peak in the beta-2 fraction (Bertazzolo et al., 2022. Vet Clin Pathol, 51:70-76). From our clinical case reviews, angiostrongylosis in dogs commonly presents with coagulation abnormalities, posing diagnostic challenges with vitamin K antagonist intoxication. However, the distinctive coagulative profile, characterized by low fibrinogen levels, sets it apart from the typical hyperfibrinogenemia resulting from inflammation due to intratissutal bleeding. Neurological cases further complicate diagnosis, often resembling other central nervous system vasculopathies. Here again, clinical pathology proves invaluable, aiding in differentiating angiostrongylosis based on coagulation profiles and the electrophoretic pattern. Considering the spectrum of atypical presentations, angiostrongylosis warrants inclusion in the differential diagnosis of complex cases, even those with nonspecific signs such as weight loss, diarrhea, anorexia, lethargy, and exercise intolerance (Colombo et al., 2021. Pathogens, 10:1372).

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# SYMPOSIA

XXXIII

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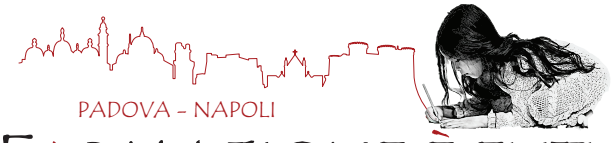
# STUDI DI VALUTAZIONE DELLA COMPETENZA VETTORIALE




FORMAZIONE È FUTURO
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# GUIDELINES FOR EXPERIMENTAL INFECTION OF MOSQUITOES AND *CULICOIDES*

Veronesi E.*

*University of Applied Science and Arts of Southern Switzerland - SUPSI, Bellinzona, Switzerland*

**Keywords**: Arthropod containment, Standard Operating Procedure, BSL3.

Operating within a biocontainment environment necessitates rigorous training and a comprehensive understanding of associated risks when handling pathogens and infected animals. Establishing and operating laboratories dedicated to the infection and manipulation of live arthropods carrying pathogens relevant to animal and human health demands meticulous biocontainment measures to safeguard personnel, prevent pathogen escape into the environment, uphold data integrity by minimising cross-contamination, and ensure standardisation and reproducibility. However, the scarcity of adequately trained personnel proficient in arthropod infection exacerbates the complexity of this endeavour, contributing to the limited number of laboratories equipped for such specialised procedures. Mosquitoes and biting midges (*Culicoides*) rank among the most significant vectors of pathogens capable of causing severe diseases in humans and animals. Assessing their potential to transmit viruses (vector competence) such as Dengue, Zika, Chikungunya, West Nile, Bluetongue, African horse sickness, and others is paramount for evaluating epidemiological risks and implementing measures to prevent epidemic spread or facilitate their control. Various aspects must be considered in experimentation assessing the vector competence of these arthropods, including not only biosafety in their containment and the standardisation of methods used to verify their vectorial role.
This presentation aims to elucidate best practices (Pondeville, et al., 2022. Pathog Glob Health, 117:293-307) and insights for conducting activities involving Risk Group 3 (RG3) pathogens and handling infected live arthropods within a biocontainment facility (CL3/BSL3).

# ARTIFICIAL SYSTEMS FOR TICKS' FEEDING AND INFECTION

Salata C.*

*University of Padova, Department of Molecular Medicine, Padova, Italy*

**Keywords**: *In vitro* feeding, Ticks, Artificial infection.

Ticks are hematophagous arthropods capable of transmitting a high number of infectious agents to humans and animals representing high risk to their health. Rearing ticks in the laboratory is essential to investigate their biology and vector competence to develop new control strategies. However, the research in this field is affected by strict limits including: i) the need of rearing animals for ticks' meal that is expensive and time-consuming, ii) the long time required for the feeding and, iii) the biosafety concerns in the presence of tick-borne pathogens (TBPs), in high biocontainment conditions (Romano et al., 2018. Acta Trop, 183:43-56). Possible solutions to some of the above-mentioned issues are the development of tick's artificial feeding devices and of approaches for artificial infection. The use of devices to artificially feed arthropod vectors would indeed leverage progresses in ticks' biology and in the characterization of TBPs-vector interaction. In the last decades, several devices have been developed. The first method used capillary tubes placed over the tick mouthparts. Several studies have been also conducted on membrane-based strategies that are more effective in mimicking the host. Both natural membranes (such as animal skin) and synthetic membranes (such as silicone membrane) have been developed. However, membrane-based methods require the use of specific stimuli promoting ticks' attachments and feeding (Romano et al., 2018. Acta Trop, 183:43-56). Regarding artificial infection, in addition to the infected animal models used to feed ticks, some animal-free strategies have been investigated. The simplest method is based on the direct injection of pathogens into the tick's body. An alternative approach requires the immersion of the tick in a solution containing the pathogen that is then easily ingested. Finally, the more physiological way to infect ticks is based on the artificial blood meal using capillaries or membrane-based approaches (Romano et al., 2018. Acta Trop, 183:43-56; Bonnet et al., 2012. Acarologia, 52:453-64). In this presentation, the main advantages and drawbacks of these procedures will be discussed. Furthermore, our recent efforts on the development of a new automatic membrane-based fluidic system for tick's feeding will be disclosed.

**Acknowledgements**: This research was supported by EU funding within the MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

# VECTOR COMPETENCE STUDIES ON *CULICOIDES*

Goffredo M.*[1], Foxi C.[2], Quaglia M.[1], Satta G.[2]

*[1]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy; [2]Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy*

**Keywords**: *Culicoides*, Vector competence, Bluetongue.

Incursions of *Culicoides*-borne viruses have occurred in Italy during the last two decades: Bluetongue (BTV) since 2000, Schmallenberg (SBV) since 2011, and recently Epizootic Haemorragic Disease (EHDV) since November 2022. Moreover, other diseases transmitted by *Culicoides* are considered at risk of introduction, such as African horse sickness (AHS). Vector competence is associated with the capability of certain species to: become abundant near susceptible animals simultaneously with virus circulation, test positive for virus detection, become infected through natural or artificial infected blood meals, and transmit the virus via saliva after an extrinsic incubation period. Italy, situated in the middle of the Mediterranean Basin, features a *Culicoides* vector fauna representative of both Southern Europe, where *C. imicola* plays a predominant role, and Central-Northern Europe, where *C. obsoletus*/*scoticus* is more significant. *C. obsoletus* and *C. scoticus* are cryptic species within a complex that can only be distinguished with molecular tools, thus often are grouped together. Apart from these crucial taxa, other species have shown competence for BTV in Italy, being abundant during outbreaks and/or testing positive for virus detection, such as *C. pulicaris*, *C. lupicaris*, *C. newsteadi*, *C. punctatus*, *C. montanus*, and *C. dewulfi*. A similar pattern was observed during the recent incursion of EHDV, with species testing positive for virus detection during outbreaks including *C. imicola*, *C. obsoletus*/*scoticus*, *C. newsteadi*, *C. pulicaris*, and *C. bysta*. Additionally, *C. imicola* and *C. obsoletus*/*scoticus* have been found positive for SBV in the field in Italy. Vector competence was assessed under laboratory conditions, by feeding Italian populations of *C. imicola* and *C. obsoletus*/*scoticus* on artificial blood meals infected with BTV (serotypes 1, 2, 4, 8, 16), SBV, EHDV (serotypes 6 and 8), and AHSV (serotypes 4 and 9). Wild-caught *Culicoides* were collected alive overnight with light traps near livestock, at selected farms based on the abundance of the target species. Midges were acclimatized in the laboratory before subjected to artificial blood feeding, conducted using a membrane or cotton pledgets. After feeding, fully engorged females were sorted and maintained on a sucrose solution in cardboard boxes, and incubated for at least 8 days. Surviving females were identified to species level, age graded, and then tested for virus detection. In recent trials, midges were exposed to honey-soaked FTA® cards after the extrinsic incubation period to evaluate the presence of virus in saliva. This overview of vector competence studies includes observations made during feeding trials, regarding feeding behaviour and abdomen pigmentation of fed midges. We particularly highlight the finding of positive FTA® cards for BTV, offered to orally infected midges after the incubation period, and discuss the perspectives of this method.

# VECTOR COMPETENCE STUDIES OF *PHORTICA* SPP. FOR ZOONOTIC THELAZIASIS

Pombi M.*

*Sapienza Università di Roma, Dipartimento di Sanità Pubblica e Malattie Infettive, Roma, Italy*

**Keywords**: Eyeworm, lachryphagy, *Thelazia*.

Thelaziasis is a vector-borne zoonotic disease caused by nematodes (Spirurida, Thelaziidae) affecting the eyes of mammals and birds, and occasionally humans. This infection can exhibit a range of symptoms from subclinical to severe, typically linked to mechanical damage in the ocular tissue, including conjunctivitis, various discharges, epiphora, keratitis, blepharospasm, blepharitis, lacrimation, photophobia, chemosis, corneal ulceration, and potentially blindness. The species known to cause infections on humans are *Thelazia callipaeda*, *Thelazia californiensis*, and *Thelazia gulosa*. *Thelazia californiensis* commonly affects domestic and wild mammals in the USA, while *T. gulosa*, typically found in cattle, has only recently been reported in human cases (Bradbury et al., 2020. Clin Inf Dis, 70:518-20; Sobotyk et al., 2021. Vet Parasitol, 24:100553). Another Thelaziidae, *Oxyspirura petrowi*, associated with Galliformes and Passeriformes birds, has shown its zoonotic potential with two human cases described in literature so far (Kalyanasundaram et al., 2020. J Parasitol, 106:623-24). However, most human cases are attributed to *T. callipaeda*. This species has become an emerging disease due to its increasing incidence and geographic spread from the Asian continent to non-endemic regions, particularly in European countries (Do Vale et al., 2019. Vet Parasitol, 275:108957). Transmission of these nematodes occurs through the lachryphagous activity of several dipteran vectors, except for *O. petrowi*, which infests birds through the predation of Blattodea and Orthoptera intermediate hosts. Vector species of zoonotic *Thelazia* spp. feed on the lacrimal secretions of hosts, ingesting L1 larvae produced by the adult worms and allowing them to develop into the infective L3 stage. Natural vectors of *T. callipaeda* are the Drosophilidae *Phortica variegata* and *Phortica okadai*, distributed in Europe and Asia, respectively. However, other species have been shown to be competent in laboratory conditions (*i.e. Phortica magna* and *Phortica oldenbergi*), indicating that a broader spectrum of vectors might contribute to the transmission of this eyeworm (Bezerra-Santos et al., 2022. Acta Trop, 233:106565). In contrast to the increasing knowledge about *T. callipaeda* vectors, information on *T. gulosa* and *T. californiensis*, for which several Muscidae such as *Musca* spp. and *Fannia* spp., are described as natural vectors, still lacks laboratory studies demonstrating the relative roles of potential vectors. Laboratory breeding protocols for potentially competent species are essential to understand their epidemiological significance as secondary vectors, which can be non-negligible in specific contexts. Establishing breeding colonies could pave the way for further studies not only to assess their vector competence but also to characterize their life history traits, which are essential for developing novel control strategies and testing vector control products.

# VECTOR COMPETENCE STUDIES OF *AEDES* AND *CULEX* SPECIES FOR DENGUE AND WEST NILE VIRUSES

Fortuna C.*[1], Severini F.[1], Marsili G.[1], Toma L.[1], Mancuso E.[1], Amendola A.[1], Venturi G.[1], Argentini C.[1], Casale F.[1], Bernardini I.[1], Boccolini D.[1], Fiorentini C.[1], Barzon L.[2], Dal Molin E.[2], Hapuarachchi H.C.[3], Montarsi F.[4], Gobbo F.[4], Di Luca M.[1]

*[1]Istituto Superiore di Sanità, Rome, Italy; [2]University of Padova, Padova, Italy; [3]Environmental Health Institute, Singapore, Singapore; [4]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy*

**Keywords**: Vector competence, Arbovirus, Mosquitoes.

**INTRODUCTION:** The role of mosquitoes in transmitting pathogens, including viruses that cause serious human diseases, is of fundamental importance (Weaver et al., 2010. Antiviral Res, 85:328-45). Several species belonging to *Culex* and *Aedes* genera are efficient vectors for several arboviruses. Experimental studies on vector competence are useful for understanding the dynamics of virus transmission and developing effective control strategies. However, these studies must face various challenges that can compromise the validity and interpretation of the results. Therefore, a thorough analysis of the main issues associated with experimental mosquito infections is crucial for a proper interpretation of the obtained data.

**MATERIALS AND METHODS:** To assess their competence in transmitting viruses, we experimentally infected Italian populations of *Aedes albopictus* with dengue virus, while populations of *Culex pipens* and *Aedes koreicus* were exposed to the West Nile virus. Mosquitoes were orally infected through an artificial feeding system and maintained under controlled laboratory conditions. Vector competence was evaluated through a series of parameters, including infection, dissemination, and transmission rates, measured at different time points following infection.

**RESULTS AND CONCLUSIONS:** The results of experimental infections of *Aedes* with dengue virus and *Culex* and *Aedes* with West Nile virus highlighted the high competence of these mosquito populations, confirming their crucial role as vector species in the transmission of these viruses. However, despite efforts to standardize experimental procedures, the intrinsic complexities of mosquito-virus interactions, along with variables related to experimental conditions, continue to pose obstacles to an accurate assessment of vector competence. The long experience gained from conducting these studies allows us to identify and address challenges related to the intrinsic limitations of these experimental systems. Evaluating the vector competence of indigenous and invasive mosquito species in non-endemic countries is of fundamental importance to understand and mitigate the risk of introduction and circulation of mosquito-borne diseases (Medlock et al., 2012. Vector Borne Zoonotic Dis, 12:435-47). This approach is useful for optimizing targeted and effective preventive measures, such as vector control strategies and public health interventions, in order to prevent the spread of pathogens responsible for possible epidemics.

# POTENTIAL ROLE OF CALLIPHORIDAE AS VECTORS FOR MULTIPLE PATHOGENS

Ceglie L.*

*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy*

**Keywords**: Calliphoridae, Pathogens, Vector capacity.

Studies on necrophagous insects that feed directly on cadaver or on fluids released from cadaver during the decay process usually help the pathologists in the determination of the post-mortem interval (PMI). However, they could also provide them with important hints about any possible pathogen present on it at death (Brundage et al., 2016. Vet Pathol, 53:898-909). Among this wide insect population, the Calliphoridae are frequently in the spotlight because of their synanthropic behavior. In fact, sharing the same environment with humans, they may carry pathogens from the cadavers where they are feeding on and contaminate human foodstuff (Sukontason et al., 2006. Parasitol Res, 98:477-481). Besides, American researchers demonstrated that Calliphoridae might play a central role as mechanical vectors of viable *Cryptosporidium* oocysts and *Giardia* cysts not only externally but also internally and among different animal species (Conn et al., 2007. Vector Borne Zoonotic Dis, 7:643-51). In 2019, British researchers described the species of bacteria (*Enterobacteriaceae*, Staphylococci, *Clostridia*, *V. cholerae*, etc.) hosted by flying insects, mainly Calliphoridae, circulating in 7 different U.K. hospitals and collected over a 16-month period (Boiocchi et al., 2019. J Med Entomol, 56:1684-97). With regard to the virus of the African swine fever (ASFV), which is highly resistant to environmental factors included the decomposition process, European scientists detected by qPCR the DNA of ASFV in larvae of *Calliphora vicina*, fed on experimentally ASFV-infected splenic tissues (Forth et al., 2018. Transbound Emerg Dis, 65:e210-e213). In Lithuania, flying insects were sampled nearby both ASFV-affected domestic pig farms and not ASFV-affected farms in ASFV-positive area: viral DNA was detected also in farms not affected by ASFV outbreak, suggesting a possible link between wild boars and domestic pigs (Turèinavièienë et al., 2021. Med Vet Entomol, 35:484-89). Japanese scientists conducted a survey focusing on blowflies, particularly on *Calliphora nigribarbis*, reporting a prevalence of 2.2% for the presence of viral HPAI-RNA on this fly species, collected during the HPAI season and suggesting that these insects should be added as a target of HPAI vector control (Fujita et al., 2024. Sci Rep, 14:10285). Moreover, an American study on the highly resistant canine parvovirus type 2 (CPV-2) analyzed flies collected inside and outside canine facilities, demonstrating that open canine facilities are potentially more affected by CPV-2 outbreaks (Bagshaw et al., 2014. Prev Vet Med, 114:276-84). In conclusion, my report aims at giving an overview on studies about the potential role of Calliphoridae as vectors of different pathogens, with special focus on the preliminary data obtained in a research project, where we tested the hypothesis that necrophagous insects can be used as alternative matrix for CPV-2 detection in advanced decay cadavers.

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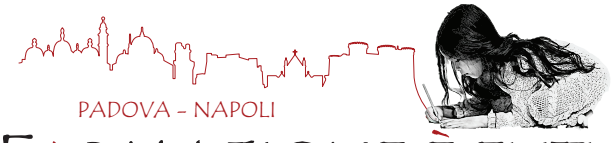
# ADVANCES IN LEISHMANIASES RESEARCH: FROM VECTOR/PARASITE BIOLOGY TO THE DISEASE CONTROL



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# PHLEBOTOMINE SAND FLIES AND BEYOND: CHANGING OUR VIEW OF *LEISHMANIA* VECTORS BY EXPERIMENTAL AND FIELD STUDIES

Dvorak V.*

*Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic*

**Keywords**: Sand fly, Leishmaniasis, Vector.

Sand fly-borne diseases rank among most important diseases transmitted by arthropod vectors and constitute a challenge to both human and veterinary medicine. Phlebotomine sand flies (Diptera: Psychodidae) vector diverse pathogens that include parasitic protozoans of the genus *Leishmania*, bacteria and viruses. Many regions suffer from emerging outbreaks of human leishmaniases, often exacerbated by poverty, natural disasters, political unrest and human migration, and evidence grows that sand fly-borne viruses, many just recently discovered, are of previously underestimated medical significance. However, in contrast to their significance, sand fly-borne diseases remain neglected.
Curiously, despite many decades of research, the knowledge of sand fly fauna diversity and species composition in Europe remains incomplete, as exposed by recent recognition and descriptions of several new species. While the expertise in classic morphology declines, alternative molecular tools emerged that foster conclusive species identification: DNA barcoding, MALDI-TOF protein profiling or wing interferential pattern using deep learning and artificial intelligence. Especially the deployment of mass spectrometry that enables conclusive, time-, labour- and cost-effective species identification of field-collected specimens from large-scale multinational entomological surveys, may provide an effective solution toward sand fly routine identification, albeit its effective dissemination faces several challenges.
Several projects (VectorNet, CLIMOS) have aimed to assess in a wider perspective the current sandfly distribution in Europe. It is generally assumed that ongoing climatic change will significantly affect their geographical range, presumably allowing expansion into nowadays non-endemic regions further north which will pose a risk of subsequent introduction of sand fly-borne pathogens. Field data that document the spread of sand flies are nevertheless so far scarce and the concept of their emergence mainly stems from models based on environmental variables. One of the goals of sand fly research is to compare these modelled predictions with real field data that can then feed epidemiological-climatic predictive mathematical models of realistic human-induced climatic changes scenarios to monitor and mitigate human-sand fly contact. Experimental infections using the laboratory-reared specimens allow testing of vector competence, an irreplaceable first step in understanding potential involvement of tested species in transmission of local as well as newly introduced strains and species of *Leishmania*. Exclusive role of sad flies as vectors of leishmaniases was recently challenged by growing numbers of autochthonous human and animal cases outside the range of their distribution. Circumstantial evidence suggests involvement of other hematophagous vectors, presumably biting midges (Diptera: Ceratopogonidae), potentially changing our understanding of epidemiology of leishmaniases.

# *LEISHMANIA* SPP. IN SAND FLIES, MAMMALS AND REPTILES: NEW EPIDEMIOLOGICAL SCENARIOS

Mendoza-Roldan J.A.*, Otranto D.

*University of Bari "Aldo Moro", Bari, Italy*

**Keywords**: *Leishmania tarentolae*, Mammals, Reptiles.

In the Mediterranean basin, *Leishmania infantum* is the main species causing zoonotic cutaneous and visceral leishmaniasis (Akhoundi et al., 2016. PLoS Negl Trop Dis, 10:e0004349). In the same area there are other species of *Leishmania* such as *Leishmania tarentolae* from the subgenus *Sauroleishmania* (Ntais et al., 2013. Am J Trop Med Hyg, 89:906; Akhoundi et al., 2016. PLoS Negl Trop Dis, 10:e0004349; Mendoza-Roldan et al., 2022. Transbound Emerg Dis, 69:e1326-e1337). *Leishmania tarentolae* has a sympatric distribution with *L. infantum* in the Mediterranean basin (Akhoundi et al., 2016. PLoS Negl Trop Dis, 10:e0004349; Klatt et al., 2019. PLoS Negl Trop Dis, 13:e0007424) 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 (Pozio et al., 1983. Acta Trop, 40:399-400) as well as other squamata reptiles (*i.e.*, lizards and snakes) in Italy (Mendoza-Roldan et al., 2024. PLoS Negl Trop Dis, 18:e0011973), and Morocco (Mendoza-Roldan et al., 2023. PLoS Negl Trop Dis, 17:e0011431). Furthermore, the molecular and serological detection of *L. tarentolae* in blood donors from central Italy (Pombi et al., 2020. Med Vet Entomol, 34:470-475), in inhabitants of the Pelagie archipelago (Iatta et al., 2021. PLoS Negl Trop Dis, 15:e0009817) and in sheltered dogs (Mendoza-Roldan et al., 2021. Parasit Vectors, 14:1-12), 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 (Pombi et al., 2020. Med Vet Entomol, 34:470-75; Abbate et al., 2019. Comp Immunol Microbiol Infect Dis, 67:101374), suggest the possibility of exposure of mammals to *L. tarentolae*. Experimental studies have corroborated the hypothesis that *L. tarentolae* can develop in *Phlebotomus perniciosus* and *P. papatasi* (Ticha et al., 2021. Microorganisms, 9:2256). 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 (Pombi et al., 2020. Med Vet Entomol, 34:470-75; Abbate et al., 2019. Comp Immunol Microbiol Infect Dis, 67:101374; Mendoza-Roldan et al., 2021. Parasit Vectors, 14:1-12). 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.

# INFLUENCE OF CLIMATE DRIVERS AND MITIGATION TOWARDS PHLEBOTOMINAE VECTORS DISTRIBUTION AND THE DISEASES THEY TRANSMIT

Bongiorno G.*[1], De Liberato C.[2], Di Muccio T.[1], Dottori M.[3], Fortuna C.[1], Montarsi F.[4], Mosca A.[5], Oliva G.[6], Satta G.[7], Vitale F.[8], Gradoni L.[1]

*[1]Department of Infectious Diseases, Vector-Borne Diseases Unit, Istituto Superiore di Sanità, Rome, Italy; [2] Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Rome, Italy; [3]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy; [4]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy; [5]Institute for Wood Plants and the Environment - I.P.L.A. S.p.A., Turin, Italy; [6]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy; [7]Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy; [8]National Reference Center for Leishmaniasis (C.Re.Na.L.), Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy*

**Keywords**: Sand flies, *Leishmania infantum*, Toscana virus.

**INTRODUCTION:** Due to climate and environmental drivers, we are assisting an increase and spreading of climate-induced vectors and zoonotic pathogens they transmit. Among them Phlebotomine sand flies (Diptera:Psychodidae) are considered to be expanding their boundaries into territories where they were not considered as endemic and lengthening their seasonal activity period. Being ectothermic insects, their presence are climate constrained, particularly minimum winter temperatures affecting larval survival and cold and rainy summers reducing adult biting period. In Italy they are vectors of zoonotic visceral and cutaneous leishmaniasis caused by *Leishmania infantum* as well as Phleboviruses endemic in our country, among which Toscana Virus, Naples and Sicilian Sand fly Fever and Fermo virus. Therefore, the aim of this study is to report sand fly species distribution, seasonal dynamics and natural infection prevalences.

**MATERIALS AND METHODS:** This longitudinal study reports data of eight Italian regions: Emilia-Romagna, Friuli Venezia Giulia, Latium, Piemonte, Sardinia, Sicily, Tuscany and Veneto from 2017 till 2023. Sampling was performed using CDC and BG-sentinel traps keeping flies frozen pending subsequent analysis. Collected specimens were morphologically identified and molecularly tested by RFLP and RT-PCR for pathogen detection, Leishmania spp. and Toscana Virus respectively. Contextually an IFA Test analysis was performed to clarify epidemiological scenario only in Latium and Sicily regions in 2023.

**RESULTS AND CONCLUSIONS:** Almost 25.000 sand fly specimens were identified representing six out of eight endemic sand fly species present in our territory, showing *Phlebotomus perfiliewi* as the most prevalent one (90%) particularly in Tuscany and Emilia Romagna. Concerning remaining regions, *Ph. perniciosus* was the prevalent species with 45.6% followed by *Ph. perfiliewi* (26.2%), *Sergentomyia minuta* (26.0%), *Ph. mascittii* 1.2%, *Ph. papatasi* 1.0% and *Ph. neglectus* (0.004%). Canine seroprevalence was higher in Sicily than in Latium ($p < 0.0001$), showing an increase in IFAT positive seroprevalences during sand flies activity season. Confirming a greater parasite circulation due vectors presence in the aforementioned activity season. Preliminary results of sand flies pathogens detection highlighted differences among regions for *Leishmania* spp. and Toscana viruses prevalences. The high positivity for *Leishmania* was recorded in Sardinia 1.9% followed by 0.3% in Veneto and 0.2% in Tuscany, mostly associated with *Ph. perniciosus* (1.3%). Otherwise, Toscana virus circulation results showed positivity in Piedmont (0.8%), Sardinia (0.7%). Latium (0.4%) and Tuscany (0.03%). Data concerning sand fly species prevalences confirm *Ph. perniciosus* as the widely distributed in Italian territory, attesting its role as primary Italian vector. Pathogens preliminary analyses improved knowledge of presence and distribution of phlebotomine-borne diseases in the Italian territory.

# MATHEMATICAL MODELS FOR THE ANALYSIS OF EMERGING VECTOR-BORNE PATHOGENS

Ferrari N.*, Fesce E.

*Department of Veterinary Medicine and Animal Sciences, Wildlife Health Lab, Università degli Studi di Milano, Lodi, Italy*

**Keywords**: Vectors, Dynamics of infection, Modelling.

Emerging pathogens are a major health concern, and vector-borne infections play an important role among them. The dynamics of vector-borne pathogens are complex, involving three components; vectors, host species responsible for maintaining the pathogens and possibly target species of interest for the sanitary outcomes. All these three components have distinct dynamics, but for the occurrence of pathogen transmission, these components have to interact. These systems turn out to have therefore complex mechanisms even more, since the several processes are affected differently by extrinsic factors such as the environment. Therefore, the interpretation of field data and prediction are cumbersome. Mathematical models, through the simulation of biological mechanisms, allow the formalization of pathogen transmission between vector and host populations including their biological life cycle and the environmental influence. These models allow thus the analyses of these systems since we quantify the contribution of the processes involved, and we can estimate the unknown number of vector/host compartments of their populations. Moreover, these models provide the opportunity to simulate scenarios as hypothetical effectiveness of alternative intervention strategies. Here, we present worked analyses on models of vector-borne infection, with the aim to disentangle the epidemiological contribution of processes and quantify the effects of control strategies.

# RECENT PROGRESS IN MUCOSAL VACCINATION: HOW TO EXPLOIT A PROTOZOAN PARASITE AS A VEHICLE FOR ANTIGEN DELIVERY

Varotto Boccazzi I.*[1], Molteni R.[1], Zuccotti G.[2], Epis S.[1], Bandi C.[1]

*[1]Department of Biosciences, University of Milan, Milan, Italy; [2]Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy*

**Keywords**: Mucosal vaccines, Vaccine platform, *Leishmania*.

The mucosal immune system represents the first line of defence against numerous infectious diseases, including those caused by respiratory viruses and intestinal parasites, and consequently it plays an important role in infection control. Administration of mucosal vaccines can trigger both cell-mediated immune response and antibody production, both at the mucosal and systemic level. Mucosal vaccines also offer advantages, in terms of the requirements for production and regulatory issues. Therefore, mucosal immunisation is considered a promising tool and a potential alternative to the classical intramuscular/subcutaneous route of administration. In the context of *Leishmania* research, only few studies on mucosal vaccination have been published so far, particularly considering the nasal route as a possible way of administration. Here, the protozoan parasite *Leishmania tarentolae* is proposed as a potential antigen vehicle for mucosal vaccination. *L. tarentolae* is non-pathogenic to mammals, human included. In the last few years this protozoon attracted a great deal of attention for its potential biotechnological applications. Furthermore, considering the propensity of *Leishmania* parasites to target antigen presenting cells (*i.e.* macrophages or dendritic cells), this parasite represents an ideal vaccine vehicle. An overview of different *L. tarentolae* applications in mucosal vaccine research will be highlighted showing examples of the use of this vaccine platform applied to SARS-CoV-2, through *in vitro* and *in vivo* studies, and applied to the pathogenic *Leishmania* species *L. infantum*.

# TYPING OF KINETOPLAST MAXICIRCLE CONSERVED REGIONS REVEALS THE PRESENCE OF PECULIAR *L. INFANTUM* STRAINS IN A HUMAN LEISHMANIASIS OUTBREAK, NORTHERN ITALY

Gritti T.*[1], Rugna G.[2], Carra E.[2], Späth G.F.[3], Bruno F.[4], Castelli G.[4], Reale S.[4], Ortalli M.[1], Morselli S.[1], Guglietta N.[1], Gaspari V.[5], Carrillo Gallego E.[6], Moreno Nuncio F.J.[6], Varani S.[1], Solana Morcillo J.C.[7]

*[1]Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy; [2]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy; [3]Institut Pasteur, Université Paris Cité, INSERM U1201, Unité de Parasitologie moléculaire et Signalisation, Paris, France; [4]Istituto Zooprofilattico Sperimentale della Sicilia, Centro di Referenza Nazionale per le Leishmaniosi (C.Re.Na.L.), WOAH Leishmania Reference Laboratory, Palermo, Italy; [5]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Unit of Dermatology, Bologna, Italy; [6]Instituto de Salud Carlos III, WHO Collaborating Centre for Leishmaniasis, National Center for Microbiology, Majadahonda, Spain; [7]Centro de Investigación Biomédica en Red de Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain*

**Keywords**: *Leishmania infantum*, Phylogenesis, Kinetoplast DNA maxicircles.

**INTRODUCTION:** *Leishmania infantum* is the etiological agent of leishmaniasis in southern Europe and can cause both human visceral leishmaniasis (VL) and localized cutaneous and mucosal forms (tegumentary leishmaniasis, TL) of the disease (Gradoni L, López-Vélez R, Mokni M, 2017. Manual on Case Management and Surveillance of the Leishmaniases in the WHO European Region, World Health Organization, Copenhagen, 2-23). Last decade showed a northward spread of this parasitic infection, with new epidemic foci in non-classical endemic areas, as in the Bologna province, where a multi-annual outbreak of human cases is ongoing (Todeschini et al., 2024. Euro Surveill, 29:2300190). In this study, we employed the complete kinetoplast maxicircle coding region (CR), a powerful phylogenetic tool, to analyze human leishmaniasis isolates from northern Italy by using Next Generation Sequencing (NGS), thus identifying the taxonomic position of the selected strains (Solana et al., 2022. Genes, 13:1070).

**MATERIALS AND METHODS:** *Leishmania* parasites were isolated from tissue biopsies or bone marrow aspirates obtained from TL or VL cases, respectively. Genomic DNA (gDNA) extraction was performed from cultured promastigotes. Library preparation was performed using Nextera DNA CD Indexes, following Nextera DNA Flex Library Prep protocol, sequencing by using V3 cartridges with the Illumina MiSeq platform, and run using a paired-end 300-cycle protocol. Whole genome sequencing (WGS) reads from sequenced isolates and from published *L. infantum* and *L. donovani* strains were used to build the CR sequences. All reads non-aligned against reference *Leishmania* nuclear genome assemblies were collected and quality filtered. *De novo* assembled contigs were selected excluding ones shorter than 500 base-pair (bp) and identified by NCBI-BLAST tool as part of the CR. The CR sequences were assessed by positions of the genes 12S rRNA (start) and ND5 (end) referring to the *L. infantum* reference strain JPCM5 maxicircle annotations. Phylogenetic analysis was conducted on MEGA11, consensus tree and measurement of phylogenetic relationship of the examined taxa was performed by applying maximum likelihood method and Tamura-Nei model, with 1000 replicates.

**RESULTS AND CONCLUSIONS:** NGS data allowed us to assemble a consensus maxicircle CR sequence of around 16000 thousand bp for all isolates. Phylogenetic analysis grouped the strains that were isolated from human leishmaniasis in northern Italy in two divergent clades. The latter were positioned outside the main reference *L. infantum* group, but close to parasitic strains from Cyprus that were previously identified as *L. infantum*/*donovani* hybrids.
These results evidenced the emergence of *L. infantum* strains with peculiar phylogenetic characteristics in human VL and TL cases from an active focus of infection in northern Italy. Future studies will evaluate clinical features and potential risk related to drug resistance of these strains.

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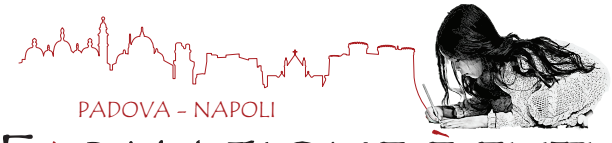
# EXTRACELLULAR VESICLES AND CIRCULATING RNAs IN PARASITE-HOST INTERPLAY




FORMAZIONE È FUTURO
IN PARASSITOLOGIA
SOIPA 2024

# EXTRACELLULAR VESICLE miRNAs IN PLASMA AS BIOMARKERS FOR DISEASE SEVERITY AND COGNITIVE IMPAIRMENT IN CHILDREN WITH SEVERE MALARIA

Cheng I., Combes V.*

*University of Technology Sydney, Sydney, Australia*

**Keywords:** Severe malaria, Extracellular vesicles, microRNA.

Cerebral malaria (CM) and severe malarial anaemia (SMA) are two major complications of *Plasmodium falciparum* severe malaria. Severe malaria results in hundreds of thousands of deaths in children under age five, with survivors at risk of developing significant motor and neurocognitive impairment (NCI). Pathogenesis of severe malaria is not entirely understood, though, among other cellular and soluble factors, increased extracellular vesicle (EV) levels are associated with disease severity in individuals with CM. Currently, there are no reliable markers for early assessment of disease severity; however, recent interest in EV cargo, such as proteins and microRNA, has identified potential biomarkers. This study investigates, for the first time, the complete microRNA profile of plasma EVs by next-generation sequencing (NGS), in children with severe malaria (n total=20, n=4/group: CM with full recovery (FR), CM with fatal outcome (FO), CM with NCI, SMA with FR, and SMA with NCI) and asymptomatic community control children (CC) (n=3). This unbiased approach identified 92 differentially expressed microRNAs and 13 microRNAs of interest. Five miRNAs of interest, hsa-miR-1-3p, hsa-miR-19a, hsa-miR-30, hsa-miR-4516, and hsa-miR-590-3p were verified via real-time quantitative polymerase chain reaction (RT-qPCR) on an additional 25 (n=5/group) children with severe malaria and 5 community children, as differentiating one or more of the 5 disease groups from another and/or from CC. Four out of the five microRNAs have high prognostic potential especially when differentiating the outcomes of CM, specifically children that developed complications and survived from those with fatal outcomes. All five miRNAs have high sensitivity and specificity for the groups they can differentiate and, when combined into a panel, have greater potential as prognostic biomarkers.

# INSIGHTS ON HOST-PARASITE IMMUNOMODULATION MEDIATED BY EXTRACELLULAR VESICLES SHED BY *LEISHMANIA* PARASITES

Weber J.[1], Freitas B.[2], Rodrigues A.[1], Valério-Bolas A.[1], Palma-Marques J.[1], Nunes T.[3], Carvalheiro M.[4], Antunes W.[5], Soares R.P.[6], Alexandre-Pires G.[7], Monteiro De Andrade H.[2], Pereira Da Fonseca I.[7], Santos-Gomes G.*[1]

*[1]Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal; [2]5 Instituto de Ciências Biológicas, ICB, Universidade Federal de Minas Gerais, UFMG - Belo Horizonte, Belo Horizonte, Bouvet Island; [3]Microscopy Center, Faculty of Sciences, University of Lisbon, Lisbon, Portugal, Lisbon, Portugal; [4]Research Institute for Medicines, iMed, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal, Lisbon, Portugal; [5]Unidade Militar Laboratorial de Defesa Biológica e Química (UMLDBQ), Lisboa, Portugal, Lisbon, Portugal; [6]Instituto René Rachou/FIOCRUZ - Belo Horizonte, Brasil, Belo Horizonte, Brazil; [7]CIISA, Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal*

**Keywords:** Cutaneous *Leishmania* spp., Parasite extracellular vesicles, Immunomodulation.

Leishmaniases are anthroponotic and zoonotic diseases of global importance caused by parasites of the genus *Leishmania* that are transmitted to humans and animals by infected sandflies. Depending on the species of *Leishmania*, the host may develop leishmaniasis with cutaneous or visceral clinical presentations. Cutaneous leishmaniasis (CL) is the more common zoonotic infection that can lead to permanent injuries and disfigurement, significantly affecting psychological, social, and economic well-being. Several species of *Leishmania* cause American cutaneous leishmaniasis (ACL). Macrophages (MØs), the definitive host cells of *Leishmania* parasites, are phagocytes that play a crucial role in the innate immune microbial defense and are antigen-presenting cells driving the activation of the acquired immune response. Extracellular vesicles (EVs) constitute a group of heterogeneous cell-derived membranous structures, naturally emitted by all mammal cells, that can modulate the activity of recipient cells. EVs can also be shed by *Leishmania* parasites and may play a role in parasite infection. These nanosized lipid vesicles carry parasite-derived macromolecules, such as glycoprotein of 63kDa (gp63), heat shock proteins (HSP), 14-3-3-like protein, lipophosphoglycan, and elongation factor 1, interfering with the immune activity of host target cells, influencing both innate and adaptive immunity. Considering the eventual importance of EVs in the communication between parasite and host immune cells, our group has been investigating the composition of EVs shed by species of *Leishmania* causing ACL, as is the case of *L. amazonensis*, *L. guyanenesis*, and *L. shawi*, as well as their immunogenic potential. EVs derived from cultured promastigotes of these Leishmania species shed vesicles with a diameter compatible with exosomes and microvesicles. EVs are specifically recognized by antibodies of experimentally infected mice and ACL patients and the cargo seems to include gp63, HSP70, and the proteasome subunit alpha, indicating similarities with previous studies. Moreover, mice MØs rapidly incorporate EVs. Depending on the EVs concentration and the duration of exposure, they can modulate innate immune receptors and induce MØs to express MHC molecules, which can be associated with antigen presentation to T cells. EVs also seem to activate the enzyme arginase of MØs which hydrolyzes arginine to produce urea. The activation of MØs' alternative route leads to increases in the production of urea and ornithine, used in the biosynthesis of polyamines, which is beneficial for the parasite. Thus, EVs from American *Leishmania* spp. that cause CL have the potential to be exploited as disease biomarkers or immunomodulators for the development of efficient prophylactic or therapeutic tools for leishmaniasis.

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# IN-DEPTH COMPARATIVE PROTEOMIC ANALYSIS OF *G. DUODENALIS* SECRETED VESICLES

Moyano S.[1], Balan B.[2], Camerini S.[3], Casella M.[3], Cecchetti S.[3], Bertuccini L.[3], Musso J.[1], Jex A.[2], Touz M. C.[1], Lalle M.*[4]

*[1]Instituto de Investigación Médica Mercedes y Martín Ferreyra, Consejo Nacional de Investigaciones Científicas y Técnicas (INIMEC-CONICET), Universidad Nacional de Córdoba, Córdoba, Argentina; [2]Population Health and Immunity Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; [3]Core Facilities, Istituto Superiore di Sanità, Roma, Italy; [4]Department of Infectious Diseases, Istituto Superiore di Sanità, Roma, Italy*

**Keywords**: *Giardia duodenalis*, Extracellular vesicles (EVs), High-resolution proteomics.

**INTRODUCTION:** The parasitic protist *Giardia duodenalis* is responsible for human and animal giardiasis, a diarrheal disease with a worldwide distribution. The human disease ranges from asymptomatic to acute and chronic diarrhea, eventually causing malnutrition and stunting in children and long-term *post*-infectious sequelae. Two *Giardia* genetic groups (Assemblages A and B) cause human infection. Differences at genomic level between and within Assemblages can account for variations in growth rate, infectivity, and pathogenicity. Extracellular vesicles (EVs) operate as cargoes from cell to cell, carrying proteins and nucleic acids, being implicated in physiological and pathological processes. *Giardia* also releases EVs. This work aimed to isolate EVs from assemblage A and B reference isolates to define and compare EVs proteomes to find a link with differences in assemblage pathogenicity.

**MATERIALS AND METHODS:** Released EVs (exosomes and micro-vesicles) and freely secreted protein (secretome) were purified from *G. duodenalis* Assemblages A (WB) and B (GS) trophozoites using serial ultracentrifugation and a modified synthetic medium. EVs were morphologically and biochemically characterized by electron microscopy and dynamic light scatter (DLS). EVs and secretome were finally characterized by high-resolution mass spectrometry and datasets relative abundance, protein-protein interaction networks and functional enrichment analysis.

**RESULTS AND CONCLUSIONS:** High-resolution mass spectrometry identified over 20% of *Giardia*'s annotated proteome extracellularly, quantifying ~4-fold more proteins than previous *Giardia* extra-cellular proteomic data. Our proteomics study unveiled a conserved extracellular proteome in *Giardia* assemblages, with distinct signatures in assemblages A and B, indicating variant pathophysiology and virulence. Furthermore, we mapped distinct protein signatures of *Giardia* EV sub-populations, namely exosomes and micro-vesicles highlighting ribosome-mediated translation, cilium, integral membrane components, Annexin-like Giardins, and Rab-GTPases crucial for endosome fusion, vesicular trafficking, and exosome secretion. An additional overview of mechanistic markers of ESCRT-dependent, ESCRT-independent, and Ectosomes pathways within the EV proteome was also identified. By exploring "conserved" and "eukaryotic-innovative" compositions in *Giardia* EV proteome relative to archaea EV proteome, we observed complex cellular processes providing insights into the origins of eukaryotic intercellular communication. Collectively, our findings advanced current comprehension of *Giardia*'s protein release dynamics, establishing novel correlations between different *Giardia* Assemblages and diverse extracellular vesicle subtypes. These revelations highlight unique protein signatures inherent to each assemblage, underscoring the complexity and diversity of proteins released by *Giardia*, expanding the scope of our understanding in this field.

# ROLE OF MOSQUITO SALIVA IN VECTOR-HOST-PATHOGEN INTERACTIONS: NOVEL INSIGHTS FROM SALIVARY miRNAs

Arcà B.*[1], Dipaola M.G.[1], Bevivino G.[1], Buezo Montero S.[1], Bertuccini L.[2], Lombardo F.[1]

*[1]Sapienza University of Rome, Department of Public Health and Infectious Diseases, Rome, Italy; [2]Istituto Superiore di Sanità, Core Facilities, Rome, Italy*

**Keywords**: Mosquito saliva, miRNAs, Host manipulation.

Mosquito saliva is a complex mixture of bioactive molecules whose primary role is to help blood meal acquisition in virtue of its antihemostatic, anti-inflammatory and immunomodulatory properties. Moreover, saliva-induced changes at the bite site may facilitate infection and establishment of pathogens as diverse as *Plasmodium* and arboviruses. A quite large number of studies focused on mosquito salivary proteins provided significant insights into complexity and functions of mosquito salivary repertoires; however, only limited attention has been paid so far to extracellular RNAs that are known to circulate in metazoan body fluids, including saliva. Using RNAseq we analyzed small RNAs from saliva and salivary glands of the malaria vector *Anopheles coluzzii* (Arcà et al, 2019. Sci Rep, 9:2955) and the arboviral vector *Aedes aegypti* (Fiorillo et al, 2022. Sci Rep, 12:9536). miRNAs were asymmetrically distributed in both mosquito species, with some enriched in saliva and others in salivary glands, suggesting a non-random occurrence and the existence of some sorting mechanism selectively conveying specific miRNA subsets to saliva. Interestingly, 11 of the most abundant miRNAs in mosquito saliva were identical to human miRNAs targeting genes involved in immune and inflammatory pathways. This was, for example, the case for miR-7-5p, miR-100-5p, miR-92a-3p or miR-200b-5p, which may target components of the NF-kB, mTOR and TLR signaling pathways. These findings suggest that miRNAs from mosquito saliva, possibly enclosed within exosome-like microvesicles, and in concerted action with salivary proteins, may contribute to vertebrate host manipulation, with potential implications for pathogen transmission. Intriguingly, among the 11 mosquito saliva miRNAs mimicking human miRNAs, 6 to 8 were also found in saliva of the evolutionary distant ticks *Ixodes ricinus* (Hackenberg et al., 2017. RNA, 23:1259-69) and *Haemaphisalis longicornus* (Malik et al., 2019. Parasit Vectors, 12:68), and in exosomal miRNAs from the parasitic nematodes *Brugia malayi* (Zamanian et al., 2015 PLoS Negl Trop Dis, 9:e0004069) and *Heligmosomoides polygirus* (Buck et al., 2014. Nat Commun, 5:5488). These observations raise the biologically fascinating hypothesis that mimicking human miRNAs may represent an evolutionary convergent strategy for host manipulation by blood feeding arthropods and parasitic nematodes, a tactic that would allow to exploit a conserved network of host miRNA target sites, as previously suggested for viral miRNAs (Kincaid et al., PLoS Pathog, 8:e1003018). We are currently using artificial feeding for mass collection of mosquito saliva and exosome-like microvesicles purification, which we plan to use for electron microscopy studies as well as for RNAseq, proteomic analyses and functional studies in suitable experimental systems (supported by EU funding within the NextGeneration EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases, Project no. PE00000007, INF-ACT).

# EXTRACELLULAR VESICLES AND HOST-PARASITE INTERACTION IN HUMAN STRONGYLOIDIASIS

Tiberti N.*[1], Deiana M.[1], Manfredi M.[2], Bisoffi Z.[1], Piubelli C.[1], Buonfrate D.[1]

*[1]IRCCS Sacro Cuore Don Calabria Hospital, Department of Infectious, Tropical Diseases and Microbiology, Negrar di Valpolicella, Italy; [2]University of Piemonte Orientale, Department of Translational Medicine, Novara, Italy*

**Keywords**: Human strongyloidiasis, Extracellular vesicles, Proteomics.

Human strongyloidiasis, caused by *Strongyloides stercoralis*, is a neglected tropical disease affecting an estimated 600 million people, particularly in tropical and sub-tropical regions. In humans, *S. stercoralis* infection can persist lifelong due to the peculiar auto-infective cycle. The difficult diagnosis and the limited knowledge of the mechanisms underpinning this chronic infection are key issues in disease management and control. To gain novel insights into the pathogenesis of human strongyloidiasis, we are studying host- and parasite-derived extracellular vesicles (EVs) *ex-vivo* and *in-vitro*. Indeed, EVs represent important players in the host-parasite cross-talk, the investigation of which might reveal novel insights into the mechanisms of maintenance of chronic strongyloidiasis. For the *ex-vivo* experiments, we investigated the systemic modulation in protein expression and serum-EVs induced by chronic strongyloidiasis. Samples (*i.e.*, serum and serum-EVs) from Italians with long-lasting chronic strongyloidiasis collected at diagnosis as well as 6 months post-ivermectin treatment were analysed using untargeted proteomics, together with samples from un-infected control subjects. Quantitative analyses revealed few proteins as significantly modulated in patients at baseline compared to *post*-treatment (n=14) or uninfected controls (n=22). Such results suggest that, at the systemic level, important mechanisms of adaptation might have established for the host to tolerate the chronic presence of *S. stercoralis*, which will be studied more in depth by extending our analyses to subjects with recently acquired infections. *In vitro* investigations were also carried out to explore the local interaction between the parasite and its host. Human intestinal epithelial cells (Caco-2) were exposed to infective larvae (iL3) isolated from a clinical sample or to iL3-derived EVs. Preliminary analyses revealed that both larvae and EVs induce the release of CXCL10 from Caco-2 cells in a time-dependent manner, while iL3 also induce a significant release of IL-8, similarly to the profile reported in patients from endemic areas. The gene and protein expression modulation induced by iL3 and iL3-EVs on human cells will be examined more in depth using 'omics approaches to expand our understanding of host-pathogen interaction in human strongyloidiasis.

# PARASITIC EVs AND ORGANOIDS AS NEW MODEL TO STUDY HOST-PARASITE INTERPLAY DURING ANISAKIASIS

Cavallero S.*[1], Bellini I.[2], Pronio A.[3], Scribano D.[2], Ambrosi C.[4], Rondón S.[2], Chiovoloni C.[2], Pietrantoni A.[5], Kashkanova A.[6], Rinaldi F.[7], Fabiano M.[7], D'Intini E.[7], D'Amelio S.[2]

*[1]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory affiliated to Pasteur Institute - Fondazione Cenci Bolognetti, Rome, Italy, Rome, Italy; [2]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy; [3]Digestive Endoscopy Unit, Department of General Surgery and Surgical Specialties "Paride Stefanini", Sapienza University of Rome, Azienda Policlinico Umberto I, Rome, Italy; [4]Laboratory of Microbiology of Chronic-Neurodegenerative Diseases, San Raffaele Open University, IRCCS, Rome, Italy; [5]Core facilities, Istituto Superiore di Sanità, Rome, Italy; [6]Max Planck Institute for the Science of Light, Erlangen, Germany; [7]Department of Drug Chemistry and Technology, Rome, Italy*

**Keywords**: Anisakiasis, Intestinal organoids, Tumorigenic potential.

Organoids are 3D *in vitro* multicellular model accounting for various differentiated cells able of self-renewal and self-organization, maintaining the functionality and architecture of the tissue of origin (Date and Sato, 2015. Annu Rev Cell Dev Biol, 31:269-289). To date, they represent the most advanced and powerful tool to study physiological and pathological condition of the gastrointestinal tract as well as pathogenesis of infectious agents. In parasitology, intestinal *in vitro* models have been mainly used to explore protozoan parasite infections (Klotz et al., 2012. Int J Med Microbiol, 302:203-209). Considering helminths' size and their intrinsic biological complexity, organoids will allow to shed light into less explored aspects, like the life cycle stages, effect of excretory/secretory factors (including extracellular vesicles EVs), immune response and tumorigenic potential (Duque-Correa et al., 2020. Trends Parasitol, 36:170-181). Animal derived gastrointestinal organoids have been successfully used for live larval nematodes of suitable size (*i.e*. *Trichuris muris*, *Teladorsagia circumcincta*, *Ostertagia ostertagi*) in order to investigate early infection events (Duque-Correa et al., 2022. Nat Commun, 13:1725; Faber et al., 2022. Front Cell Infect Microbiol, 12:904606). For larger parasites, the use of organoids combined with parasitic derived products may be of great support. This is the case of anisakiasis, for which pathogenetic features in common with other helminthiases, like immuno-modulation and potential cancer development, have been suggested (reviewed in Cavallero et al., 2022. Pathogens, 11:285). Emerging evidence supports a role for EVs as important participant in cross-phylum communication and in pathogenicity (White et al., 2023. J Extracell Vesicles, 12:e12298). In this sense, specific methodological guidelines for helminths EVs are needed, to increase knowledge (*i.e.* EVs composition, concentration, the role of internal and external cargo), rigor and reproducibility. In order to explore Anisakis tumorigenic potential, we used parasitic EVs to treat human colonic organoids. The effect was studied using comparative transcriptomics and protein estimation of key factors involved in inflammation, immunomodulation and cancer. Despite the low number of differentially expressed genes obtained between controls and *Anisakis* EV-treated organoids, a potential link to cancer processes related to alterations in cell cycle regulation and apoptosis was detected in all such transcripts. Moreover, two significantly downregulated transcripts emerged as putative target of two *Anisakis* miRNAs enriched in EVs. An overall parasitic strategy based on mitigation of the immune and inflammatory response mediated by *Anisakis* EVs was observed, reinforcing previous data on a general downregulation of type 2 innate response also by the use of miRNAs packaged into EVs, as a potential common strategy of nematodes to survive within their hosts.

# ROLE OF microRNAs FOR DIAGNOSIS AND STAGING OF CYSTIC ECHINOCOCCOSIS

Vola A.[1], Stocchero C.[2], Cialini C.[3], Pea A.[3], Manciulli T.[4], Lissandrin R.[5], D'Alessandro G.[2], Bazzocchi C.*[3], Brunetti E.[6]

*[1]Microbiology and Virology Unit, IRCCS San Matteo Hospital Foundation, Pavia, Italy; [2]Department of Clinical Surgical Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy; [3]Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy; [4]Department of Clinical and Experimental Medicine, University of Florence, Florence, Italy; [5]Infectious Diseases Unit, IRCCS San Matteo Hospital Foundation, Pavia, Italy; [6]Department of Clinical Surgical Diagnostic and Pediatric Sciences, University of Pavia/Immunology and Infectious Diseases, IRCCS San Matteo Hospital Foundation, Pavia, Italy*

**Keywords**: *Echinococcus granulosus*, microRNAs, Tapeworm.

Cystic echinococcosis (CE) is a chronic, complex and neglected helminthic zoonosis caused by the larval stage of the tapeworm *Echinococcus granulosus s.l*. The mainstay of abdominal CE diagnosis and staging is imaging, especially ultrasound (US) (Tamarozzi et al., 2018. Lancet Infect Dis, 18:769-78). The WHO Informal Working Group on Echinococcosis standardized US classification stages cysts into active (CE1 and CE2), transitional (CE3a and CE3b), and inactive (CE4 and CE5). Clinical decision-making in hepatic CE should be based on the WHO classification (Hosch et al., 2008. NMR Biomed, 21:734-54). Serological tests are limited by the lack of antigen standardization, protocols and cross-reactions with other parasites (Vola et al., 2019. Am J Trop Med Hyg, 101:1345-9). In the attempt to develop biological markers of CE to be used in clinical practice, parasite-derived extracellular vesicles (EVs) are currently being investigated. EVs are lipid bilayer-enclosed entities with a size from 30 to 1000 nm in diameter and present in most animal body fluids. EVs contain a variety of bioactive molecules, including lipids, glycans, proteins, microRNAs and DNA (Siles-Lucas et al., 2017. Vet Parasitol, 236, 22-33). In recent years, miRNAs are studied as potential markers in both neoplastic and infectious diseases. However, a clinically unambiguous and valid tool for the diagnosis of CE is not available (Casulli et al., 2020. Trends Parasitol, 36:1-4; Marcilla et al., 2014. J Extracell Vesicles, 3:25040). Previous work showed a potential role of host-derived miRNAs as markers of a specific host immune response against the infection (Cucher et al., 2023. Biology, 12:715), and that host miRNAs might also play a role in *E. multilocularis* development (Ancarola et al., 2020. PLoS, 14:e0008890). With this PRIN-MIUR 2022 project, we aim to investigate the different expression of miRNAs in cystic fluid and sera of patients with active and inactive CE and in the culture medium of isolated protoscolices, to develop marker-based diagnostic blood tests to improve diagnosis, staging and follow-up of CE.

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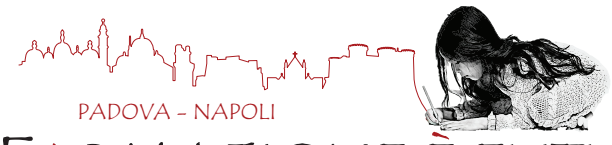
# CYSTIC AND ALVEOLAR ECHINOCOCCOSIS: LARGE-SCALE STUDIES AND THEIR IMPACT ON PUBLIC HEALTH



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SOIPA 2024

# INTERNATIONAL MULTICENTRE STUDIES ON THE DETECTION OF *ECHINOCOCCUS* SPP. IN LETTUCES, BERRIES AND DOG FECES

Umhang G.*

*Anses, Malzeville, France*

**Keywords:** Foodborne contamination, Dog infection, Risk factors.

Several multicenter studies have been conducted at European level and beyond in the MEmE project (One Health EJP consortium). These studies aimed to generate large-scale epidemiological data for the detection of *Echinococcus* spp. in different matrices. Two multicenter studies were conducted to detect *Echinococcus* spp. DNA in food and dog feces. Multicenter Study-1: A multicenter study aimed to evaluate the proportion of lettuces and berries contaminated by *E. multilocularis* (Em) and *E. granulosus s.l.* (Eg) DNA. Specific real-time TaqMan qPCR assays were able to detect DNA from both parasites in pellets obtained after washing and filtrating 1,034 lettuce samples, 300 strawberry batches and 130 blueberry batches from 13 European countries, but also Tunisia and Pakistan. Detection of Em and Eg DNA was in agreement with the known endemic areas of both parasites. The presence of Em DNA was detected in 1.2% of lettuces, 5.4% of strawberries and 7.3% of blueberries in European endemic areas. Additionally, this parasite species DNA was also detected in 56% of blueberries from Pakistan. Regarding Eg DNA, it was detected in 1.3% of lettuces, 1.5% of strawberries and 1.3% of blueberries in European countries where a domestic lifecycle is established but also in 12% of lettuces and 81% of strawberries from Tunisia. This finding is an indirect measure of environmental contamination by these zoonotic parasites. Whether these parasitic DNAs can be linked to the presence of infective eggs, and thus represent a real risk to humans, is currently unknown. Multicenter Study-2: As dogs are considered a potential source of human infection, a multicenter evaluation of the prevalence of dogs infected with *Echinococcus* spp. and other *Taeniidae* species was conducted on 1,619 faecal samples collected from seven countries (Denmark, Estonia, Italy, Finland, France, Norway, and Poland). Parasite DNA was detected in a small proportion of dogs. A global prevalence of 0.2% was obtained for Em with cases only from Poland and Latvia and 1.4% of Eg with cases reported from Estonia, Italy (Sardinia) and Poland. Regarding other *Taeniidae* species, five different species were detected from all countries except Denmark for a global prevalence of 4.0%. Questionnaires were also sent to dog owners to identify potential risk factors. Whether a relatively low prevalence in dogs may correspond to a high risk of infection for humans due to their close contact is currently unknown. The two studies provided data on potential risk factors for human infection with *Echinococcus* spp. for better understanding and prevention.

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# PILOT SURVEY OF CYSTIC ECHINOCOCCOSIS IN MASAAI LIVESTOCK-KEEPING COMMUNITIES OF NORTHERN TANZANIA

Tamarozzi F.*

*IRCCS Sacro Cuore Don Calabria Hospital, Negrar di Valpolicella, Italy*

**Keywords:** *Echinococcus granulosus*, Epidemiology, Northern Tanzania.

Cystic echinococcosis (CE) caused by *Echinococcus granulosus sensu lato* is a zoonosis prioritized for action by the WHO, with prevalence mapping listed among critical actions. Knowledge of the epidemiological situation in sub-Saharan Africa is extremely scant. Maasai communities of northern Tanzania are suffering from a very high prevalence of cerebral coenurosis (*Taenia multiceps/Coenurus cerebralis*), causing high mortality in small ruminants. Given the close similarity between the life cycle of *T. multiceps* and *E. granulosus*, this raises concerns about an increased risk of human CE. We estimated the prevalence of human and livestock *E. granulosus* infection in Maasai communities of northern Tanzania. Abdominal CE was diagnosed by ultrasound in five villages in Longido and Ngorongoro Districts. Infection in ruminants was evaluated through inspection in local abattoirs, followed by molecular identification, using PCR followed by RFLP and Multiplex PCR, and sequencing of non-*E. granulosus* samples. Ultrasound was performed on 823 volunteers. Human CE was diagnosed only in Ngorongoro (n=6; 1.3%); village-level prevalence ranged between 0-2.4%. Of 697 ruminants inspected, 34.4% had parasitic cysts. *E. granulosus s.l.* was identified in 51.4% livestock cysts, from both districts; 87.5% were *E. granulosus sensu stricto* (G1-G3 genotypes), 9.7% *E. ortleppi* (G5), and one *E. canadensis* (G6-10). Multiple *E. granulosus s.l.* species/genotypes are circulating in Maasai communities of northern Tanzania. More precise estimation of prevalence in this area and understanding of specific risk factors for CE among Maasai communities is needed. Interventions targeting transmission routes common to both *E. granulosus* and *T. multiceps* would have dual benefits for preventing both human and livestock diseases.

The paper is published at DOI: 10.1186/s13071-022-05518-x.

# FIRST CASE OF CONFIRMED AUTOCHTHONOUS HUMAN ALVEOLAR ECHINOCOCCOSIS IN ITALY

Tamarozzi F.*

*IRCCS Sacro Cuore Don Calabria Hospital, Negrar di Valpolicella, Italy*

**Keywords:** Alveolar echinococcosis, Italy, Autochthonous human case.

In September 2023, a patient from Alto Adige, Italy, who had never travelled abroad was referred for suspected hepatic cystic echinococcosis. We excluded the diagnosis of CE based on the lesions' morphology on US, which did not show any CE pathognomonic nor compatible features. Five lesions were seen, hypoechoic with irregular margins and fine and tightly packed septations. The lesions were not enhanced on contrast-enhanced imaging. Serology with WB was positive for *Echinococcus* spp., with only genus-specific bands. Further serology showed low antibody titers against crude antigen preparations of *E. multilocularis* and *E. granulosus* in indirect haemagglutination and ELISA. Em18-ELISA was negative. 18F-FDG PET showed light hypermetabolism in delayed acquisitions. Taken together, these results made the lesions strongly suspicious of AE. The only accessible lesion was biopsied and submitted to histology, immunohistochemistry (IHC) and PCR to the Bernhard-Nocht Institute for Tropical Medicine, Hamburg. Histology revealed necrotic granuloma and fibrosis; PCRs targeting cestode cytochrome oxidase and *Echinococcus*-specific 12S rDNA were negative. In contrast, IHC with the monoclonal antibody Em2G11 stained positively *E. multilocularis*, confirming the diagnosis of AE. Staging according to the WHO-IWGE was P2N0M0. The patient is currently receiving albendazole 400 mg BID.To the best of our knowledge, no previous autochthonous confirmed human AE case has been reported in Italy. An expansion of the area of endemicity of *E. multilocularis* in Europe has been observed and predicted by modeling. In Italy, records of infected foxes have been reported for 20 years in the Trentino-Alto Adige region, where autochthonous animal transmission might occur and recent estimates suggest prevalence in foxes is increasing. A survey conducted in 2017 in Liguria first detected *E. multilocularis* in fecal samples of dogs and wolves, suggesting a southward expansion of the parasite. Due to the high lethality of this disease if misdiagnosed and mistreated, it is imperative that physicians, especially of the alpine regions of Italy, become aware of this infection and its possibility also in patients who had never lived or travelled to well-known endemic areas.

The case is published at DOI: 10.3201/eid3002.231527.

# UNVEILING THE HEALTH IMPACT OF HUMAN CYSTIC AND ALVEOLAR ECHINOCOCCOSIS IN EUROPE (1997-2023)

Casulli A.*

*WHO Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis. Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; European Union Reference Laboratory for Parasites (EURL-P). Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*

**Keywords:** *Echinococcus* spp., Cystic and alveolar echinococcosis, Epidemiology.

**INTRODUCTION:** Cystic (CE) and alveolar (AE) echinococcosis affects mainly pastoral and rural communities in both low-income and upper-middle-income countries. In Europe, CE and AE should be regarded as orphan and rare diseases. Although human CE and AE are notifiable parasitic infectious diseases in most European countries, in practice they are largely under-reported by national health systems (Casulli, 2020. Lancet Glob Health, 8:e470-e471).

**MATERIALS AND METHODS:** To fill this gap, we extracted data on the number, incidence, and trend of human cases in Europe through a systematic review approach, using both the scientific and grey literature and accounting for the period of publication from 1997 to 2023. The highest number of possible human cases at the national level was calculated from various data sources to generate a descriptive model of human CE and AE in Europe.

**RESULTS AND CONCLUSIONS:** Regarding CE, we identified around 65,000 human cases from 40 European countries (Casulli et al., 2023. Lancet Infect Dis, 23:e95-e1072). The mean annual incidence from 1997 to 2020 throughout Europe was 0.64 cases per 100,000 people. Based on incidence rates and trends detected in this study, the current epicentre of CE in Europe is in the southeastern European countries, whereas historically endemic European Mediterranean countries have recorded a decrease in the number of cases over the time. Regarding AE, the ongoing research currently identified around 4,000 probable or confirmed human cases from 40 investigated European countries. The mean annual incidence, from the index case after 1997 to 2023 throughout Europe, was 0.06 cases per 100,000 people. Switzerland and Lithuania recorded the highest incidences. Based on incidence rates detected in this study, two major epicentres have been identified in Europe: the Alpine area and the Baltic area. CE and AE in Europe remain a relevant public health issue and findings from this study should be used to support the planning of surveillance and control programmes in Europe according to the WHO 2021-2030 roadmap for Neglected Tropical Diseases (Casulli, 2021. PLoS Negl Trop Dis, 15:e0009373).

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# INTERVENTION STRATEGIES AGAINST CYSTIC ECHINOCOCCOSIS IN THE MEDITERRANEAN AREA: FROM RESEARCH TO ACTION

Rinaldi L.*[1,2], Bosco A.[1,2], Pepe P.[1], Nocerino M.[1], Ciccone E.[1], Lattero N.[1,2], Maurelli M.P.[1], Boué F.[3], Umhang G.[3], Said Y.[4], Sotiraki S.[5], Laatamna A.[6], D'Orilia F.A.[2], Cringoli G.[1]

*[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy; [2]Centro di Riferimento Regionale Sanità Animale (CRESAN), Regione Campania; [3]ANSES, Annency, France; [4]Universite De La Manouba, Sidi Thabet, Tunisia, [5]Veterinary Research Institute, Hellenic Agricultural Organisation Elgo-Dimitra, Thessaloniki, Greece; [6]Ziane Achour University of Djelfa, Algeria*

**Keywords**: *Echinococcus granulosus*, Veterinary public health, Control programme.

Cystic echinococcosis (CE), caused by the larval stage of *Echinococcus granulosus*, is a public health priority due to its worldwide distribution and its impact on human and animal health. CE is on the list of the 20 neglected tropical diseases for which control measures are recommended by the World Health Organization (Casulli et al., 2023. Lancet Infect Dis, 23:e95-e1072). Control programmes against *E. granulosus* are considered long-term public health measures that require an integrated approach, involving various animal and human interventions in the areas of surveillance. Over the years, actions to control CE in the Mediterranean areas have been implemented in various countries, including southern Italy, to reduce the infection rate of the parasite in definitive and intermediate hosts (Cringoli et al., 2021. Vet Parasitol, 290:109347; Ciccone et al., 2024. Parasitology, 151:421-428). In recent decades, intervention strategies against CE have also been implemented in the Campania region of southern Italy to translate scientific studies and innovative research into policy measures in the veterinary sector. These include education of farmers and citizens, diagnosis in ruminants and dogs (*e.g*. ultrasound techniques, Mini-FLOTAC technique, organ inspection at slaughterhouses and microsatellite analysis with FTA cards), surveillance (with geographic information systems, GPS data loggers, camera traps) and control measures (EG95 vaccine, baits laced with praziquantel and drones to treat stray canids). Since 2022, the control of *E. granulosus* in animals in Campania has been officially included as a mandatory task in the annual regional planning document (DPAT) of the veterinary services (AASSLL). In the last two years, thanks to this task (*procedura documentata, CRESAN*), control measures have been carried out on over 200 farms whose animals (sheep, cattle and buffaloes) had tested positive for CE in the slaughterhouses. Over 300 dogs were treated with praziquantel on these ruminant farms and almost 1,000 lambs were vaccinated on the sheep farms. The impact of the newly developed strategies will be evaluated after a few years from the start of the interventions. The intervention strategies implemented in southern Italy were successfully transferred to other endemic areas in Algeria, Tunisia and Greece as part of the Echino-Safe-Med PRIMA project.

# MITOCHONDRIAL GENETIC DIVERSITY OF *ECHINOCOCCUS MULTILOCULARIS* IN EUROPE

Santoro A.*, Santolamazza F., Casulli A.

*Istituto Superiore di Sanità, Rome, Italy*

**Keywords:** *Echinococcus multilocularis*, Europe, Mitochondrial haplotype.

The cestode *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis, a highly fatal zoonotic parasitic disease of the northern hemisphere. Red foxes are the main reservoir hosts and, probably, the main drivers of the geographic spreading of the disease in Europe. Knowledge of genetic relationships among *E. multilocularis* isolates at European scale is a key to understand the historical and current dispersal characteristics of *E. multilocularis*. The genetic diversity of *E. multilocularis* isolates obtained from different hosts in 20 European countries was described. Based on the analysis of complete nucleotide sequences of the cob, atp6, nad2, nad1 and cox1 mitochondrial genes (4,968 bp), 44 haplotypes were inferred over 234 isolates. Four haplotypes (namely H1 to H4) represented 62.82% of the examined isolates (147/234), and one of these four haplotypes was found in each country investigated, except Svalbard (Norway). Mainland Europe appeared to be dominated by two main geographic clusters, one represented by most western-central-eastern European countries, dominated by haplotypes H1-H3, and the second represented by the Baltic countries, North-East Poland and Ukraine, dominated by the haplotype H4. Further comparison made with the cob, nad2 and cox1 global haplotypes (Nakao et al., 2009. Parasitol Int, 58:384-9) unveiled the presence of one Asian-like haplotype identified in Latvia and North-Eastern Poland. The haplotypes identified from the Svalbard archipelago were markedly different from all the other ones, rather resembling similarities with *E. multilocularis* isolates from Yakutia (Russian Far-East) previously characterized (Konyaev et al., 2013. Parasitology, 140:1637-47). Last, to better elucidate the relationships among the most frequent haplotypes we examined the almost full mitogenomes (11,705 bp) of selected isolates. The final comparison suggested that while the overall difference among haplotypes H2, H3 H4 do not increase visibly, haplotype H1 diverged markedly when wider mitogenome portions were observed, posing questions on its historical evolution. Further studies, including endemic regions not investigated in the present study, especially some Eastern European countries, are needed to better clarify the presence of Asian genetic variants of *E. multilocularis* in Europe, and to get a more comprehensive European wide coverage.

# MOLECULAR PHYLOGENETIC ANALYSIS OF *ECHINOCOCCUS GRANULOSUS SENSU LATO* INFECTING SHEEP IN ITALY

Bonelli P.*[1], Serra E.[1], Dei Giudici S.[1], Peruzzu A.[1], Crotti S.[2], Danesi P.[3], Carvelli A.[4], Piseddu T.[1], Masala G.[1]

*[1]Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy; [2]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche Perugia, Italy; [3]Istituto Zooprofilattico Sperimentale della Venezie, Legnaro, Italy; [4]Istituto Zooprofilattico Sperimentale del Lazio della Toscana, Roma, Italy*

**Keywords:** Cystic echinococcosis, *Echinococcus granulosus sensu stricto*, Sheep.

Cystic Echinococcosis (CE), caused by the larval form of *Echinococcus granulosus sensu lato* (*s.l.*), is a neglected zoonosis still threatening public health worldwide. In Italy different epidemiological scenarios were reported depending on the geographical area and associated socio-economic activities. Specifically, in northern Italy, characterized by an intensive livestock production, the occurrence of *E. granulosus sensu stricto* (*s.s.*) is considered sporadic. On the contrary, in the southern regions, where 90% of the entire Italian sheep population is extensively raised, high prevalence of CE in humans and livestock has been recorded. Apart from *E. granulosus s.s.*, a very small number of infections caused by *E. equinus* in horses (Varcasia et al., 2008. Parasitol Res, 102:815-818), *E. ortleppi* in bovines (Casulli et al., 2008. Vet Parasitol, 155:168-172) and *E. canadensis* in domestic pigs and wild boar (Genchi et al., 2021. Vet Parasitol, 23:100536; Laurimäe et al., 2019. Parasitol Res, 118:2193-2201; Varcasia et al., 2006. Parasitol Res, 98:273-277) have been described in Italy. We analysed CE cysts collected from infected sheep from various Italian regions, with the main objective to investigate intergenotypic and intragenotypic variations at national level. CE cysts were collected from slaughtered sheep following post-mortem inspection at local abattoirs. Total genomic DNA was extracted and fragments of the mitochondrial genes cox1 (691 bp) and nad5 (670 bp) were amplified and sequenced. Molecular typing based on cox1 sequences was performed by BLAST analysis against the NCBI database to identify species within *E. granulosus s.l.* complex. Bayesian evolutionary analysis of a nad5 dataset (n=260) composed of *E. granulosus* samples from this study (n=126) and all the nad5 haplotypes available in GenBank (n=134) was carried out. In addition, haplotype network, population diversity indices and neutrality tests were estimated on cox1 and nad5 sequences of Italian origin. *E. granulosus s.s.* was found to be the only *Echinococcus* species infecting sheep in Italy, mainly represented by G1 genotype (76%) and, to a lower extent, G3 genotype (24%). The phylogenetic tree inferred from nad5 dataset identified two well-resolved clades represented by *E. granulosus s.s.* G1 and G3 genotype. Network analyses revealed 40 nad5 and 33 cox1 haplotypes, and the presence of two founder haplotypes, belonging to G1 and G3 genotypes, showing 100% similarity with DNA sequences from different geographic areas. As a matter of fact, low genetic differentiation between Italian regions and between Italy and other distant countries was observed. The lack of geographical segregation, high haplotype and low nucleotide diversity coupled with significant negative values of Tajima's D and Fu's Fs tests observed in this study indicated high genetic variation and demographic expansion of *E. granulosus s.s.* in the country.

# EDUTAINMENT AND COMMUNICATION PROJECTS TO INCREASE AWARENESS ON CYSTIC ECHINOCOCCOSIS AND OTHER ZOONOSIS IN CHILDREN AND PUBLIC OPINION

Varcasia A.*[1], Cavallo L.[1], Abbas I.[1], Nonnis F.[1], Porcu F.[1], Zeinoun P.[1], Tamponi C.[1], De Luca I.[2], Brianti E.[2], Cantacessi C.[3], Scala A.[1], Gaglio G.[2]

*[1]University of Sassari, Department of Veterinary Medicine, Sassari, Italy; [2]University of Messina, Department of Veterinary Science, Messina, Italy, [3]University of Cambridge, Department of Veterinary Medicine, Cambridge, United Kingdom*

**Keywords:** Children, Edutainment, Zoonosis.

The integration of health education (HE) into school programmes is recommended by the WHO as it is widely ascertained that behaviors harmful to health perpetuated from early childhood can persist into adulthood (Eckert et al., 2001. World Organisation for Animal Health, 2001). Within HE, edutainment (= educational and entertainment) integrates pedagogy, education, and entertainment using an interdisciplinary approach, taking advantage of the assistance provided by teaching aids such as educational videos, cartoons and comics to communicate complex topics in a simple and effective way. Traditionally, scientific communication has been based on the knowledge deficit model, a top-down approach according to which information is provided to fill knowledge gaps on the assumption that people, once understood, are able to act rationally (Simis et al., 2016. Public Underst Sci, 25:400). However, this model has proven to be highly ineffective as human beings are characterized by different processes of reasoning that influence the perception of information and decision making. Therefore, presenting information in a way that leads to awareness and change of behaviors is highly warranted. Characters and stories need to be engaging for children to motivate them to maintain and replicate the behaviors implemented by the characters themselves. In particular, children identify themselves with characters through various cognitive processes, such as parasocial interactions and relationships. In addition, children establish dialogues and friendships with the characters they interact with and learn more as they become increasingly familiar with them. Within this framework, from 2019 to 2024 our research group has worked on several projects using edutainment to make research and public engagement for zoonosis prevention. The first project “Fight the parasite” was carried out for Cystic Echinococcosis (CE) prevention and involved 14 primary schools and a total of 896 schoolchildren of Sardinia Island providing 22.8% increase in CE awareness. The project developed also multilanguage teaching tools under the Creative Commons Attribution 4.0 International License, that are open access (Porcu et al., 2022. Parasit Vectors, 15:449). In 2023, we develop a PRIN PNRR project named “Carefree with our pets”, in which we develop an upgraded protocol to extend it from CE to other parasitic zoonoses transmitted by pets. The project aims to raise awareness in elementary schoolchildren of Sardinia and Sicily, through school-based participatory health education for zoonosis control, with an edutainment approach. To date, the ongoing project engaged a total of 900 children of both islands in line with the strategic emerging topic Human wellbeing of PRIN.

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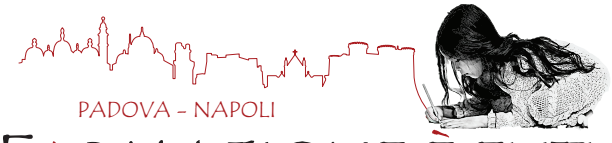
# GATTI - PARASSITI - ONE HEALTH



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# PARASITES IN CATS: ERRORS AND HORRORS IN CLINICAL PRACTICE

Venco L.*

*Ospedale Veterinario Città di Pavia, Pavia, Italy*

**Keywords:** Clinical parasitology, Faecal examination, Parasites.

Parasitology plays an extremely important area in internal medicine in terms of frequency and variety of pathologies induced by parasites. Private practitioners, however, are not familiar with diagnostic investigations, particularly those based on faecal examination, often relegating this type of investigation to students or unqualified personnel, and with the biological cycle of parasites. This comes from the fact that Parasitologists are seen as a person who works in a laboratory, mainly with a microscope and have no knowledge of the clinical aspects of medicine. Clinical Parasitology is therefore a branch not considered, both at University level and at European College level (less than 1% of graduates carry out clinical activity in the field). From all this it follows that the diagnostic sensitivity, due to the scarcity of flotation solutions used, or because they are inadequately prepared, is poor and the microscopic investigations that should be carried out “in clinic” are delegated to external laboratories, often favouring sophisticated investigations (PCR) which have lower performances than those of a microscopic examination performed correctly with a technique suitable for the parasite that should be diagnosed. To overcome all this, the only possibility is that Clinical parasitology takes on equal dignity with other sectors of internal medicine (dermatology, cardiology, and so on).

# CATS AND BROAD-SPECTRUM PARASITICIDES

Traversa D.*

*University of Teramo, Teramo, Italy*

**Keywords:** Endoparasites, Ectoparasites, Treatment.

Internal and external parasites impair health and welfare of cats throughout their life. Cats become infected/infested by direct contact, vectors, ingestion of animals, vertical transmission, or environmental exposure. The “ideal” parasiticide that suits every cat does not exist. The choice of a parasiticide requires a case-by-case evaluation relying on epidemiological scenario, age, lifestyle and compliance of cats. Other key factors are spectrum, persistence and duration of efficacy, speed/onset of action, safety. Understanding the type of owner is of importance to select the most appropriate parasiticide administration. Monoproducts or formulations with narrow activity are used for focused therapies or restricted control programs, while the management of cats with or at risk of mixed infections/infestations should rely on broad-spectrum products. Formulations effective against endoparasites contain different parasiticide classes, *e.g.* depsipeptides, imidazothiazoles, pro-benzimidazoles, tetrahydropyrimidines and isoquinolines. Broad-spectrum formulations for internal parasites and some ectoparasites may contain macrocyclic lactones (ML), while those with efficacy against ectoparasites may include fenilpirazoles, sesquiterpenoids, neonicotinoids, isoxazolines (-aner) and bispirazoles (tigolaner). Broad-spectrum formulations used for routine management do not prevent infections by intestinal nematodes, but they act with a deworming activity. Emodepside interrupts vertical transmission of cat roundworms and is effective against lungworms. Formulations containing a ML are used *vs.* intestinal and some extra-intestinal parasites, and other endo- and ecto-parasites. Praziquantel is effective against tapeworms, including *Dipylidium caninum*. Products containing ectoparasiticides provide control against (re-)infestations caused by different ectoparasites (mainly ticks and fleas) from weeks to months, depending on the molecule and formulation. When long-term control strategies are planned, the duration of action of novel ectoparasiticides (such as isoxazolines or bispyrazoles) should be taken into account. Mixed feline parasitosis occur often in those animals that are allowed to free roam. Several parasites can be transmitted through predation and consumption of small animals, like reptiles, mice, and birds. Moreover, cats with partial or full outdoor lifestyle may acquire ectoparasites and transmitted pathogens, soil-transmitted nematodes, and protozoal infections. Although indoor cats are often perceived as being less exposed to parasitic infections compared to their outdoor counterparts, they are still susceptible to many parasitic diseases. Broad spectrum formulations are useful for indoor cats as well, and veterinarians should consider their usefulness in these patients. Hence, parasite control and management should be planned on an individual basis, with the most appropriate selection of parasiticides for each single cat.

# THE ROLE OF CATS IN HUMAN VECTOR-BORNE DISEASES

Veronesi F.*

*University of Perugia, Department of Veterinary Medicine, Perugia, Italy*

**Keywords**: Cats, Zoonosis, Vector-borne pathogens.

Many infectious diseases exhibiting a zoonotic potential can be carried by cats, nevertheless the global and public health impact of the spread of these pathogens received historically a overall less attention than in dogs. A common misconception is that cats are less infested with blood-feeding periodic (ticks, fleas) and temporary vectors (mosquitoes, sandflies, flies) compared to dogs; also, the veterinarians appear relatively less unaware about the global importance of feline vector-borne diseases (FVBDs) (Hegarty et al., 2015. Parasit Vectors, 8:320). However, vector-borne pathogens (VBPs), both non-zoonotic and zoonotic, commonly occur in cats and many of the factors responsable for these emerging infectious in dogs are also relevant for cats (Lappin et al., 2018. Vet Parasitol, 254:26-29). The relevance and relative risk for each pathogen may vary widely, due to differences in the geographic range and habitat preferences of the respective vectors and significant differences in prevalence levels are known based on the cat's lifestyle (Morelli et al., 2019. CIMID 66, 101344). Most of the FVBPs having zoonoses potential consisted of bacteria transmitted by fleas and, among these, the pathogens of the *Bartonella* genus (*e.g. B. henselae*, *B. clarridgeiae* and *B. koehlerae*) are arguably the vector-borne zoonotic diseases of greatest current global interest. Cats might act as reservoirs and they are commonly referred to as "stealth organisms" because subclinical infection is common (Maggi et al., 2022. Parasit Vectors, 15:415). Further emerging flea-borne pathogens include *Rickettsia felis*, the causal agent of cat flea typhus or flea-borne spotted fever (FBSF) in humans and *Yersinia pestis* (Richter et al., 2002. Emerg Infect Dis, 8:207-208; Gage et al., 2000. Clin Infect Dis, 30:893-900). In the field of parasitic diseases, the vector-borne zoonotic protozoa affecting cats include *Leishmania infantum*, the agent of the human zoonotic visceral leishmaniasis and cat has been proved to be secondary hosts, *Trypanosoma cruzi*, the agent of American trypanosomosis and also other *Trypanosoma* spp. (*e.g*. *T. evansi*, *T. brucei brucei* and *T. congolense*) have been reported in cats. Cats can also be infected with various species of helminthes of zoonotic concern, including the cosmopolitan tapeworm *Dipylidium caninum*, the heartworm *Dirofilaria immitis*, responsible for unifocal or multifocal pulmonary nodules in humans, *Dirofilaria repens* which can cause subcutaneous nodular lesions (Maggi et al., 2022. Parasit Vectors, 15:415), *Thelazia callipaeda* and *Thelazia californiensis*, which may cause human thelaziosis with conjunctivitis, keratitis and corneal ulcers. For these two last pathogens, cats have been proven to represent an important reservoir for human infections (Spoerel et al., 2020. Parasitol Res, 119:3099-3104). The veterinarians should emphasize to cat owners and handlers the importance of vector prevention to limit zoonotic risk of VBPs transmission.

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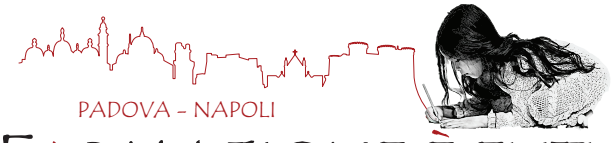
# MICROBIAL SYMBIOSIS IN ARTHROPOD VECTORS



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# LAYERS OF IMMUNITY: DECONSTRUCTING THE *DROSOPHILA* EFFECTOR RESPONSE

Lemaitre B.*

*Global Health Institute, EPFL, Lausanne, Switzerland*

Following decades of neglect where adaptive immunity captured most of the attention, innate immune mechanisms have become central to our understanding of immunology. However, the recent emphasis on innate immunity has focused on the first two phases of the immune response: recognition and signaling. In contrast, the contribution of immune effectors individually or collectively to host resistance has not yet been investigated to the same extent. We are currently dissecting the *Drosophila* innate immune response with a focus on effectors. Since immune effectors are members of multigene families, their function cannot be adequately addressed by the single mutant approach that still prevails today. Thus, we are generating flies carrying single and multiple mutations of immune effectors in a defined genetic background, which will allow comparative analysis of gene function either individually or collectively at the level of gene families. Our study will be done both at the level of individual effectors and immune modules. Recently, we have characterized how antimicrobial peptides individually or collectively contribute to host defense. Our studies reveal an unexpected level of specificality at the effector level as a single antimicrobial peptide can determine survival or death to a defined pathogen. We have also identified a family of stress proteins that protect the host against antimicrobial peptides increasing their specificity. We also address the role of immune effectors beyond immunity in contexts that have been implied but not well demonstrated, notably in the control of the gut microbiota and the elimination of tumor cells, neurodegeneration, and aging. By deciphering how immune effectors combat infectious microbes and impact non-immune processes, our work will illuminate critical aspects of *Drosophila* host defense and will be instrumental in comprehension of innate immunity in general.

# THE HISTORY OF *WOLBACHIA*: CHARACTERS, DISCOVERIES, AND ANECDOTES, FROM THE CONTROL OF VECTOR-BORNE VIRUSES TO THE CURE OF FILARIAL DISEASES

Bandi C.*

*Department of Biosciences, University of Milan, Milan, Italy*

**Keywords:** Symbiosis, Dengue control, Filariasis therapy.

September 1993 Montpellier, European Evolutionary Congress. Richard Stouthamer and Hans Breeuwer had just published a paper in Nature on *Wolbachia*. Senior author: Jack Werren. I approached Richard and Hans and asked whether my work on cockroach bacteria was worth to be sent to a high-rank journal. Richard gave me a brief lesson on *Wolbachia*. Three types of reproductive alterations in arthropods: males capable to sterilize females; parthenogenesis; feminization of genetic males. All these alterations caused by microorganisms. One single bacterium causing the three alterations: *Wolbachia*! I realized that *Wolbachia* was going to blast a new research area. There was indeed some excitement around *Wolbachia* at the congress. French groups presented their work (Solignac, Bouchon, Rousset, others), published in 1992. That 1992 had also witnessed another seminal paper, by O'Neill's group. The congress passed and months dragged by. I was just a bystander in this ongoing explosion of *Wolbachia* research. Reading on symbiosis, I came across papers on possible intracellular bacteria in filarial nematodes, published during the '70s. I thus examined adult females of the dog filaria, *Dirofilaria immitis* (thanks to my mentors Claudio Genchi and Luciano Sacchi) looking for intracellular bacteria. The bacteria were there, sound and abundant, in the ovaries, embryos and body wall of the nematodes. I went to 16S PCR identification: the bugs were closely related to *Wolbachia*! I published this first evidence for *Wolbachia* in nematodes, in 1995. Then my PhD period passed by, and my salary with it. But I started to receive e-mails and letters, form the big shots from both the *Wolbachia* and filaria fields. *Wolbachia* was going to blast also in the filarial nematode area. Within a few years we realized that most of the filarial nematodes that cause disease in humans are infected by *Wolbachia*. And the focus on *Wolbachia* provided a novel type of target to cure these infections: *Wolbachia* itself. In this short personal account, I will mention just the contributions of the groups in Liverpool and Hamburg, and a few seminal studies in that period, on the use of *Wolbachia* as a target for the therapy of filarial infections. *Wolbachia* then flourished further. Seminal basic-science studies include those on lateral gene transfer from *Wolbachia* to the host genome, the discovery of male-killing *Wolbachia*, and many others. On the side of application, I remember when Scott O'Neill, at a *Wolbachia* meeting in Crete, presented his idea of exploiting life shortening *Wolbachia* to reduce the transmission of dengue by *Aedes* mosquitoes. It seemed a crazy idea, but then the project developed, switching to "normal" *Wolbachia*, and took the wind towards field applications. On the side of filarial nematode control, the project launched and coordinated by Mark Taylor, on the anti-symbiotic chemotherapy for filariasis control, represented another amazing development in *Wolbachia* (and filaria) research.

# DETERMINATION OF THE IMMUNOSTIMULATORY ROLE OF *ASAIA* IN *AEDES AEGYPTI*: A POTENTIAL SYMBIONT-BASED CONTROL APPROACH?

Sorana S.*[1], Cappelli A.[2], Damiani C.[2], Catapano P.L.[1], Ricci I.[2], Favia G.[2]

*[1]School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy, [2]School of Biosciences and Veterinary Medicine, University of Camerino, CIRM Italian Malaria Network, Camerino, Italy*

**Keywords:** Mosquito-borne diseases, Immunity, Symbiotic control.

Recurring outbreaks of arboviruses such as dengue on a global scale, it is imperative to adopt multi-faceted strategies to limit the transmission of mosquito-borne diseases (MBDs). Insecticide and drug resistance, coupled with the absence of effective vaccines, presents a significant obstacle in combating MBDs. Recent years have seen a deeper understanding of mosquito microbiota, revealing its role in different traits of the mosquito biology such as sexual reproduction, development, nutrition, and resistance to pathogens. As a reflection of this new knowledge, exploitation of symbiotic bacteria of vector mosquitoes has emerged as a potential control-strategy of MBDs. For instance, the symbiotic bacterium *Asaia* has been shown to activate immune genes in different insect hosts including *Anopheles* where it elicits an anti-plasmodium response (Gonnella et al., 2019. Front Physiol, 10:795; Cappelli et al., 2019. Front Genet, 10:836). The present study investigates the effect of *Asaia* on the immune system of a main vector of arboviruses such as *Ae. aegypti*. A laboratory strain of *Ae. aegypti* (New Orleans 2011) was reared at standard conditions. The experimental set up included three groups of female mosquitoes (a, b and c). Groups a and b received different dietary boosts of *Asaia*, respectively 105 cell/ml and 108 cells/mL while the control group c received a normal diet. Half of the mosquitoes per each group were feed with blood meal and collected daily for 5 days post feeding. The *Asaia* amount was monitored in all the tested samples by qPCR. The expression of transcription factors (Rel 1, Rel 2) and effectors genes of IMD and Toll (Cecropin A, Defensin C, Gambicin and C-type Lectin) cascades together with two genes (Heme peroxidase 7, Superoxide dismutase) codifying enzymes involved in the degradation of Reactive oxygen Species (ROS) were evaluated by qPCR. Moreover, the effect of *Asaia* supplementation on the microbiota composition was assessed through 16S MiSeq analysis. Outcomes suggest that the analysed antimicrobial peptide genes and transcription factors are not affected by *Asaia* overloads, nonetheless the expression of two ROS genes increased concurrently with the proliferation of the bacterium on the second day post-blood meal. These observations need to be corroborated by further analysis for quantifying specific metabolites associated with oxidative stress. Microbiota analysis indicates a marked proliferation of *Asaia* following blood-feeding, emerging as the predominant bacterium. Other symbiont such as *Pantoea* exhibited a modest increase in abundance and *Pseudomonas* experienced a sharp decrease. Exploring the *Asaia-Ae. aegypti* system to investigate the influence of symbiotic bacteria on stimulating the mosquito immune response against arboviruses, holds potential. This could pave the way for the development of symbiotic-based interventions that can complement existing approaches in the field.

# THE FORCE WITHIN: *MIDICHLORIA* SYMBIONTS OF HARD TICKS

Sassera D.*

*Università di Pavia, Pavia, Italy*

**Keywords:** Ticks, Symbiosis, *Ixodes ricinus*.

**INTRODUCTION:** Ticks, as obligate hematophagous parasites, rely on bacterial symbionts to provide nutrients deficient in their diet. Different tick species established mutualistic relationships with various bacterial species through their evolution.
*Midichloria* bacteria (Rickettsiales), initially identified in *Ixodes ricinus*, have been found to act as symbionts in numerous other hard ticks. As most other Rickettsiales, *Midichloria* are intracellular bacteria, but differently from their relatives, they inhabit not just the cellular cytoplasm, they can also colonize the mitochondria within their hosts' cells. Recent efforts have been focused towards understanding the mechanisms of this unique interaction.

**MATERIALS AND METHODS:** Utilizing 3D-electron microscopy, a thorough examination of the colonized mitochondria's structure was conducted. This confirmed *Midichloria*'s positioning between the outer and inner membranes of the organelle and highlighted its capacity of influencing not only mitochondrial morphology, but also the tick's mitochondrial network, thereby potentially manipulate organelle function.
In parallel, comparative genomics provided insight into the origin and evolution of *Midichloria*'s ability to interact with mitochondria. This study determined a specific set of genes exclusive to *Midichloria* capable of mitochondrial colonization, which were absent in the members of the genus incapable of such colonization. These findings suggest the involvement of these genetic components in governing the bacteria-mitochondria interaction.

**RESULTS AND CONCLUSIONS:** Presently, leveraging dual-RNAseq data analyses, and having established an in silico structural biology pipeline for predicting protein-protein interactions, we pursue the goal of performing a high-throughput screening of *Midichloria* proteins against the entire *I. ricinus* mitochondrial proteome. The most promising *Midichloria* protein, potentially influencing mitochondrial activity, has been selected for in vivo silencing, to elucidate its function.

# THE NEGLECTED TENANTS: A GLIMPSE INTO FUNGAL SYMBIOSES OF MOSQUITOES

Ricci I.*

*School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy*

**Keywords:** Mosquito, Mycobiota, Vector control.

**INTRODUCTION:** Understanding composition and function of mosquito microbial communities can provide pivotal information on insect biology, such as their environmental adaptation or vectorial capability. While an increasing number of studies have focused on bacteria, the fungal community (mycobiota) has been largely neglected, but recent studies show the presence of an important fungal diversity in mosquitoes (Malassigné et al., 2020. Pathogens, 9:564). Mosquito-mycobiota is mainly composed of *Ascomycota*, which comprise filamentous fungi (Pezizomycotina) and yeasts (Saccharomycotina) such as *Candida*, *Meyerozyma*, *Pichia*, and *Wickerhamomyces*, that adapt to survive in the insect gut and different mutualistic yeast-insect symbioses based on trophic interdependence have been described. Yeasts generate signals of sugar resources through metabolic pathways that produce compounds, such as fermentative volatile compounds (VOCs) that attract insects. Yeasts are important not only for attraction to food, they influence oviposition sites and larval development, but also supply diet integration of adults providing organic nitrogen, essential vitamins, and lipids (Stefanini, 2018. Yeast, 35:315-330). Lastly, fungi can exert antimicrobial properties and defence of the host (Cappelli et al., 2021. Front Microbiol, 11:621605).

**MATERIALS AND METHODS:** The mycobiota of mosquitoes (*Anopheles*, *Aedes*, and *Culex*) were analysed using different approaches including molecular and culture-dependent methods, and metagenomics analysis. Larvae, adult and water samples from breeding sites were analysed providing a list of dozens fungal species associated with different mosquito species. A collection of about fifty fungal isolates was molecularly and biochemically characterised. Selected fungal strains have been processed by headspace solid-phase microextraction combined with gas chromatography-mass spectrometry to extract and analyse the yeast volatile organic compounds (VOCs). Based on the profile of VOCs yeast strains were tested for attractivity to gravid mosquitoes. Antimicrobial activity of yeast has been characterised and tested against *Plasmodium berghei* in *Anopheles stephensi*.

**RESULTS AND CONCLUSIONS:** Research outcomes are: i) Isolation of mosquito-fungal symbionts able to attract female mosquitoes; ii) Implementation of fungal blends to be combined with bio-larvicides for 'lure and kill' formulations to be released in mosquito breeding sites; iii) Identification of symbiotic fungi that impair the malaria parasite in vector mosquitoes; iv) Basic knowledge of yeast based killer activity against mosquito-borne pathogens; v) Basic knowledge of bacterial/fungal interactions in the mosquito host; vi) Formulability of fungal products to be used in field; vii) Safety assessment of fungal formulations. Such innovative fungal-based products might contribute to the control of mosquitoes and/or the diseases they transmit through very promising 'ready to use' technologies.

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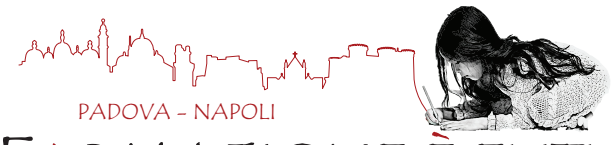
# L'INVASORE NON ARRIVA MAI DA SOLO: ASPETTI PARASSITOLOGICI DELLE INVASIONI BIOLOGICHE



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# PARASITOLOGICAL PATTERNS IN BIOLOGICAL INVASIONS

Ferrari N.*[1], Cassini R.[2]

*[1]Department of Veterinary Medicine and Animal Sciences, Wildlife Health Lab, Università degli Studi di Milano, Lodi, Italy; [2]Department of Animal Medicine, Production and Health, University of Padova, Legnaro, Italy*

**Keywords:** Alien species, Biological invasion, Emerging diseases.

Biological invasions are a new emerging phenomenon with impacts on the environment and human activities. Expansion in abundance and range of distribution of organisms has mostly been considered with respect to fauna and plants, however, pests also show these patterns, with possible repercussions such as emerging diseases for public health, domestic animals or wildlife. Multiple mechanisms can lead to parasitic biological invasions. These include: the anthropogenic introduction of allochthonous parasites into a new range, the introduction of new host and vector species that amplify the parasite population, ecological disruption with the overabundance of some species that are more competent in transmission, and finally environmental changes that favour cycles of parasite transmission. These mechanisms, and their dimensions and impacts, will be presented during the talk, to introduce the case studies presented in subsequent communications that involve different ecosystems.

# ZOONOTIC NEMATODE *ANGIOSTRONGYLUS CANTONENSIS* - A MASTER OF CO-INVASION

Modry D.*

*Masaryk University, Brno, Czech Republic; Czech University of Life Sciences, Prague, Czech Republic*

Among the plethora of metastrongylid nematodes, *A. cantonensis* attracts a lot of attention due to two reasons: association with ubiquitous invasive hosts and health consequences of zoonotic transmissions. As a typical zoonosis with strong influence of complex parasite and host ecology on infection burden in human population, *A. cantonensis* is a true “ambassador” of necessity of One Health approach. Undoubtedly, the parasite belongs among most opportunistic parasitic nematodes regarding the hosts spectrum, which results in complex circulation in ecosystems. Humans are voracious omnivorous primates, and the issue of food is central to human ecology as well as to human culture. Infection by *A. cantonensis* in humans (as purely accidental hosts) strongly depends on local cultural habits and involvement of intermediate and/or paratenic (or transport) hosts in the diet. The same way, wild and domestic animals in endemic areas enter the life cycle in various manners and consequences of “flow” of *A. cantonensis* through food chains are unpredictable. Main aim of the talk is summarizing the knowledge on the *A. cantonensis* life cycle and definition of major knowledge gaps, that hamper understanding of epidemiology in human and animals in local context. The complex life cycle and presence of *Angiostrongylus cantonensis* larvae in broad spectrum of hosts/biological materials examined in range of settings and conditions represent a continuous diagnostic challenge. Recently, qPCR, LAMP and RPA assays that target highly repeated region in the *A. cantonensis* genome were developed, that broadens the range of application in clinical diagnostics, food control and environmental studies. The diagnostic utility of the LAMP assay for detection of DNA of *A. cantonensis* was tested on aquatic and terrestrial mollusks obtained during field work in Indonesia and Laos, as well as on recently obtained experimental lineage of *A. malaysiensis*, enabling also a presentation of comparative data of detection of both *Angiostrongylus* species by AcanR3390 qPCR and LAMP.

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# WHEN THE INVADER ARRIVES BY LAND, WHAT HAPPENS TO ITS PARASITES?

Beraldo P.*

*University of Udine, Udine, Italy*

**Keywords:** Invasive species, Golden jackal, Parasitic zoonosis.

As reported in scientific literature, co-introductions of parasites with invasive hosts occur across a wide range of parasite and host taxa and often involve parasites with complex life cycles that require an alternative host in the new expansion area. Freshwater teleost parasites are particularly well-documented in the literature (likely due to the heightened susceptibility of aquatic environments to biological invasions), while information on parasites co-introduced by terrestrial invasive hosts is limited. In biological invasions, the role of parasites is intricate and diversified. While parasites can damage native species, they also regulate populations and influence ecosystem dynamics. Understanding these interactions is crucial for managing the impact of invasive species on biodiversity and ecosystems. When invasive species spread to new geographical areas, the fate of their parasites can be varied. Some may establish themselves in the new area if suitable hosts are present, while others may be lost due to environmental conditions or stress. Parasites dependent on specific hosts may face challenges if those hosts are absent. Conversely, invasive species may acquire harmful parasites from local hosts. This interaction can indirectly affect native parasites by altering competition for resources or habitat. Changes in parasite dynamics due to the introduction of invasive species can impact native species and ecosystems. Although parasites from invasive species can cause epidemics in native hosts, the extent of co-introduction and the threat to native species are poorly understood. The situation of the golden jackal (*Canis aureus moreoticus* Geoffroy Saint-Hilaire, 1835) will be analyzed, not considered an alien species, but this wild canid has experienced a rapid geographical expansion on a large scale from southeastern Europe and the Caucasus to the Balkans, and more recently, across much of Europe, settling in Friuli Venezia Giulia in the mid-1980s. Its vast geographical range, high territorial mobility, and non-selective diet underlie the numerous parasitic species that infect the golden jackal in various parts of Europe, which can be introduced into new expansion areas, increasing the risk of cross-transmission to other carnivores and humans. For instance, *Metagonimus yokogawai* is considered the most common zoonotic intestinal trematode in the Far East, therefore, golden jackals could play a significant role in environmental contamination with these parasites and be an indirect source for human transmission. Most of these parasites are also common in domestic dogs and cats. In particular, the genera *Echinococcus*, *Trichinella*, *Toxocara*, and hookworms are known to have a high zoonotic potential and therefore represent a possible public health risk. The golden jackal represents a potential reservoir of zoonotic parasites and for other animals, both wild and domestic.

# IMPACT OF ALIEN SPECIES PARASITES ON AQUATIC ECOSYSTEMS

Marchiori E.*[1], Gustinelli A.[2]

*[1]Department of Animal Medicine, Production and Health, University of Padova, Legnaro, Italy; [2]Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Ozzano dell'Emilia, Italy*

**Keywords:** Aquatic ecosystem, Red-eared slider, Blue crab.

Freshwater and marine ecosystems have been deeply transformed by invasive alien species (IAS) belonging to a number of different taxa, which, depending on their trophic role, have been proved to be able to disrupt the food web from both its base and apex. Disturbance of such fragile habitats by anthropogenic stressors, including climate change, pollution and overexploitation, make them more susceptible to biologic invasions. Some known examples of host-associated agents of transmissible diseases, such as amphibians' chytridiomycosis and crayfish plague by *Aphanomyces astaci*, introduced with *Procambarus clarki* and threatening autochthonous European crayfish, demonstrate the strong effect they may have on biodiversity conservation. The impact of introduction in European countries and Italy of further IAS, such as the red-eared slider turtle *Trachemys scripta* and the Atlantic Blue Crab *Callinectes sapidus* as reservoirs of pathogens is still strongly underestimated. The presence of *T. scripta*, native of Northern and Central America, has become increasingly consistent in Italy since the 70's, following commercialization and illegal release of pet turtles in natural environment. The species has spread across Europe, becoming a stronger competitor with the autochthonous emydid turtles. Reports of parasite spill-over events from *T. scripta* to the European pond turtle *Emys orbicularis* increased in literature in the last few years. Exotic monogeneans polystomatids, living in conjunctival sac, urinary bladder or oral cavity of chelonians, have been found in *E. orbicularis* in Spain and France, demonstrating their ability for host-switching. Most remarkably, outbreaks of fatal spirorchidiasis, sustained by cardiovascular flukes *Spirorchis* spp. of American origin have been recently reported from North Spain and Switzerland, with an important impact on the conservation of the autochthonous species. In Italy, descriptions of the parasitofauna of the two turtle species are completely lacking. Concerning *C. sapidus*, although reported in the Mediterranean around one century ago, only in a recent time it raised the attention of civil society and scientific community for its dramatic spreading on the coastal environment and for its strong predatory attitude on native population of molluscs, with a warning threat on biodiversity sustainability. Reported as susceptible host of *Hematodinum* spp., a protozoan parasite known to be responsible of devastating mortality outbreaks in several crustacean species, its role as reservoir of this parasite in the coastal areas of Italy is still to be assessed. In the present paper, results of a preliminary studies carried out in a population of *T. scripta* from a protected area in Friuli Venezia Giulia and a parasitological survey of Atlantic Blue crabs from the Adriatic coast are presented, with evidence of newly introduced parasitic species.

# ALIEN AND INVASIVE MOSQUITOES IN ITALY: WHAT IMPACT ON PUBLIC HEALTH?

Epis S.*, Gabrieli P., Naro G., Bandi C.

*University of Milan, Milan, Italy*

**Keywords:** Vector-borne diseases, Control, Monitoring.

The introduction of alien and invasive mosquito species in Italy represents a potential threat to public health. One of the most concerning species is the Asian tiger mosquito *Aedes albopictus*, which was well established in many parts of Italy and is known for its ability to transmit human and animal diseases. *Aedes koreicus* and *Aedes japonicus* are two additional invasive mosquito species that have been described in our country in recent years. The presence of these additional invasive mosquito species further complicates efforts to control mosquito-borne diseases in Italy. Their establishment increases the diversity of potential disease vectors and poses a further challenge to public health authorities, in relation with the monitoring and controlling mosquito populations. Here we described some of the key impacts on public health on the presence of invasive mosquitoes: (i) Disease transmission and increased disease risk: Invasive mosquitoes can serve as vectors for various infectious diseases. *Aedes albopictus*, for example, is capable of transmitting viruses such as dengue, chikungunya, and Zika, all of which can cause serious illness in humans. The presence of these mosquitoes increases the risk of outbreaks (as reported in our country during the last year) of these diseases in affected areas. Moreover, the spread of invasive mosquitoes expands the geographical range of the diseases they carry, putting more people at risk of infection. This is particularly concerning in regions where these diseases were previously uncommon or non-existent. (ii) Challenges for control: Invasive mosquito species can be difficult to control due to their adaptability and resilience. Traditional methods for the control of mosquitoes, at the larval or adult stage, may be less effective against invasive species, leading to increased difficulty in managing mosquito populations and reducing disease transmission. (iii) Economic impact: In several countries mosquito-borne diseases have a significant economic impact due to healthcare, costs, loss of productivity, and the burden on healthcare systems. Currently, costs of mosquito-borne diseases for the Italian health-care system are still minimal, but future further diffusion of mosquito-borne diseases might lead to an increase of these costs.(iv) Public awareness and education: The presence of invasive mosquitoes underscores the importance of public awareness and education campaigns. People need to be informed about the risks posed by these mosquitoes and the measures they can take to protect themselves, such as using insect repellent, wearing protective clothing, and eliminating mosquito breeding sites around their homes. Overall, the establishment of alien and invasive mosquito species in Italy (or in Europe in general) poses a serious threat to public health and underscores the need for proactive measures to control mosquito populations and mitigate the risk of disease transmission.

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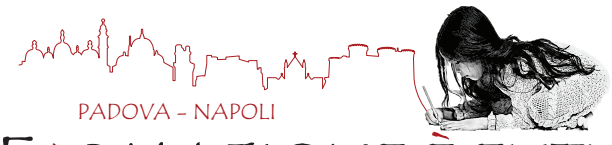
# A CENA CON I PARASSITI: LE NUOVE SFIDE DIAGNOSTICHE PER IL CLINICO



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# OLD AND NEW CANINE PARASITES: WHAT TO EXPECT IN CLINICAL PRACTICE

Traversa D.*

*University of Teramo, Teramo, Italy*

**Keywords:** Arthropods, Angiostrongylosis, Epidemiology.

The distribution of diseases transmitted by invertebrates is influenced by factors inherent to environment, animals, and people. Different drivers have recently modified the epidemiology of arthropods and transmitted diseases. Climate changes, destruction of wild habitats, landscape modification and increase in pet movements, are causing expansion and/or (re-)emergence of arthropods and transmitted diseases in many areas. Ticks have a severe direct pathogenic impact in dogs, *e.g*. they cause nuisance, anaemia, skin damages and secondary infections, and toxicosis. Additionally, ticks transmit various pathogens (mostly protozoa and bacteria) to dogs, many of which have the potential to cause diseases in humans. Leishmaniosis caused by *Leishmania infantum* is one of the most important diseases transmitted by insects (*e.g*. sandflies) in Europe. In dogs, *L. infantum* causes a multi-organ and potentially fatal disease, while in people it may induce varying clinical forms. Another major parasite transmitted by insects (*i.e.* mosquitoes) is the canine heartworm *Dirofilaria immitis*, *i.e.* the cause of a chronic illness requiring a challenging veterinary management. Though rarely, humans also become infected with *D. immitis* through mosquito bites. Global warming influences also the spatial-temporal dispersion of gastropod-borne parasites, as rising temperatures, changes in precipitations, and climate variability have an impact on life cycle, survival, and reproduction rates of invertebrates and harboured pathogens. The same wildlife acting as a source of ectoparasites and transmitted diseases may be reservoirs of heteroxenous nematodes that can infect domestic animals, especially where conurbation occurs. This is particularly the case of felid infections by lungworms or canid infections by *Angiostrongylus vasorum*, which have recently expanded in many territories and pose an important threat for the life of domestic animals living in enzootic regions. In the last decade, studies have shown important modifications in the epidemiology of the abovementioned infections/infestations in Italy. As key examples, ticks may be active throughout the year in larger regions than in the past, and autochthonous foci of leishmaniosis and dirofilariosis are now stable in Northern and Southern Italy, respectively. At the same time there has been an increase in the distribution of gastropod-borne nematodes especially where conurbation favours bridging infections between wildlife and domestic animals. The spatio-temporal occurrence of old and new parasites is constantly changing over time for different reasons, *e.g.* global warming, movements of animals, conurbation. Thus, veterinarian should be aware of these modifications for selecting appropriate diagnostic approaches even when some pathogens are unexpected and for implementing control and preventative programs to safeguard animal and human health.

# VECTORS-PATHOGENS AND IMMUNE SYSTEM: INTERACTIONS AND CLINICAL IMPLICATIONS

Furlanello T.*

*San Marco Veterinary Clinic and Laboratory, Veggiano, Italy*

**Keywords**: Vector-borne infections, Ticks' saliva, Immunopathology.

From a didactic standpoint, we could categorize vector-borne diseases based on the inciting agent, the vector, the involved species, and other details. However, clinically distinguishing between these diseases can be challenging. In practice, dogs typically present with common symptoms such as fever, anorexia, weight loss, and pain. Laboratory findings often include anemia (regenerative or non-regenerative), thrombocytopenia, elevated serum levels of C-Reactive Protein, hypoalbuminemia, hyperglobulinemia, and kidney abnormalities, including proteinuria. Approaching Canine Vector-Borne Diseases (CVBD) with a schematic mindset may hinder our understanding of their shared pathogenesis. Consequently, clinicians are often faced with varied management choices, including targeting the infectious or parasitic agent or modulating the immune system due to immunologic complications. It is crucial to recognize that tick saliva is a complex mixture containing bioactive proteins, lipids, and nucleosides. Tick saliva influences various elements of host defense mechanisms, including pain and itch sensation, hemostasis, and innate and adaptive immune responses (Wikel, 2018. Front Biosci, 23:265-283; Kitsou et al., 2021. Trends Immunol, 42:554-574). The subsequent pathogenetic step involves nonspecific primary immunity, triggering the acute phase response. This cascade has significant clinical implications, including the ubiquitous inflammation-related anemia. Sinergy between vectors and infectious agents often favors Th2-regulated humoral responses over protective Th1-regulated cellular immune responses, facilitating infection persistence and inappropriate secondary immunopathology characterized by hypergammaglobulinemia, autoantibody production, immune complex formation, and complement activation (Day, 2011. Par & Vect, 4:48). In daily practice, clinicians frequently encounter nonspecific clinical and laboratory signs. Prominent indicators of autoimmunity could be discovered, including the presence of antibodies targeting erythrocytes and/or platelets, alongside cytological findings consistent with sterile polyarthritis or meningitis. Serology often yields positive results for multiple CBVDs, making it challenging to distinguish between direct pathological activity and immune-mediated amplification or complications. Strengthening the protection of our canine population through the optimal use of available efficient antiparasitic drugs remains the primary solution to this clinical challenge.

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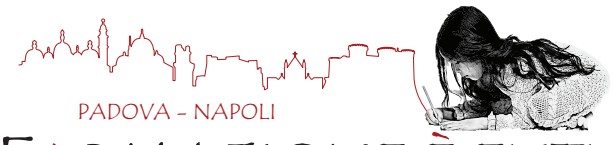
# INSIDE YOUR BRAIN: COSA SUCCEDE QUANDO I PARASSITI USANO IL CERVELLO

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# PARASITES OF THE NERVOUS SYSTEM IN FARM ANIMALS: IMPACT ON ANIMAL HEALTH AND PRODUCTION AND ZOONOTIC ASPECTS

Tamponi C.*, Cavallo L., Nonnis F., Zeinoun P., Carta C., Furqan A., Scala A., Varcasia A.

*Dipartimento di Medicina Veterinaria, Università di Sassari, Sassari, Italy*

**Keywords**: Farm animals, Neuroparasitology, CNS parasites.

Parasites can cause diseases of the nervous system of farm animals by their presence in close proximity to or within the central nervous system (CNS). Some protozoan can cause neurological disease in farm animals, like *Sarcocystis neurona* and *Neospora hughesi*, responsible of the Equine Protozoal Myeloencephalitis (EPM), with focal or multifocal signs of neurologic disease involving brain, brainstem or spinal cord (Reed et al., 2016. J Vet Intern Med, 30:491-502). *Toxoplasma gondii* and *Neospora caninum* can cause clinical neurologic disease in neonates of ruminants, horses and pigs (Nagy et al., 2004. Vet Clin Food Anim, 20:393-412; Dubey, 2009. Vet Parasitol, 164:89-103). CNS can be affected by the larval stages of cestodes, as in the case of coenurosis by *Taenia multiceps* in ruminants and cisticercosis by *Taenia solium* in pigs. Coenurosis affects the brain and spinal cord of the ovine intermediate host, causing a clinical condition known as gid, showing ataxia and behavioral changes that are likely to enhance transmission to the definitive host by means of predation (Varcasia et al., 2022. Parasit Vectors, 15:84). Pigs with neurocysticercosis (NCC) can develop clinical signs and suffer from seizures like humans with symptomatic NCC associated epilepsy (Trevisan et al., 2016. Vet Parasitol, 220:67-71). Immature stages of some parasites can exhibit neurotropic affinity, as in the case of *Hypoderma bovis* in cattle. The larvae require conditions provided by the host's CNS for their development, migrating through the spinal cord and adjacent tissues to reach its preferred site and causing neurological signs especially when killed by treatment while in these locations (Cortinas et al., 2006. Vet Clin Food Anim, 22:673-693). *Oestrus ovis* larvae, normally found in the sinus cavities in sheep and goats, may aberrantly be found in the CNS and determine a neurological syndrome (false gid) with high mortality rate (Mozaffari et al., 2013. ISRN Vet Sci, 2013:650358). Moreover, facultative parasites, normally free-living within the environment, can develop into parasites. *Halicephalobus deletrix*, a saprophytic soil nematode that is found free-living in the environment, has been reported to produce meningoencephalitis in horses and calves (Onyiche et al., 2018. Parasite Epidemiol Control, 3:36-42). Although parasites affecting CNS are a small part of farm animals parasitosis and for this reason little considered, they can have serious impact on animal health and productions, as in the case of sheep coenurosis, with economic losses consisting in loss of animal productivity, sheep fatalities and compromised genetic value, feed and farm cost of animal until the outbreak, cost of surgery or drug treatment and carcass disposal of dead sheep (Varcasia et al., 2022. Parasite Vectors, 15:84). Finally, CNS parasitosis may represent a threat for human health, as most of the parasites also have zoonotic potential (*T. gondii*, *T. multiceps*, *T. solium* and *H. deletrix*).

# NEUROTROPIC PARASITES OF SMALL COMPANION ANIMALS: THERE ARE MORE THAN *TOXOPLASMA GONDII* AND *NEOSPORA CANINUM*

Morganti G.*

*University of Perugia, Department of Veterinary Medicine, Perugia, Italy*

**Keywords**: Dogs, Cats, Neurotropic parasites.

The present work aims to give an overview of parasitic diseases affecting the central nervous system (CNS) of small companion animals. *Toxoplasma gondii* and *Neospora caninum* are traditionally associated with neurological disorders in dogs and cats. Both toxoplasmosis and neosporosis occur in dogs, while cats are just affected by toxoplasmosis. In both species, these parasite infections evolve mainly in asymptomatic form; however, when they are expressed with clinical signs, peripheral and central neurological disorders are primarily described (Calero-Bernal and Gennari, 2019. Front Vet Sci, 26:54; Silva and Machado, 2016. Vet Med, 7:59-70). Despite that, many other parasitic and fungal agents exhibit a tropism for CNS in small companion animals. Parasites could affect CNS directly displaying a natural neurotropism, as *T. gondii*, *N. caninum* and *Encephalitozoon cunicoli* in dogs, or could injury CNS indirectly through different mechanisms: i) ectopic dislocation of larvae, eggs or adult parasites/fungi due to migrations through blood or neighbouring tissues to CNS or spinal cord (*i.e.* lungworms, *Dirofilaria immitis*, *Baylisascaris procyonis*, *Aspergillus* spp., *Cryptococcus neoformans*, *Cuterebra* spp.); ii) immune-mediated reactions (*i.e. Leishmania infantum*); iii) coagulation disorders that cause bleeding/hematomas in nervous tissues (*i.e. Angiostrongylus vasorum*); iv) electrolyte imbalances that affect the transmissivity of the nervous impulse (*i.e*. due to high burden infestation by *Toxocara canis* in puppies) and v) effects of neurotoxins (*i.e.* by *Ixodes holocyclus* bite). Parasitic diseases could affect different CNS areas and frequently are linked to a significant impairment of health status (*e.g.* lethargy; behavioural changes; gait abnormalities; vestibular signs, including head tilt, ocular signs consistent with nystagmus, and circling; seizures; blindness, etc.), especially in young or immunocompromised animals. In addition, parasitic diseases injuring CNS usually cause neurological sequelae that could reduce life expectancy and quality of life (*e.g.* epileptic seizures) and frequently animals are euthanized or spontaneously die. Diagnosis can be difficult due to the complexity of parasitic life cycles and pathogenesis, commonly complicated by overlapping of clinical symptoms associated to further clinical forms. Clinical diagnosis is based on a combination of anamnestic data, neurological examination and imaging findings, cerebrospinal fluid analysis and parasitological exams (*i.e.* serological/antigenic tests). However, a definitive diagnosis, in several cases, would require a histopathological examination of the brain and/or spinal cord tissues. In conclusion, parasitic diseases of CNS could be of great impact on animals' health status and need to be suspected and included as differential diagnosis in neurological frameworks for dogs and cats.

# PARASITES AND THE NERVOUS SYSTEM: DIAGNOSTIC APPROACHES AND POSSIBLE THERAPEUTIC STRATEGIES IN THE HUMAN SETTING

Contini C.*

*Infectious Diseases, Ferrara, Italy*

**Keywords**: Brain malaria, Cisticercosis and hydatidosis, Amebiasis.

Invasion of the central nervous system (CNS) is a devastating complication of a parasitic infection. Although the most common route of CNS invasion is done through the blood and between the blood and the brain parenchyma, (blood brain barrier - BBB), the portal entry is also provided by the skin and epithelial cells of the gastrointestinal tract or by the olfactory neuroepithelium of the nasal mucosa. Neuropathologic changes depend on the type and size and virulence of the parasite, geographic parasitic strain variations and immune evasion by the parasite. Some infections may present as an expanding mass lesion. Brain parasites (BP) can cause symptomatic disease or run asymptomatically. The most common parasitic infection of the CNS is cerebral malaria (CM) followed by neurocysticercosis (NC). Other relatively common infections include toxoplasmosis, cystic and alveolar echinococcosis and schistosomiasis. Rarer BP include *Entamoeba histolytica*, free-living amoebae including *Naegleria fowleri* (NF) and *Cryptosporidium* spp. This presentation addresses some of the most important BP including CM, NC, Hydatidosis, NF. According to the Malaria Report for 2017, CM accounts for 90% of the deaths. In Europe/USA it is mainly related to travel to tropical areas and increased migration flows. The brain in patients with CM is increased from sequestration of parasitized erythrocytes. Diagnosis consists in microscopy, mRDT, and advanced PCR. Currently, the best available treatment, particularly for *P. falciparum* malaria, is artemisinin derivative combination therapy (ACT). Experimental drugs including those to prevent BBB dysfunction yet will be discussed. NC is the most common helminthic infection of the CNS and a major cause of acquired epilepsy in resource-limited countries. Imported cases are increasing in Europe. NC diagnosed by neuroimaging which is supported by immunodiagnostic tests (Western blot test with purified parasite antigens). Management of NC requires a multidisciplinary approach that includes drugs to control symptoms, anti-inflammatories, anti- antiepileptics, antiparasitic treatment and sometimes surgery.
Hydatic cyst (HC) is a zoonotic infection caused by *E. granulosus* widely endemic in regions where livestock farming is prevalent. Brain involvement is seen in only 1-2% of HC cases. 80% of patients with cerebral HC are in the pediatric age group. HC is usually diagnosed by clinical findings, serological (often false negative) and imaging methods which may give better results. NF is an amoeba commonly found in warm freshwater environments such as lakes, hot springs and poorly chlorinated swimming pools especially in United States. Commonly known as brain-eating amoeba, has mortality rate of > 90%; treatment remains problematic with common drugs such as azoles, amphotericin B and miltefosine. Modifying existing drugs using nanotechnology offers promise in the development of therapeutic interventions against these parasitic infections.

# TOXOPLASMOSIS IS FOREVER: COULD PREVENTION AND TREATMENT REDUCE THE RISK OF OCCURRENCE OF NEUROPSYCHIATRIC DISORDERS?

Bruschi F.*[1], Fabiani S.[2]

*[1]Università di Pisa, Pisa, Italy; [2]Ospedali Riuniti di Livorno, Livorno, Italy*

**Keywords**: Toxoplasmosis, Neuropsychiatric disorders, Prevention.

**INTRODUCTION:** Toxoplasmosis is a worldwide zoonosis caused by the apicomplexan protozoon *Toxoplasma gondii*. It affects about two billion people at the global level, although in industrialized countries the seroprevalence is significantly declining mostly because of changes in human habits. Infection may be transmitted by: i) ingestion of food (vegetables, fruits) or water contaminated with the oocysts eliminated within the stool of infected felines (definitive hosts); ii) consumption of raw or undercooked meat derived from infected animals; iii) vertical transmission; iv) blood transfusion; v) solid organ or hematopoietic stem cell transplantation. In immunocompetent adults, infection occurs in most cases asymptomatically or with a mild clinical picture, but in immunocompromised individuals or in the fetuses, the disease may be severe. However, immunocompetent individuals infected with more virulent strains of *T. gondii*, present in Latin America, undergo severe disease, even leading to death. The persistence of the parasite in the tissues for the duration of the host-life is a relatively recent concept. *Toxoplasma gondii* can directly and indirectly modify the function of central nervous system cells, potentially inducing occurrence of neuropsychiatric diseases in genetically predisposed individuals.

**MATERIALS AND METHODS:** Starting from these assumptions, we reviewed the literature, mainly on epidemiological and neurobiological aspects, trying to clarify some fundamental questions: i) is *Toxoplasma* seropositive individuals in the general population more susceptible to underlie neuropsychiatric diseases and behavioral changes?; ii) do prenatal exposure to *Toxoplasma* facilitate the occurrence of psychiatric disease such as autism?; iii) are anti-*Toxoplasma* drugs useful in the treatment of neuropsychiatric diseases in *Toxoplasma* seropositive patients?; iv) should asymptomatic patients also be treated with anti-*Toxoplasma* drugs, once an acute infection is serologically detected. We searched the literature on PubMed library combining the terms "*Toxoplasma gondii*" or "Toxoplasmosis" and "neuropsychiatric" "diseases" or "disorders" or "psychiatric" "diseases" or "disorders" or "neurological" "diseases" or "disorders" or "neurobehavioral disorders" or "behavioral disorders" or "schizophrenia" or "bipolar disorder" or "autism spectrum disorder" or "Parkinson's disease" or "Alzheimer's disease". We used no language or time restrictions. Search was concluded on April 30th 2024.

**RESULTS AND CONCLUSIONS:** Although the literature does not yet provide definitive answers, current data should be considered sufficient to change attitude on toxoplasmosis prevention and treatment measures, addressing them not only towards seronegative pregnant women and immunocompromised patients, as actually done, but also to subjects particularly prone to develop neuropsychiatric diseases on genetic basis and/or presenting N-methyl-d-aspartate receptor antibodies.

# RECIPROCAL DYNAMICS: EXPLORING THE BIDIRECTIONAL RELATIONSHIP BETWEEN ANIMAL BEHAVIOR AND PARASITISM

Zanet S.*, Varzandi A., Ferroglio E.

*Università degli Studi di Torino, Dip. Scienze Veterinarie, Grugliasco, Italy*

**Keywords**: Behavior, Ecology, Evolution.

Animal behavior is heavily influenced by parasites, which affect social interactions, grooming, mating displays as well as movement and foraging habits. Simultaneously, behavior drives alterations in parasite transmission and evolution. This dynamic is evident in social insects where colonies are susceptible to rapidly spreading pathogens due to high contact rates. *Nosema apis* and *N. ceranae* are typical examples (Goblirsch et al., 2019. PlosOne, 8:e58165) where individual responses to infection and collective behaviors safeguard the colony's survival. At individual level, behavior influences the infection risk of the individual by changing contact rates with parasites and susceptibility to infection upon contact. Behavior can serve as a fundamental risk factor for infection, as evidenced by sex-biased parasite infections in vertebrates. Across vertebrate taxa, males typically experience higher parasite infection rates than females due to behaviors associated with reproduction and mating, along with related physiological changes that increase exposure to parasites or susceptibility to infection (Ezenwa, 2018. Animal Behavior and Parasitism, Oxford Univ Press). An example, among many, is the sex-biased epidemiology of gastro-intestinal strongyles in Alpine ruminants (Corlatti et al., 2019. Ecol Evol, 9:8749-8758). Different contact rates and susceptibility to parasites can impact epidemic dynamics at the population level where differences in pathogen susceptibility based on social rank have been observed in several species (Sanz et al., 2020. PNAS, 117:23317-22). Variability in individuals' likelihood of becoming infected or transmitting infections has implications for epidemiological dynamics, with behavior playing a prominent role in an individual's potential to become a superspreader. The relationship between behavior and parasitism doesn't solely flow in one direction: behavior influences parasites, and parasites affect behavior. Where the relationship between hosts and parasites is deeply interconnected and heavily affects both interested parties, a better understanding of the interactions between animal behavior and parasitism will unravel many currently unknown mechanisms in the evolution of the host-pathogen dichotomy.

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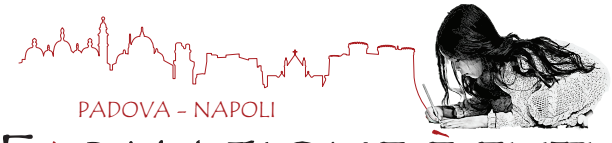
# APICOMPLEXA NEGLETTI ED EMERGENTI NEGLI ANIMALI D'AFFEZIONE, DA REDDITO E SELVATICI



PADOVA - NAPOLI

FORMAZIONE È FUTURO
IN PARASSITOLOGIA
SOIPA 2024

# *TOXOPLASMA GONDII* INFECTIONS IN DOMESTIC AND WILD ANIMALS: FROM UNNOTICED TO DEADLY

Basso W.*

*Institute of Parasitology, Vetsuisse Faculty, University of Bern, Bern, Switzerland*

**Keywords:** Toxoplasmosis, Apicomplexa, Zoonosis.

*Toxoplasma gondii* is a successful coccidian parasite able to infect all warm-blooded animals and humans, causing one of the most common zoonoses worldwide. While infections may have a subclinical course, under special circumstances they can cause life-threatening disease. This presentation will focus on recent studies carried out at the National Reference Laboratory for Toxoplasmosis in Switzerland, including diagnostic, clinical and epidemiological aspects of *T. gondii* infections in domestic and wild animals. Switzerland-wide studies in small ruminants (Basso et al., 2022. Food Waterborne Parasitol, 28:e00176) and South American camelids (Basso et al., 2020. Parasit Vectors, 13:256) showed a wide distribution of the parasite, with high seroprevalences: sheep 66.3%, goats 50.5%, alpacas 82.3% and llamas 84.8%. In addition, *T. gondii* DNA was detected in 6.1% of ovine and 6.8% of caprine abortions, highlighting the importance of this parasite in animal production. Besides, we have serologically demonstrated vertical transmission of the parasite in llamas (Rüfli et al., 2021. Animals, 11:1956). Studies on fattening pigs have shown lower seroprevalences (6%); however, pigs represent a major source of infection for humans worldwide. Therefore, a further project focused on the evaluation of ELISA and immunoblot tests to detect specific antibodies in oral fluid, collected by hanging cotton ropes in the pens for the pigs to chew on. This non-invasive approach allowed the detection of pig groups with a high exposure to *T. gondii* at the farm level. (Kauter et al., 2023. Int J Parasitol, 53:523-530). Further projects involved endangered species such as Eurasian lynx and beaver. We observed a seroprevalence of 82% and oocyst shedding in 1.7% of lynx tested, confirming for the first time this felid species as a definitive host for *T. gondii* (Scherrer et al., 2023. Int J Parasitol Parasites Wildl, 21:1-10). In addition, specific antibodies were found in 45.8% of the beavers tested. Many of the animals showed encephalitis and meningitis, underlining the clinical relevance of the infection in this rodent species (Scherrer et al., 2024. J Wildl Dis, 60:126-138). There is evidence that *T. gondii* may manipulate rodents' behaviour facilitating predation by felids. We tested this hypothesis in the natural environment by molecular analysis of cat-hunted and trapped wild small mammals of 6 different species and observed higher prevalences for *T. gondii* in cat-hunted small mammals, suggesting that infected rodents are at higher risk of predation by cats and therewith supporting the behaviour manipulation hypothesis (Pardo Gil et al., 2023. Int J Parasitol Parasites Wildl, 220:108-116). Although *T. gondii* often causes subclinical infections, in recent years we have observed several cases of disease, sometimes fatal, in both domestic (cat, goat) and wild animals (New World monkeys, lemurs, marsupials), demonstrating the clinical importance of this parasite.

# BOVINE BESNOITIOSIS: UPDATES, NOVEL INSIGHTS, AND PERSPECTIVES OF A NEGLECTED DISEASE OF CATTLE (AND OTHER ANIMALS?)

Gazzonis A.L.*

*Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Lodi, Italy*

**Keywords:** *Besnoitia besnoiti*, Bovine besnoitiosis, Apicomplexa.

*Besnoitia besnoiti*, a cyst-forming protozoan (Apicomplexa, Sarcocystidae), is the causative agent of Bovine besnoitiosis (BB), a chronic and debilitating parasitic disease affecting animal health and welfare. Despite the significant economic implications, especially for beef cattle farming, BB remains often overlooked by both veterinarians and health authorities. The clinical presentation of the infection encompasses both cutaneous and systemic manifestations, but the severity of clinical signs may vary considerably among animals, and the underlying pathogenetic mechanisms remain largely unknown (Fernández-Álvarez et al., 2024. Microorganisms, 12:586). With no chemotherapeutics and vaccines available in Europe, infection control currently relies solely on standardized diagnostic procedures and the application of corrective management measures. Furthermore, the numerous unknown or unclear aspects concerning the parasite's biology and the infection's epidemiology continue to pose significant challenges for infection control. Endemic in southern countries, in Europe BB is recognized as a (re-)emerging disease, in expansion in its geographical distribution. In Italy, the few systematic epidemiological surveys highlighted several *foci* of infection, but these studies date back a decade (Rinaldi et al., 2013. Parasitol Res, 112:1805-7; Gazzonis et al., 2014. Parasit Vectors, 10:585); hence, an update on the spread of BB is now imperative. In addition to epidemiological aspects, the interaction between the parasite and its host still requires elucidations. While cattle serve as the intermediate host (IH), the definitive (DH) and potential reservoir hosts (RH) have yet to be conclusively identified. By analogy with other Apicomplexa, wild mesocarnivores have been proposed as possible DHs (González-Barrio et al., 2021. Transbound Emerg Dis, 68:3156-3166). The identification of *B. besnoiti* in a roe deer in Spain (Arnal et al., 2017. Transbound Emerg Dis, 64:e8-e14) suggested the potential for the pathogen to infect species other than cattle. The involvement of wildlife and sympatric domestic animals as potential RH or IH should not be overlooked, particularly in regions highly endemic for *B. besnoiti*. For instance, the donkey is a potential alternative host, with cases of equine besnoitiosis reported in the USA and Europe. While the donkey is the natural host of *B. bennetti*, recent partial rDNA sequencing of parasitic DNA from skin biopsies of two donkeys suggested the involvement of *B. besnoiti*, although based on the detection of a single nucleotide variation (Villa et al., 2021. Parasitol Res, 120:1811-1819). There are therefore still many open challenges regarding both *B. besnoiti* and BB. Aiming to contribute to the development of effective control strategies, further investigation on BB should prioritize elucidating remaining uncertainties regarding its biology, pathogenetic mechanisms, host-parasite interaction, and epidemiology.

# SARCOCYSTOSIS IN MEAT-PRODUCING FARM ANIMALS AND GAME MEAT: DO WE KNOW ENOUGH?

Rubiola S.*, Chiesa F.

*Department of Veterinary Sciences, University of Turin, Grugliasco, Torino, Italy*

**Keywords**: *Sarcocystis* spp., Farm animals, Game meat.

The genus *Sarcocystis* (Protozoa, Apicomplexa) includes more than 220 species infecting mammals, reptiles, birds and fishes. The two-host life cycle of *Sarcocystis* spp. involves carnivores or omnivores as definitive hosts and herbivores, omnivores or less frequently carnivores as intermediate hosts. Humans can act as intermediate hosts of three species of *Sarcocystis*, that is *Sarcocystis hominis* and *Sarcocystis heydorni*, using cattle as intermediate hosts, and *Sarcocystis suihominis*, using wild and domestic swine as intermediate hosts. Humans can become infected after the consumption of raw or undercooked beef or pork containing viable sarcocysts. During the last decades, a growing interest has arisen around the genus *Sarcocystis* in the food industry due to the supposed association of some *Sarcocystis* spp. with gross lesions detectable at slaughter leading to carcass discard, including macroscopic cysts or cystic lesions and eosinophilic myositis, an enigmatic inflammatory myopathy characterized by the presence of green-grey diffuse or focal patches in striated muscles of the affected animals. In the present molecular era, the increasing use of genetics and genomics is allowing the detection of novel species, while also unveiling some life cycle gaps. Although *Sarcocystis* spp. seem to be highly investigated in meat-producing farm animals, due to the zoonotic potential of some species and the economic losses resulting from carcass condemnation, these protozoa could still hold some surprises; yet, studies aimed at identifying *Sarcocystis* spp. in game meat are limited. Here, I will discuss the current state of research of *Sarcocystis* spp. in some meat-producing farm animals and in game meat, including novel findings, knowledge gaps and future challenges.

# *BABESIA* INFECTIONS IN DOGS AND WILD CANIDS: WHERE DO WE STAND TODAY?

Ciuca L.*, Oliva G., Gizzarelli M., Foglia Manzillo V., Rinaldi L., Maurelli M.P.

*University of Naples Federico II (UNINA), Department of Veterinary Medicine and Animal Production, CREMOPAR, Naples, Italy*

**Keywords:** Dogs, *Babesia* spp.

Canine babesiosis is considered one of the most important tick-borne diseases of dogs worldwide (Solano-Gallego et al., 2016. Parasit Vectors, 9:1-18; Abdoli et al., 2024. Vet Med Sci, 10:e1427). Wild canids such as grey wolves and jackals are asymptomatic carriers of *Babesia* and contribute to the spread of the parasite (Milanović et al., 2020. Vet Parasitol, 282:109140). In Italy, the large *Babesia* species (*B. canis* and *B. vogeli*) are widely distributed in both the northern and in the central-southern regions (Olivieri et al., 2016. Parasit Vectors, 9:213; Maurelli et al., 2018. Parasit Vectors, 11:420; Furlanello et al., 2005. Vet Parasitol, 134:77-85; Veneziano et al., 2018. Ticks Tick Borne Dis, 9:1459-1463). However, the clinical aspects and geographical distribution of *Babesia gibsoni* (small *Babesia* species) in dogs in Italy are poorly described (Trotta et al., 2009. Vet Parasitol, 165:318-22; Carli et al., 2021. Vet Parasitol Reg Stud Reports, 25:100596). This study is aimed at providing clinical and epidemiological updates of canine babesiosis in Italy and in particular the occurrence of new cases of dogs infected with *B. gibsoni* in southern Italy. As an example, in the last three years two dogs, a 7-year-old neutered female pit bull terrier (Diana) and a 3-year-old neutered female crossbreed (Biba), were referred to the Veterinary Teaching Hospital of UNINA, for a two-week history of lethargy and urine leakage. Both dogs were found to have lethargy, anorexia, marked splenomegaly, proteinuria and haemoglobinuria. Examination of the blood smear showed piroplasms in the erythrocytes, consistent with small forms of *Babesia* spp. PCR analysis (Bajer et al., 2019. Ann Agric Environ Med, 26:538-543) also confirmed the species of *B. gibsoni*. Both dogs were monitored for a period of 6 months by blood tests, spleen and kidney examination and by PCR. Two weeks after treatment (atovaquone and azithromycin), a slight improvement of clinical conditions were observed in the two dogs and PCR was negative for *B. gibsoni* one month after the second treatment. To date, canine babesiosis remains a challenge for clinicians, primarily due to the wide spectrum of clinical presentations, the limited options for specific diagnostics and antiparasitic drugs.

# THE WILD WORLD OF APICOMPLEXA IN FELINES

Grillini M.*, Simonato G.

*Department of Animal Medicine, Production and Health, University of Padova, Legnaro, Italy*

**Keywords:** *Cytauxzoon*, *Hepatozoon*, Feline.

*Cytauxzoon* and *Hepatozoon* are apicomplexan parasites, and their life cycles involve both felid hosts and arthropod vectors. They have been detected in domestic cats worldwide (Alvarado-Rybak et al., 2016. Parasit Vectors, 9:538; Schäfer et al., 2022. J Feline Med Surg, 24:994-1000). Their prevalence depends on the distribution and abundance of competent vectors and the abundance of susceptible felid hosts, indeed both parasites are commonly found in countries where wild felids are widely present, such as Africa (Viljoen et al., 2020. Parasit Vectors, 13:220), Asia (Panda et al., 2024. Parasitol Res. 123:92), and Northern and Southern America (Reichard et al., 2021. Pathogens, 10:1170). In Europe, they were historically considered absent or rare, nevertheless recent studies have highlighted their presence in wild and domestic felids. Epidemiological surveys have detected both pathogens in European wildcats (*Felis silvestris silvestris*), lynxes (*Lynx lynx*), and in domestic cats in Romania, Hungary, Spain, Portugal, France, Switzerland, Germany, and Italy (Panait et al., 2021. Vet Parasitol, 290:109344; Willi et al., 2022. Parasit Vectors, 15:19; Schäfer et al., 2022. J Feline Med Surg, 24:994-1000; Carbonara et al., 2023. Ticks Tick Borne Dis, 14:102192; Grillini et al., 2023. Front Vet Sci, 10:1113681; Tuska-Szalay et al., 2023. Pathogens, 12:656). The distribution of *Cytauxzoon* and *Hepatozoon* infections in Italy reflects the broader pattern observed in Europe, with variable prevalence rates reported across different regions (Carli et al., 2012. Vet Parasitol, 183:343-52; Giannelli et al., 2017. Ticks Tick Borne Dis, 8:721-724). In the past, feline cytauxzoonosis and hepatozoonosis were primarily studied in relation to clinical disease (Klopfer et al., 1973. Vet Pathol, 10:185-90; Wagner, 1976. J Am Vet Med Assoc, 168:585-8), while, on wild felid populations the impact of these parasites remained poorly understood. Nowadays, the improvement in molecular diagnostics and in epidemiological surveillance have provided new insights into the prevalence and distribution of *Cytauxzoon* and *Hepatozoon* species in felids. Molecular assays targeting specific genes have enabled the detection and characterization of these parasites in blood and tissue samples from symptomatic and asymptomatic hosts (Grillini et al., 2023. Front Vet Sci, 10:1113681). Furthermore, phylogenetic analyses have revealed genetic variability within *Cytauxzoon* and *Hepatozoon* species in felids (Panait et al., 2021. Vet Parasitol, 290:109344; Harris et al., 2019. J S Afr Vet Assoc, 90:e1-e6) shedding light the need to continue surveillance and research to enhance the understanding of their impact on felid health. *Cytauxzoon* and *Hepatozoon* infections represent an emerging challenge; understanding their distribution, epidemiology, and the pathogenesis could be crucial for effective management and control.

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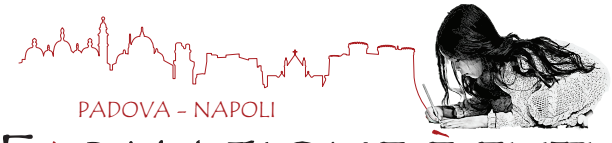
# LA PARASSITOLOGIA UMANA IN ITALIA: ESPERIENZE PLURIENNALI IN AMBITO DIAGNOSTICO



PADOVA - NAPOLI

FORMAZIONE È FUTURO
IN PARASSITOLOGIA
SOIPA 2024

# EPIDEMIOLOGY OF HUMAN INTESTINAL PARASITES: DATA FROM NATIONAL MULTICENTRE STUDIES

Crotti D.*[1], Gargiulo R.[2], Clementa L.[3], Menegotto N.[4], Oliva E.[5], Petrullo L.[6], Besutti V.[7], Bernieri F.[8], Raglio A.[9], Group C.A.[8]

*[1]Comitato Studio Parassitologia AMCLI, Perugia, Italy; [2]Ospedale di Modena Baggiovara, Modena, Italy; [3]Azienda Sanitaria Universitaria Isontina, Monfalcone, Italy; [4]Ospedale di Treviso, Treviso, Italy; [5]Ospedale Reggio Emilia, Reggio Emilia, Italy; [6]Ospedale Cotugno, Napoli, Italy; [7]Ospedale Padova, Padova, Italy; [8]Comitato di Studio Parassitologia AMCLI, Milano, Italy; [9] ASST Papa Giovanni XXIII, Bergamo, Italy*

**Keywords**: Epidemiology, Intestinal parasites, Italy.

**INTRODUCTION:** The epidemiology of intestinal parasites is not well known in non-endemic countries.The AMCLI CoSP carried out 3 national multicentre studies between 1994 and 2016 in order to understand the presence of parasites in our territory and to evaluate the diagnostic procedures adopted by the laboratories.

**MATERIALS AND METHODS:** Questionnaires were sent to the laboratories on the mailing list of AMCLI members.The following questions were included: number of patients examined per year, number of positive patients, species of parasites detected and diagnostic techniques adopted (microscopic examination after FEA concentration, permanent staining and use of clinical-epidemiological sheet).

**RESULTS AND CONCLUSIONS:** A total of 77 public and hospital laboratories answered to the 3 subsequent national investigations, 53 in Northern Italy and 12 in both Central and Southern Italy, for over 100,000 patients involved. Polycentric I took place in 1994-95 (14,880 patients), II in 2005-08 (38,450), III in 2015-16 (57,024). The percentages of positive patients in the three study periods were: for helminths 2.26%, 1.26%, 0.72% and for pathogenic protozoa 2.23%, 1.23%, 2.2%. The percentage of patients positive for helminth according the species was: *E. vermicularis* (0.30%, 0.24%, 0.33%), *S. stercoralis* (0.66%, 0.50%, 0.07%), *T. trichiura* (0.28%, 0.10%, 0.03%), *A. lumbricoides* (0.28%, 0.10%, 0.02%); *Taenia* spp. (0.56%, 0.23%, 0.12%), *H. nana* (0.09%, 0.09%. 0.03%), *D. latum* (0.01%, 0.03%, ≤ 0.01%); *S. mansoni* (≤ 0.01%, 0.08%, 0.03%), *O. felineus* (0%, 0.02%, 0%). Among the pathogenic protozoa the percentages were: *G. duodenalis* (2.02%, 1.21%, 0.8%), *D. fragilis* (0.03%, Not reported, 0.8%)); *C. belli* (0.02%, 0.02%, 0.05%). Among the laboratories that reported: the use of the Scotch test for the detection of *E. vermicularis* the percentage of positive patients was respectively: 15.8%, 12.2%, 15.5%; the use of a modified Ziehl-Neelsen stain the percentages of *Cryptosporidium* spp. was: 4.8% (over 868 patients), 2.8% (over 1.074), 0.3% (over 6.850), the use of culture or Baermann technique the percentage of *S. stercoralis* was: NR, 2.6% (over 5266 patients), 5.9% (over 511), the use of a Giemsa or Trichrome stain the percentage of *D. fragilis* was: NR, 1.88% (over 17.344 patients), 4.4% (over 21.263). Only 18% of patients were examined with 3 samples, only 62% of laboratories applied the FEA microscopy with a permanent staining and only 10% the clinical-epidemiological sheet. The data confirm a decrease of helminths and the need to apply the adequate technique for the diagnosis of *E. vermicularis*, *S. strongyloides*, *D. fragilis* and *Cryptosporidium* spp. Although the laboratories involved are numerous, they certainly cannot be representative of the Italian reality. If the diagnostic "quality" has overall proven to be lacking, this can be hypothesized to be due to a lack of resources: economic, human, cultural, professional. There is still so much to do.

# HUMAN PARASITOLOGICAL DIAGNOSIS IN LOCAL LABORATORIES: RESULT OF AMCLI, SIMET AND SoIPa SURVEY

Oliva E.*[1], Bernieri F.[2], Clemente L.[3], Petrullo L.[4], Menegotto N.[5], Raglio A.[6]

*[1]S.C. Microbiology, AUSL IRCCS Santa Maria Nuova, Reggio Emilia, Italy; [2]Consulente per i programmi di Parassitologia e Batteriologia c/o "Centro Regionale di Riferimento per il controllo di qualità" della regione Toscana, Milano, Italy; [3]SSD Laboratorio Spoke area Isontina, Azienda Sanitaria Universitaria Giuliano Isontina, Monfalcone, Italy; [4]U.O.C. Microbiologia e Virologia, Azienda Ospedaliera dei Colli, Napoli, Italy; [5]UOC Microbiologia e Virologia, ULSS2 Marca Trevigiana, Treviso, Italy; [6]ASST Papa Giovanni XXIII, UOC SMeL 1 Microbiologia e Virologia, Bergamo, Italy*

**Keywords**: Survey, Diagnosis, Network.

**INTRODUCTION:** A fragmentation and lack of homogeneity in parasitological diagnostics across the national territory has long been known. For this reason, the AMCLI Study Committee for Parasitology (CoSP) has updated the diagnostic guidelines. Furthermore, the need arose to know and support human parasitological diagnosis. Therefore, CoSP in collaboration with the Italian Society of Tropical Diseases and Global Health (SIMET) and Italian Society of Parasitology (SoIPa) they thought of developing a fact-finding a survey of which, how many and where are public or private laboratories, that can carry out adequate parasitological diagnostics.

**MATERIALS AND METHODS:** The survey formulated by CoSP AMCLI is structured in 8 modules: laboratory details, pre-analytics, microscopic and cultural examinations for fecal parasites, microscopic and cultural examinations for blood parasites and parasites of the reticuloendothelial system and other body areas, antigen research, molecular biology, parasitological serology and, finally, workloads supported by the laboratories from 2019 to 2022. The questionnaire was sent via email to all AMCLI, SoIPa and SIMET members in October 2022.

**RESULTS AND CONCLUSIONS:** Data was collected until August 2023, 96 public and private laboratories (80 and 16 respectively) located in 19 Italian regions (Abruzzo, Basilicata, Calabria, Campania, Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Liguria, Lombardy, Marche, Piemonte, Puglia, San Marino, Sardinia, Sicily, Tuscany, Trentino-Alto Adige, Umbria and Veneto). From an analysis of the main data, only 37.5% receive the information about patient's clinical history data. Furthermore, it emerged that 49% performed both a direct and post-concentration fecal parasitological examination, 71.9% performed fecal antigen research, and 46.9% performed molecular biology. As regards malaria, it is carried out by 83/96 laboratories, of which 85.5% carry out antigen research and 61.4% molecular biology. Laboratories that carry out direct microscopic research of *Leishmania* spp. they are 53.1% on bone marrow biopsy and 38.5% on skin and subcutaneous samples. To guarantee correct and effective parasitological diagnostics it is necessary to use the most reliable and targeted techniques/methods, applied according to national and international scientific guidelines. It should be highlighted that not all laboratories are able to offer adequate services and comply with the guidelines; in fact, another purpose of the survey, is to create a network of national reference laboratories for parasitological diagnosis which can be contacted for consultation and/or carrying out specialized tests.

# PCR EVALUATION FOR INTESTINAL PROTOZOA AND HELMINTHS DETECTION IN FAECAL SAMPLES

Besutti V.*[1], Di Pietra G. [2], Oliva E.[3], Schillaci N.[4], Cuntrò M.[5], Raglio A.[6]

*[1]Azienda Ospedale-Università Padova, Padova, Italy; [2]Dipartimento Medicina Molecolare, Università degli Studi di Padova, Padova, Italy; [3]AUSL -IRCCS Santa Maria Nuova, Reggio Emilia, Italy; [4]ASST-Papa Giovanni XXIII, Bergamo, Italy; [5]ASST-Papa Giovanni XXIII, Bergamo, Italy; [6]AMCLI CoSP, Italy*

**Keywords**: RT-PCR, Protozoa, Helminths.

**INTRODUCTION:** The conventional diagnostic procedure for intestinal parasites detection requires specifically skilled microbiologist. The utilisation of molecular technique has offered several advantages compared to microscopy-based procedures, including greater sensitivity and specificity, especially for protozoa. However, helminths' diagnosis based on homemade tests still appear poorly reliable. The aim of this study was to evaluate three commercial multiplex real-time (RT-PCR) assays for the detection of protozoa and helminths from faecal samples.

**MATERIALS AND METHODS:** 368 samples were collected from 12 laboratories (P1) and another 355 samples from 18 laboratories (P2) for the detection of protozoa; whereas 181 samples were gathered from 15 laboratories (E1) for helminths search. The samples were examined with traditional techniques: microscopic examination after concentration, culture for helminths, serology. In P1 and E1, DNA was extracted with the Microlab Nimbus (Hamilton, Nevada) and subjected to RT-PCR using the Allplex GI-Helmint kit (Seegene). In P2 the DNA was extracted with MagNA pure 96 System Roche and examined with multiplex tandem PCR Parasites 8-well (AusDiagnostics). Targets in P1 and P2 were: *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp., *Dientamoeba fragilis* (Df), *Blastocystis hominis* (Bh), whereas for E1 were: *A. lumbricoides* (Al), Ancylostomatidae (A/N), *Strongyloides stercoralis* (Ss), *Taenia* spp. (Ts), *Hymenolepis* spp. (Hn), *T. trichiura* (Tt) e *E. vermicularis* (Ev). Given the lack of a reference gold standard, in case of discordance between conventional diagnostic procedure and PCR, the results were re-evaluated.

**RESULTS AND CONCLUSIONS:** Compared to conventional diagnostic procedure, sensitivity and specificity for protozoa detection are greater than 93%. The molecular techniques allowed the identification of 42 Df and 20 Bh more than conventional diagnostic procedures. For helminths the specificity of RT-PCR kit was 100% while the sensitivity was 33% for Al, 91% for A/N, 63% for Ss, 92% for Ts, 85% for Hn, and 50% for Ev. Molecular techniques are more sensitive and specific than microscopy, so they can be an aid for diagnosis of protozoa infections for inexperienced parasitologists. Similar to homemade tests, the commercial kit for helminths demonstrates a species-dependent sensibility. A low sensitivity was also found for Ev, taking into account that the gold standard for these helminths is the Graham test. In general, the introduction of molecular diagnostic procedures provides the opportunity of limiting the analysis to a single sample instead of the traditional three and to improve sensitivity and specificity. However, so far, the best option is a combination of molecular tests and microscopy, since the role of the parasitologist remains crucial to properly decipher laboratory results according to clinical and epidemiological data.

# EXTERNAL QUALITY ASSESSMENT (EQA): ANALYSIS OF RESULTS AND TRAINING PROPOSALS

Bernieri F.*

*AMCLI Study Committee for Parasitology (CoSP)/CRRVEQ Consultant, Milano, Italy*

**Keywords**: EQA, CRRVEQ, Professional growth.

**INTRODUCTION:** EQA programs in parasitology, as in all other laboratory branches, are of fundamental importance to guarantee the quality of the results of the analyses carried out. The results of a program managed by the Regional Reference Center for Quality Control (CRRVEQ) of the Tuscany Region which involves laboratories from various Italian Regions are illustrated.

**MATERIALS AND METHODS:** The responses, relating to the years 2022 and 2023, of the 180 laboratories that participated in the CRRVEQ EQA program in parasitology are evacuated. The program includes both fecal samples (8 samples per year) and blood samples (4 samples per year).

**RESULTS AND CONCLUSIONS:** For samples that contain the most usual or well-known parasites (for example *Giardia duodenalis*, *Taenia* spp., *Plasmodium falciparum* in high load) the answers reach good results (> 90% of correct answers), while in the presence of less usual parasites the answers are worse, for example for *Trypanosoma cruzi* ~ 50% of correct answers, for *Chilomastix mesnili* ~ 40% of correct answers. Furthermore, difficulties have been found in the identification of *Plasmodium ovale* with a certain frequency confused with *Plasmodium vivax*, but also of *P. falciparum* if in low load, Finally, difficulties were encountered in calculating the parasitemia of *P. falciparum*. EQA in parasitology allows to experiment with samples that in many cases are rarely found in normal laboratory routine, it allows to compare your results with those of other laboratories, avoiding self-referentiality; consequently, it allows continuous professional growth even (or perhaps above all) in the event of “failures” in the answers, allowing weaknesses to be highlighted and remedied.

# SCREENING OF BLOOD DONORS FOR MALARIA AND CHAGAS DISEASE: THE EXPERIENCE OF THE UNIVERSITY HOSPITAL IN PISA DURING 8 YEARS

Bruschi F.[1], Pinto B.[1], Galli L.[2], Lupetti A.[3], Mangano V.*[1]

*[1]Dip. Ricerca Traslazionale e Nuove Tecnologie in Medicina e Chirurgia, Università di Pisa, Pisa, Italy; [2]Servizio di Medicina Trasfusionale, Ospedale di Lucca, Lucca, Italy; [3]SOD di Microbiologia Universitaria, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy*

**Keywords**: Malaria, Chagas disease, Screening.

**INTRODUCTION:** Vector-borne parasites might be transmitted through transfusion, notably *Plasmodium* spp. and *Trypanosoma cruzi*. Prevention strategies include blood donor screening, referral, and blood unit treatment by pathogen inactivation methods. In 2015 the National Blood Centre has introduced a questionnaire to identify donors at risk, and their screening by serological methods (L.219, D.M. 02/11/2015). In early 2016 the Laboratory of Parasitology of Pisa University Hospital has started the serological analysis of blood donors referring to Transfusion Services located in North Western Tuscany. The aim of the present study was to describe the prevalence of seropositive donors observed during 8 years of screening.

**MATERIALS AND METHODS:** Blood donors at risk of transmitting malaria were screened by ELISA. The DRG ELISA kit was employed until 2020, when it was substituted by the Euroimmun ELISA kit based on the results of a comparative evaluation of available commercial kits (Mangano et al., 2019. Malaria J, 18:17). Seropositive donors were offered the possibility of *Plasmodium* DNA testing by Loop-Mediated AMPlification (LAMP) to exclude current infection. Donors at risk of transmitting Chagas disease were screened by ICT employing recombinant antigen until 2021, when it was substituted by ELISA employing lysate antigen because of its higher accuracy (Mangano et al., 2021. Transfus Med, 31:63-68). Seropositive donors were tested by CLIA and in case of discordant results also by WB, according to WHO guidelines for diagnosis of chronic Chagas disease.

**RESULTS AND CONCLUSIONS:** A total of 3,651 donors were tested for anti-*Plasmodium* antibodies, revealing a 6.2% (95% CI=5.5%-7.0%) seroprevalence. Seropositivity was higher among donors from Sub-Saharan Africa (39.0%; 95% CI=31.9-46.5) and Southeast Asia (10.2%; 95% CI=5.9-16.0%). Euroimmun ELISA (4.41; 95% CI=3.48-5.59) exhibited lower seropositivity than DRG ELISA (7.44; 95% CI=6.41-8.62). Seropositivity dropped to 3.19% (95% CI=2.00-5.05) in 2020, likely because of travel restrictions during the Covid19 pandemic. None of the donors resulted positive to *Plasmodium* DNA LAMP testing. Among 4,520 donors tested for anti-*T. cruzi* antibodies seroprevalence was 0.8% (95% CI=0.6%-1.1%), aligning with national survey results (Pati et al., 2022. Pathogens, 11:1229). All seropositive donors were born in Europe or Latin America. Seropositivity was apparently lower with ELISA (0.6%, 95% CI=0.3%-1.3%) than ICT (0.9%, 95% CI=0.6%-1.2%), possibly due to ELISA higher specificity. No confirmed cases of chronic Chagas disease were identified. The study emphasizes the importance of defining the serological test employed for screening, and the need to confirm seropositive results with further testing. The low transmission risk observed in the study suggests repeating seropositive donor screening after a year to minimize referral and blood unit loss.

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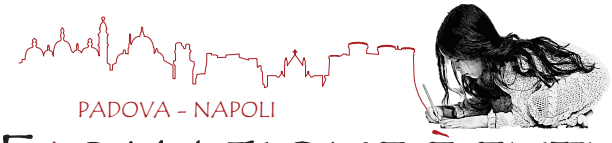
# DONNE IN PARASSITOLOGIA: DALLE PARASSITOSI "GENDER-BIASED" AL SOFFITTO DI CRISTALLO



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# WOMEN IN PARASITOLOGY: FROM "GENDER-BIASED" PARASITIC DISEASES TO THE GLASS CEILING

Cavallero S.*[1], Gabrielli S.[1], Gazzonis A.L.[2]

*[1]Sapienza University of Rome, Department of Public health and infectious diseases, Rome, Italy; [2]Università degli studi di Milano, Department of Veterinary medicine and animal sciences, Milan, Italy*

**Keywords**: STEM, Gender gap, Women empowerment.

The diversity in the scientific world represents a richness that leads to added value in terms of productivity and research impact, as different backgrounds provide greater richness in methodical approaches, insights, and expertise. However, there are still various barriers, primarily of socio-cultural nature, that hinder certain groups, discriminated for the religion, sexual orientation, or gender. Recently, the term "dream gender gap" has been coined to describe a phenomenon where, since childhood, girls seem to be deprived of the very opportunity to dream about certain perspectives: girls are not encouraged to pursue STEM interests due to prevailing norms and stereotypes. The same barriers are at the basis of the "leaky pipeline", which refers to a disproportion in terms of access and career advancement between men and women. Parasitology also aligns with the trend, with women underrepresented in academic positions and in the scientific publishing field (Calvani et al., 2023. Trends Parasitol, 39:73-9). To address some gender issues via the lens of parasitology and to create a supportive community for female parasitologists, this symposium will face different aspects concerning the gender equality in parasitology. We will open with a brief biography of an inspirational female Italian parasitologist, providing young researchers with positive role models in the STEM to break down gender stereotypes and encourage their involvement (González-Pérez et al., 2020. Front Psychol, 11). The lack of resources to support STEM education, of female scientist mentors and a patriarchal view of such fields, among other factors, will then be discussed in relation to the dearth of female representation in low- and middle-income countries. Then, the efforts proposed by European Commission in terms of gender equality in research will be discussed, highlighting key initiatives and strategies. Indeed, the Goal 5 of the United Nations' 2030 Agenda for Sustainable Development aims to achieve gender equality and empower all women and girls. Moreover, the European Commission research priority clearly encourages accounting for the gender dimension in research project development, both in terms of human resources and research content, accounting for possible differences between male and female. Finally, since women are disproportionately affected by some parasitic diseases and their sequelae, an assessment to improve public health interventions and research programmes targeting women, especially in tropical areas is needed. Efforts in increasing the training, promotion, retention, well-being and productivity of women are therefore needed, for example by incentivising systematic changes to improve the visibility and voice of women, requiring senior leaders to report on progress towards gender equality, and making research funding conditional on the availability of a transparent and adequately resourced gender equality plan (Ryan, 2022. Nature, 604.7906:403-403).

# ADDRESSING WOMEN CHALLENGES IN PAKISTAN: AN EXAMPLE FROM A PARASITOLOGIST INVESTIGATING ECHINOCOCCOSIS

Muqaddas H.*[1], Mehmood N.[2]

*[1]Department of Zoology, The Women University Multan, Multan, Punjab, Pakistan; [2]Department of Zoology, University of Sargodha, Sargodha, Punjab, Pakistan*

**Keywords**: Women parasitologists, Pakistan, Cystic echinococcosis.

A substantial gender disparity persists across all levels of science, technology, engineering, and mathematics (STEM) disciplines on a global scale. Regrettably, the aforementioned situation persists in Pakistan as well, where there is a dearth of female representation in these fields. In Pakistan, the representation of women in STEM is below 10%. Getting a university education is limited to only 5% of females in the population of 241.5 million, primarily because of cultural and familial constraints. Being a doctor is a very prestigious occupation for women. Unfortunately, according to the Pakistan Medical Council (PMC), 50% of female physicians who graduated either did not practice or left their jobs within a brief period. The situation holds true for female parasitologists as well; Pakistan has had fewer than twenty female parasitologists in the last two decades. Out of which very few are actively publishing their findings. As an example, the top-notch parasitology department at the Pakistani university “University of Veterinary & Animal Sciences (UVAS), Lahore” has no female faculty member working as a parasitologist.Cystic echinococcosis (CE) is a chronic parasitic zoonotic disease, most prevalent in poor pastoral communities globally. CE is endemic in Pakistan too; scarce and random information about the genetic patterns of *Echinococcus granulosus* is available. I am a female parasitologist investigating echinococcosis in Pakistan, trying to unravel the knowledge about the echinococcus genotypic pattern and exploring the main transmission routes infecting ruminates in Pakistan. Parasitologists like me encounter significant obstacles in three primary domains: the workplace; socio-cultural barriers; and familial constraints that may impede progress. Prevalent patriarchal societal norms entail prejudices against women who pursue careers, while early marriages obstruct their ability to do so. Field sampling and laboratory work are challenging itself. There is no trend of women going to slaughterhouses to inspect diseased animals, where men threaten them while collecting samples. Similarly, there are accounts of significant work-life conflict, with the majority of women attempting to balance socially imposed domestic responsibilities and their professional aspirations. Furthermore, women were burdened with a multitude of workplace challenges that compounded their pre-existing difficulties. Inadequate support from the administration, harassment, gender discrimination, an unsafe work environment, a deficient recruitment and selection process, and transfer restrictions all significantly contribute to the dearth of female parasitologists in Pakistan. Despite all these obstacles, initiatives are underway to bridge the gender gap and increase the participation of Pakistani women in the field of parasitology in general and in STEM in particular.

# GENDER EQUALITY IN RESEARCH AND INNOVATION AS A KEY PRIORITY IN EUROPE

La Colla F.*

*APRE - Agenzia per la Promozione della Ricerca europea, Rome, Italy*

**Keywords**: Gender, Women, Research.

Gender in research is one of the most important aspects that the European Commission is pushing in the last 10 years especially (but not exclusively) in its relation with the research and innovation. For this reason, one of the elements that will be investigated during the Panel discussion will regard the role of the gender dimension into the research environment in Europe and how the European Institutions are working to reach the gender equality also in this field. Indeed, understanding how gender plays a vital role in research and innovation allows us to address the diverse needs of EU citizens. It enhances the societal relevance of the knowledge, technologies and innovations produced, and contributes to the production of greater goods and services. Not considering the gender dimension into the research activities, means to miss important part of the analysis and to contemporary miss a key part of the population's data with its own characteristics and necessity. So, doing "wrong" research costs lives and money. Between 1997 and 2000, 10 drugs were withdrawn from the U.S. market because of life-threatening health effects. Eight of these posed "greater health risks for women than for men". Gender bias also leads to missed market opportunities. In basic research, failing to use appropriate samples of male and female cells, tissues, and animals yields faulty results. In medicine, not recognizing osteoporosis as a male disease delays diagnosis and treatment in men. At the European level, currently no Member State has achieved full gender equality and progress is slow and the gender gap is still present in the education and work sectors (wages, care, roles, and pensions). Another key aspect is the under-representation of women in STEM fields (still persistent) and this results in a low level of integration of gender issues in R&I content. One of the concrete ways in which the EC is contributing to address the gender dimension into the research activities it's represented by the introduction of the Gender Equality Plans as a mandatory requirement for the participation in EC Funding Programmes for Research and Innovation (specifically Horizon Europe).

# WOMEN AND TOXOPLASMOSIS IN ITALY AND BRAZIL

Meroni V.*

*University of Pavia, Pavia, Italy*

**Keywords**: Toxoplasmosis, Public health approach, Italy and Brazil.

**INTRODUCTION:** Toxoplasmosis is antropozoonosis caused by *Toxoplasma gondii* an apicomplexa parasite with wide world distribution. The prevalence of infection in humans varies with the climate (it is lower in very cold and very hot climates) and hygienic and dietary habits. The infection is usually asymptomatic and self-limiting but can cause severe disease in immunocompromised patients and in fetuses if transmitted from a mother with a primary infection. In Italy, first-trimester serological screening and monthly re-testing of non-immune pregnant women are offered free of charge (not mandatory) since 1998 and is still counselled by recent national guidelines despite a strong seroprevalence decrease. On the other hand, in Brazil seroprevalence range from 50% to 80% in different states. Prenatal screening is not always offered and only very recently congenital toxoplasmosis is a notifiable disease.

**MATERIALS AND METHODS:** We compare the results obtained in a multicentre study on seroprevalence of TORCH infection performed from 1 July 2019 to 30 June 2020 on childbearing aged women and italian approach to the problem to the different situation for women in Brazil.

**RESULTS AND CONCLUSIONS:** out of 111580 women of childbearing age evaluated for the presence of anti-*T. gondii* antibodies, 13% showed IgG positive and negative IgM results (which indicates a past infection) and 0.04% had acute infection. This result highlights the decreasing of seroprevalence registered in Italy that was already shown by many papers on regional data obtained in last years. In Brazil the high seroprevalence (50-80%) correlates with an incidence of congenital infection (CT) from 0.1 to 0.4 per 1000 live births and with recent dramatic outbreaks. Furthermore, the strain circulating in Brazil are more virulent (atypical strain) than strain circulating in Italy. In Italy and in many European countries, congenital toxoplasmosis treated before (via maternal screening) and after birth is a chronical ophthalmologic disease with good prognosis. On the other hands in Brazil where until 2018 no standardized guideline was set up severe cases and also death for CT have been reported (Strang et al., 2020. Acta Trop, 211:105608). In 2021 postnatal IgM serology via prick heel test started to be universally performed but many problems like implementation of public health service must be solved. As recently a Brazilian paper has clearly demonstrated the efficacy of antenatal therapy on infected babies (Gomes Ferrari Strang et al., 2023. PLosNegl Trop Dis, 17:e0011544) we hope that after a first document from Buzios meeting in 2008, the creation of a pan American *Toxoplasma* and Toxoplasmosis network/consortium during VI SIMBRATOX & III SINTOX held in Brazil on October 17th, 2023 could help in changing the approach to this problem not only in all the Brazilian states but also in many other South American countries (Gomes Ferrari Strang et al., 2023. PLosNegl Trop Dis, 17:e0011544).

# CREATING AWARENESS TO ELIMINATE NEGLECTED DISEASES: THE CASE OF FEMALE GENITAL SCHISTOSOMIASIS

Marchese V.[1], Rausche P.[1], Remkes A.[1], Kislaya I.[1], Kutz J.[1], Brito A.[1], Hey J.[1], Lorenz E.[1], Rasamoelina T.[2], Ratefiarisoa S.[3], May J.[1], Rakotoarivelo R.A.[4], Fusco D.*[1]

*[1]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany; [2]Centre d'Infectiologie Charles Mérieux, Antananarivo, Madagascar; [3]University of Mahajanga, Mahajanga, Madagascar; [4]University of Fianarantsoa, Fianarantsoa, Madagascar*

**Keywords**: Female genital schistosomiasis, Unmet medical needs, Women's health.

**INTRODUCTION:** Neglected Tropical Diseases (NTDs) are a group of pathologies distinguished by diverse aetiologies and characteristics, sharing a common denominator in their association with impoverishment, marginalised populations, and scarce attention in terms of research and development. Female genital schistosomiasis (FGS) is a neglected disease with long-term physical and psychosocial consequences, representing a major unmet medical need, which affects approximately 50 million women worldwide. FGS is the chronic manifestation of a persistent *S. haematobium* infection. However, FGS services are not routinely offered in endemic settings with only a small proportion of women at risk receiving adequate care. Despite being a significant public health concern in many parts of the world, FGS remains largely under-researched and under-diagnosed, even in Europe. The increasing movement of individuals (travellers and migrants) from schistosomiasis-endemic countries to Europe, as well as the presence of the parasite's intermediate host (*Bulinus truncatus*) in several southern European countries, has raised concerns about the emergence of the disease in thus far non-endemic areas.The scope of our study was to assess awareness and knowledge of FGS in endemic (Madagascar) and non-endemic settings (Europe) in order to highlight challenges and opportunities for a more proactive management of the disease across populations in need.

**MATERIALS AND METHODS:** Two cross-sectional surveys were conducted between August 2020 and January 2024. In Madagascar, both the general population and health care workers (HCWs) were interviewed, while in Europe the survey was limited to HCWs. In Madagascar, interviews were held in person, while in Europe the survey was conducted online. Descriptive statistics, including proportions, were calculated for the reporting of socio-demographic population characteristics. A knowledge score was calculated to describe the level of knowledge among the study population. Binary Poisson regression with robust standard errors was used to estimate crude (CPRs) and adjusted prevalence ratios (APRs) with 95% CIs.

**RESULTS AND CONCLUSIONS:** A total of 783 participants from Madagascar and 922 from Europe were included in the analysis. In Madagascar 11.3% (n=78) of the women and 53.8% (n=50) of the HCWs reported to be aware of FGS. In Europe 43.7% (n=254) of medical doctors and 12% (n=16) of nurses/midwives reported to be aware of the disease. In both contexts, none of the groups interviewed reached a knowledge score higher than intermediate, with the majority of the respondents belonging to the group with little or no FGS knowledge. In conclusion, our studies identify important gaps in knowledge and awareness of FGS in both endemic and non-endemic settings. Raising awareness and knowledge of chronic forms of schistosomiasis, as well as of other NTDs, can help to address diseases that may silently affect large numbers of people worldwide.

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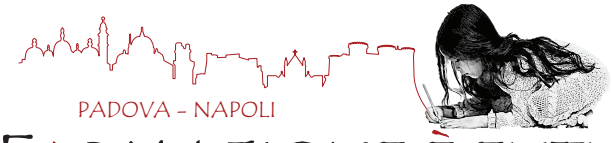
# MICOLOGIA VETERINARIA: NON SOLO DERMATOFITI



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# SPOROTRICHOSIS FROM ENDEMIC TO GLOBAL EMERGENCY

Giusiano G.*

*Instituto de Medicina Regional, Universidad Nacional del Nordeste, Resistencia, Argentina*

**Keywords:** Sporotrichosis, *Sporothrix brasiliensis*, Risk area.

Sporotrichosis, caused by species of the dimorphic genus *Sporothrix*, has emerged as a significant global health issue in recent years. This fungal infection has historically been regarded as a localized disease primarily affecting humans but also a wide range of animals in endemic regions. However, there has been a notable shift in its epidemiology and in its definition as subcutaneous mycosis. The emerging species *Sporothrix brasiliensis*, is a growing public health concern globally. Brazil is at the forefront of this worrying trend. The rise of this highly virulent species is associated with more severe clinical forms, atypical manifestations, and higher rates of dissemination, posing significant challenges for diagnosis and treatment. Transmission of *Sporothrix* spp. occurs primarily through traumatic inoculation of fungal spores into the skin or mucous membranes. While environmental exposure remains a common route of infection, zoonotic transmission (bites or scratches from infected animals) is one of the main routes for sporotrichosis brasiliensis, but a possible route of transmission through respiratory droplets is currently being considered. The most common clinical presentations are cutaneous and lymphocutaneous but extracutaneous, immunoreactive and ocular forms associated to *S. brasiliensis* infections are not easily recognized, delayed diagnosis leads to worse outcomes. The endemic nature of sporotrichosis was once confined to specific geographical regions; however, changes in environmental conditions and the emergence of a highly virulent species, as well as social and demographic factors, have contributed to its spread beyond traditional boundaries. Climatic and anthropogenic changes, as well as population migrations, have created new ecological niches for the fungus, facilitating its spread to previously unaffected areas. *S. brasiliensis*, first confined to Brazil, has spread to South and Central America and Europe. Enhanced surveillance and diagnostic capabilities are essential for early detection. Healthcare professionals must be vigilant for atypical presentations, particularly where sporotrichosis may not be immediately considered in the differential diagnosis. Efforts are needed to better understand the epidemiology and pathogenesis, particularly in the context of emerging agents like *S. brasiliensis*. The global emergence of sporotrichosis poses a significant challenge for healthcare systems worldwide. Sporotrichosis can manifest often mimicking other diseases. Its diversity in clinical presentation can lead to diagnostic delays and inappropriate treatment, exacerbating morbidity and mortality. Only through concerted global action can we hope to mitigate the impact and prevent its further spread. Part of this process is to raise awareness of the zoonotic potential of sporotrichosis, not only among healthcare professionals, but also among the general public who own or interact with pets.

# NEW EMERGENCIES IN MEDICAL AND VETERINARY MYCOLOGY

Cafarchia C.*

*University of Bari Aldo Moro, Department of Veterinary Medicine, Bari, Italy*

**Keywords:** *Candida auris*, *Candida blankii*, *Malassezia* spp.

Nowadays, fungal infections pose a threat to public health by causing infections characterized by high morbidity and mortality (Fisher et al., 2020. mBio, 11:e00449-20; Chowdhary et al., 2020. Clin Microbiol Infect, 26:648; Geremia et al., 2023. Healthcare, 11:425). Yeasts of the genus *Malassezia* and some species of *Candida non albicans* (*i.e.*, *C. auris* and *C. blankii*) are considered new emerging pathogens, as they are associated with a high mortality rate mainly in nosocomial settings (Rhimi et al., 2020. Front Cell Infect Microbiol, 10:370; Chowdhary et al., 2020. Clin Microbiol Infect, 26:648.e5-648.e8; Geremia et al., 2023. Healthcare, 11:425). Briefly, *Malassezia* spp. yeasts are well long-known organisms inhabiting the skin and mucosa of humans and animals which are involved in a variety of skin disorders in humans, in animals and in bloodstream infections in severely immunocompromised patients (Rhimi et al., 2020. Front Cell Infect Microbiol, 10:370). *C. auris* infections were reported in 2009 and the first European outbreaks date back to 2015 in France and to 2019 in Italy (Geremia et al., 2023. Healthcare, 11:425), whereas for *C. blankii*, the first report goes back to 1968 as a lethal pathogen in mink (Buckley and van Uden, 1968. Mycopathol Mycol Appl, 36:257-266) but it was recognized as cause of severe infections in immunocompromised patients only from 2014 (Chowdhary et al., 2020. Clin Microbiol Infect, 26:648.e5-648.e8). All these yeasts are characterized by a high virulence profile, multidrug resistance phenomena, biofilm development, and the ability to evade the response of the innate immune system. However, many gups in the epidemiology, transmission route, diagnosis and treatment need to better address. Herein, the most recent literature on epidemiology, biology, virulence factors, drug-resistance phenomena, therapy and prophylaxis procedures of *Malassezia* spp., *C. auris* and *C. blankii* infections will be summarized and discussed with the general goal of arousing the interest of clinicians, mycologists and operators to better manage these emerging infections.

# *GALLERIA MELLONELLA* LARVAE AS A MODEL FOR INVESTIGATING *ASPERGILLUS*-HOST INTERACTIONS

Danesi P.*, Sgubin S., Foiani G.

*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy*

**Keywords:** *Galleria mellonella*, *Aspergillus*, Alternative model.

The principles of the 3Rs (Replacement, Reduction and Refinement) embedded in international legislation aim to guarantee the welfare of animals used for scientific purposes and encourage researchers to replace traditional rodent models with alternative, non-mammalian ones (Scully et al., 2006. FEMS Microbiol Lett, 263:1-9). *Galleria mellonella* has become one of the most popular invertebrate models (with more than 2,200 scientific articles published) used to study microorganisms (mostly bacteria and fungi) and host-microbe interactions. *Galleria* larvae are naturally exposed to pathogens and have developed immune defense systems that share several similarities with the innate immune system of vertebrates. The wax moth innate immune system, mediated by haemocytes, is able to fight against a large spectrum of pathogens via phagocytosis, melanization and the secretion of antimicrobial peptides (Trevijano-Contador et al., 2018. J Fungi, 5:1) *Aspergillus* genus is one of the most pathogenic fungal groups able to cause life-threatening diseases in both humans and animals. The main objective of the study was to test *G. mellonella* as animal model to assess *Aspergillus* spp. virulence. Larvae of *G. mellonella* were experimentally infected with six strains of *Aspergillus* (*A. flavus*, n=2; *A. fumigatus*, n=2; *A. terreus*, n=2) with different spore concentrations (10^3 to 10^7). Survival curves of larvae monitored for 7 days at 37°C showed different pathogenicity. All strains were isolated from clinically symptomatic animals and/or after necropsy. At the highest inoculum concentration (10^7) *A. flavus*, *A. fumigatus* (3696/18) and *A. terreus* (3996/20) ($p$<0.05) were significantly more pathogenic than the control groups. Compared with the other isolates, *A. flavus* (3996/19) showed a higher pathogenetic trend already at low inoculum concentration (10^3) starting from the second day *post*-infection ($p$=0.05238) to the end of the trial ($p$<0.05). We investigated the progress of *A. fumigatus* infection in larvae after 3 and 7 days of spore inoculation by histological preparations. Melanized nodules and granulomas containing both conidia and hyphae have been detected in the larvae. The number and size of the nodules and granulomas, which are distributed all over the larva, increased over time after the infection. These preliminary results, in line with previous studies, showed that *G. mellonella* larvae represent a reliable model for the analysis of pathogenesis and virulence factors of *Aspergillus* strains.

# EXOTIC FUNGAL PATHOGENS AND WHERE TO FIND THEM

Peano A.*

*Dipartimento di Scienze Veterinarie, Università di Torino, Grugliasco, Italy*

**Keywords:** Animals, Invasive mycoses, Fungi.

Fungi that infect animals and humans include monocellular (yeasts), filamentous, or thermally-dependent dimorphic organisms (Kozel and Wickes, 2014. Cold Spring Harb Perspect Med, 4:a019299). They are responsible for diseases involving superficial tissues up to life-threatening infections. Some fungi, such as *Aspergillus* spp. and *Cryptococcus* spp., are distributed worldwide and are opportunistic pathogens. Other fungal infections, such as those due to some dimorphic fungi (*e.g.* histoplasmosis, coccidioidomycosis, blastomycosis, talaromycosis), are classically considered to have a more restricted geographical distribution. These fungi are primary infection agents and a significant cause of human morbidity and mortality. Furthermore, epidemiology is still incomplete for some fungi, such as the newly described *Emergomyces* species (Zerbato et al., 2023. Mycopathologia, 188:307-334). Apart from the "true" fungi, other organisms known as pseudo-fungi (since they present structures similar to those of fungal organisms) are occasionally involved in infection episodes in mammals. An example of such an infection is pythiosis, caused by organisms having an aquatic life cycle (Peano et al., 2023. Emerg Infect Dis, 29:1447-50). In recent years, 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). Moreover, the spectrum of fungi causing infections is expanding thanks to more discriminating tools for identification. These tools are revealing new fungal species involved in various infective forms and better defining the borders between formerly recognized species with consequent changes in taxonomy and nomenclature (Kozel and Wickes, 2014. Cold Spring Harb Perspect Med, 4:a019299). Cases of invasive mycosis are more rarely reported in animals principally because the situations leading to immunosuppression in human patients are not mirrored in veterinary medicine. Moreover, the diagnostic procedures are often less advanced, which leads to the possibility that cases occur but are overlooked (Elad and Segal, 2018. Front Microbiol. 9:1303).Over the past few decades, there have been increasing reports of unexpected infections due to fungi that are considered not endemic in certain areas. There are numerous potential reasons for the appearance of these "exotic" pathogens. Beyond the considerations above on the increased number of immunosuppressed people, global factors such as migration, increased travel, and climate change may play a role (Ashraf et al., 2020. Mycopathol, 185:843-865). The talk connected to the present abstract focuses on these epidemiological considerations regarding animals, with the description of some unusual cases found in Italy (for example, a case due to *Emergomyces pasteurianus* in a cat, and cases due to *Pythium periculosum* in dogs) (Peano et al., 2023. Emerg Infect Dis 29:1447-50) and literature analysis.

# FUNGAL RHINITIS IN COMPANION ANIMALS

Matteucci G.*

*AbLab srls, Veterinary Diagnostic Laboratory, Sarzana (SP), Italy*

**Keywords:** Fungi, Rhinitis, Companion animals.

The most common fungal respiratory infection in dogs involves sino-nasal cavities. It mostly affects young otherwise healthy dolichocephalic and mesocephalic dogs, with *Aspergillus* spp., particularly *Aspergillus fumigatus*, most commonly reported. Other causes of canine mycotic rhinitis are occasionally reported (*e.g*. *Penicillium* spp., *Cryptococcus* spp., *Blastomyces dermatitidis*, *Rhinosporidium seeberi*, *Entomophthorales*) (Ostrzeszewicz et al., 2015. Pol J Vet Sci, 18:683-8; Jaffey et al., 2021. Front Vet Sci, 8:633695). Incidence of *Fusarium* spp., *Talaromyces* spp., and *Scedosporium* spp. as cause of fungal rhinitis in dogs is often underestimated and is now becoming clinically relevant (Matteucci et al., 2017. Veterinaria, 31:333-7). Infection with fungi other than *Aspergillus* spp. should be considered as differential diagnosis in dogs with fungal rhinitis, and a fungal culture should be performed even if aspergillosis is suspected. Various mycotic infections can be confused with *Aspergillus* spp. due to similar clinical presentation and morphologic features at histopathological examination. Correct identification of the organism through culture and molecular tools is imperative for prognosis and treatment as different fungal species have different behaviour and antifungal susceptibility. *Cryptococcus neoformans* is the most frequent species causing sino-nasal mycoses in cats (Pennisi et al., 2013. J Feline Med Surg, 15:611-618), while sino-orbital mycoses are primarily caused by *Aspergillus felis*. This fungus is often refractory to aggressive antifungal therapy and brachycephalic cats are at a higher risk (Hartmann et al., 2013. J Feline Med Surg. 15:605-10). Although very rare, blastomycosis and histoplasmosis should be included in the differential diagnoses for feline nasal mycosis if in endemic areas (Grinstead et al., 2021. JFMS Open Rep, 7:2055116921993385), together with other fungal species (*e.g*. *Aspergillus* cryptic species, *Penicillium* spp., *Paecilomyces* spp., phaeohyphomycetes, *Microsporum canis*) (Giordano et al., 2010. J Feline Med Surg, 12:714-23; Ziglioli et al., 2016. J Small Anim Pract, 57:327-331). Among non-traditional companion animals, birds and reptiles are more prone to fungal rhinitis (Galosi et al., 2022. Front Vet Sci, 9:883276; Nardoni et al., 2023. J Fungi, 9:518). Treatment of fungal rhinitis is highly specific, expensive and often invasive. Therefore, precise diagnosis is mandatory to start the therapy. Culture of nasal biopsies collected during rhinoscopy leads to molecular identification and in vitro susceptibility data. In addition, the gold standard requires to submit biopsies for histopathology to detect the fungus and determinate if it invades the nasal mucosa or is a secondary colonizer (Ostrzeszewicz et al., 2015. Pol J Vet Sci, 18:683-8).

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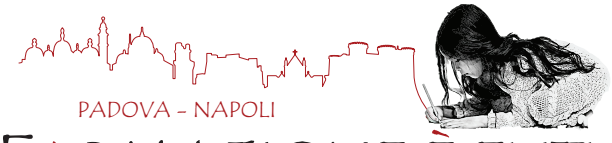
# ONE HEALTH: UNA SALUTE UNICA E UNA SOLA SCIENZA. L'ESPERIENZA DELLA REGIONE VENETO PER LA PREVENZIONE E IL CONTRASTO DELLE ZOONOSI DA VETTORE



PADOVA - NAPOLI

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# ONE HEALTH AND ONE SCIENCES: THE EXPERIENCE OF THE VENETO REGION IN THE PREVENTION OF VECTOR-BORNE ZOONOTIC DISEASES

Lazzarini L.[1], Montarsi F.[2], Cassini R.[3], Simonato G.[3], Scaggiante R.[4], Colombo L.[5]

*[1]UOC Malattie Infettive, Ospedale San Bortolo di Vicenza; [2]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy; [3]Dipartimento di Medicina Animale Produzioni e Salute (MAPS), Università di Padova; [4]UOC Malattie Infettive, Ospedale San Martino di Belluno; [5]MSD Animal Health*

Vector-borne diseases in Europe are becoming increasingly important and pose serious problems for animal health and public health. The World Health Organization (WHO) estimates that they cause over 1 billion human cases and 1 million deaths each year, accounting for about 17% of total cases of communicable diseases. Various factors contribute to the emergence or re-emergence of certain zoonotic diseases. Global climate change is part of these factors that impact on the physiology, life cycle, and geographic distribution of vectors and reservoir species, influencing the appearance and increased prevalence of climate-sensitive diseases. The interactions among temperature, vector, and pathogen can change the risk of human-to-human disease spread and of spillover to humans from reservoir hosts. The Intergovernmental Panel on Climate Change Reported that the prevalence of vector-borne diseases has increased in recent decades and that the prevalence of malaria, dengue, Lyme disease, and West Nile virus infection in particular, are expected to further increase during the next 80 years if measures are not taken to adapt and strengthen control strategies (Thomson and Stanberry, 2022. N Engl J Med, 387:21). Lyme disease (which is caused by the *Borrelia burgdorferi sensu lato* complex) is the most common tick-borne illness worldwide, with an estimated seroprevalence of 14.5%; the reported prevalence is highest in the temperate regions of central and western Europe with an average of 128,888 cases reported annually from the countries that have surveillance systems. The absence of surveillance of data in some European countries, like Italy, represent an important gap in our understanding of the geographic extent of endemic areas (Burn et al., 2023. Vector Borne Zoonotic Dis, 23:156-171). Italy is one of the countries in the Mediterranean basin traditionally considered endemic for leishmaniasis, a neglected vector-borne zoonotic disease caused, in this area, by *Leishmania infantum*, responsible for both cutaneous and visceral forms, with dogs recognized as the main reservoir. According to data reported to WHO, less than 100 cases of both human cutaneous (CL) and visceral leishmaniasis (VL) were reported in Italy in 2020 most of which were autochthonous. However, although case notification for human leishmaniasis is mandatory in Italy, the disease is generally underreported, especially CL that do not require hospitalization and several cases in illegal migrants might remain undiagnosed. These data suggest the need for education and training at university and post-graduate levels to increase the awareness of NTDs among healthcare professionals, as well as for targeted public health interventions (Casulli et al., 2023. Parasitology, 150:1082-1088). In Veneto, a sufficient knowledge has been achieved on the distribution of well-established species of ticks (*e.g. Ixodes ricinus*) and on the control of the associated endemic zoonotic diseases (*e.g*. Lyme diseases, Tick-Borne Encephalitis). However, climate change and animal movements can facilitate the spread of tick species already present in the territory or newly introduced, leading to an increased risk for the transmission of emerging zoonotic diseases (*e.g.* SENLAT, human babesiosis, tularemia, Crimean-Congo haemorrhagic fever) (Hartemink and Takken, 2016. Exp Appl Acarol, 68:269-278). The direct impact of tick-borne pathogens on domestic animals has likely a minor relevance, although some pathogens circulating mainly in wild carnivores may cause clinical diseases in domestic animals. In contrast, few scattered Canine leishmaniosis (CanL) autochthonous infections were reported in Veneto region in the past. However, more recently, CanL cases are expanding due to the resulting expansion and increased density of competent vector (sand flies) favoured by global warming, in association with travelling/relocation of infected dogs from the southern Italy. Moreover, the occurrence of phlebotomies has led to the emergence of Phlebovirus infections in humans posing new One Health challenges (Gradoni et al., 2022. Vet Parasitol Reg Stud Reports, 27:100676). Tick and sandfly-borne diseases such as Lyme disease, babesiosis and leishmaniosis can also affect dogs causing a wide range of symptoms and posing severe health issues. Preventive measures are essential to protect dogs from these pathogens. Mechanical actions

such as keeping the dog's environment clean, avoiding areas with high vector activity, and the regular use of chemical repellents and insecticides represent key strategies (Gálvez et al., 2018. Res Vet Sci, 121:94-103). Additionally, routine veterinary check-ups and vaccinations, when available (WSAVA guidelines 2024), help in early detection and control, ensuring the health and well-being of dogs. In addition, since these diseases are potentially zoonotic, these measures are crucial not only to safeguard the canine but also human health. A robust monitoring and prevention system based on collaboration between institutions is necessary to control and contain zoonotic vector-borne diseases. Furthermore, a close interprofessional synergy between the human medicine sector (emergency physicians, internal medicine doctors, general practitioners, infectious disease specialists, pediatricians, geriatricians) and the veterinary sector (private practice veterinarians and prevention departments of Local Health Authorities) is recommended, without forgetting the support of other professionals such as biologists involved in entomological surveillance and parasitologists. One Health professionals are therefore the most important asset for infection detection and health protection in the territory. The symposium, organized by HPS - AboutPharma and MSD Animal Health, aims to deepen, from a One Health perspective, the "*status quo*" of some of the most common arthropod-borne diseases in the Veneto region, with a focus on leishmaniasis and Lyme disease. The symposium will analyze the epidemiological, environmental, and climatic aspects, as well as surveillance and containment measures through a discussion among the main stakeholders involved.

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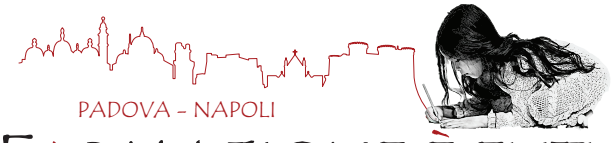
# LA DIDATTICA PARASSITOLOGICA IN ITALIA



PADOVA - NAPOLI

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# TEACHING PARASITOLOGY AND PARASITIC DISEASES IN ITALY

Cringoli G.[1], Bruschi F.[2], Cassini R.[3], Varcasia A.[4]

*[1]Dipartimento di Medicina Veterinaria e PA, Università di Napoli Federico II, Napoli, Italy; [2]Dipartimento di Ricerca Traslazionale e delle Nuove Tecnologie in Medicina e Chirurgia, Università di Pisa, Pisa, Italy; [3]Dipartimento di Medicina Animale Produzioni e Salute - MAPS, Università di Padova, Italy; [4]Dipartimento di Medicina Veterinaria, Università di Sassari, Sassari, Italy*

In the curricula of the medical sciences degrees the teaching of subjects related to Parasitology and parasitic diseases act as a link between the basic sciences knowledge and the clinical approach, playing an important role also in the field of public health. As a consequence, these subjects have an intrinsic complex and interdisciplinary nature and this calls for a continuous update of the teaching methods, aiming at a better learning at all levels (e.g., concepts knowledge, topics connection, problem analysis, practical skills).
The continuous improvement of the quality of the teaching methods and the teaching materials in the Italian context is therefore the aim of this symposium, whose first part is dedicated to the presentation of the last editions of different textbooks, which are a fundamental component of the teaching material. In the second part, the experiences of two Italian Universities in two highly relevant aspects of teaching innovation will be presented. Specifically, the use of tools and methods based on a student-centred approach on one side, and the influence of video communication and artificial intelligence in teaching and research on the other side, will be delineated as briefly described here below.
The European Union strategy for the Higher Education Institutions () encourages Universities and university faculties to experiment new student-centred teaching and learning strategies. The University of Padova has funded different projects aimed at the implementation of this new kind of approach, promoting the introduction of the Problem-Based Learning (PBL) and the Team-Based Learning (TBL) in different Degrees of the veterinary and animal health area (Broseghini et al., 2024, Vet. Sci. 11:104). These methodologies foster the development of important transversal skills, such as analytical thinking, problem solving, interpretation, synthesis, collaboration and self-directed learning. Furthermore, during plenary sessions, student skills in effective communication are also enhanced. A peculiar case is represented by an international project started in 2021, and now named i-PLEX, that consists of an international Blended Intensive Course with veterinary students from three European Universities (University of Padova, University of Porto, Estonian University of Life Sciences). Students are requested to solve different clinical and public health case-studies using the PBL approach, the added value being represented by the international and multicultural learning environment. These experiences also contribute to the creation of teaching communities and foster interdisciplinarity, promoting discussion on teaching practices within the academic staff.
In recent years, the use of new media, video communication and artificial intelligence (AI) has improved the way in which parasitology and more generally science is taught to undergraduate and postgraduate students. Digital content, such as videos, has been used extensively for teaching, to support articles, but also as visual summaries in scientific papers. The use of summaries in addition to abstracts has been increasingly required by journals in order to disseminate them on the Internet and provide concrete public engagement, which has been widely evaluated by Governmental agencies, like ANVUR in VQR. Video tutorials on the most common parasitological techniques have also been included in the latest parasitology books (Otranto and Wall, Wiley 2024) in the form of QRs. The incorporation of AI into most social networks and YouTube has also overcome language barriers by providing content with subtitles/captions in all languages, which also helps individuals with specific learning disabilities. In relation to this last aspect, AI has also improved the use of CamelCase and AltText descriptions - both of which are important from a Diversity and Inclusion (D&I) perspective. AltText descriptions can be created quickly and easily for images using AI such as ChatGPT. The use of AI copilots for veterinary medicine has been pioneered by AITEM for student training, also thanks to collaboration with Italian research groups.

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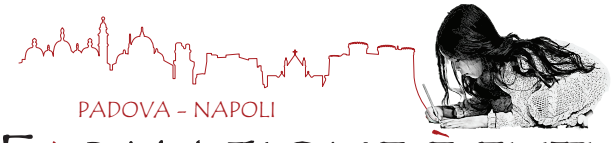
# ARTROPODI VETTORI E PATOGENI TRASMESSI: L'APPROCCIO INTEGRATO DEL PROGETTO PNRR INF-ACT

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# DISEASE SURVEILLANCE IN A DATA SCIENCE CONTEXT: THE H2020 MOOD PROJECT

Rizzoli A.*[1], Dub T.[2], Arsevska E.[3], Dagostin F.[1], Marini G.[1], Tagliapietra V.[1], Cataldo C.[4], Busani L.[4]

*[1]Fondazione Edmund Mach, San Michele all'Adige (TN), Italy; [2]THL, Helsinki, Finland; [3]UMR-ASTRE, CIRAD, Montferrier-sur-Lez, France; [4]Istituto Superiore di Sanità, Roma, Italy*

**Keywords:** Diseases intelligence, Risk modelling, Tick borne diseases.

The MOOD project aims to develop innovative tools and services for the early detection, assessment, and monitoring of current and future infectious disease threats across Europe in the context of ongoing environmental and socio-economic global changes. MOOD innovations aim to increase the operational capabilities of epidemic intelligence systems to face new disease threats, including emerging diseases of known or unknown origins, and antimicrobial-resistant pathogens. The MOOD project addresses the challenges of cross-sectoral data sharing and valorization in a One Health framework based on multi-disciplinary collaboration for animal, human, and environmental health through big data and disease modelling innovations. The end-users are human and veterinary public health agencies responsible for designing and implementing strategies to mitigate the identified risks. The technical innovations envisioned by MOOD are based on the constant involvement and co-design with users to ensure they meet their needs. The MOOD case studies establish a close collaborative space where MOOD researchers and end-users discuss together the development of Epidemic Intelligence (EI) tools for routine use. The case studies focus on model pathogens based on (1) their current impact on European public health in terms of burden in humans and animals; (2) the economic cost related to their medical care and for outbreak monitoring and control; (3) their sensitivity to climate and other environmental changes and the potential to further emerge; (4) their representativeness of different disease systems (transmission routes) for which different data streams are needed to monitor and early detect possible outbreaks. Five study cases on airborne, vector-borne, multiple transmission routes diseases, including AMR and disease X have been implemented. In this presentation, the research activities and modelling framework specifically developed for TBE case study will be presented and discussed.

# CURRENT DISTRIBUTION OF ARTHROPOD OF PUBLIC HEALTH RELEVANCE IN ITALY: RESULTS FROM SYSTEMATIC REVIEWS

Severini F.*[1], Bongiorno G.[1], Salata C.[2], Montarsi F.[3], Toma L.[1], Maioli G.[4], Di Luca M.[1], Gentili D.[5], Bertola M.[6], Mosquitoes/ticks/sandflies W.G.[7]

*[1]Istituto Superiore di Sanità, Dipartimento Malattie Infettive, Roma, Italy; [2]Università di Padova, Dipartimento di Medicina Molecolare, Padova, Italy; [3]Istituto Zooprofilattico Sperimentale delle Venezie, Laboratorio entomologia sanitaria e patogeni trasmessi da vettori, Padova, Italy; [4]Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Brescia, Italy; [5]Istituto Superiore di Sanità, Servizio Comunicazione/Biblioteca, Roma, Italy; [6]Istituto Zooprofilattico Sperimentale delle Venezie, UO veterinaria centralizzata protezione animali utilizzati a fini scientifici, Padova, Italy; [7]Mosquitoes/Ticks/Sandflies Working Group, Italy*

**Keywords:** Mosquitoes, Sand flies, Ticks.

**INTRODUCTION:** Within the INF-ACT project task 2.3.1. PE13 - RESEARCH NODE2 (Gathering of data on vector presence/abundance/resistance to insecticide in Italy from scientific and grey literature) three different working groups (one for each arthropod group) were selected for mosquitoes (M), sand flies (S) and ticks (T). The aim of the work is to systematically review the existing published literature of the last 22 years related to the occurrence (geographic presence and distribution) of mosquito, sand fly and tick species in Italy.

**MATERIALS AND METHODS:** For the screening of publications, we considered all studies published in peer-reviewed journals obtained from the literature search on major databases (Scopus, CABA, EMBASE, WoS, MEDLINE). The selection for relevance and eligibility of these studies (title and abstract) has been carried out by working groups that included volunteers (M: 28, S: 18, T: 24), gathered in pairs and working independently, solving the potential disagreements on study eligibility, if necessary, with the examination of the studies by a third reviewer. Only publications with Italian or English full text were considered for the first screening of relevance, therefore publications without available full text in these two languages were excluded. In order to avoid double counting of the studies published more than once, the papers were compared juxtaposing author names, geographic area of the studies, the sample sizes and the outcomes.

**RESULTS AND CONCLUSIONS:** The results of the first phase are shown below sorted by systematic group. The volunteers received in total 6,909 (M), 1,981 (S), 12,018 (T) selected records, and extracted by all five used databases. 751 (M), 440 (S), 1,494 (T) of them resulted accepted, 515 (M), 597 (S) doubtful and 5,643 (M), 944 (S), 10,524 (T) rejected. It should be noted that for ticks group no doubtful papers are present because of the already screened abstracts. In conclusion, hitherto 12.8% of the papers screened from the literature were found to be suitable for the eligibility criteria and will be subjected to full-text screening. The aim of the next working step will be the creation of a unique database shared among involved groups (mosquitoes, sand flies and ticks) and, after full-text retrieval, reviewers will extract data from text, tables, or figures and will gather them into pre-defined tabular forms. As previously explained, extracted data will pass a double cross-checking procedure, operated by two reviewers independently. An additional comparison of all extracted data will be scheduled with the original selected studies. Full-text examination is still ongoing and as soon as data collection phase will be completed, thematic systematic review manuscript will be produced.

# MOSQUITO SALIVA AND THE BLOOD MEAL: BEHAVIOR AND IMMUNE RESPONSE

Arnoldi I.*[1], Villa M.[1], Mancini G.[2], Varotto-Boccazzi I.[1], Bandi C.[1], Epis S.[1], Forneris F.[2], Gabrieli P.[1]

*[1]University of Milan, Milan, Italy, [2]University of Pavia, Pavia, Italy*

**Keywords:** *Aedes albopictus*, LIPS-2, Mosquito allergy.

Mosquitoes shape the life of billions of people yearly, acting as vectors of multiple pathogens, causing potentially deadly vector-borne diseases, through mosquito saliva injection into the host skin during blood feeding, a process necessary to complete egg development in female mosquitoes. Saliva has a heterogeneous composition and multiple functions. First, salivary proteins counteract hemostasis and inflammation at the bite site, where they act as allergens. Indeed, mosquito bites usually have a mild and self-resolving outcome which can evolve to severe hypersensitivity reactions associated with Immunoglobulin (Ig) - E production, defined as mosquito allergy, in some subjects. Secondly, during the bite saliva is partially re-engorged by mosquitoes, suggesting an effect on the insect itself during this process. During last years, three research topics were faced to investigate the multiple roles of the saliva of *Aedes albopictus*, an invasive mosquito species diffused worldwide and highly spread in Europe and in Italy. *Ae. albopictus* is a competent vector of many arboviruses and filarial nematodes, and it is often associated with allergic reactions to mosquito bites. First, we described the modulation of intradermal probing (IP), the initial phase of blood feeding oriented to the search of a blood vessel into the host skin, by the salivary protein Labrum Interacting Protein of the Saliva (LIPS) - 2 (Arnoldi and Mancini et al., 2022. Curr Biol, 32:3493-3504). LIPS-2 is both secreted into the skin and interacting with the cuticular protein Cp19 at the tip of the labrum, the stylet acting as food canal in mosquito proboscis. This interaction was characterized by multiple biochemical and biophysical assays and was suggested to have a role in the regulation of the IP process. Secondly, to develop an appropriate test for the diagnosis of mosquito allergy, the recombinant form of LIPS-2 and of the salivary protein Antigen5-3 were used to detect the serum IgE of subjects affected by the disease by in-house ELISA, alternatively to salivary gland extract as antigenic source (Arnoldi et al., 2023. World Allergy Organ J, 16:100836). For an epidemiological purpose, the proteins were employed also to assess serum IgE in an extended group of people not affected by mosquito allergy. Finally, we investigated the role of mannose receptor, found expressed on host immune cells and recognizing mannose glycosylation, typical of allergens, in mediating immunomodulation at the bite site. Indeed, salivary proteins inhibited the production of pro-inflammatory mediators by macrophages also stimulated with lipopolysaccharide and RNA interference targeting mannose receptor expression in these cells reverted this effect.

# REPELLENCE VS ATTRACTION OF ARTHROPOD VECTORS: THE ROLE OF VOLATILE ORGANIC COMPOUNDS

Bezerra-Santos M.A.*, Otranto D.

*University of Bari, Department of Veterinary Medicine, Bari, Italy*

**Keywords**: Attraction, Arthropod vectors, VOCs.

Volatile organic compounds (VOCs) are chemicals emitted as products of the cell metabolism, which reflects the physiological and pathological conditions of any living organisms (Carapito et al., 2024. Talanta, 268:125296). Several VOCs are emitted by vertebrate hosts; however, a relatively small amount of them have an influence on the behavior of arthropod vectors, being defined as 'allelochemicals', which are further classified into kairomones (attractants) and allomones (repellents) (Poldy, 2020. Animals, 10:1984). In this sense, kairomones could serve as selective tools for studying population abundance, surveillance of invasive species and vector-borne pathogens, as well as for predicting pathogen outbreaks. In addition, the repellence effect of allomones represents a potential tool for controlling arthropod vectors (Dormont et al., 2021. J Chem Ecol, 47:351-93). Indeed, VOCs play a key role as olfactory cues for arthropods of medical and veterinary importance (*e.g*. mosquitoes, sand flies and ticks) influencing their behavioral choices, such as host preference and selection of oviposition sites for gravid females (Bezerra-Santos et al., 2024. Submitted manuscript). Moreover, deadly vector borne pathogens such as *Plasmodium falciparum*, and *Leishmania infantum* have been suggested to manipulate the VOCs profile of the host cells to make them more attractive to mosquitoes and sand fly vectors, respectively (Correa et al., 2017. Parasit Vectors, 10:215; Magalhaes-Junior et al., 2014. Anal Bioanal Chem, 406:6691-6700). Under the above circumstances, studies on these compounds have demonstrated their potential usefulness as attractants or repellents for ticks, mosquitoes, and sand flies, as well as for the diagnosis of vector borne diseases (VBDs), such as malaria and leishmaniasis. Here, we provide an account for scientific data available on VOCs to study the host seeking behavior of arthropod vectors, and their usefulness as attractants, repellents, or tools for early diagnosis of VBDs.

# THE CONTRIBUTION OF CITIZEN SCIENCE APPROACHES TO THE STUDY, MONITORING AND CONTROL OF MOSQUITOES IN ITALY

della Torre A.*[1], Caputo B.[1], Salvemini M.[2]

*[1]Università Sapienza, Rome, Italy; [2]Università di Napoli Federico II, Napoli, Italy*

**Keywords:** Citizen Science, Mosquito Alert, Procida.

**INTRODUCTION:** Citizen Science refers to scientific work undertaken by members of the general public under the direction or in collaboration of professional scientists and scientific institutions. In the latest years, Citizen Science has been particularly effective in gathering reliable, timely, large-scale data on the presence, ranges, and distributions of animal species, including mosquito species that are vectors of arboviruses, such as Dengue, Chikungunya and Zika. Also, it is widely recognised that citizens' active contribution is an instrumental element for effective mosquito control. Here, we present the results of 3 years' participation of citizens to Mosquito Alert Italia project, as well as of Stop Tigre project in Procida Island (Naples gulf).

**MATERIALS AND METHODS:** Mosquito Alert is a Citizen Science project (active in Italy since October 2020) that aims to contribute to the surveillance and study of vector mosquitoes using expert-validated photographic records of targeted mosquitoes, as well as records of bites and breeding sites, sent by citizen scientists through a Mosquito Alert app for smartphones. Stop Tigre project aims to eliminate the invasive species *Aedes albopictus* and from Procida island through the use of eco-sustainable technologies and through the active participation of the island community.

**RESULTS AND CONCLUSIONS:** From October 2020 to the end of 2023, >20,000 citizens downloaded Mosquito Alert app in Italy and sent thousands of mosquito photos and reports of bites, and hundreds of photos of breeding sites. The results, the potential, the limitations and the difficulties encountered in involving Italian citizens in the project and in exploiting data obtained to improve knowledge on mosquito vectors will be presented and discussed. Stop Tigre project started in 2016 with the help of Procida's administration to study the spatial distribution and the temporal dynamics of *Ae. albopictus* in the island and evolved since then with increased community engagement and the instrumental contribution of artists of fine arts academy of Naples. Results create the bases for the evidence-based planning of a season-long *Ae. albopictus* reduction trial by biweekly releases of hundreds of thousands of sterile males in progress in 2024.
Citizen Science has a high potential to improve knowledge on mosquitoes at national scale level, as well to complement entomological monitoring and contribute to successful mosquito control.

# LEISHMANIOSIS: THE EPIDEMIOLOGICAL, DIAGNOSTIC AND THERAPEUTIC CHALLENGES IN THE ERA OF CLIMATE CHANGES

De Pascali A.M.*[1], Varani S.[1], Longoni S.S.[2], Varotto-Boccazzi I.[3], D'Alessandro S.[4], Basilico N.[4], Sambri V.[1]

*[1]Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy; [2]Department of Infectious, Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Negrar di Valpolicella, Italy; [3]Department of Biosciences, University of Milan, Milan, Italy; [4]Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy*

**Keywords:** *Leishmania*, INF-ACT, Diagnostic and therapeutic gaps.

**INTRODUCTION:** The distribution of leishmaniasis is dynamic, subject to social and environmental factors, such as movement of infected animals and people, urbanization, and climate change. The diagnosis of leishmaniasis is based on clinical manifestations, epidemiological and laboratory data such as microscopic examination, *in vitro* culture, serology, and molecular. tests. Serological tests have several difficulties associated with the interpretation of data: (i) nonspecific cross-reactions; (ii) delay between infection and seroconversion; (iii) not permanent seroconversion. On the other side, microscopy has limited sensitivity, while *in vitro* cultures are laborious, expensive, and susceptible to contamination. Recently, several diagnostic tools have been implemented, in particular molecular tests, as tool for sensitive diagnosis and tool for drug discovery. Europe currently lacks international standardization for diagnosis and treatment limiting disease control, compromising epidemiological data collection, and increasing mortality rates. The aim of this study is to understand the gaps in order to address the research activity of the PNRR INF-ACT project.

**MATERIALS AND METHODS:** The review was conducted on Scopus using the following search string: ((TITLE-ABS-KEY (leishmania) OR TITLE-ABS-KEY (leishmaniasis) AND TITLE-ABS-KEY (gap)) AND PUBYEAR > 2013 AND PUBYEAR < 2025 AND (LIMIT-TO (PUBSTAGE, "final")) AND (LIMIT-TO (LANGUAGE, "English")) AND (LIMIT-TO (OA, "publisherfullgold"))). Titles and abstracts were reviewed and linked to the WHO's Target Product Profile for Leishmaniasis in order to understand whether the priorities defined in the scientific literature matched the priorities defined by WHO. The priorities found were then aligned with the results obtained within the PNRR INF-ACT project with the aim of defining a strategy to be pursued to fill the gaps in the diagnosis and treatment of Leishmaniasis.

**RESULTS:** The systematic review reported 151 articles of whom 80% were published within the last 5 years. Gaps emerged were: i) serology difficult to interpret, ii) lack of point-of-care; iii) lack of methods for *Leishmania* species identification; iv) expensive and toxic drugs. Within INF-ACT, the research groups focused their activities on: 1) mechanisms of *Leishmania*-macrophage interaction, 2) protective role of *Leishmania tarentolae*, 3) genomic characterization of circulating strains of *Leishmania*, 4) drug discovery on natural compounds, 5) identification of *Leishmania* species by multi-target PCR.

**CONCLUSIONS:** Research priorities should be geared to filling knowledge gaps related to the epidemiology, developing rapid diagnostic tests for the detection of leishmaniasis and its drug resistance, research into new therapies and vaccines. INF-ACT project is working in the right direction by providing encouraging and innovative strategies to respond to the challenges of an emerging and increasingly widespread disease.

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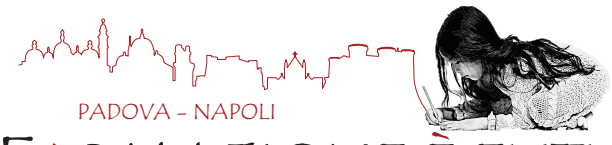
# PARASSITI, ZOONOSI E SPILLOVER, NELLA STORIA ANTICA E RECENTE DI *HOMO SAPIENS*



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# HISTORY AND STORIES OF HUMANS AND INFECTIONS

Galli M.*

*University of Milan, Milan, Italy*

**Keywords:** Zoonosis, Agricultural transition, Historical epidemiology.

During the Neolithic, agricultural transition and animal domestication determined a profound change in the life of humans, and in the human-animal interaction. In general, the invention of agriculture (with a transition to a more sedentary lifestyle), changes in the diet, and the establishment of larger villages represented an evolutionary mismatch for humans and domesticated animals. It is a general thought that some of the main zoonoses that accompanied *Homo sapiens* in his history originated in this context. On the other hand, humans became a source of infections for animals. Infectious diseases have then accompanied humans during our successive history, with a deep impact on historical events, and or our culture and thought.

# CATTLE BREEDING AND THE ORIGIN AND SPREAD OF HUMAN CRYPTOSPORIDIOSIS

Castelli M.*[1], Bellinzona G.[1], Nardi T.[1], Batisti Biffignandi G.[1], Bandi C.[3], Sassera D.[1], Cacciò S.M.[2]

*[1]University of Pavia, Pavia, Italy; [2]Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; [3]Department of Biosciences, University of Milan, Milan, Italy*

**Keywords**: *Cryptosporidium*, Zoonosis, Genomics.

**INTRODUCTION:** The genus *Cryptosporidium* (Apicomplexa) encompasses over 40 different species, affecting multiple vertebrate hosts. Several *Cryptosporidium* lineages infect humans, and the prominent zoonotic member is *Cryptosporidium parvum*, representing a global cause of gastrointestinal disease in humans and ruminants. In this study, we aimed to investigate by a genomic approach the epidemiology and history of *C. parvum*, which a focus on the role of zoonotic transmission.

**MATERIALS AND METHODS:** We generated whole genome sequence (WGS) data from 123 human- and ruminant-derived isolates collected in 13 European countries and included other available WGS data from Europe, Egypt, China and the USA (n=72). We applied rigorous filters to exclude mixed infections and analysed a dataset from 141 isolates showing a total of 28,047 high-quality SNPs. By this this dataset, we inferred phylogenetic relationships, investigated the population structure, population genetics and recombination events.

**RESULTS AND CONCLUSIONS:** Our results indicate that several important steps in the geographical and genetic separation of *C. parvum* lineages can be linked to historical events related to breeding of livestock, in particular cattle. First, we identified three distinct and strongly supported populations: population 1 (China and Egypt), population 2 (a minority of European isolates), and population 3 (most European isolates and all isolates from the USA). The common origin of all those populations dates back to around 10,000 years ago, thus overlapping with cattle domestication in the Middle East. Population 3 has emerged more recently from population 2 and expanded throughout Europe, and possibly after a single introduction event from the UK, reached the USA and expanded also therein. As compared to population 2, population 3 is more frequently involved in epidemic outbreaks. Although the reason(s) for this successful spread of population 3 remain elusive, genes under selective pressure uniquely in this population were identified. Taken together, our results, together with previous studies, suggest a probable preferential role of cattle and other livestock as vehicles for the geographical spread of *C. parvum*, and possibly as determinants for its genetic diversification. In particular, it is highlighted that the geographical origin of *C. parvum* affecting livestock and humans most likely was the “fertile crescent”, where its ruminant hosts were initially domesticated. Further analyses, from currently under-represented countries, will be necessary to shed light on this scenario and to further investigate the role of cattle breeding on the geographical spread and evolution of *C. parvum*, in particular intensive breeding approaches.

# *HOMO SAPIENS* AND THE DOMESTICATION OF SHEEP, DOG AND… *ECHINOCOCCUS GRANULOSUS*

Casulli A.*

*WHO Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis. Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; European Union Reference Laboratory for Parasites (EURL-P). Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*

**Keywords:** Domestication, *Echinococcus granulosus*, Archeoparasitology.

**INTRODUCTION:** Domestication is an anthropogenic-driven evolutionary process by artificial selection. It is a multi-generational mutualistic relationship between humans and other organisms in which humans take over control and care of another organism to gain a supply of resources. Domestication events resulted in a higher density of human populations which, however, provided ripe conditions for different pathogens to evolve, increase fitness, spread, and eventually find a new host in humans. In fact, a side effect of domestication has been an increase of zoonotic diseases. As an example, cattle have given humanity various viral poxes, measles, and tuberculosis, while pigs and ducks have contributed with influenza and horses with rhinoviruses. Parasites, including helminths, were not excluded from the above-mentioned process. In fact, cystic echinococcosis (CE) is a neglected disease caused by *Echinococcus granulosus sensu lato (s.l.)*, a complex of species most of which infecting livestock. In strict terms, CE should be considered as global endemicity and its worldwide distribution could be due to long lasting evolutionary events of domestication of different livestock species occurred approximately 12,000-14,000 years ago. This presentation is aiming to support such theories on co-evolution of human beings and pathogens in the context of ancient domestication events.

**MATERIALS AND METHODS:** We searched in the PubMed database for papers on the genetic diversity, phylogeny, evolution, paleoparasitology, archeoparasitology and paleogenetic of *Echinococcus* spp.

**RESULTS AND CONCLUSIONS:** Little evidence has been found in the literature to support coevolution events between *Echinococcus* spp. and its animal hosts. Taeniid eggs have been identified in ancient dogs from Iran (8,100 B.C.). Since eggs of the genus *Taenia* and *Echinococcus* are morphologically indistinguishable, we cannot ascertain the presence of *Echinococcus* during the domestication events of dog. On the contrary, due to the natural evolution of *E. granulosus s.l.* metacestode, that can calcify at the end of its life, several cysts have been found in many ancient burial sites. Calcified echinococcal cysts have been identified in many different areas (Denmark, England, France, Greece, Iceland, Ireland, Italy, Poland, Russia, Spain, Scotland and Switzerland), from medieval time to Roman time up to early Neolithic (8,000 years ago). Globally, vast majority of CE human infections are caused by *E. granulosus sensu stricto (s.s.)*, a species that is mainly transmitted by sheep. Therefore, the genetic variability of *E. granulosus s.s.* has been used to infer the impact of sheep domestication on this parasite. Only two studies suggested that the higher genetic diversity of *E. granulosus s.s.* in the middle east, pairing with higher genetic diversity of sheep in this area (compared to the more distant areas), may reflect coevolution events occurred in this area.

# THE IMPACT OF HUMAN MIGRATION ON PARASITE DISTRIBUTION

D'Amelio S.*

*Sapienza University of Rome, Rome, Italy*

**Keywords:** Migration, Parasite dispersal, Epidemiological transfer.

Human and animal migration has been crucial in shaping environments, societies and cultures throughout history. Moreover, the consequences of migration include significant effects on public health, particularly in the spread and prevalence of various parasitic infections, given that the global movement of individuals from endemic regions to non-endemic areas can introduce parasitic pathogens to new environments, leading to new endemic scenarios. Human migration can be roughly subdivided in prehistoric and historical. Limiting to *Homo sapiens*, humans started to leave Africa around 125000 years ago with a coastal migration to Asia and Oceania and a series of later migration flows led to Europe (40000 years ago) and to Americas (15000 years ago). These phenomena contributed to the spread of several helminthic diseases such as STHs all over the world, with findings in China (2300 years ago), Canada (3700 BC), Brazil (6000 BC), among many others. More recent historical examples of parasite dispersal facilitated by human migration are due to: 1) The Silk Road (2nd century BCE - 14th century CE) being a network of trade routes connecting the East and West. Along these routes, parasites such as *Plasmodium* spp. could be transmitted between different regions. 2) The Columbian Exchange (15th-16th centuries). Besides to the military destruction of the indigenous populations, the European colonizers and settlers introduced diseases and parasites such as smallpox, measles, and intestinal worms to indigenous populations, causing devastating epidemics and long-term health impacts. An outstanding example of this phenomenon is the introduction of *Aedes aegypti* and the yellow fever from endemic areas in Africa to South America (Lavagnino, 1993. Zanzare). 3) The European Colonization and the Slave Trade (15th-19th centuries). For example, the introduction of African slaves to the Americas led to the introduction of parasitic infections endemic to Africa, such as schistosomiasis, to the New World. However, even recently a high level of concern grew up after the report of an outbreak of urinary cases in the southern part of Corsica (Boissier et al., 2016. The Lancet Inf Dis, 16:971-979). The combination of human migration and availability of the intermediate host made possible the establishment of the life cycle of *Schistosoma haematobium/bovis*, suggesting the need for an efficient control in areas where the competent snails are present. As for malaria vectors, a recent estimate of divergence suggests two independent transfer of *P. falciparum* in South America, linked to slave trade (Yalcindag et al., 2011. PNAS, 109:511-516). Human migration may also affect the distribution of specific feature related to malaria, such as drug resistance. The increasing movements from South-East Asia to Africa may expand the spread of artemisinin-resistant variants, with unpredictable and potentially devastating effect (Lubell at al., 2014. Malaria J, 13:452).

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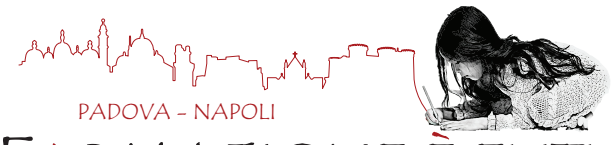
# PASSATO, PRESENTE E FUTURO DELLA FORMAZIONE E DELLA RICERCA SULLE MALATTIE PARASSITARIE DELLE API DA MIELE



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# HONEYBEE PARASITOLOGY TEACHING IN EUROPE: FROM BEEKEEPERS TO VETERINARIANS

Meana Mañes A.*

*Facultad Veterinaria UCM, Madrid, Spain*

**Keywords**: Honeybee veterinary parasitology, Training, Research.

The intervention of veterinarians in beehive research and management is crucial to prevent bee extinction and strengthen the Spanish beekeeping sector, which spans numerous regions and produces high-quality products. Veterinarians work with beekeepers and agri-food experts to protect this vital species. In beehives, they provide technical advice, diagnose and treat diseases, establish prevention and control programs for endemic diseases, and perform clinical diagnoses. Additionally, they offer consultancy on nutrition, hive management, products, and legislation. Strengthening honeybee veterinary education is beneficial. Veterinary establishments should prepare veterinarians to practice science-based honeybee medicine by incorporating relevant teachings into undergraduate curricula and offering postgraduate opportunities to enhance skills. A recent study shows that 75% of establishments teach honeybee veterinary medicine, with clear geographical differences. In northwestern countries, only half include it in undergraduate curricula, while in eastern, central, and southern countries, the majority incorporate it. Of these establishments, 86% include it in their core curriculum, either as a separate subject or integrated into other subjects. Additionally, 25% organize postgraduate training courses in this field. Beekeeping knowledge has been orally transmitted within families and communities for centuries, with Spanish treatises on beekeeping dating back to medieval times and included in veterinary curricula in the 19th century. Since 1998, beekeeping training has been part of PNA programs, with talks on varroosis control at fairs and meetings. However, an agreement on the academic background for technicians or advisors in the current Sectoral Beekeeping Intervention (ISA 2023-2027) has not been reached. Data from the Association of Veterinarians Specialized in Apicultural Health and Production (AVESPA) show an increase in clinical veterinarians in advisory services for beekeeper associations. A recent survey of veterinary parasitology professors in Spanish faculties indicated that all respondents cover common parasites, diseases, and pests affecting apiculture, such as *Varroa destructor* and *Vespa velutina*.

# ADVANCEMENTS IN THE DIAGNOSIS OF *VARROA*

Mortarino M.*

*Università degli Studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali, Milano, Italy*

**Keywords**: Honey bee, *Varroa destructor*, Diagnostics.

The mite *Varroa destructor* poses a significant threat to honey bee colonies worldwide (Warner et al., 2024. Sci Total Environ, 906:1674921). This parasite exerts direct damage, as it feeds on hemolymph and fat body taken from larvae, nymphs, and adult honeybees, and can also transmit some pathogens, including various viruses, which are generally responsible for the most evident symptoms during the infestation in the colony (Traynor et al., 2020. Trends Parasitol, 36:592-606). Traditional methods for diagnosing *Varroa* infestations involve visual inspections of honey bee brood and adult honey bees, or using sticky boards to monitor mite drop, with inherent limitations in terms of accuracy, invasiveness and labor intensity (Gregorc et al., 2019. Diversity, 11:243. Pietropaoli et al., 2021. Appl Sci, 11:4458). In recent years, there has been a shift towards more accurate and/or sustainable diagnostic techniques leveraging advancements in technology for early detection of parasite load and *Varroa*-induced damages. In particular, non-invasive, high-resolution image collection and analysis, *e.g*. through Computer Tomography approaches and machine learning detection algorithms, may improve speed and accuracy of in-hive diagnostics to assess honey bee brood health and *Varroa* load on adult honey bees, during infestation and following antiparasitic treatment (Facchini et al., 2019. Sci Rep, 9:10614; Keszthelyi et al., 2021. Apidologie, 52:155-162; Bilik et al., 2024. Comput Electron Agric, 217:108560). Biochemical and molecular techniques also offer sensitive and specific detection of varroa biomarkers on non-invasive matrices, and a robust toolbox for investigating drug resistance in the mites and supporting the development of diagnostic tests for sustainable control strategies (Ribani et al., 2020. Vet Sci 7:113; Benito-Murcia et al., 2022. Res Vet Sci, 152:34-37). Overall, ongoing research aims to develop cost-effective and sustainable solutions to monitor and mitigate *Varroa* impact on honey bee populations. In veterinary medicine educational programs, apicultural health and parasitology are typically taught through specialized courses covering diagnostic methods, treatment strategies, and preventive measures for honey bee diseases and parasites like *Varroa* (D'Ascenzi et al., 2023. Animals, 13:1795). Integrating the above topics into university teaching programs for veterinary apiculture would provide students with comprehensive and up-to-date training on challenges and solutions in the field of honey bee health and parasitology.

# UPDATES ON *VARROA* TREATMENT WITH NATURAL PRODUCTS

Bava R.*[1], Castagna F.[3], Lupia C.[2], Britti D.[1], Marrelli M.[4], Statti G.[4], Palma E.[1], Musella V.[1]

*[1]Department of Health Sciences, University of Catanzaro Magna Græcia, Catanzaro, Italy, Catanzaro, Italy; [2]Mediterranean Ethnobotanical Conservatory, Sersale (CZ), 88054 Catanzaro, Italy, Sersale (CZ), Italy; [3]Mediterranean Ethnobotanical Conservatory, Sersale (CZ), 88054 Catanzaro, Italy, Sersale, Italy; [4]Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende, Cosenza, Italy, Cosenza, Italy*

**Keywords**: *Varroa destructor*, Essential oils, Acaricidal efficacy tests.

**INTRODUCTION:** *Varroa destructor* mite is the most important ectoparasite of *Apis mellifera*. By feeding on the hemolymph and fat body cells/cellular components, the mites cause physical harm to their hosts. Additionally, mites act as a vector for fatal bee viruses. The control of varroosis is mainly based on the use of synthetic drugs, which are often ineffective due to the spread of resistance phenomena (Traynor et al., 2020. Parasitol Trends, 7:592-606). Therefore, the search for new control treatments is mandatory. Due to their low toxicity to the environment and non-targets, and the complex chemical composition that make it difficult for pest to develop resistance, essential oils (EOs) are promising compounds for the control. In this study, the acaricidal efficacy of various EOs has been evaluated through several tests.

**MATERIALS AND METHODS:** For contact tests, Eppendorf tubes were filled with 50 µL of acetone and EOs dilutions. To coat the walls with the EOs and allow acetone evaporation, the tubes were frequently rotated. Once the acetone had evaporated, five *V. destructor* were inserted into each test tube. The tubes were sealed tightly and placed in an incubator. One hour later, mortality was assessed (Bava et al., 2021. Pathogens, 10:1182). Two different methods were used for the fumigation tests. In the first, the “closed chamber”, a cotton ball was inserted into the cap of the Eppendorf tubes. Then, five *V. destructor* were transferred to the bottom of the test tubes. To avoid contact between the mites and the cap, a piece of tulle was inserted. 40 µL of EO diluted in distilled water was poured onto the cotton ball (Castagna et al., 2022. Vet Sci, 9:124). In the second, the “open chamber”, double-level cages were set up. Ten adult bees, with ten *V. destructor* attached, were placed in the upper part of the cage. EOs, diluted in distilled water, were used to soak filter papers placed in the lower compartment. Mite mortality was assessed at different times in both methods (Bava et al., 2022. Vet Sci, 9:684).

**RESULTS AND CONCLUSIONS:** In contact tests, the EOs showed a mite neutralization above 92%, 82% and 77% for the EOs of *O. heracleoticum*, *C. limon* and *C. bergamia*, respectively. Fumigation tests in closed chamber showed good acaricidal efficacy of 84% for *O. haeracleoticum*, 76% for *C. limon* and 68% for EOs of *C. bergamia*. In open chamber tests, much higher concentrations (up to 20 times) of EOs were required to achieve similar rates of mite neutralization. EOs have proven to be extremely effective. However, these studies highlight some critical issues of the different types of tests used. Therefore, to employ EOs in beekeeping practice, it is necessary to conduct semi-field studies on the doses to be used, while also going to carefully evaluate the toxicity to bees. Consideration should be given to nanoencapsulation in nanoparticle systems that can effectively retain bioactive and release them in a controlled manner over time.

# GASTROINTESTINAL PARASITES OF HONEY BEES

Power K.*[1], De Vico G.[1], Maiolino P.[2]

*[1]Department of Biology, University of Naples Federico II, Naples, Italy; [2]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy*

**Keywords**: Nosemosis, Trypanosomatida, *Ascosphaera apis*.

Honey bees can be infected with a great number of ecto and endoparasites, and their role in honey bee colony impairment is well known. Also, honey bee parasites are among the factors associated to decreased productions, thus contributing to the crisis of the beekeeping sector. Microsporidia and Trypanosomatida are the most frequent endoparasites of honey bees showing high tropism for the gastrointestinal tract. *Nosema* sp. is a widespread Microsporidia of *Apis mellifera* and it is considered the second most prevalent pathogen connected to colony loss (Grupe et al., 2020. PLoS Pathog, 16:e1008580). *N. apis* is responsible for nosemosis type A, which is associated to diarrhea, crawling bees, and winter losses, while *N. ceranae* is responsible for nosemosis type C, which progresses with no evident clinical signs leading in the end to colony mortality (Martín-Hernández et al., 2012. Environ Microbiol. 14:2127-38). *Ascosphaera apis* is also an important mycotic agent which affects the developing brood, causing the so called “Chalkbrood disease”. The disease is characterized by the death of honey bee larvae and their transformation into “chalkbrood mummies”: dehydrated white, gray or black larvae of hard consistency (Aronstein and Murray, 2010. J Invertebr Pathol, 1:S20-9). Honey bee trypanosomatids are the least-studied group of bee parasites. *Crithidia mellificae*, *C. bombi* and *Lotmaria passim* are increasing their prevalence in honey bees and have been associated to impairment of colony wellbeing (Michalczyk et al., 2022. J Apic Res, 63:287-296). While *C. mellificae* and *C. bombi* are less frequently identified in *A. mellifera* populations, *L. passim* is currently the predominant trypanosomatid species identified in honey bee colonies (Schwarz et al., 2015. J Eukaryot Microbiol, 62:567-83). All trypanosomatids are associated with increased lethality and reduction in honey bee lifespan, as they colonize the gut lumen and induce microbiome and immune alterations (Liu et al., 2020. Commun Biol, 3:51). Understanding the epidemiology, pathogenesis, and clinical signs of honey bee parasitic diseases, also through the development and application of new techniques (Power et al., 2020. Vet Pathol, 57:200-201), can help set measures to control and reduce damage to colonies, leading to new flourishing of the beekeeping sector.

# EXPERIENCES FROM INTERNATIONAL NETWORKS FOR RESEARCH AND EDUCATION ON BIOSECURITY IN BEEKEEPING: NOT ONLY *VARROA*, NOT ONLY WESTERN HONEY BEE

Formato G.*

*Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "Mariano Aleandri", Rome, Italy*

**Keywords**: Pollinator, Research, Network.

Bees and other pollinators play a fundamental role in ecosystems by ensuring the survival of many plants. They have a primary importance in terms of biodiversity, but also in providing a varied diet to humans and animals: about 75 percent of agricultural production of fruits and seeds for human consumption depends on pollination activity. Pollinators enable crops of agricultural interest to improve productivity from a qualitative and quantitative point of view. They also represent a valuable means of combating climate change and regenerating degraded and wild environments. When we think of bees, our minds often turn to the Western honeybee, *Apis mellifera*. However, various species (and subspecies) of bees are kept worldwide. In Europe, America, and West Asia, the Western honeybees (*Apis mellifera*) are common, while in East and South Asia beekeepers maintain the indigenous Eastern or Asiatic honeybee (*Apis cerana*). In tropical regions, other species of social bees such as stingless bees (*Melipona* and other genera) are kept, primarily for honey production or medicinal use. Additionally, bumblebees (genus *Bombus*) are maintained globally for their pollination services. Some other species are kept in specific regions (*e.g.*, *Apis dorsata* and *Apis laboriosa* in Nepal and India, and *Apis florea* and *Apis andreniformis* in Southwest Asia). Many advancements are being made in understanding the biology, pathologies, prevention, and control of these bees, but much remains to be done. The report will provide examples of these findings and how the study of these pollinators can translate into operational projects, thanks also to synergies of cooperation between international organizations and research institutions.

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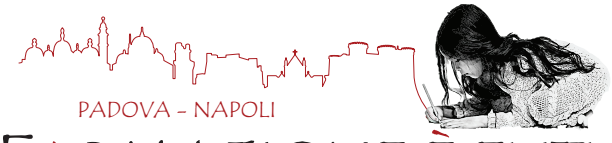

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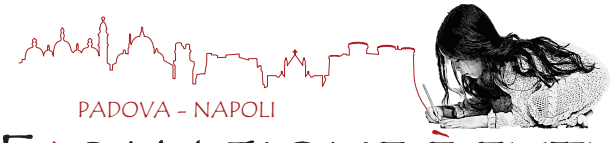
# ALTERNATIVE METHODS FOR PARASITE CONTROL



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# *IN VITRO* EFFICACY OF DIFFERENT CONCENTRATIONS OF *BEAUVERIA BASSIANA* "NATIVE STRAIN" ON *PHLEBOTOMUS PERNICIOSUS* EGGS HATCHING RATE

Perles L.*, Bezerra-Santos M.A., Otranto D., Cafarchia C.

*Department of Veterinary Medicine, University of Bari, Valenzano, Italy*

**Keywords:** Sand fly, Entomopathogenic fungi, *Phlebotomus perniciosus*.

**INTRODUCTION:** *Phlebotomus perniciosus* (Diptera, Psicodidae) is the primary vector of *Leishmania infantum*, a protozoan that causes major cutaneous, mucosal, and visceral leishmaniasis worldwide. The use of chemical insecticides (i.e., pyrethroids) for the control of sand flies is crucial tool for the prevention of canine leishmaniasis, though it may cause environmental and food contamination, as well as the development of drug resistance. Entomopathogenic fungi have been investigated for their potential in the biological control of arthropods. In particular, *Beauveria bassiana sensu lato* strains isolated from hosts or environment are effective against many arthropod species, including ticks and mites (Cafarchia et al., 2015. Parasit Vectors, 8:1-7; Immediato et al., 2015. Vet Parasitol, 212:478-82), with limited data available for sand flies (El-Shazly et al., 2012. Enviromn Res, 41:11-19; Figueiredo et al., 2020. J Med Entomol, 57:2025-29) and never for *P. perniciosus*. The study reports the pathogenicity of a native strain of *B. bassiana* on the egg hatching rate of *P. perniciosus*.

**MATERIALS AND METHODS:** Eggs of *P. perniciosus* were placed in a sterile Petri dish on filter paper soaked with different concentration of *Beauveria bassiana* [5×106 conidia/ml (n= 368); 1- 5×107 conidia/ml (n= 358) and 1-5×108 conidia/ml (n= 388) - Bb CIS]diluted in distillated water plus 0.1% tween 80 (TGs). Untreated control eggs (n= 401) were exposed only to filter paper soaked with distillated water plus 0.1% tween 80 (CG). All experiments were repeated in two independent experiments with three replicates for each group within the TGs and CG. Petri dishes were incubated at 28°C and 90% of humidity. The egg hatching was counted every two days, and total larvae were quantified until 16 days post-treatment to obtain egg hatching rate (EHR) [(number of eggs hatched/total number of eggs in group) x 100].

**RESULTS AND CONCLUSIONS:** Egg hatching was observed in all groups from day 4 to day 12, except for TG with Bb CIS 108 conidia/ml, in which no egg hatching was observed at any time point. The EHR of TG with Bb CIS 107 conidia/ml was statistically lower than CG, starting from 6 days post infections. The EHR of TG with Bb CIS 106 conidia/ml were not significantly different from that CG during at any time points. Bb CIS 108 conidia/ml was highly virulent towards eggs of *P. pernicious*, thus suggesting that this fungus may be effective in decreasing the number of viable eggs in the environment. The high susceptibility of sand fly eggs to Bb suggests that Bb might be a potential alternative to the chemical control to reduce sand fly populations and therefore the risk of *L. infantum* transmission.

# *IN VITRO* ANTHELMINTIC ACTIVITY OF AGRO-INDUSTRIAL BY-PRODUCTS AGAINST *TRICHOSTRONGYLUS COLUBRIFORMIS* IN SHEEP

Bosco A.*[1], Nappa A.[1], Capezzuto G.[1], Nocerino M.[1], Di Donato L.[1], Vastolo A.[1], Amato R.[1], Kiatti D.D.[1], Calabrò S.[1], Cutrignelli M.I.[1], Sotiraki S.[2], Rinaldi L.[1]

*[1]University of Naples Federico II, Department of Veterinary Medicine and Animal Production, Naples, Italy; [2]Veterinary Research Institute, Hellenic Agricultural Organisation ELGO-DIMITRA, Thessaloniki, Greece*

**Keywords:** *Trichostrongylus colubriformis*, Agro-industrial by-products, *In vitro* tests.

**INTRODUCTION:** *Trichostrongylus colubriformis*, an intestinal parasite of small ruminants, is the second nematode to *Haemonchus contortus* in terms of distribution and magnitude in several regions (Kaba et al., 2023. Trop Anim Health Prod, 55(3):177). The control of this helminth is traditionally achieved with the use of anthelmintic drugs, however due to regulations in organic farming and the rise in anthelmintic resistance (AR), alternatives are sought after. The aim of the present study was to evaluate the *in vitro* anthelmintic effects of olive (*Olea Europaea*), carob (*Ceratonia siliqua*), pomegranate juice (*Punica granatum*), wine (*Vitis vibifera*), citrus (*Citrus Senensis*), hazelnut (*Corylys avellana*) and tomato (*Solanum lycopersicum*) by-products extracts on *T. colubriformis* of sheep.

**MATERIALS AND METHODS:** Extracts of seven by-products were tested *in vitro* on two development stages of *T. colubriformis* (eggs and infective larvae) using the Egg Hatch Assay (EHA) and the Larval Exsheathment Inhibition Assay (LEIA). The egg hatching rate was measured after incubation with each by-product extract (concentrations: 150, 300, 600, 1200 µg/mL) for 48 h at 26 °C. Ensheathed infective larvae were incubated for 3h at 20 °C with each by-product extract (concentrations: 150, 300, 600, 1200 µg/mL). Artificial exsheathment was induced *in vitro* by adding sodium hypocloride solution (2%w/v) diluted in 1 to 300 in PBS to the larval suspension. The progress of exsheathment over time was measured by repeated observations at 20-min intervals for 60 min (Moreno-Gonzalo et al., 2013. Vet Parasitol, 197:235-43).

**RESULTS AND CONCLUSIONS:** Among the 7 extracts tested using the two *in vitro* tests (EHA and LEIA), those with the greatest anthelmintic potential against *T. colubriformis* were the by-product extracts of wine, pomegranate and hazelnut. In particular, the inhibition in the development of eggs using EHA was 2.3-48.5% for wine, 6.1-48.1% for hazelnut and 18.6-42.4% for pomegranate by-product extracts. The inhibition of exsheathment using LEIA was 87.5%-100% for wine, 54.5-98.7% for hazelnut and 62.3-100% for pomegranate by-product extracts. Results showed that by-product extracts of wine, pomegranate and hazelnut exhibits *in vitro* anthelmintic activity, suggesting that, these by-products can also be an ally for *T. colubriformis* control in sheep.

# EFFECTS OF GIARDIAVIRUS INFECTION IN THE COURSE OF *GIARDIA DUODENALIS*/HUMAN ENTEROCYTE *IN VITRO* INTERACTION

Cecchetti S.[1], Nunziata G.*[2], Pedini F.[3], Coscione R.[1], Marucci G.[2], Lalle M.[2]

*[1]Core Facilities, Istituto Superiore di Sanità, Roma, Italy; [2]Unit of Foodborne and Neglected Parasitic Disease, Department of Infectious Diseases, Istituto Superiore di Sanità, Roma, Italy; [3]Department of Oncology and Molecular Medicine, Istituto Superiore di Sanità, Roma, Italy*

**Keywords:** *Giardia duodenalis*, Giardiavirus, Human enterocytes.

**INTRODUCTION:** *Giardia duodenalis* is a protozoan parasite able to infect the upper-small intestine of mammals causing giardiasis, a diarrheal disease. Symptoms of human giardiasis range from asymptomatic to acute or chronic. The molecular mechanisms underlying the variability of the clinical manifestations of giardiasis are yet to be fully understood, partially due to lack of appropriate *in vitro* models that can recapitulate gut environment. Among factors potentially affecting giardiasis outcomes the parasite infection with Giardia lamblia virus (GLV) must be considered. GLV is a dsRNA non-enveloped virus of the family Totiviridae, encoding only for the capsid protein and the RNA-dependent RNA polymerase. However, the correlation between the presence of GLV in *G. duodenalis* and the pathogenicity of the parasite is unknown. The aim of this study was to evaluate the impact of GLV infection on the interaction between *G. duodenalis* and enterocytes using an advanced in vitro model.

**MATERIALS AND METHODS:** *G. duodenalis* WBC6 isolate (Ass. A) infected with or w/o GLVHP or GLVCAT viral strain, was used. To *in vitro* reproduce the complex model of the intestinal barrier, a co-culture system was established using human colon adenocarcinoma cell lines Caco-2/TC7 and HT29-MTX, seeded into the apical compartment of “track-etched PET membranes” inserts. Differentiated Caco-2/HT29 cells were then incubated with *G. duodenalis* trophozoites in 90%DMEM/10%TYIS33 medium. Parameters such as trans-epithelial electrical resistance (TEER), paracellular apparent permeability (Paap), trophozoite replication and cellular viability were evaluated every 24 hours within 3 days. Localization of tight junction proteins was investigated by immunofluorescence microscopy, and expression of specific genes was evaluated by qPCR.

**RESULTS AND CONCLUSIONS:** We successfully adapted a previously developed *in vitro* co-culture model of epithelial cells that can sustain up to 3 days interaction with *G. duodenalis*. We demonstrated that the model replicates certain pathogenetic mechanisms of *G. duodenalis*, such as reduction of TEER, increase of Paap and disruption of tight junction. In contrast, GLV infection mitigates *G. duodenalis* induced intestinal barrier damages in a viral strain-dependent mode. Moreover, a differential response at gene level in enterocytes in relation to the GLV presence has been detected. Our data provide the first experimental evidences pointing on GLV infection as a factor potentially attenuating giardiasis outcome by reducing *G. duodenalis*-induced alteration of the intestinal barrier. In this perspective, our results might support the use of GLV as a bio-therapeutics against *G. duodenalis* for the development of an alternative approach in the treatment of giardiasis, thus helping in overcame the increased report of metronidazole treatment refractory cases.

# GIARDIAVIRUS AND MORE: ENHANCING CURRENT KNOWLEDGE ON VIRUSES INHABITING THE PROTOZOAN PARASITE *GIARDIA DUODENALIS*

Marucci G.*[1], Lucas P.[2], Cherchi S.[1], Cecchetti S.[3], Nunziata G.[1], Blanchard Y.[2], Lalle M.[1]

*[1]Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; [2]Viral Genetic and Biosecurity Unit, Ploufragan-Plouzané-Niort Laboratory, ANSES, Ploufragan, France; [3]Core Facilities, Istituto Superiore di Sanità, Rome, Italy*

**Keywords:** *Giardia duodenalis*, Giardiavirus, viral discovery.

**INTRODUCTION:** The protozoan *Giardia duodenalis* causes giardiasis, a globally distributed parasitic diarrheal disease. A small dsRNA virus comprising two ORFs (capsid protein and RNA-dependent RNA polymerase), referred to as Giardiavirus (GLV, *G. lamblia* virus), family Totiviridae, might inhabit the cytoplasm of many human and animal isolates of *G. duodenalis*. Recently, high throughput sequencing of few GLV genomes combined with experimental infections showed the occurrence of two GLV subtypes, namely GLVHP and GLVCAT, with limited differences at genomic and protein level, but showing remarkable divergence in infection phenotype. In addition, a new dsRNA virus was found to co-infect GLV positive *Giardia* isolates. To support GLV clustering and expand current knowledge of RNA virus inhabiting *G. duodenalis*, a viral discovery approaches was undertaken on *G. duodenalis* isolates naturally infected with GLV.

**MATERIALS AND METHODS:** Cultures of *G. duodenalis* isolates positive for GLV were obtained from collections worldwide. Total RNA was extracted and RNAseq was conducted on Illumina platform. Ad hoc pipeline was used for the identification of viral contigs, extension, control of assembly quality, and taxonomic classification. Biological properties of the identified viral strains, including infection efficiency, viral effect on parasite replication and encystation, were also investigated by experimental infection of the naïve WBC6 *Giardia* isolate.

**RESULTS AND CONCLUSIONS:** We sequenced 13 GLV sequences and identified a new, unclassified RNA viral-like sequence related to Ormycovirus in three *G. duodenalis* isolates. Phylogenetic analysis and experimental infection confirmed the occurrence of the two previously described GLV distinct subtypes, GLVHP and GLVCAT, which display different phenotypes and transmissibility in experimental infections of a GLV naïve *Giardia* isolate. We observed a different susceptibility of *G. duodenalis* Assemblages to GLV infection in particular Assemblages AII and B have proven to be resistant to the infection while AI and E were susceptible. Experimental infection showed that viruses of the GLVHP subtype have a greater effect in reducing the growth of the parasite as well as its ability to form cysts. Our study provided new evidence on GLV genome organization and biology by confirming the potential diversity of viral infections in the protozoan parasite *Giardia* and strengthens the possibility that GLV, or other endosymbiont virus infections, cause alteration of particular *Giardia* phenotypic traits, including virulence, posing themselves as possible candidates in advanced antiparasitic treatment strategies.

# *IN VITRO* EFFICACY OF *DUDDINGTONIA FLAGRANS* FOR THE CONTROL OF GASTROINTESTINAL STRONGYLES OF SHEEP

Paoletti B.*[1], Astuti C.[1], Morelli S.[1], Iorio R.[1], Bartolini R.[1], De Angelis E.[2], Traversa D.[1], Colombo M.[1], Di Teodoro L.[1], Di Cesare A.[1]

*[1]University of Teramo, Department of Veterinary Medicine, Teramo, Italy; [2]Vet Practitioner, Teramo, Italy*

**Keywords:** *Duddingtonia flagrans*, Strongyles, Biological control.

**INTRODUCTION:** *Duddingtonia flagrans* is a nematode-trapping fungus used as biological controller of gastrointestinal nematodes (GIN) affecting livestock. Chlamydospores of *D. flagrans* survive in the digestive tract and capture nematode larvae in faeces. Nevertheless, the quantity of chlamydospores required for the reduction of GIN third-stage larvae (L3) is still under investigation (Zegbi et al., 2021. Exp Parasitol, 230:108156). This experiment has evaluated the *in vitro* activity of varying concentrations of *D. flagrans* chlamydospores against different amounts of GIN L3 and their efficacy on the reduction of the larval count of different GIN.

**MATERIALS AND METHODS:** GIN L3 were cultured from a pool of fresh faeces of sheep and recovered by Baermann technique. An aliquot of L3s was used for morphological and molecular identification. The growth of *D. flagrans* and interaction with L3 were examined on enriched Sabouraud agar plates sown with 1 ml of fungal suspension containing 1000, 3000, 6250 or 11000 chlamydospores respectively and cultivated for 7 days at 25°C. On the 7th day, 1 mL of culture suspension containing respectively 500, 1000 and 1500 L3 were poured into each plate. Three series for each chlamydospores concentrations were set up with and without *D. flagrans*. After 7 days of incubation, L3 were collected using Baermann technique. The L3 were counted and identified. Kruskal-Wallis test was used for statistical analysis of results. The fungal efficacy was estimated using as previously described (Terril et al., 2004. Vet Parasitol, 120:85-296).

**RESULTS AND CONCLUSIONS:** Identified GIN were *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus colubriformis* and *Chabertia ovina*. Data analysis showed that L3 reductions were 93%, 98.5%, 99% and 100 % compared to the 500 L3-control group; 97%, 99%, 100% and 100% compared to the 1000 L3-control group; 99%, 99%, 100% and 100% compared to 1500 L3-control group. Comparison between chlamydospore concentrations revealed statistically significant differences ($p \leq 0.05$) between 1000 and 11000 chlamydospore for the 500 L3 and 1000 L3 cultures, and between 1000 and 6250 chlamydospores for the 1500 L3 culture. These results confirm the efficacy of *D. flagrans* in GIN L3 reduction and that the number of larvae is not a determining factor on the efficacy against GIN as previously suggested (Zegbi et al., 2021. Exp Parasitol, 230:108156). In fact, *D. flagrans* showed the same efficacy against all GIN species retrieved. Thus, its potential use as an alternative control of GIN is here confirmed. Such a potential alternative approach is of great importance considering the spreading of anthelmintic-resistant GIN in many geographical regions (Charlier et al., 2023. Parasite, 30:E1).

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# EARLY RESULTS OF THE MOSQUITO LARVICIDAL ACTIVITY OF *HYSSOPUS OFFICINALIS* HYDROLATE ON THREE MEDICAL IMPORTANCE MOSQUITO SPECIES

Toma L.*[1], Garzoli S.[2], Severini F.[1], Casale F.[1], Sarleti N.[1], Di Luca M.[1]

*[1]Istituto Superiore di Sanità, Rome, Italy; [2]Department of Chemistry and Technologies of Drug, Sapienza University Rome, Rome, Italy*

**Keywords:** Essential oils, Mosquito control, Larvicidal.

**INTRODUCTION:** Several essential oils (EOs) and hydrolates (Hys) have larvicidal activity against mosquitoes of medical importance mainly *Aedes* and *Culex*. However, Eos have been widely tested as control agents for vectors of important diseases, such as dengue, chikungunya, and Zika as alternative to synthetic chemical insecticides. In this study, we exploited the potential larvicidal effect of *Hyssopus officinalis*, still poorly known, on the mosquito species *Aedes albopictus*, *Aedes aegypti* and *Culex pipiens*. We used hydrolate being an aromatic water obtained during the extraction process of essential oil using steam distillation. In particular, our *H. officinalis* Hy (IHy), has 5% EO concentration. This choice is because hydrolates are more soluble in water and they could give a first indication about efficacy on mosquito larvae. The larvae were reared in a climatic chamber at a temperature of 27.0 ± 1.0 C, 80.0 ± 10.0 % relative humidity, and a photoperiod of 14:10 h light:dark, until reaching adulthood.

**MATERIALS AND METHODS:** In this study, in order to describe the volatile chemical profile of HHy, it was subjected to chemical analysis by means headspace solid phase microextraction (HS-SPME) for the sampling phase and gas-chromatography/mass spectrometry (GC-MS) technique for the separation and identification of the components. The larvae were reared in a climatic chamber at a temperature of 27.0 ± 1.0 C, 80.0 ± 10.0% relative humidity, and a photoperiod of 14:10 h light:dark, until reaching adulthood. The bioassays were performed according to the procedure of the WHO, using 60 larvae in 200ml for each test. Negative and positive control tests were performed in parallel for comparison. The positive control contained an aqueous solution 0.05 mg/L of the organothiophosphate insecticide Fenthion (LD99=0.05ppm). A log-probit regression model for the three oils was obtained and toxicity was reported as lethal concentrations LC50, and LC90 after 24 and 48 hours.

**RESULTS AND CONCLUSIONS:** The carried out chemical analyses highlighted the presence of eighteen components. Among these, carvacrol (53.3%) was the major compound followed by α-terpineol (16.2%). At 24h the results were: *Ae. albopictus* (LD50=2.7%), *Ae. aegypti* (LD50=2.2), *Cx. pipiens* (LD50=2.5). In all the three species LD99 was around 3.5-4%. The semi-field assays will allow to test the efficacy of the HHy outdoor on *Ae. albopictus* and *Cx. pipiens*. The qualitative and semi-quantitative chemical characterization allow to identify the molecules which probably may be mainly responsible for the larvicidal effect.

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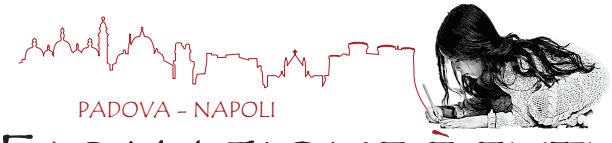
# DIAGNOSTICS, PHYLOGENY AND GENOMICS

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# (DO YOU) "SEEME"? IDENTIFICATION OF PARASITES IN METAGENOMIC SAMPLES: AN AUTOMATED WORKFLOW INDEPENDENT FROM RIBOSOMAL SEQUENCES

Vatta P.*, Cacciò S.M.

*Istituto Superiore di Sanità, Rome, Italy*

**Keywords:** Metagenomics, Parasites, Automated detection workflow.

**INTRODUCTION:** Targeted detection of eukaryotic pathogens in environmental and food matrices is normally achieved by molecular methods, such as qPCR. However, untargeted methodologies, such as shotgun NGS-based sequencing, are a promising alternative, as they allow describing the entire community of eukaryotic organisms present in a matrix. In this study, we analysed both spiked and real matrices with the goal of identifying the crucial parameters affecting the sensitivity and specificity of detection of protozoan pathogens. We propose an optimized and automated workflow and show how removal of ribosomal sequences and reliance upon genomic sequences produce trustable results.

**MATERIALS AND METHODS:** Spiked samples were prepared by adding known amounts of *Cryptosporidium* DNA to DNA extracted from lettuce. About 1 µg of DNA was used to prepare and sequence Illumina NGS libraries, generating an average of 3 Gb per sample. Additional samples (n=36) were collected in the course of a project (MicroSafeRisk) funded by the Italian Ministry of Health. Genomic DNA was extracted and quantified by Qubit. About 1 µg of DNA was used to prepare and sequence IonTorrent NGS libraries, generating an average of 2 Gb of data per sample. NGS data were analysed using an automated workflow we developed and named SEEME (SEarch Eukaryotes in MEtagenomes). The workflow includes six steps: 1) reads are aligned to a reference genome at high stringency, 2) extracted and 3) re-aligned against a database of eukaryotic ribosomal sequences (SILVA), then 4) unmapped reads are recovered and 5) used for BLAST analyses to 6) identify reads originating from the reference genome used in step 1.

**RESULTS AND CONCLUSIONS:** Analysis of the shotgun NGS data from spiked samples showed that the high similarity between *Cryptosporidium* and plant ribosomal sequences is the major confounding effect. Indeed, BLAST analyses of the sequencing reads showed that these are not derived from *Cryptosporidium*, yet align to their ribosomal genes even when high stringency (i.e., by setting the K parameter to 100) is used. We therefore adjusted the workflow to include a step that filters ribosomal reads and then processes non-ribosomal reads to confirm their specific origin by BLAST. We show that this workflow detects *Giardia*, *Cryptosporidium*, *Eimeria* and *Blastocystis* in various faecal and environmental matrices. A novel analytical workflow (SEEME) for the detection of parasites in metagenomic NGS samples has been implemented. The workflow deliberately excludes ribosomal sequences and only relies upon non-ribosomal genomic sequences for detection. We show application of this workflow to lettuce samples spiked with *Cryptosporidium* as a test case, and then to 36 real metagenomes from various matrices.

# THE GENETIC STRUCTURE OF *TRICHINELLA BRITOVI* ISOLATES IS DEFINED BY INFECTIOUS DOSE AND MIXED INFECTIONS: EXPERIMENTAL EVIDENCE

Santoro A.*, Cherchi S., La Rosa G.

*Istituto Superiore di Sanità, Rome, Italy*

**Keywords:** *Trichinella britovi*, Inbreeding, Genetic structure.

**INTRODUCTION:** *Trichinella* spp. displays a very peculiar ecology with a genetic pool partitioned over a multitude of independent demes enclosed in susceptible hosts. The genetic relatedness among demes depends on several factors e.g. the number of generations occurred and the number of breeding pairs who generated them. The higher the number of generations and the lower the number of parent pairs, the higher will be the degree of inbreeding a deme shows. Long-time isolation and high degree of inbreeding of a deme should promote speciation. Gene flow and gene recombination oppose to speciation. In *Trichinella*, gene recombination is ensured by the occurrence of multiple infections in the same host. Understanding the ecological significance of this scenario would offer precious hints to define the epidemiological rules followed by these parasites (La Rosa et al., 2018. Int J Parasitol, 48:543-53).

**MATERIALS AND METHODS:** A controlled experiment was conducted to evaluate the changes occurring in the genetic structure of *Trichinella* isolates through successive passages in mice. Briefly, two *T. britovi* isolates were identified as belonging to distinct genetic clusters based on allele frequencies of five microsatellites (single larva analysis, La Rosa et al., 2012. Infect Genet Evol,12:369-76), and selected to be used as parental strains (P1 and P2). Two mice were then orally infected with 200 larvae collected from P1 and P2 (100 larvae each) to generate the F1 in two separate lineages (A and B). Forty-five dpi, muscle larvae were recovered separately from each mouse and 200 of them were used to generate the F2. Generations from F2 to F9 received a decreasing number of infecting larvae, from 200 (F2) to 6 (F9). The genetic structure at each generation was evaluated by STRUCTURE (ver.2.3.4) algorithm.

**RESULTS AND CONCLUSIONS:** Both F1 lineages (A and B) showed an admixed pattern where each larva was assigned to two alternative clusters. From generation F2 to F8 a homogeneous genetic structure in which no clustering was detectable was observed. Finally, the F9 generation, derived from very low infectious dose, showed a strong loss of genetic variability producing genetically divergent mouse cohorts. The genetic structure of *Trichinella britovi* strongly depends on the number of reproductive individuals at each passage: many parental pairs (F2-F8) kept the genetic structure of the cohorts stable while passages with few pairs (F9) determined a drastic reduction of genetic variability and the birth of new evolutionary lines independent of the ancestors they derived from. From an epidemiological point of view, it is demonstrated that, in conditions of genetic stability, an admixed genetic structure discloses the presence of a recent mixed infection event, therefore the incidence of cohorts with admixed structure in a territory may be assumed as an indicator of the true prevalence of this parasite in nature.

# VALIDATION OF CLSH-ABBY IMHOTEP, A NEW RAPID IMMUNOCHROMATOGRAPHIC TEST FOR CANINE LEISHMANIOSIS DIAGNOSIS

Pugliese M., Culoma C.E., Sturiale E., Catone G., Passantino A., Brianti E., Napoli E.*

*Department of Veterinary Sciences, University of Messina, Messina, Italy*

**Keywords:** Canine Leishmaniosis, Diagnosis, Immunochromatographic test.

**INTRODUCTION:** Canine Leishmaniosis (CanL) is a vector-borne disease caused by *Leishmania infantum* and vectored by flebotiomine sand flies. Infected and/or diseased dogs serve as one of the main reservoir of the infection in the Mediterranean countries. CanL diagnosis CanL is still a challenge due to the variety of clinical signs and to the high percentage of asymptomatic dogs; therefore availability of sensitive and reliable diagnostic tools is of paramount importance to achive a definitive diagnosis. The gold standard for the CanL diagnosis is the detection of anti-*Leishmania* antibodies using serological tests (i.e., IFA and ELISA). Commercial rapid detection assays for anti-*Leishmania* antibodies in dogs have been reported to show lower sensitivity and specificity when compared to conventional serological methods, although these commercial tests are very attractive for practitioner as screening tests for their simple and rapid use. Recently, a new tool, the cLSH-Abby Imhotep (DongGuan Medical Industry Investment Co, LTD), based on immunofluorescence chromatography technology using of microspheres wrapped with Europium (Eu) lanthanide as a marker, has been launched in the market for the diagnosis of CanL. The diagnostic performance of the LSH-Abby Imhotep and of another immunochromatographic test (ICT) the *Leishmania* IgG/IgM Rapid Test (Cassette, Citest Diagnostic imminc.) were evaluated using as reference a commercial indirect ELISA test (ID Screen® Leishmaniasis Indirect Test, VET- Innovate ID Diagnostics).

**MATERIALS AND METHODS:** A total of 61 sera of privately owned adult dogs (34 seropositive, 22 seronegative, and 5 doubts), diagnosed by the commercial indirect ELISA test, were used. The samples were submitted to both ICTs following the manufacturers' instructions. Statistical analysis was performed to evaluate the diagnostic performance of each ICT in comparison with the ELISA and Cohen's kappa coefficient was calculated for the accuracy of the tests.

**RESULTS AND CONCLUSIONS:** Both ICTs scored 97.06% for sensitivity while specificity was 81.81% and 90.90% for *Leishmania* IgG/IgM Rapid Test and cLSH-Abby Imhotep, respectively. Cohen's kappa coefficient underlined a perfect agreement between cLSH-Abby Imhotep and ELISA results (i.e., Cohen's k: 0.81), while a substantial agreement between *Leishmania* IgG/IgM and ELISA results was detected (Cohen's k: 0.73). Rapid tests are a valuable tool for the diagnosis of CanL, due to their simplicity, low cost, and practical results. The screening of dogs in *Leishmania* endemic areas is a fundamental act of public health surveillance policy, which can be implemented through the use of rapid tests coupled with a thorough clinca evaluation before the decision-making. The tests evaluated in this study showed good performances, and, according to the results, the cLSH-Abby Imhotep seems a reliable diagnostic tool that can be used in clinical and epidemiological investigations.

# POPULATION GENOMICS OF *BORRELIA BURGDORFERI SENSU LATO* IN ITALY

Melis S.*[1], Hizo-Teufel C.[2], Prati P.[3], Sambri V.[4], Bandi C.[5], Fingerle V.[2], Sassera D.[1]

*[1]University of Pavia, Pavia, Italy; [2]National Reference Centre for Borrelia at the Bavarian Health and Food Safety Authority, Oberschleissheim, Germany; [3]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Pavia, Italy; [4]Unit of Microbiology, University of Bologna, Bologna, Italy; [5]Department of Bioscience, University of Milano, Milano, Italy*

**Keywords:** *Borrelia burgdoferi*, Genome, Long-read sequencing.

**INTRODUCTION:** *Borrelia burgdorferi sensu lato* is a bacterial complex that include all spirochete that cause Lyme disease, the tick-borne illness with the highest incidence in Europe and North America. The early-stage symptoms of Lyme disease include malaise, fatigue, headache, arthralgia, myalgias and fever. In some cases, the disease can then develop into a chronic and debilitating illness characterised by skin lesions, neurological disorders, carditis and arthritis. The most commonly reported genospecies in Europe are *B. afzelii* and *B. garinii*, which are transmitted by the tick vector *Ixodes ricinus*. Despite the clinical importance of the pathogen, little is known about its genomics. Short-read sequencing technologies have been applied to small and large datasets. However, due to the highly fragmented genome architecture, they have proven not to be entirely effective in assembling the numerous plasmids.

**MATERIALS AND METHODS:** The main objective is to isolate *B. burgdorferi s.l.* from ticks and patients in Northern Italy, as there are currently no Italian genomes available, and to obtain high-quality genomes by using both short- and long-read sequencing technologies (ILLUMINA and NANOPORE). Since it has been suggested that different genospecies and genotypes can give rise to different clinical manifestation, our second aim is to investigate the presence of genetic markers responsible for infection and pathogenesis in humans.

**RESULTS AND CONCLUSIONS:** So far, we obtained six genomes of isolates obtained from single ticks collected in Italy, five *B. garinii* and one *B. burgdorferi s.s.* and we performed preliminary bioinformatic analyses. We obtained genomic-based phylogenies and applied a method to compare plasmids of the newly sequenced isolated to those retrieved from public databases. Our results show a limited level of species-specificity in the plasmid contents. Ongoing work is underway for the isolation and sequencing of other *Borrelia* cultures and for more advanced bioinformatic analyses.

# LABORATORY DIAGNOSIS OF THE ZOONOTIC TAPEWORM *DIPYLIDIUM CANINUM* IN CATS

Morelli S.*[1], Di Cesare A.[1], Traversa D.[1], Colombo M.[1], Paoletti B.[1], Ghietti A.[1], Beall M.[2], Davenport K.[2], Buch J.[2], Iorio R.[1], Marchiori E.[3], Frangipane Di Regalbono A.[3], Diakou A.[4]

*[1]Department of Veterinary Medicine, University of Teramo, Teramo, Italy; [2]IDEXX Laboratories Inc., 04092 Westbrook, ME, USA, Westbrook, United States of America; [3]Department of Animal Medicine, Production and Health, University of Padova, Padova, Italy; [4]School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece*

**Keywords:** *Dipylidium caninum*, Diagnosis, PCR.

**INTRODUCTION:** *Dipylidium caninum* is a common zoonotic cestode infecting cats and dogs worldwide. Animals become infected ingesting fleas or lice that contain cysticercoid larvae. In cats dipylidiosis is often subclinical, even though gastrointestinal signs are observed especially in heavy infections or young animals (Rousseau et al., 2022. Parasit Vectors, 15:131). Diagnosis of *D. caninum* infections can be challenging, due to the low sensitivity of classic copromicroscopic tests, e.g. floatation (Little et al., 2023. Vet Parasitol, 324:110073). This study was carried out to evaluate the diagnostic performance of different microscopic and molecular techniques for the detection of *D. caninum* in cats.

**MATERIALS AND METHODS:** Individual stool, rectal swab and perianal scotch tape test samples were obtained from 100 cats from endemic areas of Italy and Greece. All fecal samples underwent macroscopic inspection and flotation. Based on the results, cats were divided in three groups, i.e. Group A composed by 50 cats positive for *D. caninum* regardless of presence of other endoparasites, and Groups B and C, of 25 cats each, in which cats negative for *D. caninum* but infected by other helminths and cats negative for intestinal endoparasites were included, respectively. Aliquots of feces, flotation supernatant, scotch tape test, and rectal swabs were subjected to PCR using a previously validated protocol (Beugnet et al., 2014. Vet Parasitol, 205:300-6)

**RESULTS AND CONCLUSIONS:** Of the 50 samples of cats enrolled in Group A, 45 were positive to visual inspection and microscopic identification of proglottids, while 5 were positive at a conventional floatation. In the same group, the scotch test was positive for *D. caninum* in 8 cats. At least one sample type tested positive at PCR for 33 cats of Group A. Of them, all tested positive at PCR on floatation aliquot, while 9 and 1 cats scored positive at PCR on stool aliquot and scotch tape test, respectively. No samples from Groups B and C were positive to any PCRs. The sequences derived from the amplicons of Italian samples had 99-100% homology with *D. caninum* feline genotype. These findings suggest that PCR may be a useful diagnostic tool under certain circumstances, though with certain limitations. At present, the direct visualization and microscopic identification of proglottids in feces remain the preferred option in clinical settings. This is supported by the fact that this procedure demonstrated the highest sensitivity for detecting *D. caninum* infections among all other methods of the present study. A recently developed coproantigen immunoassay (Elsemore et al., 2023. J Vet Diagn Invest, 35:671-78; Little et al., 2023. Vet Parasitol, 324:110073) showed a high potential for the diagnosis of dipylidiosis. Further studies are advocated to ultimately demonstrate that this coproantigen immunoassay, with or without a combination of traditional techniques, would be the best choice for diagnosing feline dipylidiosis.

# LUNG MICROBIOME IN PATIENTS WITH *PNEUMOCYSTIS JIROVECII* PNEUMONIA

Del Prete V.*, Berrilli F., Di Cave D.

*Universita degli Studi di Roma Tor Vergata, Dipartimento di Scienze Cliniche e Medicina Traslazionale, Roma, Italy*

**Keywords:** *Pneumocystis jirovecii* Pneumonia, Lung microbiome, Next Generation Sequencing.

**INTRODUCTION:** *Pneumocystis jirovecii* pneumonia (PCP) is an opportunistic fungal infection linked to high morbidity and mortality in immunocompromised individuals. Understanding the intricate interplay among pathogens, host immune responses, and the microbiome is crucial in elucidating the etiology of infectious and inflammatory diseases. Thus, our study aims to analyze the lower respiratory tract microbiome in PCP patients with different underlying diseases.

**MATERIALS AND METHODS:** We analyzed bronchoalveolar fluid samples from 48 patients (27 PCP-positive and 21 PCP-negative) at the parasitology laboratory of Fondazione Policlinico Tor Vergata, Rome, Italy. PCP-positive samples were collected between April 2020 and December 2021, while PCP-negative samples were selected from those matching primary characteristics of of PCP-positive patients, including age, sex, underlying disease, and infectious comorbidity. Next-generation sequencing of the bacterial 16S rRNA gene V3/V4 regions was employed to investigate microbiome composition.

**RESULTS AND CONCLUSIONS:** Among the 27 PCP-positive patients (61% males, median age 62 years), hematological malignancies (HM, 12 cases), HIV (9 cases), solid tumors (4 cases), and autoimmune disorders (2 patients) were prevalent underlying diseases. PCP-negative patients (median age 61 years, 57% males) exhibited similar conditions: HM (10 cases), HIV (8 cases), solid tumors (2 cases), autoimmune disorders (1 case). Although microbial profiles varied across patients with different underlying diseases, the microbiome differences between PCP-positive and PCP-negative cohorts were not statistically significant. In particular, *Streptococcu*s and *Pseudomonas* were prevalent genera in patients with solid tumors and HM, while *Haemophilus* and *Neisseria* were more frequent among HIV patients. In conclusions, our findings emphasize the close association between the lower respiratory tract microbiome and underlying diseases in PCP patients. Further investigations could elucidate specific microbiome interactions, enhancing our understanding of PCP pathogenesis and guiding therapeutic strategies.

# *STRONGYLOIDES STERCORALIS* GENOTYPING FROM HUMAN CLINICAL SAMPLES

Deiana M.*, Piubelli C., Malagò S., Degani M., Rizzi E., Buonfrate D.

*Department of Infectious-Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Negrar di Valpolicella, Verona, Italy*

**Keywords:** *Strongyloides stercoralis*, Genotyping, NGS.

**INTRODUCTION:** *Strongyloides stercoralis*, a soil-transmitted helminth, presents a significant global health burden (1), challenging both veterinary and human public health sectors (2). Despite morphological similarities between *S. stercoralis* found humans in and in other hosts, molecular studies have revealed distinct lineages (3). Multiple *S. stercoralis* populations have been reported in zoonotic infections, but due to the complexity of the sample, currently they cannot be distinguished from those found in humans (4). As of today, the presence and the role of different genotypes in human infections is still unexplored. Therefore, a comprehensive genomic analysis, encompassing genotyping, is warranted to explore the presence and potential clinical associations of various haplotypes in humans across different geographical areas. Ideally, the genomic characterization of single adult male worms would produce clean genomic data, for a precise depiction of the genotypes infecting humans. Thus, we aimed to develop a comprehensive method for extracting DNA, amplifying the entire genome, and preparing a sequencing library suitable for long-read sequencing from a single *S. stercoralis* adult male.

**MATERIALS AND METHODS:** Fecal samples from human patients were cultured, and a variable number (from 1 to 10) of *S. stercoralis* adult male worms was collected from each positive plate. DNA extraction was performed using the Monarch® Genomic DNA Purification Kit (NEB), followed by whole genome amplification (WGA) using the REPLI-G Midi Kit (Qiagen). The amplified DNA underwent quality assessment, and library preparation was carried out using the Native Barcoding Kit 24 V14 (Oxford Nanopore Technologies) for subsequent sequencing on the MinION Mk1C Sequencing Device. Reads were aligned to the Reference Genome GCA_029582065.1, and data from different worm quantities were compared.

**RESULTS AND CONCLUSIONS:** Read numbers and qualities across samples were compared for library preparation and sequencing protocol optimization. Sequencing metrics will be evaluated in order to establish whether it will be possible to genotype a single helminth, or the analyses would better be conducted on batches of multiple worms. Sequencing of single worms will ensure greater accuracy in genotyping and a better understanding of the genetic diversity of *S. stercoralis* among host species, as it will provide a more precise indication of the genetic variants present while reducing the risk of cross-contamination or chimera genome. In conclusion, the methodology, if succeed, will enable the comprehensive genomic analysis of *S. stercoralis* from a single adult male specimen, overcoming the technical challenges associated with working with such small and complex organisms.

# SEROLOGICAL IGG DIAGNOSIS BY IMMUNOBLOT ASSAY FOR TOXOCARIASIS IN PIEDMONT

Di Domenico C.*, Tozzini M., Aliberti S., Pellò M.G., Faolotto G., Mercandino A., Quaglia V., Andreoni S.

*Maggiore della Carità Hospital, Microbiology and Virology Laboratory, Novara, Italy*

**Keywords:** *Toxocara*, Piedmont, Seroprevalence.

**INTRODUCTION:** Toxocariasis is a tissue helminthiasis due to nematode larvae belonging to the genus *Toxocara*. The most common agent is *Toxocara canis* (dog roundworm) and sometimes *Toxocara cati* (cat roundworm) (Eberhard et al., 1998. Am J Trop Med Hyg, 59:404-6). Contamination of humans, the accidental host, occurs by ingestion of embryonated eggs or larvae (Magnaval et al., 2001. Korean J Parasitol, 39:1-11). The purpose of this study was to analyze the distribution of positive cases due to contact with *Toxocara canis* in the provinces of Eastern Piedmont.

**MATERIALS AND METHODS:** The study was conducted on serum samples received at the Novara Microbiology and Virology Laboratory. The samples were tested for specific IgG antibodies directed against the ES (excretory-secretory) antigen of *Toxocara canis* by western blot method, a laboratory technique designed to identify proteins of interest in a mixture by antigen/antibody reaction. The principle of western blotting is based on the identification, with a dedicated antibody, of a specific antigen (protein) present within a complex mixture of antigens (or proteins) separated in a polyacrylamide gel by molecular weight and immobilized on a nitrocellulose membrane.

**RESULTS AND CONCLUSIONS:** A total of 109 serum samples were analyzed during a time span from January to November 2022. 48 samples were from the province of Novara (NO), 16 from the province of Biella (BI), 18 from Verbano-Cusio-Ossola (VCO), 23 from Vercelli (VC), and 4 samples were from outside Eastern Piedmont (EP). The prevalence of positives was 35.8% (39), with the following distribution by province: NO 35.9% (14), BI 20.5% (8), VCO 17.9% (7), VC 23.1% (9), outside EP 2.6% (1). The results show the distribution of cases of contact with *Toxocara canis*. Although this is a preliminary study, rates of some significance were found, especially in the provinces of Novara and Vercelli. The high positivity of contact with the parasite in these areas may be attributable to the intensive presence of agricultural crops. Further investigations will follow to confirm this hypothesis.

# APPLICATION OF A ddPCR ASSAY FOR RAPID IDENTIFICATION AND QUANTIFICATION OF *HAEMONCHUS CONTORTUS* IN SMALL RUMINANT FECES

Zoccola R.[1], Moroni B.*[1], Maurizio A.[2], Dotto G.[2], Zoppi S.[1], Goria M.[1]

*[1]Istituto Zooprofilattico Sperimentale di Piemonte, Liguria e Valle d'Aosta, Turin, Italy; [2]Department of Animal Medicine, Production and Health (MAPS), University of Padua, Legnaro, Padova, Italy*

**Keywords:** Gastrointestinal nematodes, Small ruminants, ddPCR.

**INTRODUCTION:** Identification and quantification of gastrointestinal nematodes (GIN) of small ruminants is of paramount importance for a correct and timely treatment. Nevertheless, traditional copromicroscopical methods based on egg count and larval culture of GIN are time-consuming and need the expertise of specialized operators for the morphological identification of larvae. Novel droplet digital PCR (ddPCR)-based applications have been recently validated to identify and quantify mixtures of genomic DNA extracted from different species of adult worms (Elmahalawy et al., 2018. Vet Parasitol, 261:1-8), although in-field applications of ddPCR should be tested to quantify parasitic egg-derived DNA in the feces, rather than in cultured adult worms. The aim of this pilot study was to test a new ddPCR protocol for *Haemonchus contortus* eggs collected from sheep and goat feces with symtomps compatible with haemonchosis.

**MATERIALS AND METHODS:** First, *Haemonchus contortus* was isolated and morphologically identified from a goat that was necropsied at the Istituto Zooprofilattico Sperimentale in Turin. Then, from the same animal, feces were collected, and parasitic load was quantified using a concentration McMaster technique with a sensibility of 100 EPG (Roepstorff et al., 1998. FAO Animal Health Manual No. 3. Food and Agriculture Organization of the United Nations, Rome, pp. 51-56). Two solutions were obtained from McMaster chambers with 5 and 10 eggs, respectively, DNA was extracted from both using the QIAamp Fast DNA Stool Mini Kit (QIAGEN Inc.) and serial dilutions were prepared. All the dilutions were tested in triplicate and the amount of DNA from strongylides and from *Haemochus contortus* was quantified in multiplex ddPCR with two different sets of primers and probes as described by Elmahalawy et al., 2018. As a positive reaction control was used an extract obtained from an adult parasite morphologically identified as belonging to the genus *Haemonchus*. Then, the same protocol was tested in feces of animals that presented symptoms compatible with haemonchosis.

**RESULTS AND CONCLUSIONS:** Preliminary tests correctly detected the presence of the target DNA in extracts in both the solutions (5 and 10 eggs), but only at the lowest dilution. Both assays tested (ITS2 in the ribosomal DNA gene array present in any strongylid nematode and the internal transcribed spacer region 2 of the ribosomal RNA gene array of *Haemonchus*) worked correctly with good repeatability of results. The copy number detected with the two ddPCR assays did not correspond proportionally to the number of eggs in the starting matrices. This result is probably due to a non-optimised extraction procedure. The results of this pilot study indicate that ddPCR can be regarded as a useful diagnostic tool to identify *Haemonchus* spp. in small ruminant feces, bypassing time-consuming and laborious techniques such as larval culture. Preliminary results on the quantification of the eggload was not successful, although this will be object of further investigation.

# LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY AS A RAPID DIAGNOSTIC TOOL FOR *TRITRICHOMONAS FOETUS* IN CATTLE AND CATS

Moroni B.*, Guglielmetti C., Pontei A., Petruccelli G., Rossi F., Zoppi S.

*Istituto Zooprofilattico Sperimentale di Piemonte, Liguria e Valle d'Aosta, Turin, Italy*

**Keywords:** *Tritrichomonas foetus*, LAMP, Trichomoniasis.

**INTRODUCTION:** *Tritrichomonas foetus* is a worldwide distributed flagellate protozoan parasite responsible for disease in cattle as well in cats. Bovine trichomoniasis is a venereal disease associated with long intercalving periods and low conception rates, while cats usually show gastrointestinal symptoms such as chronic large-bowel diarrhea and abdominal pain. Prompt and accurate diagnosis of t trichomoniasis could be helpful to remove infected bulls as well as properly treat diseased cats. Loop-mediated isothermal amplification (LAMP) is a highly specific amplification technique due to the use of two to three pairs of primers (internal, external, and loop), is carried out at isothermal temperature (60-70°C) and, unlike traditional PCR, have shown high tolerance to biological fluids (Mori et al., 2020. J Infect Chemoter, 26:13-17). In our study, preliminary tests are conducted on bulls preputial washings and feline faecal samples to evaluate the performances of the methods as screening test in our laboratory.

**MATERIALS AND METHODS:** Two bovine preputial washes, previously negative by bacteriology, were used as negative samples, while ATCC Number: 30003™ *Tritrichomonas foetus* BP-4:Beltsville was used as positive sample. Four aliquots of preputial washes (25 ml) were spiked with ATCC strains using 1 and 10 microliter inoculating loops. After a centrifugation (10' at 1,300rpm), supernatants were discarded. DNA extraction was carried out both with Promega ReliaPrep gDNA Tissue Miniprep System and by simple extraction, with centrifugation (5' at 12,000g), resuspension of pellet in 500 µl of Nuclease-Free Water, lysis (10' at 95°C) and centrifugation (3' at 2,000g). Two different sets of primers were used, for the elongation factor 1a1 (tf-ef1a1) and the beta tubulin 2 (tf-btub2) (Morero et al., 2021. Vet Parasitol, 295:109462). For amplification, two different commercial lamp kits were tested [LavaLAMP DNA Master Mix (Biosearch Technologies) - SuperScript IV RT-LAMP Master Mix (Thermo Fisher Scientific)], using the miniaturized Hyris bCUBE System by setting two different programs for tf-ef1a1 (45' at 62°C) and tf-btub2 (60' at 65°C) primers.

**RESULTS AND CONCLUSIONS:** Preliminary results on preputial washing appear promising. Amplification signals were obtained on positive control and from spiked samples extracted with both methods. The two commercial amplification kits tested led to the amplification of the positive control and spiked samples. Primers concentration was, at the beginning, too high giving non-specific signals in the non-template control (NTC) and in the extraction blank (bE). The 10 fold dilution of tf-btub2 primers allowed the amplification of the spiked samples and positive controls and the absence of non-specific signals in the NTC and bE wells. Authors are also working on the extraction procedures for feline faecal samples to offer an economic, quickly and accurate diagnosis of Trichomoniasis in both, bovine and feline samples.

# REDESCRIPTION AND MOLECULAR INVESTIGATION OF *SCLERODERMUS DOMESTICUS* LATREILLE AND *SCLERODERMUS CEREICOLLIS* KIEFFER (HYMENOPTERA, BETHYLIDAE)

Masini P.*[1], Rebora M.[2], Salerno G.[1], Azevedo C.O.[3]

*[1]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy; [2]Department of Chemistry, Biology e Biotechnology, University of Perugia, Perugia, Italy; [3]Federal University of Espírito Santo, Department of Biological Sciences, Vitória (ES), Brazil*

**Keywords:** *Sclerodermus domesticus*, *Sclerodermus cereicollis*, Mitochondrial DNA.

**INTRODUCTION:** *Sclerodermus domesticus* and *Sclerodermus cereicollis* are two ectoparasitoid flat wasps of wood-boring beetle larvae (Gordh & Móczár, 1990. Mem Am Entomol Inst, 46:1-364). The importance of various species of *Sclerodermus* as biological control agents against insect pests (Yang et al., 2014. Biol Control, 68:117-28), together with their role as sting dermatitis agents in humans, make their study very valuable (Azevedo et al., 2022. Zootaxa, 5124:501-19). However, the *Sclerodermus* taxonomy is still confounded: the descriptions of the European species of *Sclerodermus* are old and incomplete (Azevedo et al., 2018. Zootaxa, 4489:1-294). Given this scenario, we here propose a morphological redescription and the first molecular analysis of these two species, investigating specimens from two different populations collected in Italy and bred in the laboratory of the University of Perugia.

**MATERIALS AND METHODS:** The morphological and molecular investigations were performed at the Núcleo de Excelência em Sistemática de Bethylidae (NESB) and at the Núcleo de Genética Aplicada Conservação da Biodiversidade (NGACB), Universidade Federal do Espírito Santo, Brazil. We compared our specimens to the type material of these species deposited at Museum für Naturkunde (Germany) and Museo Civico di Storia Naturale Giacomo Doria (Italy). The pictures of the species were taken with a Leica Z16 APO stereomicroscope coupled to a Leica DFC 2 video camera (Leica Microsystems, Switzerland). Helicon Focus was used to combine the images (HeliconSoft). The line drawings were made with a camera lucida adapted to a Leica DM 2500 microscope. The DNA was extracted from the metasoma and mesosoma of eleven specimens (7 ♀ and 5 ♂) of our insects by using the NucleoSpin Tissue Kit®. Standard PCR procedures and primers proposed by Folmer et al. 1994 (Mol Mar Biol Biotechnol, 3:294-9) were used to amplify the mitochondrial gene Cytochrome c oxidase subunit I (COI). The amplified products were purified using an enzymatic procedure with the ExoSAP-IT kit (USB Corporation) and then sequenced in a T3500 Genetic Analyzer.

**RESULTS AND CONCLUSIONS:** It was possible to differentiate the two species for the following morphological characteristics: *S. domesticus* is a larger species (2.56-4.03 mm) than *S. cereicolllis* (2.10-3.68 mm). Moreover, the median clypeal lobe is much longer than the lateral ones, with an evident median carina on *S. domesticus*, while in *S. cereicollis* it is clearly as long as the lateral lobes. The first and second flagellomeres are longer than wide in *S. domesticus*, while in *S. cereicollis* they are as long as wide. The eyes are instead relatively smaller in *S. domesticus*. The metapectal propodeal complex is very different between the two species; its lateral profile is sharp-edged in *S. domesticus* and rounded in *S. cereicollis*. Finally, the genitalia are markedly different in the shape of the dorsal arms, aedeagus, and cuspis. This study represents a starting point for proposing a synopsis of the Palaearctic *Sclerodermus* species.

# XXXIII

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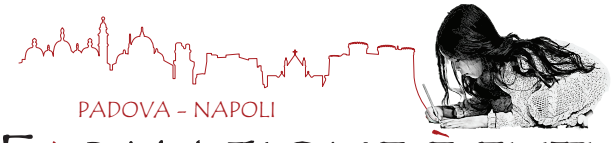
# ECO-PARASITOLOGY AND WILDLIFE



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# THE ENDOSYMBIONT *MIDICHLORIA MITOCHONDRII*: A TROJAN HORSE FOR ASSESSING RED DEER EXPOSURE TO *IXODES RICINUS* TICKS

Cafiso A.*[1], Nava M.[1], Cialini C.[1], Corlatti L.[2], Stocchero C.[3], Pedrotti L.[2], Gugiatti A.[2], Luzzago C.[1], Bazzocchi C.[1]

*[1]University of Milan, Department of Veterinary Medicine and Animal Sciences, Lodi, Italy; [2]Stelvio National Park, ERSAF Lombardia, Bormio, Italy; [3]University of Pavia, Department of Clinical-Surgical, Diagnostic, and Pediatric Sciences, Pavia, Italy*

**Keywords:** Tick bite, Wildlife, Serological analysis.

**INTRODUCTION:** The tick *Ixodes ricinus* is currently expanding into previously unaffected European areas, driven by factors such as climate change and alterations in land management practices. This species is a vector for microorganisms with medical and veterinary significance and additionally hosts the endosymbiont *Midichloria mitochondrii* (order Rickettsiales; hereafter 'Mm'). While no pathological role has been attributed to Mm thus far, the bacterium has been observed to be transmitted during blood meals of *I. ricinus* in several vertebrate hosts (e.g., dog, sheep, roe deer, human, rabbit; Cafiso et al., 2019. Ticks Tick Borne Dis, 10:5-12). Wildlife surveillance provides the opportunity to collect samples on culled individuals and data regarding the potential geographic distribution of ticks and their transmitted pathogens, particularly compared to the expensive and time-consuming procedures associated with field collection. In the Lombardy sector of the Stelvio National Park (SNP), information about tick presence is limited and poorly investigated. Since a considerable increase in red deer density occurred over the past decades, a culling program has been started in SNP since 2011. To indirectly assess the occurrence of *I. ricinus* in the study area, a serological evaluation was conducted to determine the presence of antibodies against Mm in red deer.

**MATERIALS AND METHODS:** Sera from culled red deer were obtained in 2017-2019 in SNP. Data including sex, age, and culling coordinates were collected. To assess the exposure of red deer to *I. ricinus*, an in-house ELISA assay was conducted to detect IgG produced against the flagellar protein FliD of Mm, using a recombinant form of the antigen (Mariconti et al., 2012. Microbiology, 1677-83). A generalized additive model was performed to investigate the probability of exposure to Mm. In the global model the optical density (OD) was the response variable, as a function of sex in interaction with all the other variables collected, plus a spatial trend surface. Animals were subdivided into age-classes that would best reflect the ecology and behavior of the species: calves 0 (0.5 yrs), yearlings 1 (0.5-1.5 yrs), subadults 2 (2-7 yrs) and adults 3 (8+ yrs).

**RESULTS AND CONCLUSIONS:** A total of 201 individuals were sampled, which included 59 animals of age class 0, 34 of class 1, 83 of class 2 and 25 of class 3. The final model included the additive effect of age-class and trend surface. Based on the OD values, the probability of tick bite increased significantly in age-classes 1, 2 and 3 compared to that of age-class 0. Higher OD values were observed in older animals. Arguably, individuals of age-classes >0 may have been continuously exposed to *I. ricinus* bite compared to fawns.
The present work shows indirect evidence of the risk of exposure to *I. ricinus* bite in SNP. Red deer from the eastern area presented a higher probability of exposure to Mm antigens than those from the western area, suggesting an increasing expansion of *I. ricinus* in SNP and at higher altitudes.

# LONG TERM SURVEY ON *TRICHINELLA* SPP. IN WILD CANIDS OF SOUTHERN ITALY

Scarcelli S.*[1], Sgroi G.[2], D'Alessio N.[2], Rea S.[2], Locantore F.[3], Rufrano D.[4], Toscano V.[5], Fioretti A.[1], Modrý D.[6], Veneziano V.[1]

*[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy; [2]Experimental Zooprophylactic Institute of southern Italy, Department of Animal Health, Portici, Italy; [3]Department of Veterinary Medicine, University of Bari, Valenzano, Bari, Italy; [4]Dipartimento di prevenzione area della sanità pubblica veterinaria e della sicurezza alimentare, Azienda Sanitaria Locale Salerno, Salerno, Italy; [5]CRIUV, Centro di riferimento regionale per l'Igiene Urbana Veterinaria, Presidio ospedaliero veterinario dell'ASL Napoli 1 centro, via Cupa del principe ex. O.P. Frullone, Napoli, Italy; [6]Department of Veterinary Sciences, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*

**Keywords:** Trichinellosis, Wildlife, Wild carnivores.

**INTRODUCTION:** *Trichinella* spp. are worldwide nematodes infecting a wide range of hosts such as domestic and wild mammals, including humans, by the ingestion of raw or undercooked infected meat (Pozio, 1998. Parasitol Today, 14:35-8). In Italy, wild canids (i.e., red foxes, *Vulpes vulpes* and wolves, *Canis lupus*) due to their ecology are the main hosts of these helminths (Pozio, 2009. Int J Parasitol, 39:71-9). This survey aims to assess the presence of *Trichinella* spp. in these canids and their epidemiological role in southern Italy.

**MATERIALS AND METHODS:** From 2017 to 2023, as part of a health monitoring plan of wildlife in Campania region, southern Italy, carcasses of red foxes (n = 352) and wolves (n = 41) were collected. At postmortem examination, data including sex, age, place of collection and altitude were reported and 10 grams of tibial or diaphragm muscles were sampled. *Trichinella* spp. larvae were detected and counted using the HCl-pepsin digestion method (European Union, 2015) in order to assess average number of larvae per gram (lpg) of muscle. Recovered larvae were molecularly identified (Marucci et al., 2022. Food Waterborne Parasitol, 27:e00156) by Istituto Superiore di Sanità (Rome, Italy). Univariate statistical analysis was performed on sex, age classes, sampling years, province and altitude; a p-value ≤0.05 was considered statistically significant.

**RESULTS AND CONCLUSIONS:** On a total of 393 wild canids, 13 (3.3%) tested positive, in detail 4 (1.1%) red foxes and 9 (21.9%) wolves. All isolated larvae were molecularly identified as *Trichinella britovi* by multiplex-PCR. The HCl-pepsin digestion method revealed in red foxes a total average value of 5.9 lpg (2.7 lpg in diaphragm pillar and 9.0 lpg in tibial) and in wolves a total average value of 22.8 lpg (8.7 lpg in diaphragm pillar and 36.9 lpg in tibial). The higher prevalence and lpg values in wolves compared to red foxes may be due to different foraging strategies (Boitani et al., 2003. Mammalia III. Calderini, Bologna), influenced also by the altitude (Pozio, 1998. Parasitol Today, 14:35-8), dispersal behaviour of young wolves (Badagliacca et al., 2016. Vet Parasitol, 231:124-27) and wolf's longer life expectancy (Martínez-Carrasco et al., 2023. Vet Sci, 10:206). Statistically significant difference of prevalence was found by province ($p$=0.05) for red foxes, with higher positivity in Salerno probably related to lower urbanization of this province, favouring carnivorous feeding behaviours. While for wolves, the sampling years showed a $p$=0.01 and the higher prevalence in 2020 may be due to natural fluctuation or to COVID lockdown, reducing hunting pressure and increasing prey availability. This study assessed for the first time *Trichinella* spp. epidemiology of wild canids from southern Italy, showing notable *T. britovi* prevalence and confirming their crucial role. Further research is needed in the study area to fully understand the parasite eco-epidemiology, including the role of other hosts like mustelids.

# CHASING *ECHINOCOCCUS MULTILOCULARIS* IN WILD CARNIVORES FROM NORTHERN TUSCANY, ITALY

Cafiero S.A.*[1], Casulli A.[2], Rossi C.[3], Wassermann M.[4], Romig T.[4], Hauffe H.C.[3], Massolo A.[5]

*[1]Ethology Unit, Department of Biology, University of Pisa, Pisa, Italy; [2]European Union Reference Laboratory for Parasites (EURL-P). Department of Infectious Diseases, Istituto Superiore di Sanità; WHO Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis; [3]Conservation Genomics Research Unit, Centre for Research and Innovation, Fondazione Edmund Mach, San Michele all'Adige, Italy; [4]Institute of Zoology, Parasitology Unit, University of Hohenheim, Stuttgart, Germany; [5]University of Calgary, Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, Calgary, AB, Canada; UMR CNRS 6249 Chrono-environnement, Université Bourgogne Franche-Comté, Besançon, France; Ethology Unit, Department of Biology, University of Pisa, Pisa, Italy*

**Keywords:** *Echinococcus multilocularis*, Italy, Parasite distribution expansion.

**INTRODUCTION:** *Echinococcus multilocularis* (Em) is a Taeniidae cestode, spread across the Northern hemisphere, circulating among carnivores as definitive hosts and voles as intermediate, respectively (Romig et al., 2017. Adv Parasitol Part A, 95:213-314). Moreover, following egg ingestions humans can develop alveolar echinococcosis (Conraths et al., 2017. PLOS NTD, 11:1-15). In Italy, the first Em-positive foxes were found about 25 years ago in the Trentino Alto Adige region (Manfredi et al., 2002. Vet Rec, 150:757) in an autochthonous and highly endemic focus (Casulli et al., 2005. Int J Par, 35:1079-1083; Obber et al., 2022. PLOS ONE, 17:e0268045). Recently, Em eggs were extracted from shepherd dog and wolf faeces in the Ligurian Alps (Massolo et al., 2018. IJP-PAW, 7:309-316), suggesting a southern expansion of Em distribution. We aimed to investigate the Em presence in the Apuan Alps, an Apennine protected area close to the Ligurian Alps.

**MATERIALS AND METHODS:** Faeces of wild carnivores (wolves, foxes and mustelids) were collected from 2020 to 2023 on a quarterly basis along 52 fixed pathways, and stored at -80°C for at least five days for safety (Veit et al., 1995. Parasitology, 110:79-86) and then at -20°C until analysis. A total of 148 scats (from 10 mustelids, 58 foxes and 80 wolves) were processed by two distinct procedures. First, flotation and sieving technique (FST) with ZnCl2 solution (Mathis et al., 1996. J Helminthol, 70:219-22) for taeniid egg harvest was implemented. DNA extraction, nested PCR and sequencing of portions of nad1 and cox1 genes were conducted on individual eggs (Hüttner et al., 2008. IJP, 38:861-68; Štefanić et al., 2004. Parasitol Res, 92:347-51). Secondly, two Em-specific copro-qPCRs were then used directly on each fecal sample. The former followed Knapp et al.'s (2014. Vet Parasitol, 201:40-7) with minor modifications (Obber et al., 2022) targeting the mtDNA marker rrnL; the latter targeted primers Nad234_F and Nad234_R (Santa et al., 2018. IJP-PAW, 7:111-15).

**RESULTS AND CONCLUSIONS:** Cestode eggs were successfully detected by FST and sequenced from 1/9 mustelids, 4/41 foxes and 16/60 of wolves. Em DNA was detected in 1 fox and 3 wolf samples. Nonetheless, the modified Knapp et al.'s copro-qPCR on the same samples did not yield any positive result, whereas Santa et al.'s qPCR is yet to be carried out. *Taenia hydatigena* and *Taenia krabbei* were identified in wolves, whereas *Taenia polyacantha*, *Mesocestoides litteratus*, *Mesocestoides* sp. and *Dipylidium caninum* occurred in foxes. One mustelid harboured *M. litteratus* and *T. polyacantha*. If furtherly confirmed by qPCR, these findings would open for a new scenarios for Em expansion to the Apennines, which were so far considered Em-free (Crotti et al., 2023. IJP-PAW, 21:11-16). Different timelines, sample sizes and techniques specificity might have contributed to negative results.

# WILDLIFE RELATED PATHOGEN SURVEILLANCE WITH NANOPORE SEQUENCING OF EDNA SAMPLES: COMPARING *IN VITRO* AND *IN SILICA* TARGET ENRICHMENT APPROACHES

Varzandi A.R.*[1], Reska T.[2], Zanet S.[1], Pastori I.[1], Rubele E.[1], Vada R.[1], Benatti F.[1], Fenn A.[2], Urban L.[2], Ferroglio E.[1]

*[1]University of Turin, Grugliasco, Italy; [2]Helmholtz Pioneer Campus, Munich, Germany*

**Keywords:** Wildlife, Targeted surveillance, Nanopore sequencing.

**INTRODUCTION:** Surveillance of wildlife populations for diseases is crucial for the early detection of emerging epidemiological situations, protecting animal conservation efforts and safeguarding the public and animal health. Environmental DNA/RNA (eDNA/eRNA) techniques have gained popularity due to their non-invasive nature and efficiency, making them a valuable instrument for monitoring wildlife-related pathogens (WRP). eDNA offers distinct advantages over conventional direct sampling methods, particularly in the detection of parasitic diseases, where the environment serves as a common reservoir for various parasitic life stages and hosts. With the emergence of portable sequencing technologies, eDNA sequencing has become a leading method for early detection of parasitic infections in wildlife.

**MATERIALS AND METHODS:** This study focuses on eDNA collected from lotic waters in and around La Mandria Regional Park's fenced-off area (Piedmont region), recognized as one of the primary European hotspots of *Fascioloides magna*, an invasive trematode of wild and domestic ruminants (Bassi, 1875). We employed active targeted surveillance using Nanopore MinION sequencing and evaluated two enrichment strategies: long-range metabarcoding of protist ribosomal subunits (PCR-dependent in vitro target enrichment) and Nanopore's Adaptive Sampling (PCR-free real-time *in silica* target enrichment).

**RESULTS AND CONCLUSIONS:** Our results demonstrate the potential of Nanopore's Adaptive Sampling for efficient, fast, and cost-effective targeted surveillance of parasitic diseases, as evidenced by the difference in detection rates between the two approaches. This suggests a path towards streamlined, real-time in situ genomic-informed surveillance programs.

# DIROFILARIOSIS IN ITALIAN WOLF POPULATION (*CANIS LUPUS ITALICUS*): A PERSISTENT VECTOR-BORNE DISEASE IN NORTHEN ITALY

Dini F.M.*[1], Musto C.[1], Moroni B.[2], Fiorentini L.[3], Bassi P.[3], Bianchi A.[3], Pupillo G.[3], Delogu M.[1], Galuppi R.[1]

*[1]Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy; [2]Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy; [3]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy*

**Keywords:** *Dirofilaria immitis*, *Canis lupus italicus*, Italy.

**INTRODUCTION:** Heartworm disease, caused by *Dirofilaria immitis*, affects domestic and wild carnivores and it is transmitted by various species of mosquitoes. Untreated dogs serve as the primary reservoir for this parasite, usually showing high microfilaraemia and high parasitic burden. In north-western Italy, heartworm infection is prevalent, particularly in humid lowlands. The role of wildlife in maintaining and spreading heartworm is debated, and information on heartworm infection in wolves is limited. However recent studies suggest that this species can act as a competent host in the *D. immitis* lifecycle. In northwestern Italy, infection rates in wolves recolonizing Alpine mountains appear to be similar to those in untreated dog populations endemic to the area (Moroni et al., 2021. Parasit Vectors, 13:482). The present study aimed to assess the prevalence of *D. immitis* in a large sample of Italian wolves from central-northern Italy, an area experiencing a recent increase in wolf population, and to genetically characterize the helminths found.

**MATERIALS AND METHODS:** From January 2021 to February 2024, a total of 452 wolves were examined for the presence of *D. immitis* in the pulmonary trunk and heart. All individuals were aged using 3 categories as follows: class 1: ≤12 months; class 2: 1-2 years; class 3: > 2 years. When filarial nematodes were visualized, they were collected, fixed in 70% ethanol, and prepared for morphological evaluation. Additionally, at least one worm from each wolf underwent genetic characterization through sequencing of 12S rDNA, 18S rDNA, and COI mtDNA. The Knott technique was applied to the blood (whenever possible based on carcass preservation) in cases of positive wolves to assess microfilaremia.

**RESULTS AND CONCLUSIONS:** The wolves sampled came from 38 provinces across six different regions in central northern Italy (Piedmont, Lombardy, Emilia-Romagna, Tuscany, Marche, Umbria). The presence of *D. immitis* adults was observed in 14 individuals (3%), comprising 9 males and 5 females from age classes 2 and 3. Positive wolves came from Emilia-Romagna Region (5% of prevalence), Piedmont (3%) and in one case from Lombardy (3%). Adults were collected and processed for the morphological identification in 12 cases showing a mean intensity of 3 worms. Additionally, blood analysis conducted on two individuals, revealed the presence of microfilaremia. Sequencing of the different molecular markers confirmed the morphological identification of *D. immitis*, with sequence similarities ranging from 99.4% to 100%. Phylogenetic analysis is currently underway to position our sequences relative to those in available databases from other host species. The prevalence observed in the wolves studied aligns with the seroprevalence estimated in dogs in northern Italy (Mendoza Roldan et al., 2020. Parasit Vectors, 13:193). Furthermore, the presence of microfilaremia in wolves confirms their hypothesized role as competent hosts and sheds light on their potential role as a source of *D. immitis* transmission.

# DOUBLE TROUBLE: CO-INFECTION OF *ANGYOSTRONGYLUS VASORUM* AND *DIROFILARIA IMMITIS* IN THE GOLDEN JACKAL (*CANIS AUREUS MOEOTICUS*) IN FRIULI VENEZIA GIULIA, ITALY

Fabbri D.*[1], Orioles M.[1], Miani G.[1], Pesaro S.[1], Dorigo L.[2], Bregoli M.[3], Saccà E.[1], Beraldo P.[1]

*[1]Università degli Studi di Udine, Udine, Italy; [2]Museo Friulano di Storia Naturale (MFSN), Udine, Italy; [3]Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro (PD), Italy*

**Keywords:** Wild canid, Angiostrongylosis, Dirofilariosis.

**INTRODUCTION:** In Italy, the golden jackal (*Canis aureus*) was confirmed as early as 1985, with numerous stable meta-populations currently established in Friuli Venezia Giulia (FVG), making the development of a regional health monitoring and surveillance network necessary. While co-infection by cardiopulmonary nematodes *Dirofilaria immitis* and *Angyostrongylus vasorum* in golden jackal was previously reported in Europe (Gavrilović et al., 2017. Acta Parasitol, 62:880-84), their role as sylvatic hosts in Italy remains unreported and unclear. In this study, we report the first case of co-infection with *A. vasorum* and *D. immitis* identified in golden jackals from Friuli Venezia Giulia, Italy, describing parasitological and anatomo-pathological findings and discussing epidemiological implications.

**MATERIALS AND METHODS:** Data regarding golden jackal corpses retrieved in FVG were recorded in the InfoFaunaFVG regional wildlife surveillance network. From 2020 to date, 109 carcasses underwent necropsy at University of Udine, jointly with the IZSVe and MFSN (Udine), to ascertain the death causes and for anatomopathological and parasitological exams. Of 109 animals, 57 were deemed suitable for cardiopulmonary parasitological examinations using a total worm count approach. Parasites were morphologically identified and then DNA extraction, amplification, and sequencing were performed for confirmation of species identity.

**RESULTS AND CONCLUSIONS:** Road-killed jackals consistently showed fractures of appendicular skeleton, haemorrhagic abdominal and thoracic effusions. Prevalence of *A. vasorum* and *D. immitis* was 29.8% (95%CI: 18.8-43.6%) and 7% (95%CI: 2.3-17.8%) respectively, and mean intensity was 6 (range 1-56) and 4 (range 3-6). *D. immitis* adults were mainly located in the heart and, in one case, both in the heart and pulmonary arteries. In the last two years, 3 out of 57 animals (5.3%, 95%CI: 1.8-14.4) were found co-infected with both *A. vasorum* and *D. immitis*. Histologically, the lung parenchyma was multifocally effaced by clusters of granulomas, containing small central deposits of necrotic tissue and occasionally small, calcified areas. Primitive and embryonated nematode eggs and larvae were embedded within the inflammatory foci. The alveolar septa were thickened by lymphohistiocytic infiltration. In addition, adult nematodes were found in blood vessels concurrently with thrombotic lesions. To the best of the authors' knowledge, this is the first report of *A. vasorum* and *D. immitis* co-infection in golden jackals in Italy (second in Europe). Wild carnivores such as this species are recognized hosts of *D. immitis* and *A. vasorum*, indicating the existence of their sylvatic cycle. Similarly to dogs, golden jackals are susceptible to potentially fatal cardiovascular and pulmonary complications due to these nematodes. Their adaptability and potential presence in urban contexts raise concern for increased parasite transmission, especially to non-endemic regions.

# CARDIOPULMONARY NEMATODES OF THE IBERIAN WOLF (*CANIS LUPUS SIGNATUS*) IN CANTABRIA (NORTHERN SPAIN)

Napoli E.*[1], Fayos M.[2], Racioppi V.[3], Velarde R.[4], Brianti E.[1], Martínez-Carrasco Pleite C.[3]

*[1]Department of Veterinary Sciences, University of Messina, Messina, Italy; [2]Centro de Recuperación de fauna Silvestre de Cantabria, Obregón, Spain; [3]Department of Animal Health. Faculty of Veterinary, University of Murcia, Murcia, Spain; [4]Wildlife Ecology and Health Group (WE&H), Departament de Medicina i Cirurgia Animals, Universitat Autònoma de Barcelona, Bellaterra, Spain*

**Keywords:** Cardiopulmonary nematodes, *Canis lupus signatus*, Spain.

**INTRODUCTION:** The Iberian wolf (*Canis lupus signatus*) is an endemic wolf subspecies of the Iberian Peninsula. The northwest of Spain has the largest population of wolves in Western Europe. Because of its predatory habits, the Government of Cantabria established in 2016 a wolf population control for livestock damage prevention, although nowadays the wolf is a protected species. This population control may be favorable, despite controversial, to implement an adequate parasite sampling protocol for health surveillance and/or research purposes. The wolf, in fact, is host of a wide variety of parasites, being able to participate in their transmission to wild and sympatric domestic animals and viceversa including zoonotic agents. The present work was performed to investigate the cardiopulmonary nematodes of the Iberian wolf.

**MATERIALS AND METHODS:** The study was performed in the Community of Cantabria, northern Spain, from April 2016 to December 2020, where a total of 46 wolves (25 females and 21 males; 21 juveniles, 25 adults) were hunted for management purposes. The carcasses were immediately transported to the Wildlife Recovery Center of Cantabria and the necropsies performed. Thoracic viscera were sent to the Veterinary Faculty of the University of Murcia (Spain) for the analysis. In the laboratory, the heart, lungs and trachea were examined separately. All helminths encountered were washed with distilled water and fixed in 70% ethanol until morphologic identification.

**RESULTS AND CONCLUSIONS:** Out of the 46 wolf carcasses examined, 12 were positive for at least one cardiopulmonary nematode species resulting in an overall prevalence of 26.1%. Three nematode species were detected, namely *Capillaria aerophila* (6/46, 13.0%), *Angiostrongylus vasorum* (5/46, 10.9%) and *Crenosoma vulpis* (3/46, 6.5%). Single infections (9/12) were more common than co-infections (3/12), being observed simultaneously the presence of two different species (i.e., *A. vasorum* and *C. aerophila*; *A. vasorum* and *C. vulpis*; *C. vulpis* and *C. aerophila*). The highest mean parasite intensity was observed for *A. vasorum*, being 14 (S.D. ±22.38), followed by *C. aerophila* and *C. vulpis*, with 4.2 (S.D. ±4.9) and 3 (S.D. ±2) nematodes per host, respectively. Overall, any statistical difference was observed in the parasite presence between sex (i.e., male 3/21, 9/25 female; P-value = 0.0948) or age category (i.e., juvenile 4/21, adult 8/25; P-value= 0.993). Concretely, *A. vasorum*, *C. aerophila* and *C. vulpis* are considered the most important lungworm species of wild and domestic canids in Europe. Their prevalence here reported is higher than in other similar studies conducted in Spain. These parasites are also emerging in dogs and fox populations in the Iberian Peninsula, and the high prevalence herein observed could be the result of possible transmission from dogs or red foxes to wolves. Hence lungworms are pathogenic and emerging and could represent a health issue for the Iberian wolf.

# OLFACTORY CUES IN THE HOST-LOCATION OF THE ECTO-PARASITOIDS *SCLERODERMUS DOMESTICUS* LATREILLE AND *SCLERODERMUS CEREICOLLIS* KIEFFER (HYMENOPTERA: BETHYLIDAE)

Masini P.*[1], Austeri L.[1], Rebora M.[2], Piersanti S.[2], De Francesco F.[3], Salerno G.[1]

*[1]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy; [2]Department of Chemistry, Biology e Biotechnology, University of Perugia, Perugia, Italy; [3]National Research Council (NRC), Institute of BioEconomy (IBE), Sesto Fiorentino (FI), Italy*

**Keywords:** Parasitoid, Chemical ecology, *Sclerodermus*.

**INTRODUCTION:** *Sclerodermus domesticus* is a cosmopolitan ectoparasitoid of longhorn beetle larvae, but it can also naturally develop on other woodboring species belonging to the family Ptinidae (Gordh & Móczár, 1990. Mem Am Entomol Inst, 46:1-364). *Sclerodermus cereicollis* is a species described for the first time by Kieffer on the base of specimens from Annobón island (Equatorial Guinea) and Giglio island (Italy) and then poorly investigated until now regard its taxonomy and biology (Kieffer, 1904. Annali del Museo civico di storia Naturale di Genova. Serle 3. 1(41): 351-412). Chemical ecology regarding the species *S. cereicollis* and *S. domesticus* is a central issue that should be deeply investigated in order to improve the efficacy of these bethylid species as biological control agents (Yang et al., 2014. Biol Control, 68:117-28) and, moreover, to understand the mechanisms underlying their role as sting dermatitis agents in humans (Azevedo & Colombo, 2022. Zootaxa, 5124(5): 501-19).

**MATERIALS AND METHODS:** In order to better understand the host location in *S. cereicollis* and *S. domesticus* species, we have evaluated their behavioural responses in a Y tube olfactometer and in a still air olfactometer towards volatile stimuli deriving from the host microhabitat, the sawdust of the host woody plant, and those indirectly associated with the presence of the host, frass, silk and kairomones deriving from larvae and adults. We divided these chemical stimuli into three major groups, those coming from *Trichoferus holosericeus*, from *Hylotrupes bajulus*, and from the factitious host *Corcyra cephalonica*.

**RESULTS AND CONCLUSIONS:** A total of 454 insects were tested in olfactometer. Naïve micropterous females of *S. cereicollis* and *S. domesticus* responded to volatile stimuli from sawdust from the host woody plant in the two natural host groups (*T. holosericeus* and *H. bajulus*). A further statistically significant response was recorded towards semivolatile stimuli derived from the host's frass in all major groups. Mainly in still air olfactometer *S. cereicollis* partially responds to stimuli from singular and grouped larvae of *H. bajulus* and *S. domesticus* towards stimuli from *T. holosericeus* larvae. No significant responses were recorded towards stimuli coming from the *H. bajulus* adult.

# INTESTINAL PARASITES FROM WILD BOAR POPULATIONS IN ITALY: BASELINE DATA IN THE FRAMEWORK OF THE PRIN PNRR 2022 PROJECT

Berrilli F.*[1], Rondon S.[2], Malaspina P.[3], Di Cave D.[1], Rossi L.[1], Guadano Procesi I.[1], Pace F.[1], Cignini B.[3], D'Amelio S.[2], Cavallero S.[2], Bellini I.[2], Chiovoloni C.[2], Gavaudan S.[4], Morandi B.[4], Gobbi M.[4], Di Lullo S.[4], Massolo A.[5], Petroni L.[5], Cafiero S.[5], Calderola S.[6], Orazi V.[7], Belardi I.[7], Calosi M.[7], Lazzeri L.[7], Fattorini N.[7], Ferretti F.[7]

*[1]Department of Clinical Sciences and Translational Medicine, University of Rome Tor Vergata, Rome, Italy; [2]Department of Public Health and Infectious Diseases, Sapienza, University of Rome, Rome, Italy; [3]Department of Biology, University of Rome Tor Vergata, Rome, Italy; [4]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Ancona, Italy; [5]Department of Biology, University of Pisa, Pisa, Italy; [6]Parco Nazionale Gran Paradiso, Servizio Biodiversità e Ricerca scientifica, Ufficio Fauna, Noasca (TO), Italy; [7]Department of Life Sciences, University of Siena, Siena, Italy*

**Keywords:** Wildlife management, Intestinal parasites, Host-parasite relationships.

**INTRODUCTION:** The recent expansion of wild boar (*Sus scrofa*) populations has raised significant concerns due to its potential negative consequences for biodiversity conservation and economically relevant human activities. While wild boar play essential roles in key ecological processes, such as predator-prey and species-habitat dynamics, our understanding of this expansion is currently fragmented, lacking integration across ecology, disease, and anthropogenic drivers. Also, data on intestinal parasites are crucial for understanding wild boar biology and ecology. Within the framework of the PRIN PNRR 2022 project a specific objective will be to identify intestinal parasites on wild boar populations from different areas and analyse their prevalence and diversity.

**MATERIALS AND METHODS:** From November 2023 to April 2024, 61 faecal samples from wild boar were collected: n=7 from hunting districts of the Marche Region (MR), n=23 from the Maremma Regional Park, Tuscany, n=31 from Gran Paradiso National Park, Val d'Aosta. Moreover, in a previous investigation during 2023, 46 faecal samples from the MR were analysed. Samples were collected directly from rectum or from the ground. Intestinal parasites were determined by the Mini-FLOTAC technique. Standard copro-parasitological methods (direct smear and flotation) were performed on the 46 MR faecal samples. Oocysts, cysts, eggs and larvae were photographed, measured and identified.

**RESULTS AND CONCLUSIONS:** Overall, 90.3% of the 61 samples scored microscopically positive to parasites with different taxa detected (*Eimeria* spp., *Eimeria suis*, *Eimeria scabra*, *Eimeria perminuta*, *Ascaris* sp., *Trichuris* sp., gastrointestinal strongyles, *Metastrongylus* spp.). Gastrointestinal strongyles and *Eimeria* spp. were found predominant, although differences in prevalence and intensity were observed among wild boar populations. Moreover, 47.8% of the 46 MR samples previously analysed was positive for intestinal parasites (*Entamoeba coli*, *Iodamoeba bütschlii*, *Balantidium/Buxtonella* sp., *Isospora* sp., *Eimeria* sp., *Giardia* sp., *Blastocystis* sp., *Ascaris* sp., *Trichuris* sp., gastrointestinal strongyles, *Strongyloides* sp., *Metastrongylus* spp.). Our results showed a non-negligible prevalence of intestinal parasites in wild boar. Parasites play a significant role in driving ecological dynamics within ecosystems, impacting host fitness even when clinical signs are not evident. At local scale, the varying effects of infections can differently modulate both inter- and intra- specific dynamics. This study represents a first-step approach toward understanding the ecological roles of parasites within wild boar populations across various geographical areas in Italy.

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# COUPLING MORPHOLOGY WITH MOLECULAR CHARACTERIZATION OF *EIMERIA* SPECIES IN ALPINE MARMOTS

Zanet S.*[1], Toppino C.[1], Varzandi A.R.[1], Ferrari C.[2], Bassano B.[2], Ferroglio E.[1]

*[1]Università degli Studi di Torino, Dip. Scienze Veterinarie, Grugliasco, Italy; [2]Parco Nazionale Gran Paradiso, Noasca, Italy*

**Keywords:** *Marmota marmota*, Coccidia, Oocysts.

**INTRODUCTION:** The identification of *Eimeria* species in the Alpine marmot (*Marmota marmota*), and in general in the Tribe Marmotini (Sciuridae), is based on morphological description of sporulated oocysts. The presence of morphologically indistinguishable species and the lack of information on host-specificity led, over time to the generation of redundant records, with obsolete descriptions. This study aimed at associating the morphological description of oocysts, historically reported in the Alpine marmot to molecular data.

**MATERIALS AND METHODS:** Single *Eimeria* oocysts, from naturally infected marmots were isolated from freshly collected fecal samples and sporulated under laboratory conditions. Morphometric and morphological description of single oocysts (n=160 oocysts) were carried out. Total genomic DNA was extracted from individual oocysts and a specific portion of the 18S rDNA was amplified to associate morphological characters to genetic information.

**RESULTS AND CONCLUSIONS:** Phylogenetic analysis confirmed the presence of at least three species of *Eimeria* in *M. marmota* which were identified as *E. lateralis*, *E. monacis* and *E. callospermophili*.

# MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF *REIGHARDIA STERNAE* IN A YELLOW LEGGED GULL (*LARUS MICHAELLIS*)

Ceccherelli R.[1], Ebani V.V.[2], Rossi G.[3], Perrucci S.*[2]

*[1]CRUMA-LIPU, Livorno, Italy; [2]University of Pisa, Department of Veterinary Sciences, Pisa, Italy; [3]University of Camerino, School of Biosciences and Veterinary Medicine, Matelica, Italy*

**Keywords:** *Reighardia sternae*, *Larus michaellis*, Italy.

**INTRODUCTION:** The ubiquitous pentasomid species *Reighardia sternae* is a blood feeding parasite that can be found in the body cavity, respiratory system and air sacs of the host sea birds, namely gulls and terns and other larids, as well as skuas (Literák et al., 2017. J Parasitol, 103:588-92). This study reports the identification of *R. sternae* in a yellow legged gull (*Larus michaellis*) in Italy.

**MATERIALS AND METHODS:** An adult male yellow legged gull (*Larus michaellis*) found crippled and deceased after four days from the hospitalization in the Marine and Aquatic Bird Recovery Center of Livorno (CRUMA), Tuscany, central Italy, was submitted to necropsy. Generalized aerosacculitis and cardiac enlargement were evidenced in the deceased gull. Moreover, seven morphologically similar female pentastomes were found in the interclavicular air sac. Collected parasites were cleaned in saline solution and fixed in 70% ethanol. On five specimens, morphological and metrical features of body length and width, parasite internal organs, anterior and posterior hooks, and oral apparatus were assessed. Morphological identification was performed according to the description given in previous studies (Kanarek et al., 2005. Fragm Faun, 48:101-6; Naupay et al., 2016. Parasitol Int, 65:288-90; Literák et al., 2017. J Parasitol, 103:588-92). For molecular analysis, two parasites were submitted to DNA extraction. PCR was carried out using the primers targeting a 383 bp fragment of the nuclear 18S rDNA locus, Pent629F (CGGTTAAAAAGCTCGTAGTTGG) and Pent101IR (GGCATCGTTTATGGTTAGA ACTAGGG) (Brookins et al., 2009. Vet Pathol, 46:460-63) and the obtained products were sequenced.

**RESULTS AND CONCLUSIONS:** According to morphological features, all pentastomes were identified as *R. sternae* adult females as all morphometric characteristics were in good agreements with those previously reported for *R. sternae* by (Kanarek et al., 2005. Fragm Faun, 48:101-6; Naupay et al., 2016. Parasitol Int, 65:288-90; Literák et al., 2017. J Parasitol, 103:588-92). Molecular analysis confirmed the morphological identification and both sequenced specimens showed a 100% sequence homology to *R. sternae* previously found in *Larus ridibundus* in Spain, *L. michaellis* in Portugal, and *Larus belcheri* in Peru (Naupay et al., 2016. Parasitol Int, 65:288-90; Literák et al., 2017. J Parasitol, 103:588-92). This study is the first record of *R. sternae* in the yellow legged gull (*Larus michaellis*) in Italy.

# URBAN WILDLIFE, A SPIKY ISSUE: FIRST DETECTION OF *GIARDIA DUODENALIS* IN ITALIAN EUROPEAN HEDGEHOGS (*ERINACEUS EUROPAEUS*)

Brustenga L.[1], Rigamonti G.*[1], Moretta I.[1], Morganti G.[1], Calgaro V.[1], Diaferia M.[1], Lepri E.[1], Lucentini L.[2], Veronesi F.[1]

*[1]University of Perugia, Department of Veterinary Medicine, Perugia, Italy; [2]University of Perugia, Department of Chemistry, Biology and Biotechnology, Perugia, Italy*

**Keywords:** *Giardia duodenalis*, European hedgehogs, Wildlife parasitology.

**INTRODUCTION:** *Giardia duodenalis* is a widespread protozoan responsible for giardiasis, a disease that can cause gastrointestinal symptoms. Basing on the genetic analysis, *G. duodenalis* is classified in eight assemblages (A to H) that display different host affinity (Cacciò et al., 2018. Infect Genet Evol, 66:335-45); assemblage A and B specifically infect humans with select sub-genotypes showing the capacity for zoonotic transmission (Rojas-López et al., 2022. Trends Parasitol, 38, 605-6). Among the many species of wild animals that inhabit urban settlements, the European hedgehog (*Erinaceus europaeus*) is one of the few species that has adapted to live in close contact with humans (Pettett et al., 2017. Eur J Wildl Res, 63:54); therefore, survey of potential zoonotic parasites shared from hedgehogs and humans could be of great public health concern, especially in urban areas with high hedgehog density (Jota Baptista et al., 2021. Biologics, 1:61-9).

**MATERIALS AND METHODS:** Fecal flotations for coprological examination are routinely carried out on symptomatic and asymptomatic hedgehogs admitted to a Wildlife Rescue Center in Central Italy. To confirm the suspect of infection with *G. duodenalis*, feces were also destined to a direct immunofluorescence assay (MERIFLUOR) and DNA extraction followed by Nested PCR amplification of a 511 bp fragment of the beta-giardin gene to be used in a PCR-RFLP protocol that allow for assemblage and sub-genotype characterization (Lalle et al., 2005. Int J Parasitol, 35:207-13). Furthermore, one of the two hedgehogs that tested positive to *G. duodenalis* died due to a severe diffuse interstitial and granulomatous pneumonia; samples of the small intestine were formalin fixed, processed with routine histological techniques, and stained with Hematoxylin and Eosin.

**RESULTS AND CONCLUSIONS:** Cysts of *G. duodenalis* were detected in the fecal flotations of two hedgehogs, both the immunofluorescence assays and the PCR amplification confirmed the presence of the cysts and DNA respectively. The RFLP protocol attributed the sample to the assemblage A1. Trophozoites were also found on the histologic slides, confirming once more the presence of the parasite. *G. duodenalis* has been previously found in hedgehogs from other European Countries and New Zealand but this is the first description of assemblage A1 from European hedgehogs in Italy. A1 has the potential to infect humans as well as a range of other mammals (Zajaczkowski et al., 2021. Curr Res Parasitol Vector Borne Dis, 1:1000055). Is therefore important not to leave available food and water sources that can promote the spreading of zoonotic parasites from wildlife to humans and pets. The detection of a highly pathogenic zoonotic assemblage in hedgehogs can bridge the transmission of the parasite from the wild to the domestic environment and underlines the importance of maintaining appropriate hygiene measures when interacting with both symptomatic and asymptomatic animals.

# OCCURRENCE OF TICK-BORNE PATHOGENS IN TICKS COLLECTED FROM WILDLIFE AND DOMESTIC ANIMALS IN NORTHEASTERN ITALY

Toniolo F.*, Gradoni F., Sgubin S., Manzi S., Danesi P., Montarsi F., Gobbo F.

*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy*

**Keywords:** TBP, Reservoir, *Ixodes ricinus*.

**INTRODUCTION:** Northeastern Italy is considered endemic for tick-borne pathogens (TBPs) and wildlife and domestic hosts play an important ecological role in their maintenance. 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:** Ticks (adults, nymphs and larvae) were collected from both wildlife and domestic hosts through passive surveillance in endemic areas of northeaster Italy during 2019-2023. They were morphologically identified and screened for TBPs individually in case of adults or pooled for nymphs and larvae. Nucleic acids extraction was performed automatically and TBPs detection (i.e., TBE virus, *Anaplasma phagocitophylum*, *Babesia* spp., *Borrelia* spp. and *Rickettsia* spp.) was carried out with a SYBR Green Real Time PCR followed by sequencing.

**RESULTS AND CONCLUSIONS:** Ticks were removed from 98 animal hosts belonging to 13 species. A total of 537 ticks were collected: 312 *Ixodes ricinus* (58.1%), 147 *I. hexagonus* (27.3%), 1 *I. canisuga* (0.18%), 1 *D. marginatus* (0.18%), 1 *Rhipicephalus sanguineus* (0.18%), 2 *Ixodes* spp. (0.37%) and 2 *Dermacentor* spp. (0.37%). *Ixodes ricinus* was found on all wild and domestic host species; *I. hexagonus* from badger, hedgehog and fox; *I. canisuga* from a badger; *D. marginatus* and *Dermacentor* spp. from wild boar, and *R. sanguineus* from hedgehog. The host species with the higher number of removed ticks were hedgehog (31.3%), roe deer (23%) and wolf (23%). None tick sample resulted positive for TBE virus, whereas 12 TBPs were detected in 110 out of 466 pools (420 adults, 40 nymphs and 4 larvae). *Ixodes ricinus* was found positive for at least one TBP in 99/318 samples (31.1%): *A. phagocitophylum* 60 pools (12.9%); *R. helvetica* 31 pools (6.6%); *R. monacensis* 14 pools (3%); *Borrelia* spp. 11 pools (10%) (7 *Borrelia miyamotoi*, 3 *Borrelia afzelii* and 1 *Borrelia burgdorferi*) and 4 pools for *Babesia* spp. One *Dermacentor* spp. specimen was found positive for *R. slovaca* and seven *I. hexagonus* for *Rickettsia* spp. Chamois and roe deer resulted the host species with the highest occurrence of multiple TBPs coinfections in *I. ricinus* (3.5%). The most common coinfection (11/19 ticks) was between *A. phagocitophylum* and *R. helvetica*; in addition, two samples from roe deer showed triple coinfection (*A. phagocitophylum-R. monacensis-B. venatorum* and *A. phagocitophylum-B. miyamotoi-B. azfelii*). According to these data, the main tick species in this study was *I. ricinus* and one third of these resulted positive at least for one pathogen highlighting the occurrence of a high diversity of pathogen species in this area. Moreover, in the study area there is a high occurrence of wild species such as roe deer and chamois (Vicenza, Belluno and Bolzano) underlining their role in the epidemiology of TBDs.

# ENTOMOFAUNA COMPOSITION AND *POST-MORTEM* INTERVAL IN WILD BOARS -PRELIMINARY RESULTS IN THE FRAMEWORK OF A PREPAREDNESS STRATEGY AGAINST AFRICAN SWINE FEVER

Carlin S.[1], Obber F.[1], Celva R.[1], Poletto E.[1], Chiarello G.[1], Da Rold G.[1], Assirelli G.[1], Franzoso A.[1], Mian G.[1], Casara A.[3], Verin R.[3], Gallo M.[2], Michelotto E.[2], Rocca G.[2], Montarsi F.*[1], Pozzato N.[1], Citterio C.V.[1]

*[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy; [2]Parco Regionale dei Colli Euganei, Este, Italy; [3]Department of Comparative Biomedicine and Food Science, University of Padova, Padova, Italy*

**Keywords:** African swine fever, Wild boars, Necrophagous insects.

**INTRODUCTION:** African swine fever (ASF) is an infectious viral disease that affects suids. There are currently no vaccines, and lethality can reach 100% in *Sus scrofa*. The high resistance of the virus in the environment is the key for its transmission cycle in wild boars in Europe (https://www.fao.org/documents/card/en?details=cc0785en). In case of an ASF outbreak, knowledge on the postmortem interval (PMI) and the decomposition process of wild boar cadavers in different microhabitats is essential for disease management (Probst et al., 2020. Vet Sci, 7(1):6). Here we report the preliminary results about the entomofauna composition in relation to the PMI on wild boar cadavers, in the framework of an ongoing research project focused on ASF passive surveillance in the Parco Regionale dei Colli Euganei (Veneto Region, Italy).

**MATERIALS AND METHODS:** Two sampling sites, representative of the environmental and climatic features of the study area, have been identified. Three wild boar cadavers were placed in each site and monitored during four sampling sessions, lasting about two months each. Environmental temperature and humidity in each site were monitored using external data loggers; in addition, data loggers were placed inside each cadaver, allowing to monitor changes in body conditions. Entomological samplings were periodically carried out for each cadaver. Moreover, the stage of degradation was recorded, in order to evaluate the successions of cadaveric entomofauna in relation to the stages of decomposition.

**RESULTS AND CONCLUSIONS:** During the first and second sampling sessions, 491 and 323 entomological samples were collected, respectively. To date, taxonomic identification has been carried out using dichotomous keys (Szpila et al., 2023. Forensic Sci Int, 354:111889) for adults, 3rd instar larvae and pupae from the summer session, while samples collected during the fall and spring session are currently being processed. During the first session, the predominant orders of insects were Diptera (7 families, 11 genera) and Coleoptera (7 families, 9 orders). A high number of adult flies belonging to Calliphoridae were attracted to the corpses within a few minutes from placement, with consequent egg deposition observed after two hours. Following morphological identification of larvae, most of them were found to belong to the genera *Lucilia* and *Calliphora*. In chronological order, the Dermestidae family was the first detected within the Coleoptera order (adults from day two). The data collected so far are in line with previous literature, allowing to validate the sampling method. Once all entomological and environmental data are analyzed, the current research project will provide more robust information to estimate PMI of wild boar cadavers. Such an information will be strategic to plan, implement and review the scanning surveillance in both “peace time” and in case of ASF introduction.
The present work is part of the research project IZSVE RC 06/2022-DEATHBOARS funded by the Italian Ministry of Health.

# WILD UNGULATE UNCONTROLLED GROWTH IN SICILY: A REGIONAL PROJECT FOR THE EARLY DETECTION, SURVEILLANCE AND PREVENTION OF WILDLIFE-RELATED ZOONOSES

Napoli E.*[1], Migliore S.[2], Galluzzo P.[2], Gucciardi F.[2], Brianti E.[1], Nalbone L.[1], Loria G.R.[2], Dara S.[2], Ciprì V.[2], Grippi F.[2], Guercio A.[2], Blanda V.[2]

*[1]Department of Veterinary Sciences, University of Messina, Messina, Italy; [2]Istituto Zooprofilattico Sperimentale della Sicilia Mirri, Palermo, Italy*

**Keywords:** Wild boar, TBPs, Risk map.

**INTRODUCTION:** Wild ungulates play a crucial role in transmission and maintenance of several zoonotic pathogens. In addition, wild boars, feral pigs and fallow deer are regarded as the most important hosts for several tick species and tick-borne pathogens some of which zoonotic concerns. In the last decades, a demographic growth of the populations of wild ungulates has been observed in Sicily. This increase in number poses eco-pathological issues and may represent a threat for both human and animal health. Therefore, considering the relevance of wild ungulates in the epidemiology of some infectious and parasitic agents of zoonotic concern surveillance activities in these species is strongly advocated. The research project Wild Ungulate Uncontrolled Growth in Sicily, funded by the Ministry of Health (GR-2021-12373930), aims to investigate the potential health risk posed by the increase in number of wild ungulate populations in Sicily.

**MATERIALS AND METHODS:** Tick-borne pathogens (TBPs), and food-borne agents in areas where ticks, wild, domestic animals and humans live in sympatry will be investigated. A risk analysis with a set of parameters to evaluate the potential hot spots in Sicily for the spread of TBPs associated with wild ungulates was performed in the first phase of the project. Risk factors included climatic-ecological conditions, wild ungulate population dynamics (uncontrolled growth of fallow deer and/or wild boar population, culling plans), tick abundance, human activities (presence of urban centres/small towns, recreational areas, grazing areas) were assessed and analysed and transferred onto a map using a Geographic Information Systems (GIS).

**RESULTS AND CONCLUSIONS:** A risk ranking was elaborated, and five different potential hot-spot areas were identified, namely: S1 (Madonie Alte), S2 (Madonie Basse), S3 (Ficuzza/Corleone), S4 (Nebrodi) and S5 (Peloritani). All the study sites are characterised by the presence of natural areas, grazing area for livestock and the presence of a large number of wild ungulates. In particular, the S1, S2 and S3 are characterized by a large increase of wild boar and fallow deer populations, while in S4 and in S5 only wild boar are present. In the second phase of the project, a monthly tick sampling from the environment and the molecular detection and identification of pathogens in the collected ticks will be performed. Moreover, blood, faeces and tissue samples form hunted animals and/or from animals undergoing selective control will be performed in the same sites. Results of this project will be useful to address future mitigation measures in the highest risk areas and the dissemination of the obtained results will increase the awareness of people and workers daily at risk of pathogen transmission.

# *LETHOCERUS PATRUELIS*, STÅL, 1854 (HEMIPTERA: BELOSTOMATIDAE) LINKED TO HUMAN ATTACK (ITALY, APULIA REGION)

Cariglia M.G.*[1], Raele D.A.[1], Grimaldi S.P.[1], Speranzoso F.[1], Ettore F.[2], Cafiero M.A.[1]

*[1]Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy: [2] Azienda Sanitaria Locale Taranto, Servizi Veterinari, Manduria, Italy*

**Keywords:** *Lethocerus patruelis*, Bites, Italy.

**INTRODUCTION:** Giant water bugs are widespread and well-known freshwater insects distributed in tropical and subtropical regions (Lauck & Menke,1961. Ann Entomol Soc Am, 54:644-57), with the only species *Lethocerus (L.) patruelis* reported in Europe (Davranoglou & Karaouzas, 2021. Ecol Monten, 41:56-61). These hemipteran insects normally inhabit ponds, marshes, lakes, slow moving rivers where they feed on a large range of preys, such as amphibians, fish and reptiles, including turtles (Ribeiro et al., 2017. Zool J Linn Soc,182:319-59). The victim is immobilized with hooked limbs and penetrated using a robust rostrum to inject saliva contains several enzymes with paralyzing and necrotizing activity (Cardoso et al., 2009. J Exp Biol, 213:3305-10). Although these arthropods are not currently considered of medical interest, they can occasionally inflict paiful bites to humans if they are disturbed (Haddad et al.,2010. Wild Environ Med, 21:130-33). We report the presence of *L. patruelis* closeness to humans and information on the status of this species in Italy.

**MATERIALS AND METHODS:** In August 2023, the local Health Agency (ASL) delivered to the Istituto Zooprofilattico di Puglia e Basilicata a big bug with identification request. The specimen was collected in the act of biting a man staying on the beach of San Pietro in Bevagna (TA). Following morphological identification according to Novoselsky's keys (Novoselsky et al., 2018. Isr J Entomol, 48:119-41), a conventional PCR targeting COX1 gene was also performed (Folmer et al., 1994. Mol Mar Biol Biotechnol, 3(5):294-99). The obtained ampliclon was sequenced, compared with nucleotide sequences in GenBank database and the phylogenetic tree constructed using the ClustalW algorithm (Tamura et al., 2011. Mol Biol Evol, 28(10):2731-39).

**RESULTS AND CONCLUSIONS:** The specimen was identified as *L. patruelis*, female (80mm long and 20mm large). The yielded amplicon was 98,96% identical to the corresponding region of the NCBI sequences of the *L. patruelis* COX1 gene. The obtained sequence clusters with Asian group of *L. patruelis*. In the last decades, records of *L. patruelis* have become frequent on the Italian coasts of south-eastern regions, mainly of Apulia (Lo Parrino & Tomasi, 2021. Biogeographia, 36:s005) where also the one only Italian case of human bite attributable to *Lethocerus* spp, probably *L. patruelis*, occurred in a swimmers in Polignano a Mare (BA) (http//www.entomologitaliani.net). Evidences show that *L patruelis* is spreading in Italy, mainly in Apulia region where it can reasonable be assumed that the species is breeding (Cianferoni and Mazza, 2023. REDIA, 106:161-66). To study *L. patruelis*, molecular tools are used herein for the first time in our country. Due to the lacking of data in literature, the molecular approach can be useful to get knowledge on the origin of this species, potentially harmful to humans. At the date, this is the first Italian case of human attack attributable to the *L. patruelis*.

# INVESTIGATION ON RED FOX ECTOPARASITES IN THREE PROVINCES OF EMILIA ROMAGNA REGION

Plazzotta K.[1], Rugna G.[2], Maioli G.[2], Pupillo G.[2], Dini F.M.[1], Galuppi R.*[1]

*[1]Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy; [2]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy*

**Keywords:** *Vulpes vulpes*, Ectoparasites, Italy.

**INTRODUCTION:** Sarcoptic mange has a prominent role in fox mortality, exerting a potential constraint on population density (Pisano et al., 2019. Parasit Vectors, 12:521-36). Nonetheless, comprehensive data regarding the dissemination of other species of ectoparasites among these carnivores remain scarce. Given the increase and urban encroachment of red fox populations, the objective of the current investigation was to assess the ectoparasites in red foxes across three provinces of the Emilia-Romagna Region.

**MATERIALS AND METHODS:** A total of 67 red foxes, either shot or found dead between January and September 2020, were examined through collaboration with the Zooprophylactic Institutes of Bologna, Modena, and Reggio-Emilia, where these foxes were acquired in accordance with wildlife monitoring plans. During necropsy, skin samples measuring about 6x6 cm were collected from two specific regions: the axillary/thoracic area and the base of the tail (Nimmervoll et al., 2013. J Wildl Dis, 49:91-102). Additionally, samples from areas with alopecic lesions were also collected. The hair was trimmed using scissors, ensuring the removal of any macroscopically visible parasites. Subsequently, the skin flaps were submerged in a 10% aqueous solution of sodium hydroxide (NaOH) and incubated for 4 hours at 37 °C to facilitate dissolution. Following the removal of coarse material, the resulting suspension underwent washing cycles via centrifugation and final flotation using a 1300 PS solution (Di Felice & Ferretti, 1962, Nuovi ann Ig Microbiol, 13:414-21), followed by microscopic examination.

**RESULTS AND CONCLUSIONS:** Ectoparasites were detected in 43 out of 67 foxes (64.17%). *Sarcoptes scabiei* was identified in 25 individuals (37.31%), predominantly without observable lesions. No significant differences were observed across provinces, altitude (plain, hill, mountain), sex, or age groups, although a slightly higher prevalence was noted among younger foxes compared to adults (48.48% vs. 26.47%, X2= 2.59; *p*=0.06). Ixodidae ticks were discovered in 12 foxes (17.91%), in particular *Ixodes ricinus* in 9 individuals and *Ixodes hexagonus* in 3. Fleas were noted in 7 foxes (10.44%), with *Pulex irritans* affecting 6 animals and *Ctenocephalides* spp. observed in one. Additionally, both *Trombicula autumnalis* and *Felicola* (*Trichodectes*) *vulpis* were found in 6 (8.95%) foxes; notably, this is the first documentation of *F. vulpis* in Italy. Red foxes can thus serve as reservoirs for various ectoparasites, including species capable of affecting both domestic dogs and humans (Perrucci et al., 2016. Parasite Epidemiol Control, 1:66-71). Finally, flotation techniques allowed the observation in 9 foxes of tapeworm eggs that were morphologically identified as Anoplocephalidae. Although their presence on fox fur could stem from contamination, it nonetheless underscores the potential role of foxes in the spread of helminth eggs, thus emphasizing their importance in the disease ecology.

# SYNANTROPIC RODENTS AND THEIR ECTOPARASITES: A FOCUS ON FIVE PROVINCES OF NORTHERN-CENTRAL ITALY

Crucitti S., Dini F.M.*, Bordoni T., Galuppi R.

*Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy*

**Keywords:** Rodents, Ectoparasites, Synantropism.

**INTRODUCTION:** Synanthropic rodents, such as mice and rats, rank among the 100 most invasive and harmful species worldwide, according to the IUCN list. These species can alter ecosystems, change vegetation, and cause the decline or extinction of native species, both through direct predation and by depleting trophic resources (Gotti et al., 2022. L' eradicazione del Ratto nero (*Rattus rattus*) dalle isole del Mediterraneo: linee guida, buone pratiche, casi di studio» Ispra, Manuali e Linee Guida 199). Furthermore, due to their close contact with humans, have always been a concern because of their substantial impact on public health. This study aims to investigate the presence of ectoparasites in rodents captured during pest control campaigns in various Italian provinces (Bologna, Forlì-Cesena, Rimini, Ravenna, Arezzo).

**MATERIALS AND METHODS:** A total of 140 samples of skin from synanthropic rodents were examined, specifically 49 *Rattus rattus*, 81 *Rattus norvegicus*, and 10 *Mus musculus*, originating from the provinces of Bologna, Forlì-Cesena, Ravenna, Rimini, and Arezzo. During the necropsies, the skin was visually examined to collect macroscopic ectoparasites and skin samples measuring about 3x3 cm were collected from the caudal-dorsal area. The skin flaps were submerged in a 10% aqueous solution of sodium hydroxide (NaOH) and incubated for 4 hours at 37 °C to facilitate dissolution. Following the removal of coarse material, the resulting suspension underwent washing cycles via centrifugation and final flotation using a 1300 PS solution (Di Felice and Ferretti, 1962, Nuovi Ann Ig Microbiol, 13:414-21), followed by microscopic examination.

**RESULTS AND CONCLUSIONS:** A total of 61 rodents (44%) tested positive for ectoparasites. Specifically, *Polyplax spinulosa* lice were found in 36 subjects (26%), comprising 22 *R. norvegicus*, 13 *R. rattus*, and 1 *M. musculus*. Myobiidae mites were detected in 15 subjects (11%), including 10 *R. norvegicus*, 3 *R. rattus*, and 2 *M. musculus*. Laelapidae mites, particularly *Echinolaelaps echidninus*, were identified in 7 subjects (5%) (6 *R. norvegicus* and 1 *R. rattus*). Mites causing scabies, notably *Notoedres muris*, were found in 7 *R. norvegicus*, and other mite genera such as *Steatonyssus* sp. were discovered in 1 *R. norvegicus* and 2 *R. rattus*. *Liponyssoides muris* (syn. *Dermanyssus muris*) was observed in a single *R. rattus*. Additionally, *Nosopsylla fasciatus*, known for its potential role as a vector of *Yersinia pestis*, was found in one *R. norvegicus*. Other insects and mites, likely due to environmental contamination, were sporadically detected. This research provides a comprehensive overview of the arthropods present on the fur of synanthropic mice and rats in the surveyed provinces. The study represents a valuable baseline investigation, particularly given the lack of nationwide data.

# PRELIMINARY RESULTS ON COMPREHENSIVE PARASITOLOGICAL EVALUATION OF EUROPEAN HEDGEHOG (*ERINACEUS EUROPAEUS*) IN EMILIA-ROMAGNA REGION

Siviglia Y., Dini F.M.*, Bordoni T., Caffara M., Galuppi R.

*Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy*

**Keywords:** *Erinaceus europaeus*, Endoparasites, Ectoparasites.

**INTRODUCTION:** The European hedgehog (*Erinaceus europaeus*) is a widespread mammal in Europe. In Italy, it is present throughout the peninsula and is particularly common in suburban and rural areas. The frequent presence of hedgehogs near public parks and gardens (Mizgajska-Wiktor et al., 2010. Wiad Parazytol, 56:329-32) has led to a high level of interaction with humans. This species is an important component of the epidemiology of various parasites, including zoonotic ones (Ruszkowski et al., 2021. Animals, 11:1754). The aim of this study is to obtain an overview of the parasitic agents that most frequently affect the European hedgehog, investigating their epidemiological aspects and zoonotic potential.

**MATERIALS AND METHODS:** A total of 21 specimens of *E. europaeus*, collected from roadsides in the provinces of Bologna and Ferrara between 2006 and 2023 following road accidents or poisoning. During necropsy, tissue samples from the brain, heart, and tongue were subjected to qPCR and nested PCR targeting the B1 gene of *Toxoplasma gondii*. Ears, liver, spleen, and popliteal lymph nodes were used for qPCR targeting a fragment of kinetoplast minicircles DNA and nested PCR targeting the cysteine peptidase B (cpb) gene for *Leishmania* spp. Spleen tissue was utilized for PCR targeting the 18S rDNA of pyroplasms. The trachea and lungs were collected to search for pulmonary parasites using macroscopic dissection and the Baermann technique. The intestines were opened and examined for gastrointestinal helminths, whose identification was confirmed through sequencing different molecular markers (18S rDNA and COI). Lastly, skin samples were collected to search for ectoparasites through maceration in sodium hydrate followed by the flotation technique.

**RESULTS AND CONCLUSIONS:** Four (19%) hedgehogs tested positive for *T. gondii*, and seven (33.3%) tested positive for *L. infantum*, showing the presence of the two strains circulating in the Emilia-Romagna region (Magri et al., 2022. Int J Parasitol, 52:745-50). Additionally, nine hedgehogs (42.8%) tested positive for *Crenosoma striatum*, and seven (33.3%) had gastrointestinal parasites, identified as *Brachylaemus erinacei* and *Capillaria erinacei*, similarly to previous studies in Emilia-Romagna region, while a greater helminth species richness was found in Central Italy and the Islands (Poglayen et al., 2003. Vet Rec, 152:22-4; Mariacher et al., 2021. Animals, 11:3171). Furthermore, eleven specimens (52.4%) tested positive for ectoparasites, including fleas (*Archaeopsylla erinacei*), mites (*Caparinia tripilis*), previously reported in southern Italy (Bezerra-Santos et al., 2021. Int J Parasitol: Parasites and Wildlife, 15:95-104), and ticks (*Ixodes ricinus* and *Ixodes hexagonus*). This study confirms the high prevalence of multiple endoparasites in *E. europaeus*, some of which are highly pathogenic for hedgehogs and important in wildlife rescue centers, such as *C. striatum*, while others with potential zoonotic risks.

# SARCOCYSTIDAE INFECTION IN RODENTS FROM PERIURBAN CONTEXTS

Dini F.M.*, Caffara M., Cantori A., Luci V., Monno A., Galuppi R.

*Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy*

**Keywords:** *Toxoplasma gondii*, Synantropic rodents, Sarcocystidae.

**INTRODUCTION:** Rodents constitute a notably significant group of mammals, particularly in terms of serving as reservoirs for various pathogens, some of which are of zoonotic concern (Han et al., 2015. Proc Natl Acad Sci U S A, 112:7039-44). Their biological attributes, characterized by elevated reproductive rates, opportunistic behaviors, adaptability, and worldwide distribution, position them strategically, thereby enhancing the likelihood of disease transmission among wildlife, domestic animals, and human populations (Luis et al., 2013. Proc Biol Sci, 280:20122753; Han et al., 2015. Proc Natl Acad Sci U S A, 112:7039-44). *Rattus* spp. and *M. musculus* exemplify species capable of coexisting within anthropogenically influenced environments. This coexistence raises concerns for potential human health risks due to the close proximity between these species and human habitats. Within the scope of this research, we examined the occurrence of Sarcocystidae infections in synanthropic rodents using a broad-spectrum PCR assay targeting the 18S rRNA of Coccidia.

**MATERIALS AND METHODS:** We examined 97 brown rats (*Rattus norvegicus*), 67 black rats (*R. rattus*), 47 house mice (*Mus musculus*), and 1 common shrew (*Sorex araneus*) collected during pest control programs from urban and rural areas of northern-central Italy, in the provinces of Ferrara, Forlì-Cesena, Ravenna, Bologna (Emilia Romagna Region) and Arezzo (Toscana Region). During necropsies, tongue, central nervous system and heart muscle were collected and processed by PCR targeting the 18S rDNA gene, which is generic for Coccidia, followed by sequencing.

**RESULTS AND CONCLUSIONS:** PCR testing yielded positive results in at least one of the examined tissues in 26 *R. norvegicus* (26.8%), 13 *R. rattus* (19.4%), and 13 *M. musculus* (27.6%). Sequencing comparisons using BLAST allowed us to identify four different species of cyst-forming Apicomplexa. In *R. norvegicus*, the most frequent species was *H. hammondi* (including *H. hammondi*-like, characterized by a SNP in the analysed sequence) with a prevalence of 17.5%, followed by *Besnoitia* sp. at 7.2%; one sequence showed 100% similarity with *B. besnoiti*. Trailing behind in prevalence were *T. gondii* at 4% and *Sarcocystis gigantea* at 2%. For *R. rattus*, only two Apicomplexan were molecularly identified in the analyzed tissues: *H. hammondi/H. hammondi*-like (11.9%) and *T. gondii* (9%). Regarding *M. musculus*, the most prevalent parasite was *H. hammondi/H. hammondi*-like (23.4%), followed by *T. gondii* (4.2%). In one instance, *B. besnoiti* was detected in a heart sample with 100% sequence similarity. *S. araneus* tested positive for *T. gondii*. Rodents from peri-urban and urban environments can act as indicators of environmental contamination by oocysts of apicomplexan parasites with cats as definitive hosts, such as *T. gondii*, *H. hammondi*, and *S. gigantea*, the latter of which has never been previously recorded in rodents. Moreover, the presence of *B. besnoiti*, a parasite with an unidentified definitive host in Europe, sheds light on the potential role of these hosts as infection sentinels.

# SURVEY ON SARCOPTIC MANGE IN IBEX (*CAPRA IBEX L.* 1758) OF THE FRIULIAN DOLOMITES REGIONAL NATURE PARK

Frega B.*[1], Favalli M.[2], Dini F.M.[1], Bordoni T.[1], Galuppi R.[1]

*[1]Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy; [2]Parco Naturale Regionale delle Dolomiti Friulane, Udine, Italy*

**Keywords:** *Capra ibex*, *Sarcoptes scabiei*, Friulan Dolomites.

**INTRODUCTION:** The establishment of the ibex (*Capra ibex*) colony within the Friulian Dolomites Nature Park dates back to 1985-87 and it started with the release of 26 specimens sourced from the surviving population of the western Alps. Initially, the colony experienced steady growth, but in 2011, the onset of sarcoptic mange led to a progressive decline in population numbers. In fact, the inherent coevolutionary dynamics between the parasite and its host gives rise to a cyclical pattern characterized by periodic epidemic outbreaks, although with reduced incidence rates affecting the population. This study aimed to determine the current population size and the trend of the sarcoptic mange epidemic within the ibex colony of the Friulian Dolomites Nature Park.

**MATERIALS AND METHODS:** The study began with a retrospective analysis of data collected from annual censuses between 2012 and 2023. Additionally, it involved a longitudinal survey conducted from February to October 2023 in the Salta-Borgà area, located in the southwest sector of the Park. This survey is the result of monthly monitoring of ibex through direct observation aided by optical instruments along established transects. These observations were carried out daily for several consecutive days, spaced one month apart. Each observation session included photographic documentation for subsequent health analysis of the animals, resulting in classification into four degrees of mange severity based on skin lesions. During the survey, biological samples were collected from the environment and from carcasses exhibiting mange-related lesions. These samples underwent laboratory analysis to detect Sarcoptes scabiei using a maceration in sodium hydrate followed by a flotation technique.

**RESULTS AND CONCLUSIONS:** The findings confirmed the presence of a second sarcoptic mange outbreak, commencing in 2019 within the Salta-Borgà area. While resulting in a reduced demographic decline compared to the initial outbreak, this recovery failed to facilitate a positive population trend. Laboratory analysis of samples revealed *Sarcoptes scabiei* presence both in carcasses and environmental samples, indicating potential transmission through various indirect sources. Despite milder cutaneous lesions, this secondary wave of sarcoptic mange propagated throughout the Park's ibex colony. Considering the population´s characteristics - relatively young, genetically restricted and territorially isolated, future cyclical waves with low incidence can be foreseen due to the disease´s natural evolution. Hence, the recommendation from this study is to enhance genetic variability through reintroducing individuals from colonies with prolonged exposure to sarcoptic mange, potentially exhibiting an efficient coevolutionary response to the parasite.

# HEMOPARASITES IN FIRE SALAMANDERS (*SALAMANDRA SALAMANDRA*) FROM THE REGIONAL NATURE RESERVE "FONTANA DEL GUERCIO", COMO DISTRICT

Bigoni F.*[1], Epis S.[2], Cattaneo G.M.[2], Ficetola F.[3], Manenti R.[3], Mendoza-Roldan J.A.[4], Bandi C.[2], Alvaro A.[2]

*[1]Department of Veterinary Medicine and Animals Sciences, University of Milan, Milan, Italy; [2]EntoPar lab, Department of Biosciences, University of Milan, Milan, Italy; [3]Department of Environmental Science and Policy, University of Milan, Milan, Italy; [4]Department of Veterinary Medicine, University of Bari, Bari, Italy*

**Keywords:** *Salamandra salamandra, Dactylosoma, Leishmania.*

**INTRODUCTION:** The fire salamander (*Salamandra salamandra*) is an urodelan amphibian widely distributed in Europe and particularly in Italy, in the Alps and throughout the Apennines. The European population of *S. salamandra* experienced a dramatic decline because of several threats, including habitat loss and emerging infectious diseases. The aim of this research was to determine the presence of hemoparasites in individuals of *S. salamandra* collected in the Regional Nature Reserve "Fontana del Guercio' located in the municipalities of Carugo and Inverigo (Como district). We emphasize that limited information has so been reported on hemoparasites infecting *S. salamandra*.

**MATERIALS AND METHODS:** During the year 2022, 71 individuals of fire salamanders were captured along transects within the reserve. Blood samples were collected through venipuncture, and a drop was used to obtain blood smears. Cloacal swabs were also collected. DNA was extracted from the obtained samples, and screened for the presence of apicomplexan protozoans and *Leishmania* spp., via PCR and qPCR. Samples were screened for apicomplexan DNA with primers targeting the 18S rRNA gene (Pinheiro et al., 2020. J Microbiol Methods, 175:105985), and for *Leishmania* spp. through a qPCR targeted to the ITS-1 ribosomal gene region (el Tai et al., 2000. Trans R Soc Trop Med Hyg, 94:575-79). The blood smears were observed under a light microscope at 100X magnification.

**RESULTS AND CONCLUSIONS:** No successful detection of apicomplexan DNA was obtained with the employed primers. However, after thorough microscopic observation of the blood smears, the presence of intra- and extra-cellular hemoparasites was observed, that were then identified as belonging to the genus *Dactylosoma*. At least one of the developmental forms of this parasite was observed in every blood smear. The 15% of the blood samples and the 38% of cloacal samples resulted positive for *Leishmania* DNA. Nevertheless, only one sample provided a good quality sequence, that clustered with those of *Leishmania tarentolae*. In the blood smears, a few extracellular forms of flagellated parasites comparable to *Leishmania* promastigotes were also observed. To the best of our knowledge, our study reports the first documented *Dactylosoma* sp. parasite infection in fire salamanders, and the first evidence for *Leishmania* sp. presence in an amphibian. Further experiments and analyses are needed to shed light on the biology and the taxonomy of hemoparasites of this population of fire salamanders.

# HIGH ALTITUDE PARASITES: AN EPIDEMIOLOGICAL UPDATE ON THE STATUS OF GASTRO-INTESTINAL PARASITES OF ALPINE GALLIFORMES

Pasquetti M.[1], Meneguz P.G.[1], Molinar Min A.R.[1], Beltramino M.[1], Rossi L.[1], Moroni B.*[2], Catala-Barrasetas M.[1], Deriu S.[3], Borgna V.[4], Tizzani P.[1]

*[1]Dipartimento di Scienze Veterinarie, Università di Torino, Grugliasco, Turin, Italy; [2]Istituto Zooprofilattico Sperimentale di Piemonte, Liguria e Valle d'Aosta, Turin, Italy; [3]Comparto Alpino valle Stura CA CN 4, Cuneo, Italy; [4]Comparto Alpino valli Gesso e Vermenagna CA CN 5, Cuneo, Italy*

**Keywords:** Alps, Galliformes, Parasite-host community.

**INTRODUCTION:** The Italian Alps are home to five Galliformes species: *Tetrao tetrix tetrix, Lagopus muta helvetica, Bonasa bonasia styriaca, Tetrao urogallus crassirostri, Alectoris graeca saxatilis* (Brichetti, De Franceschi, Bacetti, 1992, Fauna d'Italia, XXIX. Aves. I, Gavidaee-Phasianidae, Calderini, Bologna, 964). Few studies investigate their health status (e.g. Formenti et al., 2013. Eur J Wildl Res, 59(3): 351-58), although recent works are filling this gap (Fanelli et al., 2020. Parasitology, 147(4): 471-77; Fanelli et al., 2020. Parasitology, 147:828-34; Tizzani et al., 2020. Grouse new, 59:16-19). However, for their conservation, it would be essential to obtain more information on their parasite community, in relation to the range occupied by the host species. Further investigation in the Maritime Alps is of great interest because this area: i) represents the southern limit of the alpine range occupied by *T.t. tetrix* (Black Grouse), *L.m. helvetica* (Rock Ptarmigan), and *A.g. saxatilis* (Rock Partridge); ii) is very important from a management point of view because it has been interested by releases of "game ready hunting", with potential sanitary impact; iii) there are no previous data on the parasitic community of Alpine Galliformes in this area.

**MATERIALS AND METHODS:** In order to fill this gap in knowledge, an initial sampling effort was carried out during the 2021 hunting season, resulting in the collection of 72 intestinal packets (32 Black Grouse, 36 Rock Partridge, 4 Rock Ptarmigan). Following the sampling activity in 2021, follow-up sampling was carried out during the 2022 and 2023 hunting seasons, with the collection of 81 intestinal packets in 2022 (53 Black Grouse, 35 Rock Partridge, 3 Rock Ptarmigan), and 38 in 2023 (14 Black Grouse, 24 Rock Partridge).

**RESULTS AND CONCLUSIONS:** During the first sampling session six genera of parasites were detected: four nematodes (*Ascaridia* sp., *Cheilospirura* sp., *Spiruridae* sp., *Heterakis* sp.), one trematode (*Corrigia* sp.), one cestode. Prevalence of positive animals was 16.6% in Black grouse, 41.2% in Rock partridge, and 25% in Rock ptarmigan, with average parasite abundance (number of parasite / sampled animals) equal to 0.25 in Black grouse, 1.08 in Rock partridge, and 0.25 in Rock ptarmigan. Prevalence in following year remained stable in Black grouse (18%) and in Rock ptarmigan (33%), and increased in Rock partridge (95%).
This work provides information on the Galliformes parasite community in the Maritime Alps. Only few parasite species were detected, with *A.g. saxatilis* being the host with highest parasite prevalence. The global low parasite community richness is in line with previous studies. Further studies should be conducted, focusing not only on parasites but including other diseases that may impact on population dynamics. A better understanding of the health status of Alpine Galliformes could help to improve future management and conservation strategies.

# XXXIII

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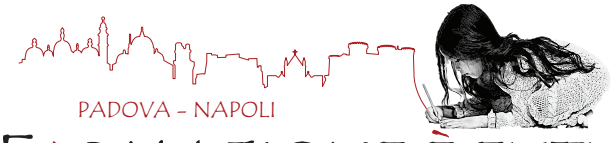
# MEDICAL TROPICAL PARASITIC DISEASES



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# CEREBRAL CYSTIC ECHINOCOCCOSIS IN A CHILD FROM ROMAN COUNTRYSIDE (SOURCE ATTRIBUTION AND SCOPING REVIEW)

Casulli A.*, Santoro A., Santolamazza F.

*WHO Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis; European Union Reference Laboratory for Parasites, Department of Infectious Diseases, Istituto Superiore Di Sanita', Rome, Italy*

**Keywords:** *Echinococcus granulosus sensu lato*, Cerebral cystic echinococcosis, Source attribution.

**INTRODUCTION:** Human cystic echinococcosis (CE) is a zoonotic parasitic infection caused by the larval stage of the species belonging to the *Echinococcus granulosus sensu lato* (*s.l.*) complex. Parasitic cysts causing human CE are mainly localized in the liver and in the lungs. In a smaller number of cases, larvae may establish in any organ or tissue, including the central nervous system (CNS) (Stojkovic et al., 2013. Handb Clin Neurol, 114:327-34). Cerebral CE (CCE) is rare but poses serious clinical challenges.

**MATERIALS AND METHODS:** This study presents a case of CCE in a child living in the countryside near Rome (Italy), along with a comparative molecular analysis of the isolated cyst specimens from the patient and sheep of local farms. We also systematically searched the literature to summarize the most relevant epidemiological and clinical aspects of this uncommon localization.

**RESULTS AND CONCLUSIONS:** The comparative molecular analysis confirmed that the infection was caused by *E. granulosus sensu stricto* (*s.s.*) (G3 genotype). The infection was most likely acquired in the family farm since data showed a clear and direct genetic relationship between the child's cyst and the cysts belonging to sheep of his own family's farm. In particular two sheep cyst sequences were identical (100%) to those of the child. The literature search identified 2,238 cases of CCE. In 80.51% of cases, brain was the only localization and single CCE cysts were present in 84.07% of cases. Mean patients' age was 20 years and 70.46% were children. Cyst rupture was reported in 12.96% and recurrence of CCE after treatment in 9.61% of cases. Permanent disability was reported in 7.86% of cases, while death occurred in 6.21%. In the few reports that identified at molecular level the CCE cyst, *E. granulosus s.s.* was found in 40% and *E. canadensis* in 60% of cases. When considering clinical centres reporting all anatomical sites of CE, liver represented 70%, lungs 19%, and unusual localizations 11% of all CE cases. In case series reporting all CE localization, CCE represented 1.5% of all CE cases. The proportions of CE cases with uncommon localizations and with high impact on patients' lives have been globally neglected and should be included in the computation of the global burden of CE. This research was funded by the MEmE project from the EU's Horizon 2020 Research and Innovation programme under grant agreement number 773830: One Health European Joint Programme (https://onehealthejp.eu/projects/emerging-threats/jrp-meme).

# THE PREVENTION OF TRANSMISSION OF PARASITIC INFECTIONS FROM SOLID ORGAN TRANSPLANTATION

Petrullo L.*[1], Ascierto M.[2], Panariello M.V.[1], Fioretti A.[1], Coppola M.G.[1], Galdiero M.[2]

*[1]Azienda dei Colli of Naples "D. Cotugno" Hospital, UOC Microbiology and Virology, Naples, Italy; [2]Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy*

**Keywords:** Strongyloidiasis, Screening, Prevention.

**INTRODUCTION:** The safety and increase of organs available for transplantation is one of the priority objectives of the National Transplantation Network. Information flows make it possible to validate and/or modify guidelines that represent a dynamic tool for new epidemiological challenges. *Strongyloides stercoralis* infects over 300 million people globally. *Strongyloides* transmission from the donor has been reported after heart, kidney, kidney-pancreas and liver transplantation. The lethality rate of *Strongyloides* exceeds 50%. A recent epidemiological study conducted in our country showed seropositivity rates of 8% in the Italian population and 17% in the foreign population. It is therefore essential to perform serology for *Strongyloides stercoralis* on all donors, the result of which is not necessary for allocation purposes but may allow early treatment of recipients.

**MATERIALS AND METHODS:** Strongyloidiasis was diagnosed by antibody detection by an enzyme immunoassay, standard copro-parasitological examination, culture examination and by molecular biology in filmarray using the Novodiag instrument: a random-access molecular test for the qualitative detection of 25 human parasites including protozoa, helminths and microsporidia.

**RESULTS AND CONCLUSIONS:** Clinical case: O.J., Patient from Sudan, male 35 years old, in Italy (Campania Region) for 15 years, admitted to the UOSD "Circulatory Mechanics and Transplantation" for dilated myocarditis, candidate for heart transplant. During admission, the patient underwent a TransThoracic echocardiogram showing severely reduced global sistolic function (FE: 30%) and Cardiac MRI showing severely increased left ventricle size (FE: 32%). On 30/11/2022 standard copro parasitological examination sent positive for *Strongyloides stercoralis* larvae; *Strongyloides* antibodies positive at 35. The clinical case described reveals the importance of searching for *Strongyloides* antibodies in a particolar category of patients, from endemic areas, who in the absence of such a diagnosis could have undergone transplantation, reactivating a deadly *Strongyloides* hyperinfestation following immunodepressant therapy. From 2019 to 2024, the UOS of Parasitology of the P.O. 'D. Cotugno' in Naples, Regional Reference Centre for Parasitological Diagnoses for Research and Screening of *Strongyloides stercoralis* infection, carried out 228 serological tests, 684 copro-parasitological tests, 153 molecular biology tests in filmarray, and set up 100 cultures. In conclusion the importance of the presence in Italy of reference centres for the diagnosis and treatment of parasitic diseases is fundamental, as is the high and constant teamwork expertise.

# INTERACTIONS BETWEEN MALARIA PIGMENT AND ARTEMISININ DERIVATIVES IN AN *IN VITRO* ENDOTHELIAL CELL MODEL

Sambin A.*[1], D'Alessandro S.[2], Parapini S.[3], Misiano P.[2], Taramelli D.[2], Basilico N.[1]

*[1]University of Milan, Department of Biomedical, Surgical and Dental Sciences, Milan, Italy; [2]University of Milan, Department of Pharmacological and Biomolecular Sciences, Milan, Italy; [3]University of Milan, Department of Biomedical Sciences for Health, Milan, Italy*

**Keywords:** *Plasmodium falciparum*, Hemozoin, Dihydroartemisinin.

**INTRODUCTION:** The endothelium plays a crucial role in the pathogenesis of severe malaria due to the phenomenon of cytoadherence of parasitized erythrocytes to the small vessels. During the erythrocytic stage of malaria, schizonts release merozoites and parasite products such as hemozoin (HZ), derived from the catalysis of hemoglobin (Venugopal et al., 2020. Nat Rev Microbiol, 18:177-89). This crystal of Fe (III) protoporphyrin IX, bound to lipids, parasite DNA, and plasma proteins, possesses immunomodulatory properties on monocytes, macrophages, and dendritic cells, while its effects on endothelial cells are less known. Artemisinin-based combination therapies are the first line treatment for uncomplicated *P. falciparum* malaria. The immunomodulatory role of artemisinin derivatives is also known, but their activity on endothelium in the context of malaria is not yet fully understood.

**MATERIALS AND METHODS:** HMEC-1 microvascular endothelial cells were treated with TNF-α (100 U/mL) and IL-1β (100 U/mL), two cytokines involved in severe malaria pathogenesis, or HZ (20-10-5 µg/mL), either alone or in the presence of dihydroartemisinin (DHA) (1-0.5 µM) or parthenolide (10 µM). HZ was isolated from *P. falciparum* cultures, maintained in medium containing human plasma or AlbuMax, using Percoll gradient centrifugation. In some experiments, cells were treated with fibrinogen (200-50-10 µg/mL). Levels of cytokines (IL-6 and CXCL8) in supernatants were determined by ELISA.

**RESULTS AND CONCLUSIONS:** As expected, inflammatory cytokines significantly up regulated the production of both IL-6 and CXCL8. DHA, alone and in association with TNF-α, stimulated IL-6, but not CXCL8 production. HZ stimulated CXCL8 production, which was inhibited by DHA, but had no effect on IL-6 production. The release of CXCL8 was approximately two-fold higher in HZ-treated cells than in controls. Since HZ is associated to host plasma components, two different HZ preparations were compared: HZ isolated from *P. falciparum* cultures in a medium containing human plasma vs. HZ isolated from cultures containing AlbuMAX, a synthetic derivative of albumin. Only plasma-derived HZ induced CXCL8 secretion by HMEC-1, indicating that plasma components were involved in the chemokine secretion. HMEC-1 cells were thus treated with fibrinogen, a plasma component known to be bound to HZ. Indeed, fibrinogen enhanced by two-three times CXCL8 production, which was inhibited by DHA, but no effect was seen on IL-6. Since DHA is an inhibitor of the nuclear transcription factor NF-κB, which is activated by HZ, another NF-κB inhibitor, parthenolide, was used. As expected, parthenolide inhibited both IL-6 and CXCL8 production induced by TNF-α, but increased CXCL8 production in the presence of HZ.
This study confirm that DHA and HZ play an immunomodulatory role in endothelial activation. Moreover, it appears that part of the activity ascribed to HZ is indeed shared with plasma components, such as fibrinogen, which are known to be associated with HZ crystals.

# FIRST EVIDENCE OF A QUORUM SENSING MECHANISM IN *GIARDIA DUODENALIS* LINKED TO 14-3-3 PROTEIN POLYGLYCYLATION LEVEL

Camerini S.[1], Salzano A.M.[2], Marlow M.[3], Cecchetti S.[1], Yee J.[3], Lalle M.*[4]

*[1]Core Facilities Technical-Scientific Service, Istituto Superiore di Sanità, Roma, Italy; [2]Institute for the Animal Production System in the Mediterranean Environment, CNR, Naples, Italy; [3]Department of Biology, Trent University, Peterborough, Canada; [4]Unit of Foodborne and Neglected Parasitic Diseases, Dep. Infectious Diseases, Istituto Superiore di Sanità, Roma, Italy*

**Keywords:** *Giardia duodenalis*, 14-3-3 proteins, Polygycylation.

**INTRODUCTION:** The highly conserved dimeric 14-3-3s are a family of eukaryotic proteins that regulates a plethora of cellular processes by interacting with hundreds of post-translational modifications client proteins via the recognition of conserved Ser/Thr phosphorylated binding motifs. The flagellated protozoan *Giardia duodenalis* encodes for a single 14-3-3 isoform constitutively phosphorylated at Thr214. In addition, the C-terminal Glu246 is subject to polyglycylation (polyGly), a common tubulin polymodification consisting of the addition of multiple glycine residues to the γ-carboxyl group of the glutamate. Mutational analysis have demonstrated that polyGly length affects the nuclear localization of g14-3-3 and encystations timing. Here we further explore how 14-3-3 and its post-translational modifications (PTMs) levels change during cell-cycle and parasite proliferation.

**MATERIALS AND METHODS:** *G. duodenalis* isolates WBC6 (Ass. A), GS/M (Ass. B) and P15 (Ass. E) were used. Enrichment of trophozoites at different cell cycle stages was achieved using counterflow centrifugal elutriation and verified by flow cytometry. PTMs of 14-3-3 were analysed combining protein affinity purification and MALDI-TOF mass fingerprinting. Expression of target genes was evaluated by qPCR. Density assay was performed on trophozoites growth on 24 well plate, coated with low-melting agarose medium, in microaerophilic condition for the desired time.

**RESULTS AND CONCLUSIONS:** Using specific pAbs, we observed a distinct alteration of the polyglycylation level for 14-3-3, as well as tubulin, in *Giardia* trophozoites throughout 72 h of growth. The variations of the polyglycine chain length were further confirmed by 14-3-3 affinity chromatography and MALDI-TOF analysis. PTMs alteration during parasite proliferation correlate with differential gene expression of the polyglycylase and the two deglycylases enzymes. We did not detect changes in the level of the higher polyglycylated form relative to the lower polyglycylated form of 14-3-3 during cell cycle progression in log phase *Giardia* cultures Intriguingly, cell density, and to a less extent medium depletion, affected 14-3-3 expression and PTMs level. When trophozoites of the reference Assemblage A, B and E were compared after 48 h of growth, the 14-3-3 intracellular distribution and PTMs extent were markedly different. In conclusion, the results presented herein suggest that in *Giardia* trophozoites polyglycylation, in addition to its role in preventing g14-3-3 oligomerization and in regulating g14-3-3 nuclear localization during encystation, could be part of a not yet defined transduction mechanism integrating both nutrient and quorum sensing. Indeed, g14-3-3 with long polyglycine chain are present in cell approaching the stationary phase as well as cell growing at high density. Our observations clearly indicate that, as in bacteria and other protozoa, a quorum sensing mechanism might occur also in *Giardia* and deserve further studies.

# HUMAN LEISHMANIASIS IN ITALY: EPIDEMIOLOGICAL TRENDS FROM 2015 TO 2022

Di Muccio T.*[1], Scalone A.[1], Fiorentino E.[1], Orsini S.[1], Gradoni L.[1], Maraglino F.[2], Ferraro F.[2], Gramiccia M.[1]

*[1]Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Rome, Italy; [2]Ministero della Salute, Direzione Generale della prevenzione Sanitaria, Rome, Italy*

**Keywords:** Human Leishmaniasis, Italy, Epidemiology.

**INTRODUCTION:** Leishmaniases are *Phlebotomus*-borne diseases endemic in over 98 countries. According to the WHO, 700 000 to 1.3 million new cases of visceral and cutaneous leishmaniasis (VL and CL) occur every year. The WHO Europe regional incidence of leishmaniasis is estimated to be around 2% of the global burden but it is considered underestimated especially for CL. These diseases are likely to re-emerge, due to the movement of humans and dogs, immunosuppressive conditions, climate and human-mediated environmental changes. Systematic collection and analysis of data associated with leishmaniasis occurrence are necessary for implementing public health practice (Gradoni et al, WHO Regional Office Europe; 2017). In Italy, the disease is endemic, present as zoonotic VL and sporadic CL caused by *Leishmania infantum*. Animal reservoir hosts are dogs and some wildlife species. As previously reported, a VL epidemic involved all Italian regions, during 1990-2012 period, with a peak of 200 cases/year in 2000-2004, to which an outbreak in Campania region and an outbreak affecting human immunodeficiency virus -infected individuals throughout the country have contributed (Gramiccia et al., 2013. Euro Surveill, 18:20535).

**MATERIALS AND METHODS:** Both VL and CL are compulsory notifiable diseases; notifications are gathered at regional level and centralized at the Ministry of Health (Infectious Disease Reporting System, PREMAL, D.M.07.03.2022). ISS performs surveillance of the human trends of leishmaniasis. Patient records was anonymized prior to analysis. Preliminarily, the following variables were considered: (i) year of diagnosis, (ii) age at time of diagnosis, (iii) sex, (iv) type of disease (CL, VL), (v) autochthonous or imported. One hundred seventy positive cases were genotyped at ISS.

**RESULTS AND CONCLUSIONS:** The human database analysis, referred to 2015-2022 period, confirms the stable endemic trend of leishmaniasis that reaches on average of 108 cases/year (cases range: 55-152), with cumulative 871 cases, 460 VL and 411 CL. The number of notified CL cases became quite similar to that of VL cases, likely due to the improvement of CL notification in some territories. The epidemiological analysis shows epidemic determinants comparable in both VL and CL forms and for that analyzed together. In detail, 728 cases (about 84%) were adults (>17 years, yrs) of witch the elderly group (>70 yrs) for 151 cases (17%), whereas 143 cases (16%) were pediatric (0-16 yrs) of which infants (<2 yrs) were the most numerous group (71 cases) but just 8% of all cases, and about twice as many cases were male respect to female (605 vs 266). *L. infantum* was the only autochthonous species among the cases that were analyzed. Our analysis confirms endemic status of VL and CL throughout Italy and shows that most cases consisted of adult individuals. There are no specific risk groups for *Leishmania* infection, the same applies to imported cases, but adult males appear more exposed to the vector contact probably referred to different behaviours.

# FOUR YEARS OF ACTIVITIES AT THE WHO COLLABORATING CENTRE ITA-116

Maurelli M.P.*, Pepe P., Nocerino M., Gualdieri L., Bosco A., Cringoli G., Rinaldi L.

*University of Naples Federico II, WHO Collaborating Centre ITA-116, Department of Veterinary Medicine and Animal Production, CREMOPAR, Naples, Italy*

**Keywords:** Intestinal parasites, Humans, Web-GIS.

**INTRODUCTION:** In February 2020, the World Health Organization (WHO) designated the Laboratories of Parasitology and Parasitic Diseases (University of Naples Federico II) as collaborating centre for the diagnosis of intestinal helminths and protozoa (WHO CC ITA-116; https://maps.parassitologia.unina.it//). The terms of reference (TOR) of the centre were to: 1) participate in collaborative activities to improve the performance of qualitative and quantitative diagnostic techniques for intestinal parasites; 2) provide specialized training courses on diagnostic techniques for laboratory technicians in endemic countries; 3) support endemic countries in monitoring preventive chemotherapy (PC) programmes. Moreover, during these years parasitological surveillance was performed for migrants arriving in southern Italy to assist health care decision makers in the management and control of intestinal parasites in non-endemic areas and to ensure access to care for the poorest and the most marginalized people. In this study we report the goals achieved for each activity in the first four years of designation.

**MATERIALS AND METHODS:** Innovative diagnostic tools (i.e., Mini-FLOTAC, Kubic FLOTAC Microscope, KFM) (Cringoli et al., 2017. Nat Protoc, 12:1723-32; Cringoli et al., 2021. Parasitology, 148:427-34) were used to achieve the objectives of TOR1, TOR2 and for parasitological monitoring of migrants. For TOR3, a WebGIS was developed to support PC programmes to monitor the impact of soil-transmitted helminths (STH) control in the six WHO regions (Maurelli et al., 2021. Geospat Health, 16:2021).

**RESULTS AND CONCLUSIONS:** TOR1: the centre validated the KFM for automated identification and counting of STH (Maurelli et al., 2023. Curr Trop Med Rep, 10:17-25) and *Fasciola hepatica* (Capuozzo et al., in press) eggs, based on machine learning and artificial intelligence in stool samples. TOR2: the WHO CC staff produced manuals, guidelines, brochures, videos and other materials for interactive trainings. TOR 3: the public WebGIS with four different sets of maps (Progress of implementation; Impact of intervention on STH prevalence; Impact of intervention on STH morbidity; Drugs donated) developed to assist countries in conducting surveys under the PC programmes has been constantly updated. Moreover, two semestral newsletters per year have been published to describe the improvement of PC programmes in virtuous countries. Finally, out of the 732 migrants monitored in southern Italy, 140 (19.1%) resulted positive for at least one intestinal parasite. The most common parasites found were hookworms (29.6%) and *Trichiuris trichiura* (12.0%) among helminths and *G. duodenalis* (7.4%) and *Entamoeba histolytica/dispar* (4.6%) among pathogenic protozoa.
All the results obtained in these first four years will be useful to achieve the WHO main goals of the NTD 2021-2030 roadmap for the control and elimination of intestinal parasites. Moreover, new TOR will be realized in the next years based on these findings.

# UROGENITAL SCHISTOSOMIASIS INCREASES THE PROSPECTIVE RISK OF INFECTION WITH *PLASMODIUM FALCIPARUM*

Ouedraogo M.[1], Hilt S.[2], Kabore Y.[1], Ouedraogo I.N.[1], Van Dam G.[2], Bruschi F.[3], Corstjens P.[2], Modiano D.[4], Mangano V.*[3]

*[1]Centre National de Recherche et Formation sur le Paludisme, Ouagadougou, Burkina Faso; [2]Leiden University Medical Center, Leiden, Netherlands; [3]University of Pisa, Pisa, Italy; [4]University La Sapienza of Rome, Rome, Italy*

**Keywords:** Malaria, Schistosomiasis, Sub-Saharan Africa.

**INTRODUCTION:** There is significant overlap in the global distribution of malaria and neglected tropical diseases (NTDs). The largest health burden is observed in Sub-Saharan Africa, where many areas are endemic for both malaria and schistosomiasis, soil-transmitted helminths or lymphatic filariasis. Several observations support the hypothesis that helminths modulate immune responses towards plasmodia with a negative impact on immunity. Indeed, some epidemiological studies suggest that helminth infection including schistosomiasis increases susceptibility to malaria, but evidence is limited as it is based on single cross-sectional surveys. The aim of the present study was to investigate the impact of urogenital schistosomiasis on the prospective risk of *Plasmodium falciparum* infection in populations living in rural villages of Burkina Faso previously shown to be areas of co-endemicity.

**MATERIALS AND METHODS:** The study included a cohort of 424 subjects who participated in five surveys. Active infection with *Schistosoma haematobium* was diagnosed at the first survey (baseline) by plasma detection of Circulating Anodic Antigen (CAA), while infection with *P. falciparum* was diagnosed at each survey by microscopic examination of thick and thin blood smears. A longitudinal analysis of the association between urogenital schistosomiasis at baseline and the risk of malaria infection over time was then conducted, using regression models that included gender, age group, village of residence, and hemoglobin genotype.

**RESULTS AND CONCLUSIONS:** The prevalence of active urogenital schistosomiasis was 28.3% and that of malaria was 49.5%. Coinfection between *S. haematobium* and *P. falciparum* was observed in 15.8% of subjects and was highest among school aged children and adolescents (19.6% among 5-9 years old and 36.4% among 10-19 years old). In subjects with active urogenital schistosomiasis there was an increase of ≈ 25% in the cumulative incidence of *P. falciparum* infections (IRR=1.26, 95%CI=1.08-1.46, *p*-value=0.004). Furthermore, a non-significant trend of increase was observed in the average parasite density (Expβ=1.12, 95%CI=0.96-1.31, *p*-value=0.133) as well as in the odds of infection over the 5 investigations (OR=1.79, 95%CI=0.89-3.59, *p*-value=0.104). Similarly, it was observed that higher intensity of infection with urogenital schistosomiasis increased the cumulative incidence of *P. falciparum* infections (IRR=1.12, 95%CI=1.05-1.19, *p*-value=0.001) and the mean parasite density (Expβ=1.08, 95%CI =1.01-1.15, *p*-value=0.026), with a trend in the same direction also for the odds of infection (OR=1.28, 95%CI=0.91-1.80, *p*-value=0.159). Overall, these results provide the first evidence from a longitudinal study that urogenital schistosomiasis increases susceptibility to *P. falciparum* infection. Such evidence supports the need to implement integrated control strategies for malaria and schistosomiasis and in Sub-Saharan Africa, with a special emphasis on school-aged children and adolescents.

# FROM INVASION TO IMMUNE EVASION: THE IMMUNOMODULATORY INFLUENCE OF *LEISHMANIA INFANTUM* PARASITES ON *L. TARENTOLAE* SURVIVAL AND PERSISTENCE

Calvo-Alvarez E.*[1], Dolci M.[2], Ghezzi S.[3], D'Alessandro S.[1], Parapini S.[4], Vicenzi E.[3], Taramelli D.[1], Poli G.[5], Basilico N.[2]

*[1]Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy; [2]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy; [3]Viral Pathogens and Biosafety Unit, Division of Immunology, Transplantation, and Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy; [4]Department of Biomedical Sciences for Health, University of Milan, Milan, Italy; [5]Vita-Salute San Raffaele University School of Medicine, Milan, Italy*

**Keywords:** *Leishmania infantum*, *Leishmania tarentolae*, Immunomodulation.

**INTRODUCTION:** *Leishmania infantum* (Li), the main causative agent of human and canine visceral leishmaniasis, is a protozoan parasite with worldwide distribution, and prevalent in southern Europe. In Italy, Li coexists with *L. tarentolae* (Ltar), a reptile-infecting species classically regarded as non-infectious to mammals, yet recent evidence has shown potential for Ltar pathogenicity (Bandi et al., 2023. Parasit Vectors, 16:35). Given Li-Ltar co-circulation and Li's immunosuppressive strategies to hijack host myeloid cells for immune escape (Olivier et al., 2005. Clin Microbiol Rev, 18:293), we hypothesize that Li's immunomodulatory tactics might promote Ltar's survival in host cells, introducing novel interspecies interactions that could impact the dynamics of the disease. Besides, this research may also reveal hidden pathogenic traits of Ltar under certain immunological conditions.

**MATERIALS AND METHODS:** We used both WT Li and Ltar parasites, and generated engineered strains stably overexpressing the red fluorescent reporter tdTomato (tdT-Li), and the green fluorescent marker Citrine (Citrine-Ltar). These strains allowed us to monitor and quantify parasite phagocytosis and replication within host cells using fluorescence microscopy in real time. For infection studies, we isolated primary human monocytes from the peripheral blood of healthy donors and differentiated them into monocyte-derived macrophages (MDMs). A subset of MDM was polarized to an "M1" pro-inflammatory phenotype using LPS and IFN-γ. We performed single and sequential infections (Li pre-infection followed by Ltar) at a 1:10 cell-to-parasite ratio for up to 96 h, assessing parasite proliferation via EdU incorporation. The inflammatory response was evaluated by ELISA, WB and immunofluorescence.

**RESULTS AND CONCLUSIONS:** Our findings demonstrate that MDMs internalize tdT-Li and Citrine-Ltar at similar rates within the first 6 hours post infection (hpi). Yet, by 24 hpi, Ltar's survival significantly decreased, irrespective of Li pre-exposure. Interestingly, Li pre-infection enhanced MDM permissiveness to Ltar at chronic infection times (96 hpi), correlating with increased Ltar proliferation. In contrast, single infections with Ltar resulted in scarce, non-replicative persisters, indicative of potent anti-*Leishmania* macrophage activity. Notably, Li pre-infection modulated the inflammatory milieu, decreasing NF-κB (p65) levels and nuclear translocation, and reducing pro-inflammatory IL-1β and IL-6, even in M1-polarized MDMs. Elevated TNFα levels, however, were measured in dually-infected cells, reflecting a complex immunomodulatory landscape. Collectively, this study uncovers unanticipated Li's immunosuppressive abilities in facilitating Ltar's intracellular survival within human MDMs, suggesting complex multi-species dynamics with consequences for disease progression. These findings also highlight the capacity of Ltar to develop pathogenic traits in the context of Li-Ltar co-infections and, potentially, under broader immunosuppressed scenarios.

# DIAGNOSIS OF HUMAN LEISHMANIASIS: ANTIGEN RECOGNITION PATTERN BY WESTERN-BLOT ANALYSIS

Agnoli C.[1], Varani S.[2], Rugna G.[3], Rizzi E.[1], Granozzi B.[5], Ortalli M.[4], Piubelli C.[1], Longoni S.S.*[1]

*[1]Department of Infectious, Tropical Diseases and Microbiology IRCCS Sacro Cuore, Don Calabria Hospital, Verona, Italy; [2]Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy; [3]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Modena, Italy; [4]Unit of Microbiology, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy; [5]Infectious Diseases Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy*

**Keywords:** Leishmaniasis, Western-blot, Immunogenic profile.

**INTRODUCTION:** The prompt and correct diagnosis of leishmaniasis is of crucial importance for the correct management of the patient, preventing disabilities and death, as well as to monitor the parasite spread. Leishmaniasis diagnosis relies on parasitological, molecular, and serological tests. Parasitological or molecular assays usually require biopsy, equipped facilities and trained professionals. The serological methods represent the most suitable methods, particularly for screening. Different serological kits are commercially available, nevertheless their performance present high variability in sensitivity and specificity (van Griensven et al., 2019. Infect Dis Clin North Am, 33:79-99; Ortalli et al., 2020. Microorganisms, 8:1847). This variability is due to the *Leishmania* specie infecting the patients and to the antigen used to develop the tests. In this work, we analyze the antigenic pattern from the WHO referral strains of *Leishmania infantum* and *L. donovani*, and the putative *L. infantum/L. donovani* hybrid isolated in Emilia Romagna (Bruno et al., 2023. bioRxiv 2023.08.09.552585), to identify a possible antigenic candidate common to different *Leishmania* species.

**MATERIALS AND METHODS:** *L. infantum* strain IPT1 (MHOM/TN/80/IPT1), *L. donovani* strain DD8 (MHOM/IN/80/DD8) and putative *L. infantum/L. donovani* strain MO/38 (MHOM/IT/2016/IZSLER-MO038) were cultured in MEM medium supplemented with FBS. Promastigote of each *Leishmania* spp. were lysate using PhosphoSafe Extraction Reagent, and secreted proteins were obtained culturing the promastigotes in MEM non-supplemented with FBS for 24h and then the soluble protein were precipitated by ice-cold ammonium sulphate. Final concentration of 10µg proteins/well were loaded to an SDS-PAGE gel, blotted and the immunogenic profiles were analyzed by Western blot testing 11 laboratory confirmed *Leishmania* positive human sera and 30 negative human sera. Negativity was ensured performing two different serological tests, *LEISHMANIA* ELISA IgG+IgM (Vircell) and *LEISHMANIA* Western Blot IgG (LdBIO diagnostics).

**RESULTS AND CONCLUSIONS:** *Leishmania*-positive sera showed different immunogenic profile when tested on promastigotes' lysate of three *Leishmania* spp., with IPT1 and MO/38 showing similar patterns. These results are in accordance with literature, being the currently available test *Leishmania* specie-dependent. The strength of our analysis is confirmed by the presence of 14kDa and/or 16kDa bands in all positive samples using the lysate fractions. It is worth to remind that the positivity of the IVD CE Western-blot is based on the presence of one of those two bands. The immunogenic profile obtained from the lysates of promastigotes present a great number of non-specific bands that are not present in the secreted fraction. Indeed, data from this last antigenic fraction represent a promising possibility to unveil a successful pattern for serodiagnosis of leishmaniasis. Cross-reaction with other infectious disease, i.e. Chagas disease, malaria, TB and leprosy, will be checked.

# SCREENING FOR HUMAN NEGLECTED TROPICAL DISEASES (NTDs) CAUSED BY *STRONGYLOIDES STERCORALIS* AND *SCHISTOSOMA* SPP. IN PATIENTS OF COTUGNO HOSPITAL

Ascierto M.*[1], Panariello M.V.[2], Fioretti A.[2], Petrullo L.[2], Coppola M.G.[2], Galdiero M.[1]

*[1]Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy; [2]Azienda dei Colli of Naples, "D. Cotugno" Hospital, UOC Microbiology and Virology, Naples, Italy*

**Keywords:** Screening, Strongyloidiasis, Schistosomiasis.

**INTRODUCTION:** Strongyloidiasis and Schistosomiasis are serius problems of global health for humans, caused by *Strongyloides stercoralis* and *Schistosoma* spp, respectively. They are highly prevalent in tropical and subtropical areas, however they are widespread due to immigration and/or international travels. They are classified by WHO like two of 21 Neglected Tropical Diseases (NTDs) that if they are not suddnely diagnosed can cause mortality. It is important the screening of Strongyloidiasis and Schistosomiasis in immigrants patients or those returning from travels in endemic areas. The aim of this study, conducted between 2019 and 2024 at UOS of Parasitology of Cotugno hospital of Naples, is to analyze the number of positive patients screened for Strongyloidiasis and Schistosomiasis.

**MATERIALS AND METHODS:** The screening of *Strongyloides stercoralis* and *Schistosoma mansoni* infections were carried out through immuno-enzymatic assay (EIA). For the direct search for *Strongyloides stercoralis* and *Schistosoma mansoni*, feces of patients serologically positive were analyzed by Anglosaxon Ova and Parasites method (O&P), PCR+microarray (Novodiag Stool Parasites cartrige). The parasitological exam of urine was detected for diagnosis of *Schistosoma hematobium*. In addition, Agar plate culture was made for detection of *Strongyloides stercoralis*.

**RESULTS AND CONCLUSIONS:** Between January 2019 and January 2024, a total of 228 serum samples were screened for *Strongyloides stercoralis* antiboby: 18 resulted positives from immigrants patients (11 Africans, 1 Bengali, 1 Indian, 1 Messican, 1 Dutch, 2 Romanian, 1 Spanish) and 2 from Italian: the first backed home by travel, the second a patient candidate for renal transplant. For the detection of *Schistosoma mansoni* antibody a total of 216 samples were screened, of which 36 patients tested positive (29 Africans, 1 Bengali, 1 Dutch, 1 Italian, 1 Malesian, 2 Pakistan and 1 from Sri Lanka). Furthermore, 7 patients from Africa resulted positive for *Schistosoma hematobium*. The screening of Strongyloidiasis and Schistosomiasis is important for patients borned or residents in endemics areas for these NTDs and in those patients with risk factors, candidates for solid organ donors and/or transplantation.

# COMPARISON BETWEEN STANDARD REFERENCE AND INNOVATIVE TECHNIQUES FOR THE DIAGNOSIS OF INTESTINAL PARASITOSIS

Ascierto M.*[1], Panariello M.V.[2], Fioretti A.[2], Petrullo L.[2], Coppola M.G.[2], Galdiero M.[1]

*[1]Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy; [2]Azienda dei Colli of Naples, "D. Cotugno" Hospital, UOC Microbiology and Virology, Naples, Italy*

**Keywords:** O&P, Novodiag, Parasitosis.

**INTRODUCTION:** The diagnosis of intestinal parasitic infections still represents an important challenge for clinical microbiologists. For this reason, several companies, supported by the expertise of qualified parasitologists, have implemented the panorama of diagnostic tests, trying to standardize the methods and improve the accuracy and precision of the diagnosis. An easy-to-use solution is Novodiag Stool Parasites (NVD), a molecular test for the qualitative detection of human parasites. The aim of this work, conducted at the Parasitology laboratory of the P.O. "D. Cotugno", Regional Reference Center for the diagnosis of parasitic intestinal infections, was to compare the Reference Standard (O&P) techniques for the detection of intestinal parasites and a molecular test in random access microarray.

**MATERIALS AND METHODS:** For the detection of intestinal parasites, the exams were performed by: microscopic observation, Standard coproparasitological examination, ICT (*Cryptosporidium* spp., *Giardia duodenalis* and *Entamoeba histolytica*), IFI (*Cryptosporidium* spp. and *Giardia duodenalis*), Giemsa staining (intestinal flagellates), Kinyoun staining (coccidia), compared with Molecular Biology in microarray for the qualitative identification of 25 parasites including protozoa, helminths and microsporidia, performed on stool samples without preservatives, fresh or frozen.

**RESULTS AND CONCLUSIONS:** Of 536 samples of copros analyzed, 120 (22.3%) samples were positive for parasites: 65 (12.7%) *Blastocystis hominis*-83%sensivity(O&P) / 100%sensivity(NVD) + 100%specificity(O&P) / 100%specificity(NVD); 19(3.5%) *Giardia duodenalis* - 83%sensivity(O&P) / 100%sensivity(NVD) + 100%specificity(O&P) / 100%specificity(NVD); 19(3.5%) *Dientamoeba fragilis* - 38%sensivity(O&P) / 100%sensivity(NVD) + 100%specificity(O&P) / 100%specificity(NVD); 10(1.8%) *Cryptosporidium* spp. - 33.3%sensivity(O&P) / 100%sensivity(NVD) + 100%specificity(O&P) / 100%specificity(NVD); 1(0.1%) *Cystoisospora belli* - 0%sensivity(O&P) / 100%sensivity(NVD) + 100%specificity(O&P) / 100%specificity(NVD); 1(0,1%) *Entamoeba histolytica* - 100%sensivity(O&P) / 100%sensivity(NVD) + 100%specificity(O&P) / 100%specificity(NVD); 1(0.1%) *Enterobius vermicularis* - 100%sensivity(O&P) / 100%sensivity(NVD) + 100%specificity(O&P) / 100%specificity(NVD); 1(0.1%) *Strongyloides stercoralis* - 50%sensivity(O&P) / 100%sensivity(NVD) + 100%specificity(O&P) / 100%specificity(NVD); 2(0.3%) *Schistosoma mansoni* - 100%sensivity(O&P) / 100%sensivity(NVD) + 100%specificity(O&P)/100%specificity(NVD) and 1(0.1%) *Enterocytozoon bieuneusi* - 0%sensivity(O&P) / 100%sensivity(NVD) + 100%specificity(O&P) / 100%specificity(NVD).
The Novodiag Stool Parasites test provides a valuable strategy in parasitological diagnostics, but combined with Traditional Methods, as parasitologist experience and Microscopic confirmation remain fundamental.

# PARASITIC DISEASES AMONG ASYLUM SEEKERS IN ITALY: PRELIMINARY RESULTS FROM A CROSS-SECTIONAL STUDY

Gabrielli S.*, Maddaloni L., Gentilini E., Filippi V., D'Errico R., Ceccarelli G., Mastroianni C., Dettorre G.

*Sapienza University of Rome, Department of Public Health and Infectious Diseases, Rome, Italy*

**Keywords:** Asylum seekers, Migrations, Diagnosis.

**INTRODUCTION:** After the significant reduction in migratory flows observed during the first phase of the pandemic, the arrivals of migrants in southern Europe have progressively grown, putting the reception systems under pressure. A general screening based on a syndromic approach is applied in a wide range of reception centers, while the nonspecific symptoms of the parasitic diseases could be easily misreported (Ciccozzi et al., 2017. Trav Med Infect Dis, 1:8). The purpose of this study is to evaluate the usefulness of parasitological screening in migrant populations to intercept parasitic diseases that are often misunderstood and relevant from a public health point of view.

**MATERIALS AND METHODS:** From January to June 2023, we collected data on symptoms and physical examination of newly arrived migrants, hosted at the Asylum Seekers Centre "Mondo Migliore", Rome, Italy. Stool and urine samples collected from each subjects included in the study were analyzed with different methods to assess the presence of intestinal or urinal parasites. Sera were also tested for *Leishmania*, *Strongyloides* and *Schistosoma* spp. antibodies using commercial test (Bordier).

**RESULTS AND CONCLUSIONS:** A total of 87 migrants out 276 consented to participate in the study. The median age was 30.5 (26-40) years and a large majority (90.2%) were male. The most represented nationalities were Bangladesh (21.5%), Egypt (17.9%), and Pakistan (10.7%). Seven patients referred abdominal and/or pelvic discomfort, while 87.6% were asymptomatic. Intestinal parasites were found in 10.3% of the participants. *Entamoeba dispar* was identified in 3.4% of the samples, followed by *E. coli* (2.3%) and *Blastocystis* spp. (2.3%). One participant was infected with both *Blastocystis* spp. and *Endolimax nana*, and eggs of Taenidae were found in the stool of one subject, which were further molecularly identified as *Taenia solium*. Urine samples tested negative for *Schistosoma haematobium* eggs, while IgG antibodies against *Schistosoma* were found in two subjects (2.3%). Similarly, none stool samples resulted positive for *Strongyloides* larvae but anti-*Strongyloides* IgG antibodies were detected in one participant (1.2%). Finally, all the enrolled subjects tested negative for *Leishmania* antibodies. A surprisingly low prevalence for intestinal parasites (20.6%), which were mostly protozoa, has been reported by this study aimed to perform a parasitological screening in N=87 newly arrived migrants. The species mainly identified were the common lumen intestinal parasitic protists found also in non-tropical areas (*Blastocystis* spp., *E. coli* and *E. nana*). However, serological tests revealed hidden *Schistosoma* and *Strongyloides* infections, highlighting the importance of performing parasitological surveys in order to evaluate the reliable risk of parasites introduction in hosting countries.

# EXPLOITING A XENOSURVEILLANCE APPROACH ON NON-VECTOR MOSQUITOES TO DETECT MALARIA PATHOGENS IN DJIBOUTI CITY

Manzi S.*[1], Zaccaria O.[2], Abbate V.[2], Pazienza M.[5], Micocci M.[3], Perugini E.[3], Pichler V.[3], Montarsi F.[1], Caforio R.[2], De Santis R.[4], Lista F.[4], Pombi M.[3]

*[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy; [2]Stato Maggiore della Difesa, Rome, Italy; [3]Sapienza University of Rome, Department of Public Health and Infectious Diseases Rome, Italy; [4]Istituto di Scienze Biomediche Della Difesa, Rome, Italy; [5] Comando Tecnico dell'Esercito, Rome, Italy*

**Keywords:** Malaria, Xenosurveillance, *Anopheles stephensi*.

**INTRODUCTION:** Entomological surveillance is broadly applied to detect pathogens in mosquito vectors and estimate the risk of exposure to mosquito-borne diseases. Irrespective to vector competence, blood-fed mosquitoes could also be used as "biological syringes" to highlight pathogen circulation in human and animal populations (xenosurveillance) (Grubaugh et al., 2015. PLoS Negl Trop Dis, 9:e0003628). In this study, a xenosurveillance approach was applied in Djibouti to detect malaria parasites in blood-fed non-vector mosquitoes. After the introduction of the Asian vector, *Anopheles stephensi*, an increase in malaria cases is occurring in this area (Seyfarth et al., 2019. Parasitol Res, 118:725-32). The implementation of surveillance strategies is essential to prevent malaria outbreaks and a xenosurveillance approach could enhance early pathogen detection in areas where *Anopheles* vectors might be at low densities.

**MATERIALS AND METHODS:** From January to February 2020, 11 sticky resting box (SRB) (Pombi et al., 2014. Parasit Vectors, 7:247) and 12 BG-sentinel (BG-S) traps modified with a sugar feeding system (Manzi et al., 2023. Sci Rep, 13:12840) were deployed in Djibouti City. SRBs were serviced weekly, while BG-S worked for four consecutive days. Collected mosquitoes were morphologically identified to define species and gonotrophic stage. All abdomens from fed culicine females (e. g., *Culex* and *Aedes* spp.) were analysed through DNA extraction (Rider et al., 2012. Malar J, 11:193) and PCR to define the blood meal host (Kent and Norris, 2005. Am J Trop Med Hyg, 73:336-42); additionally *Anopheles* females were subjected to DNA extraction from head and thorax. DNA extracted from each mosquito genus was further processed to detect *Plasmodium* spp. through PCR and sequencing (Calzetta et al., 2018. Med Vet Entomol, 32:372-77).

**RESULTS AND CONCLUSIONS:** Overall, 14,378 mosquitoes were sampled during the study period; of these the 92.5% was collected with BG-S traps. Culicinae represented almost all of the total sample and included *Cx. quinquefasciatus* (96.7%), *Ae. aegypti* (2.6%) and *Cx. sitiens* (0.2%). Collected Anophelinae were *An. stephensi* (0.5%) and *An. dthali* (0.1%). The blood meal source was successfully identified in 26.4% of sample showing blood evidence (N: 500) and 46.9% of these fed on human hosts. No malaria parasites were detected in *Anopheles* species, which could be explained by the low number of collected females (N: 36). Conversely, *P. falciparum* was detected in different dates (31$^{th}$Jan, 15$^{th}$ and 20$^{th}$ Feb) from six human-fed culicine mosquitoes (*Cx. quinquefasciatus*: 3 and *Ae. aegypti*: 3). According to our findings, molecular detection of pathogens in blood fed non-vector mosquitoes make malaria surveillance more feasible at low vector density. Enhancing surveillance is needed to reduce malaria burden and a xenosurveillance approach could be more effective in a low transmission context, such as in areas of new introduction or where eradication plans occur.

# SURVEILLANCE OF IMPORTED MALARIA IN ITALY: UPDATE OF THE LAST SEVEN YEARS, 2017-2023

Boccolini D.[1], L'Episcopia M.*[1], Menegon M.[1], Caraglia A.[2], Ferraro F.[2], Maraglino F.[2], Severini C.[1]

*[1]Istituto Superiore di Sanità, Dipartimento Malattie Infettive, Rome, Italy; [2]Ministero della Salute, Direzione Generale Prevenzione Sanitaria, Ufficio 5 Prevenzione delle Malattie Trasmissibili e Profilassi Internazionale, Rome, Italy*

**Keywords:** Mandatory notifiable disease, Epidemiology, Drug-resistance.

**INTRODUCTION:** In tropical and sub-tropical areas, particularly in Sub-Saharan Africa, malaria still represents the most important vector-borne disease. Despite the efforts made by the international community to control this disease, the latest World Malaria Report (WHO, 2023) records 249 million of malaria cases worldwide in 2022, 5 million more than in the previous two years, and 608,000 deceases, the majority of whom are African children under five ages. The lack of a not yet fully effective vaccine, the spread of artemisinin-resistant *Plasmodium falciparum* strains and of insecticide-resistant *Anopheles* vector populations, currently represent the major issues in the fight against this disease. Furthermore, malaria represents a substantial problem also in non-endemic countries. In 2022, in Europe approximately 6,000 imported cases are reported, accounting the highest number of cases in France, Germany, Spain, and Italy respectively. This study provides an update of the relevant epidemiological peculiarities of the imported malaria cases in Italy in the period 2017-2023.

**MATERIALS AND METHODS:** Analyses on imported malaria were based on computerized data of the cases notified by the Local Health Services and microscopically confirmed (0.3% also molecularly confirmed) by the National Malaria Surveillance System (i.e. Ministry of Health and Istituto Superiore di Sanità).

**RESULTS AND CONCLUSIONS:** In the study period, the general trend was particularly fluctuant due to the drop in the number of cases in 2020-2021 for COVID-19 pandemic. Overall, 4,295 total cases, including 13 non-traveled related, were reported, resulting in an annual average of about 600 cases. Consistent with national data of the previous years and with the European epidemiology, in 2017-2023 the imported cases were mainly caused by *P. falciparum* of African origin (99%); males (70%) and 25-44 age (45%) were the most represented groups; reason of travel included Visiting Relatives and Friends for 68%. The deaths were 18 falciparum cases, with a fatality rate increased (0.5%) respect to previous years (<0.1%). In the notification forms with reported chemoprophylaxis (14%), adherence was mainly incomplete. Therapeutic failures were observed in 17 notified *P. falciparum* cases (0.4%) for which molecular analysis of antimalarial drug resistance markers were conducted. No mutations in *P. falciparum* kelch13 gene associated with artemisinin delayed parasite clearance were detected. In 2023, molecular analysis of antimalarial drug resistance-associated genes in an imported case from Africa revealed the I356T mutation in Pfcrt gene, assumed to be one of the background genetic changes for artemisinin resistance. These data show how the global raising concern for drug-resistant malarial parasites, associated with the suspected failure of artemisinin combination therapies, has increasingly led clinicians to request prompt molecular investigations and to opt for prolonged or alternative treatments.

# IDENTIFICATION OF DIPTERA LARVAE CAUSING OPPORTUNISTIC MYASIS IN PATIENTS FROM BOLOGNA, NORTH EASTERN ITALY

Dini F.M.[1], Galuppi R.[1], Fioravanti M.[1], Varani S.[2], Ortalli M.[3], Liguori G.[3], Gustinelli A.*[1]

*[1]Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy; [2]Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy; [3]Unit of Microbiology, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy*

**Keywords:** Myasis, *Sarcophaga*, *Lucilia*.

**INTRODUCTION:** Myiasis is a condition in which fly maggots feed on and develop within living organisms' tissues. There are two forms of myiasis: obligate, where maggots require living tissues to feed on, and facultative, where flies opportunistically use wounds, necrotic tissues, or body cavities for larval development. Among predisposing factors for myiasis are diabetes, immobility, poor hygiene, and compromised immune status (Verettas et al., 2008. Trans R Soc Trop Med Hyg, 102:950-52). These opportunistic parasites pose a significant challenge, particularly in disadvantaged settings, poverty-stricken areas, and healthcare settings. Accurate identification of the myiasis-causing larvae is crucial not only for therapeutic intervention, but also to determine the source of infestation and implement prompt control and disinfection measures. Here, we present the identification of Diptera larvae that were collected from seven different cases of opportunistic myiasis. Infested patients were visited or admitted to clinical centers of Bologna, north eastern Italy.

**MATERIALS AND METHODS:** Larvae were collected from 6 patients ranging in age from 1 to 96 years. The parasites were fixed in 70% ethanol and processed for identification. Morphological identification of the third-stage larvae was conducted using identification keys (Zumpt, 1965, Myasis in Man and Animals in the Old World, Butter Worths, London; Szpila, 2009, in Amendt et al., Current Concepts in Forensic Entomology. Springer, dordrecht, 43-56). Additionally, one specimen from each patient was processed for molecular identification: internal organs were dissected to extract DNA, and identification was performed by sequencing a 710 bp segment of the mitochondrial COI gene, commonly used as a molecular target for insect barcoding.

**RESULTS AND CONCLUSIONS:** Larvae causing myiasis were found in different body sites: three cases involved wounds, all located in the lower limbs (mostly affecting the feet); in one case larvae were collected in the skin of the pubic area; in one case derived from nasal myiasis; and in the last case larvae were found in the drainage of an intra-abdominal collection. *Sarcophaga* sp., molecularly identified as *S. argyrostoma*, was found in the cases of heel ulcers and drainage colonization. *Lucilia sericata*, confirmed by COI sequencing, was identified in two cases of lower limb wounds and one case of pubic skin colonization. Additionally, *Megaselia scalaris* was identified through sequencing in a case of nasal myiasis in a 1-year-old child, as only first-stage larvae were present. Overall, the results indicate that opportunistic myiasis are parasitic condition that warrants consideration, particularly for individuals who are not self-sufficient, including those in healthcare settings as well as in case of poor hygienic conditions.

# *SCHISTOSOMA HAEMATOBIUM* TETRASPANINS TSP-2 AND TSP-6 INDUCE DENDRITIC CELLS MATURATION, CYTOKINE PRODUCTION AND T HELPER CELLS DIFFERENTIATION *IN VITRO*

Silvano A.[1], Sotillo J.[2], Cecchi M.[1], Loukas A.[3], Ouedraogo M.[4], Parenti A.[1], Bruschi F.[5], Torcia M.G.[1], Mangano V.*[5]

*[1]University of Florence, Florence, Italy; [2]Instituto de Salud Carlos III, Madrid, Spain; [3]James Cook University, Cairns, Australia; [4]Centre National de Recherche et Formation sur le Paludisme, Ouagadougou, Burkina Faso; [5]University of Pisa, Pisa, Italy*

**Keywords:** *Schistosoma haematobium*, Tetraspanins, Dendritic Cells.

**INTRODUCTION:** Urogenital schistosomiasis caused by *Schistosoma haematobium* is a major cause of disability in endemic areas, mostly in Africa and the Middle East. Despite the socio-economic impact of the disease, no vaccine is available as yet and knowledge regarding this parasite immunobiology is limited. Annotation of *S. haematobium* genomes has revealed over 40 different genes encoding for tetraspanins, transmembrane proteins that have recently shown immunomodulatory properties in other plathelminthes. In particular, Sh-TSP-2, Sh-TSP-6 and Sh-TSP-23 are expressed in tegument and extracellular vesicles (EVs) and have been shown to be recognised by the immune system.

**MATERIALS AND METHODS:** Immature dendritic cells (DCs) from unexposed healthy donors were stimulated with each of the three proteins and the expression of maturation makers as well as was the production of cytokines was quantified. Furthermore, the DCs supernatant was used to stimulate pre-activated T CD4+ cells, and cytokine gene expression was evaluated.

**RESULTS AND CONCLUSIONS:** Results show that the Sh-TSP-2 and Sh-TSP-6 were able to induce to expression of maturation markers and the production of cytokines by DCs. In particular, Sh-TSP-2 increased the expression of maturation markers CD80 and CD83 as well as the production of pro-inflammatory (IL-6, TNFα) and regulatory (IL-10) cytokines in DCs. A dose-response effect was observed for Sh-TSP-2, with an optimal concentration of 10μg/ml. Additionally, the supernatant from Sh-TSP-2 stimulated DCs induced the expression of Th1 (IFNɣ) and regulatory (IL-10) cytokines in CD4+ T cells, while Sh-TSP-6 induced the expression of Th2 (IL-4, IL-13) cytokines. These results provide the first evidence that tetraspanins from *S. haematobium* have an immunomodulatory effect on human DCs and CD4+ T cells *in vitro*, contributing to the understanding on the complex interplay between the parasite and the human host, and support the potential of Sh-TSP-2 as a vaccine candidate against urogenital schistosomiasis.

# COMPARATIVE EVALUATION OF PLASMA BIOMARKERS OF *SCHISTOSOMA HAEMATOBIUM* INFECTION IN ENDEMIC POPULATIONS FROM BURKINA FASO

Ouedraogo M.[1], Hey J.C.[2], Hilt S.[3], Rodriguez Fernandez V.[4], Winter D.[2], Razafindrakoto R.[5], Hoekstra P.[3], Kabore Y.[1], Fornili M.[4], Baglietto L.[4], Ouedraogo I.N.[1], Van Dam G.[3], Corstjens P.[3], Fusco D.[6], Modiano D.[7], Bruschi F.[4], Mangano V.*[4]

*[1]Centre National de Recherche et Formation sur le Paludisme, Ouagadougou, Burkina Faso; [2]Bernard Nocht Institute for Tropical Medicine, Hamburg, Germany; [3]Leiden University Medical Center, Leiden, Netherlands; [4]University of Pisa, Pisa, Italy; [5]Centre d'infectiologie Charles Merieux, Antananarivo, Madagascar; [6]Bernard Nocht Institute for Tropical Medicine, Hambourg, Germany; [7]University La Sapienza of Rome, Rome, Italy*

**Keywords:** *Schistosoma haematobium*, Plasma biomarkers, Diagnosis.

**INTRODUCTION:** Infection with *Schistosoma haematobium* causes urogenital disease associated with organ disfunction, bleeding, pain, and higher susceptibility to infections and cancer. Timely and accurate diagnosis is crucial for prompt and appropriate treatment as well as surveillance efforts, and the use of plasma biomarkers offers important advantages over parasitological examination of urine, including increased sensitivity and the possibility to use the same specimen for multiple investigations. The present study aims to evaluate the diagnostic performance of different plasma biomarkers in endemic populations from Burkina Faso, West Africa.

**MATERIALS AND METHODS:** *Schistosoma* spp. Circulating Anodic Antigen (CAA), cell free *S. haematobium* DNA (cfDNA), class M and G antibodies against *S. haematobium* Soluble Worm Antigen Preparation (SWAP) and Soluble Egg Antigen (SEA) were measured in 406 plasma samples. Results of each biomarker test were compared to those of CAA, a Composite Reference Standard (CRS) and Latent Class Analysis (LCA).

**RESULTS AND CONCLUSIONS:** An identical proportion of positive samples (29%) was observed as a result of CAA and cfDNA testing, with a substantial agreement (84%, Cohen k=0.62) between the results of the two tests, and a comparable agreement with the results of CRS and LCA. A higher positivity was observed, as expected, as a result of specific antibody testing (47%-72%), with IgG showing a higher agreement than IgM with the three references. Also, higher IgG levels were observed in current vs past infection, and ROC analysis identified optimal cutoff values for improved testing accuracy.
This study provides compelling evidence that can inform the choice of the most appropriate diagnostic plasma biomarker for urogenital schistosomiasis in endemic areas, depending on the purpose, context, and available resources for testing. Either CAA or cfDNA testing can be used for the diagnosis of patients and for epidemiological investigations, even in absence of urine filtration microscopy, whereas anti-SWAP or anti-SEA IgG can be employed for surveillance and integrated monitoring of control interventions against poverty-associated diseases.

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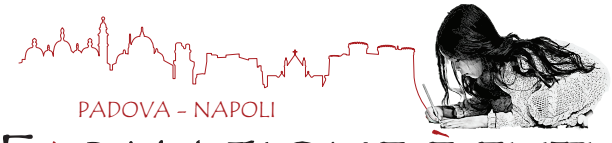
# MYCOTIC DISEASES



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# PROSPECTIVE SINGLE-ARM STUDY ON THE EFFECT, SAFETY AND TOLERABILITY OF TOPICAL 0.1% TAZAROTENE GEL IN ONYCHOMYCOSIS TREATMENT: FROM *IN SILICO* TO *IN VIVO* RESULTS

Cosio T.*, Campione E., Pistoia E.S., Gaziano R.

*University of Rome Tor Vergata, Rome, Italy*

**Keywords:** Tazarotene, Onychomycosis, Translational research.

**INTRODUCTION:** Onychomycosis is a fungal infection of the nail unit frequently observed in clinical practice. To date, we are witnessing a progressive increase in cases of resistant dermatomycosis, which is becoming a public health problem related to the One-Health perspective. Despite the availability of several antifungal drugs for clinical use, multidrug-resistant (MDR) fungi are observed in daily clinical practice. It is, therefore, necessary to identify new molecules capable of blocking or preventing fungal infection, reducing cases of drug resistance. In this regard, previous studies about the role of retinoids (vitamin A derivates) against *Pneumocystis jirovecii* human infections have been reported, as antifungal agents. To confirm the role of retinoids in mycotic diseases, we conducted a prospective, single-arm study on the effect, safety and tolerability of topical 0.1% tazarotene gel, a vitamin a derivative, in treating onychomycosis and *in vitro* and *in silico* studies to clarify its mechanism of action.

**MATERIALS AND METHODS:** Thirty patients were treated with topical 0.1% tazarotene gel once daily for 12 months, with 3-month follow-up visits. The primary objective concerned the evaluation of clinical efficacy by changing the in Onychomycosis Severity Index (OSI), in onycholysis score and subungual hyperkeratosis. The secondary objective concerned the assessment of tolerability, safety, and improving the quality of life as per the dermatology life quality index (DLQI). The potential targets of the tazarotene were evaluated by *in silico* analyses by molecular docking and Dynamic Cross-Correlation Matrix (DCCM) analysis, and the *in vitro* antifungal activity was assessed against clinically isolated strains by the broth microdilution method, the biofilm quantification by Crystal Violet and XTT assays, the visualization and quantification of cells vitality by Calcofluor White (CW) and Propidium Iodide (PI).

**RESULTS AND CONCLUSIONS:** The mycological examination revealed the presence of *Trichophyton* spp. (65%), *Candida* spp. (15%), *Aspergillus* spp. (10%) *Fusarium* spp. (10%) and *Scopulariopsis brevicaulis* (10%). The average OSI score at baseline (T0) was 6.7, diminishing to 1.8 after 12 months of treatment ($p<.001$). Hyperkeratosis changes from an average thickness of 1.4 mm to 0.6 mm after 12 months. Onycholysis was improved in the entire population, passing from an average value of 2.04 to 0.04. The DLQI score decreased from 19.2 to 2.5. At 15 months follow-up, no local reinfections were reported. Protein-ligand molecular docking and DCCM have demonstrated that tazarotene can fit the Heat Shock Protein (HSP) 90 ATP-binding site in the N-terminal domain, indicating that this compound may be considered a competitive inhibitor. In vitro results demonstrated that tazarotene negatively affects fungal growth and biofilm formation in terms of biomass, metabolic activity and morphology in a dose-dependent manner, and its efficacy was comparable to that of amphotericin B (AmB) (2 - 0.12 µg/mL).

# MYCOLOGICAL SURVEY AND ANTIFUNGAL SUSCEPTIBILITY OF *CANDIDA ALBICANS* ISOLATED FROM EUROPEAN HEDGEHOGS (*ERINACEUS EUROPAEUS*) RESCUED IN CENTRAL ITALY

Morganti G.*[1], Brustenga L.[1], Gobbi M.[2], Ranucci A.[2], Rigamonti G.[1], Moretta I.[1], Calgaro V.[1], Cruciani D.[2], Crotti S.[2]

*[1]University of Perugia, Department of Veterinary Medicine, Perugia, Italy; [2] Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy*

**Keywords:** Parasitic fungi, European hedgehog, *Candida albicans*.

**INTRODUCTION:** The European hedgehog (*Erinaceus europaeus*) is a small mammal widely distributed throughout Europe with nocturnal habits and an omnivorous diet, mostly feeding on small invertebrates (Mitchell-Jones et al., 1999. The atlas of European mammals, Poyser, London, 38-39). European hedgehogs are an important reservoir for many species of fungi of parasitological interest that can inhabits their integument and gastrointestinal system. Among these, dermatophytes like *Trichophyton mentagrophytes* var. *erinacei* can play a substantial role in superficial mycoses (Gnat et al., 2021. Microb Ecol, 84:363-75) whereas yeasts like *Candida albicans* can cause both superficial and systemic mycoses (Mayer et al., 2013. Virulence, 4:119-28). European hedgehogs have adapted to live in close contact with humans, that provide them with food and water, thus encouraging the use of human spaces. Therefore, a survey of potential zoonotic fungi shared from hedgehogs and humans could be of great public health concern, especially in urban areas with high hedgehog density.

**MATERIALS AND METHODS:** A total of 134 hedgehogs admitted to a Wildlife Rescue Center in Central Italy were enrolled in the survey from 2020 to 2023. To avoid stressful and invasive procedures, live animals were brushed with a sterile toothbrush to sample the integument fungal population, whereas, along with brush tests, oral and rectal swabs were taken from deceased animals. Dermatophytes were searched using Dermasel agar and proceeding with macro- and micromorphological evaluation of colonies and biomolecular identification following a PCR-sequencing approach (Crotti et al., 2023. J Fungi, 9:865). Yeasts were recovered by plating samples on Sabouraud agar additioned with chloramphenicol; yeast-like colonies were also cultured on CHROMagarTM *Candida* and identified using a MALDI-TOF approach. Minimal Inibitory Concentrations (MICs) of a panel of antimycotics were also determined for *C. albicans* isolated in 2023.

**RESULTS AND CONCLUSIONS:** Dermatophytes were detected in just one of the 134 hedgehogs sampled (0.8%, 95%CI: 0-0.04) and the isolate was genetically identified as *Paraphyton cookei*. Yeasts were detected in 22 out of 134 hedgehogs (16.4%, 95%CI: 0.11-0.24); from them a total of 25 isolates were obtained and identified as: 21 *Candida albicans*, 2 *Yarrowia lipolitica*, 1 *Rhodotorula mucilaginosa* and 1 *Meyerozyma guilliermondii*. All the MICs values showed high susceptibility of *C. albicans* isolates to the whole panel of the antimycotics tested. The detection of dermatophytes in just one hedgehog was rather unexpected as higher infection rates were described in scientific literature. On the other hand, the results of the MIC assays are comforting, showing that the available antimycotic drugs would be effective in case of human infections. A monitoring effort of the zoonotic fungi harbored by European hedgehogs, along with public awareness on the topic, can therefore be of great importance in a public health framework.

# SAMPLING AND ISOLATION OF *MALASSEZIA* SPP. IN PETS AND THEIR OWNERS

Miglianti M.*, Rizzo A., Cafarchia C., Otranto D.

*Department of Veterinary Medicine, University of Bari, Valenzano, Italy*

**Keywords:** *Malassezia* spp., Sampling methods, Isolation media.

**INTRODUCTION:** *Malassezia* spp. are lipid-dependent yeasts commensals of human and animal skin (Ugochukwu et al., 2023. Expert Rev Anti Infect Ther, 21:1327-38). The *Malassezia* spp. occurrence and distribution on animal and human skin vary according to the methods employed for their sampling and isolation in different cultural media, thus causing heterogeneity in culture-based epidemiological studies (Findley et al., 2013. Nature, 498:367-70; Abdillah et al., 2020. J Fungi, 6:350). Recently the FastFung medium was proposed as the most efficacious for the detection of *Malassezia* spp. on human skin (Abdillah et al., 2020. J Fungi, 6:350; Atsü et al., 2022. Mycoses, 65:704-8). Since few studies have been performed to compare the performance of different isolation methods, this study aimed to compare the performances of two methods of skin sampling and two culture media for the detection of *Malassezia* yeasts from healthy dogs and their owners.

**MATERIALS AND METHODS:** Two skin-sampling methods, namely sterile gauzes and dry swabs, were applied on three different skin sites of 12 healthy dogs (external ear channels, perianal and perioral areas) and on the palmar hands of 12 respective dog owners. A total of 96 samples were collected and cultured onto either the novel FastFung medium and the reference Dixon agar for the detection of *Malassezia* spp. by culture.

**RESULTS AND CONCLUSIONS:** A total of 45/96 (46.9%) samples were positive for *Malassezia*. A higher occurrence of *Malassezia* spp. was recorded in samples collected by using sterile gauze (54.2%) than those registered by using swabs (39.6%). *Malassezia* spp. was isolated from human skin only by using sterile gauze (16.7%). The highest occurrence and population size of *Malassezia* spp. was recorded by using FastFung medium (42.7%) compared to Dixon agar (35.4%). Human samples were positive only when FastFung was employed as medium. Our results showed that sterile gauze rubbing skin sampling followed by inoculation on FastFung medium is the best procedure to detect the presence and the abundance of *Malassezia* spp. both in human and in animal skin. These procedures should be implemented in the routine clinical laboratory for *Malassezia* spp. detection by using cultural procedure.

# FIRST DESCRIPTION OF FELINE PYTHIOSIS IN EUROPE

Sgubin S.[1], Orlandini P.[2], De Lucia M.[2], Cagnin V.[1], Pasqualotto S.[1], Matteucci G.[3], Peano A.[4], Danesi P.*[1]

*[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy; [2]San Marco Veterinary Clinic and Laboratory, Veggiano, Italy; [3]AbLab Veterinary Diagnostic Laboratory, Sarzana, Italy; [4]Dipartimento di Scienze Veterinarie, Università di Torino, Grugliasco, Italy*

**Keywords:** *Pythium insidiosum*, Cat, Europe.

**INTRODUCTION:** Pythiosis is a granulomatous and frequently life-threatening infection caused by two species of oomycete (fungus-like) organisms, *Pythium insidiosum* and *Pythium periculosum*. They occur worldwide and affect several animal species and humans, although most cases occur in immunocompetent horses and dogs in tropical and subtropical regions. To date, only a few cases of pythiosis have been reported in cats in the USA (n=15) and Brazil (n=4; Souto et al., 2020. J Mycol Med, 30(3):101005; Dowst et al., 2019. Med Mycol Case Rep, 26:57-60), mainly affecting the skin, nasal subcutaneous tissue, retrobulbar regions, oral cavity and intestines. In Europe, only a few cases have been reported in humans in Spain (Del Castillo-Jiménez et al., 2013; Bernheim et al., 2019. Int J Infect Dis, 86:40-43) and dogs in Italy (Peano et al., 2023. Emerg Infect Dis, 29:1447-50). To our knowledge, this study describes the first European case of cutaneous Pythiosis in a cat.

**MATERIALS AND METHODS:** A six-month-old male domestic shorthair cat was presented for a five-month history of progressive, not-painful swelling of the scrotal region. Physical examination showed a mass with intact overlying skin involving the perineal area and scrotum. The cat was otherwise healthy. CT scan revealed extensive involvement of the subcutaneous tissue and severe enlargement of the regional lymph nodes. Fine needle aspiration of the ischiatic lymph nodes showed granulomatous inflammation with fungal hyphae. After Grocott staining, histopathological examination of the subcutaneous tissue revealed multifocal to diffuse granulomatous panniculitis with non-pigmented and slightly septate fungal hyphae. Swabs and biopsy tissue were tested by mycological culture on Sabouraud dextrose agar at the Parasitology and Mycology Unit of IZSVE. Identification was molecularly confirmed by sequencing of the ITS1/2 and 28SLSU rRNA DNA extracted from culture and biopsy.

**RESULTS AND CONCLUSIONS:** Blasting on the GenBank database confirmed *Pythium* insidiosum from both culture and biopsy with ITS (similarity 98.5%; PP338270) and 28S LSU (similarity 99.5%; PP338776) sequences. A significant reduction of the subcutaneous mass was not observed after ten months of systemic antifungal treatment with terbinafine (30 mg/kg/24h) and itraconazole (10 mg/kg/24h) or azithromycin (10 mg/kg/24h). The general clinical conditions remained unaltered. *Pythium* species living in water and moist soil occur mainly as plant pathogens, and only *P. insidiosum* shows strong tropism for animal hair and tissue. Microbiological culture is occasionally negative; therefore, definitive diagnosis should rely on molecular tool directly from tissue or immunohistochemistry when available. *Pythium* infection should be considered a differential diagnosis of non-painful subcutaneous perineal nodules or masses in young cats.

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# CUTANEOUS FUSARIOSIS IN LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*): EFFICACY OF A TREATMENT WITH A MIXTURE OF ESSENTIAL OILS

Marchiori E.*[1], Marcer F.[1], Zoroaster A.[1], Segati S.[2], Tentoni E.[3], Scozzoli M.[3], Danesi P.[4]

*[1]Department of Animal Medicine, Production and Health, University of Padova, Legnaro (PD), Italy; [2]Centro Sperimentale per la Tutela degli Habitat (CESTHA), Marina di Ravenna (RA), Italy; [3]Centro ricerca sperimentale APA-CT srl, Forlì, Italy; [4]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy*

**Keywords:** Fusariosis, Essential oils, Loggerhead Sea Turtles.

**INTRODUCTION:** Fusariosis is a mycotic disease caused by filamentous fungi of the genus *Fusarium*. Among marine animals, skin and pulmonary infections have been described in pinnipeds and sea turtle species, both free-ranging and in captivity. In rescue centres, the risk of opportunistic infection is increased by the presence of fungal spores in the water circuit and by immunosuppression due to stressful conditions (Cafarchia et al., 2020. Vet Pathol, 57:139-46). In most studies, *Fusarium solani* species complex is reported as the main etiological agent in turtles. In this study, we report the preliminary results of essential oils (EOs) use for treating superficial fusariosis in loggerhead sea turtles hosted at the CESTHA rescue centre (Marina di Ravenna, Italy).

**MATERIALS AND METHODS:** Over the period December 2022-July 2023, 15 juvenile and subadult loggerhead sea turtles, either newly admitted or already undergoing rehabilitation, showed erosive to ulcerative lesions on carapace, head and/or limbs skin. In most severe cases, exposition of underlying bones and involvement of more than 50% body surface were observed. Samples of skin lesions' scrapings for mycological culture were obtained from the lesion periphery and the isolates were identified by morphology and PCR as belonging to *Fusarium solani* species-complex. After ineffective treatment with iodopovidone ointment, a mixture of EOs and herbal ingredients was used (B-Red Oil, Greenvet®), both through dilution in tanks water (30 ml to a maximum of 45 ml per 1,000 water liters) and topical application once daily after sunbathing for 30 minutes sessions, before returning the turtles in the water.

**RESULTS AND CONCLUSIONS:** Complete clinical recovery was achieved in all turtles in an average of one month, though most severe cases required more than 4 months for complete lesions disappearance. In vitro tests to assess the efficacy of single compounds of the EOs mixture and their synergistic activity are ongoing. The antifungal activities of EOs in a formulated mixture represent a promising alternative for the therapy of fusariosis, avoiding the toxicity of classical antifungal drugs. In order to limit the spread of fungal infection in rescue centres, prompt treatment of infected, ill animals entering the facility and disinfection of tank filters and water is advisable.

# MOLECULAR DATA OF *ENTEROCYTOZOON BIENEUSI* FROM HUMAN AND ANIMAL HOSTS IN ITALY

Guadano Procesi I.*[1], Berrilli F.[1], Rinaldi L.[2], Bosco A.[2], Petrullo L.[3], Ascierto M.[3], Di Ieso I.[1], Di Cave D.[1]

*[1]Department of Clinical Sciences and Translational Medicine, Faculty of Medicine, University of "Tor Vergata", Rome, Italy; [2]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy; [3]Azienda dei Colli of Naples, "D. Cotugno" Hospital, UOC Microbiology and Virology, Naples, Italy*

**Keywords:** Microsporidia, *Enterocytozoon bieneusi*, One Health.

**INTRODUCTION:** *Enterocytozoon bieneusi*, one of 17 microsporidian species harmful to humans in low- and high-income countries, causes symptomatic and asymptomatic intestinal infections in immunocompetent and immunocompromised individuals. Faecal-oral transmission occurs in several hosts, including various animal species, although the parasite's zoonotic potential is still unknown (Guadano-Procesi et al., 2024. Acta Trop, 252:107236). Few studies are available in Italy regarding *E. bieneusi* presence and diversity in humans, and no data on its genetic variability in animal hosts are so far reported. Through ITS rRNA sequence analysis, we presented the first *E. bieneusi* molecular data from animal hosts and updated data from humans in Italy.

**MATERIALS AND METHODS:** The commercial panel Novodiag® Stool Parasites screened stool samples from 376 patients submitted for parasitological analyses to the Azienda Ospedaliera Universitaria Policlinico Tor Vergata, Rome (July 2023-March 2024). One sample from Ospedale Cotugno, Azienda Ospedaliera dei Colli (Campania region) was also examined using the same commercial panel. Genomic DNA from *E. bieneusi*-positive samples was extracted by EZ1 automatic extractor. Moreover, from September to December 2023, 34 fresh faecal samples from buffalo calves and sheep were collected and DNA extracted using a commercial kit (QIAamp DNA Stool Mini Kit, QIAGEN). Nested amplification of the ITS rRNA region was performed (Buckholt et al., 2002. Appl Environ Microbiol, 68:2595-99). Allocation was done by comparing the obtained sequences to reference sequences from NCBI GenBank using Aliview. A Maximum Likelihood phylogenetic tree was generated using the IQ-TREE software to assess *E. bieneusi* genotypes and genetic diversity.

**RESULTS AND CONCLUSIONS:** Three human samples out of 377 resulted as positive to the cartridge-based molecular test for the qualitative detection of *E. bieneusi* (frequency of infection 0.8%). Isolates were molecularly characterized and assigned to genotype A and ITA-1, a new genotype identified during the present study. Animal samples showed positivity in 4 buffalo calves (<8 months) with an infection rate of 11.8%. Genotype A was assigned to two samples and genotype I to one sample. For one sample, the genotype obtained resulted to have 100% of identity with sequences deposited in GenBank but not already associated to a described genotype. All genotypes detected in humans and animal hosts belong to the Phylogenetic Groups 1 and 2, which presumably cause most zoonotic or cross-species infections (Li et al., 2019. Infect Genet Evol, 75:107236).
This study updates *E. bieneusi* prevalence and genetic diversity data in Italian symptomatic patients and offers the first animal host data. Additional research is required on a national scale to fully comprehend *E. bieneusi* zoonotic potential and public health impacts in our country.

# ZOONOTIC DERMATOPHYTE *TRICHOPHYTON MENTAGROPHYTES* IN WILDLIFE: FIRST REPORT IN THE ALPINE CHAMOIS (*RUPICAPRA RUPICAPRA*)

Moroni B.*[1], Zoccola R.[1], Pontei A.[1], Trabunella E.[1], Peano A.[2]

*[1]Istituto Zooprofilattico Sperimentale di Piemonte, Liguria e Valle d'Aosta, Turin, Italy; [2]Department of Veterinary Sciences, University of Turin, Turin, Italy*

**Keywords:** *Trichophyton mentagrophytes*, Dermaptophyte, Wildlife.

**INTRODUCTION:** *Trichophyton mentagrophytes* is a cosmopolitan fungal species belonging to the dermatophytes, zoonotic fungi with a predilection for non-viable keratinized portion of the skin, and can affect different mammal species, including wildlife. The main reservoir for this pathogenic fungus is considered rodent species, but it has also been frequently isolated from carnivore species (Chermette et al, 2008. Mycopathologia, 166:385-405). This report aims to describe the first case of *T. mentagrophytes* infection in an Alpine chamois (*Rupicapra rupicapra*).

**MATERIALS AND METHODS:** In August 2022, an adult male Alpine chamois was found dead in Val Vigezzo (Santa Maria Maggiore, Verbania, Italy) and transported at the Istituto Zooprofilattico Sperimentale Piemonte, Liguria, Valle d'Aosta in Turin (Italy) to determine the cause of death within the regional passive surveillance plan for wild animals. The chamois was severely cachectic, with an old fracture on the right horn. It also presented diffuse, generalized scaly skin lesions with patchy alopecia. The cause of death was attributed to starvation. Skin scrapings and hair were collected and directly examined at the stereomicroscope. No macroscopic ectoparasites were seen except for ticks located in the inguinal area. Fungal cultures were performed using selective media for dermatophytes (Sabouraud Dextrose Agar with antibiotics and cycloheximide; Dermatophyte Test Medium [DTM]). They were incubated for ten days at room temperature.

**RESULTS AND CONCLUSIONS:** White-coloured, granular colonies grew on both the media, with a red colour change of the DTM. Microscopical examination using the lactophenol cotton blue staining technique allowed us to visualize septate branched hyphae with abundant spherical microconidia, suggestive of *T. mentagrophytes*. The identification was confirmed by PCR and sequencing of the ribosomal internal transcribed spacer (ITS) region. Analyzing the ITS region allows species attribution and simultaneously strain typing since various "ITS Types" have been described thus far (Taghipour et al., 2019. Mycoses, 62:1084-91). The isolate from the chamois belonged to ITS Type III*. Dermatophytosis is a relatively common condition in livestock, while it is still considered a rare condition in wildlife, especially in ungulates. To our knowledge, this case is the first concerning an Alpine chamois. Previously, an infection by *Trichophyton verrucosum* was described in an animal from the same region (Peano et al., 2008. Eur J Wild Res, 54:153-56). Two cases due to *T. mentagrophytes* were instead reported in the Southern Chamois (*Rupicapra pyrenaica*) in the Eastern Pyrenees (Marco et al., 2007. Zoonoses Public Health, 54:278-80). The genotype (ITS Type III*) is commonly found in European animals (Taghipour et al., 2019. Mycoses, 62:1084-91), but data regard mainly domestic animals. Increasing wildlife sampling to obtain and analyze more isolates will likely contribute to a better knowledge of the "sylvatic biological cycle" of this fungus.

# AN UNUSUAL *ASPERGILLUS* SP SECTION TERREI ISOLATE IN A CASE OF SYSTEMIC ASPERGILLOSIS IN DOG

Bordoni T.*, Dini F.M., Okonji S., Cola V., Gandini G., Pisoni L., Galuppi R.

*Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy*

**Keywords:** Sistemic aspergillosis, Dog, *Aspergillus floccosus*.

**INTRODUCTION:** Canine aspergillosis in its systemic form represents a serious disease often resulting in a fatal outcome. *Aspergillus terreus* has been reported as the most frequent agent, with the German Shepherd being the most commonly affected breed. Occasionally, *A. versicolor*, *A. alabamensis*, and *A. deflectus* are reported in some cases. We report a case of discospondylitis in an 8-year-old female German Shepherd with severe neck pain, lameness of the forelimb, and progressive pelvic limb proprioceptive ataxia and paresis.

**MATERIALS AND METHODS:** The dog was referred to the University Veterinary Hospital of Alma Mater Studiorum - University of Bologna. The MRI revealed severe and diffuse changes in signal intensity of the intervertebral discs and multiple lytic lesions, confirming the suspicion of discospondylitis and C6-C7 epidural empyema. Urinary sediment was obtained for both microscopic examinations using MGG staining and culture on Sabouraud medium with Chloramphenicol (SAB-CAF) at 26°C for several weeks. The colonies were observed both macroscopically and microscopically and processed for DNA extraction. Sequencing of ITS rDNA and benA genes was performed for molecular identification.

**RESULTS AND CONCLUSIONS:** Microscopic examination of the urinary sediment stained with MGG revealed the presence of short hyphae with chlamydospores. Cultures exhibited slow growth and an atypical rugose appearance, with hyphae bearing numerous terminals and intercalary chlamydospores, but no conidial structures were observed. Sequencing of the ITS region identified the genus as *Aspergillus*, while benA sequences showed 100% similarity with *Aspergillus floccosus* (section Terrei). Subsequent passages on SAB CAF led to a morphological mutation in the peripheral sector, resulting in flat colonies with a dusty cinnamon brown surface. Microscopic examination revealed septate hyphae bearing solitary conidia or short conidiophores, with biseriate phialides on the upper half of vesicles and chains of round conidia, typical features of *A. terreus*. This transformation persisted in subsequent subcultures. Antifungal therapy with Itraconazole was started. After 16 months the dog presented with non-ambulatory paraparesis due to suspension of therapy, and euthanasia was performed due to a rapid worsening of clinical signs. This case highlighted that urine specimens can be highly indicative and minimally invasive. Additionally, the identification of *A. floccosus*, not previously described in systemic mycoses of dogs, confirms that *Aspergillus* spp. involved in canine systemic aspergillosis often belong to the section Terrei. Furthermore, the atypical morphology of the fungus made identification challenging. Previous studies have described the presence of morphological mutations in *A. terreus* as an adaptation of the fungus to changed environmental conditions, which significantly differ from laboratory growth conditions in terms of nutrient availability and physiological factors (Jukic et al., 2017. Antimicrob Agents Chemother, 61:12).

# XXXIII

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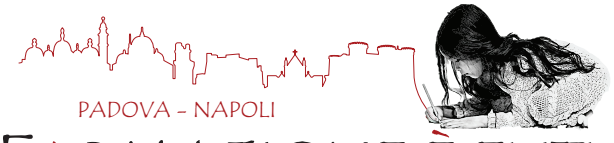
# PARASITE TREATMENT AND DRUG RESISTANCE



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# MONITORING ANTHELMINTIC TREATMENT EFFICACY IN GOAT AND SHEEP FARMS OF NORTH-EASTERN ITALY

Maurizio A.[1], Dotto G.[1], Fasoli A.[2], Tessarin C.[1], Dossi P.[1], Roman A.L.[1], Gaio F.[2], Pertile A.[2], Sommacal M.[2], Petratti S.[2], Menegozzo P.[2], Obber F.[3], Dellamaria D.[3], Beraldo P.[4], Cassini R.*[1]

*[1]University of Padova, Padova, Italy; [2]Veterinary Practitioner, NE, Italy; [3]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy; [4]University of Udine, Udine, Italy*

**Keywords:** Gastrointestinal nematodes, Faecal egg count reduction test, Small ruminants.

**INTRODUCTION:** Anthelmintic resistance (AR) is one of the major threats for grazing livestock farming and numerous reports of AR thoughout Europe were recorded in the last decades. Italy seemed to be less affected by the phenomenon, but studies are still lacking in some areas of the country (e.g., Northern Italy). The faecal egg count reduction test (FECRT) is the method of choice for assessing anthelmintic efficacy against gastrointestinal nematodes (GIN) in the field. This method is very simple, but its interpretation is potentially affected by several confounding factors (e.g., host, parasite, management, technical factors). Furthermore, the FECRT is usually implemented at high taxonomic level, considering strongylid species jointly and therefore the differences among genera are lost in the results interpretation. The aim of this study was to monitor AR in sheep and goat farms of Northern Italy, including genera identification and more specifically focusing on the quantification of the Haemoncus contortus contribution to the pre- and post-treatment burden.

**MATERIALS AND METHODS:** The study was carried out between October 2021 and November 2023, including overall 11 sheep and 11 goat farms, where a single FECRT trial was performed according to the WAAVP guidelines (Coles et al., 1992. Vet Parasitol, 44:35-44; Kaplan et al., 2023. Vet Parasitol, 318:109936). Between 10 and 15 animals were included in each trial and their faecal samples analysed with either McMaster or Mini-Flotac techniques. The contribution of *H. contortus* to the GIN burden was estimated in the first phase of the research (n farms=12) through morphological identification of cultured third-stage larvae (L3), and in the second phase (n farms=10) through molecular analysis. Two realtime PCRs were developed to quantify overall GIN and *H. contortus* eggs. The calculation and interpretation of FECRT results was performed both for GIN and specifically for *H. contortus*, as described in Maurizio et al., 2024 (Vet Parasitol, 327:110146). In the first phase, the assessment of anthelmintic efficacy was also performed for the other GIN genera.

**RESULTS AND CONCLUSIONS:** Treatments were fully effective only in 4/11 goat and in 2/11 sheep farms. In trials of the first phase *Haemonchus* displayed the worst results among the genera considered, obtaining the lowest reduction in 8/9 ineffective trials. The molecular approach used in the second phase allowed to obtain a similar information while reducing the processing time and the uncertainty in the morphological identification. The study allowed to detect an overall situation of reduced treatment efficacy against the whole GIN community in many farms and a high prevalence of treatment inefficacy towards *H. contortus*, which might become increasingly prevalent if no preventive action against AR is taken in the field. Finally, our results showed the usefulness of a genus-specific approach in monitoring and interpreting GIN burden and treatment efficacy, which are key to sustainable parasite control.

# PROTEOLYSIS TARGETING CHIMERAS (PROTACS) TO TREAT LEISHMANIASIS: DEVELOPMENT OF AN INNOVATIVE PHARMACOLOGICAL THERAPY

Di Muccio T.*[1], Fiorentino E.[1], Exertier C.[2], Fiorillo A.[2], Pasieka A.M.[3], Orsini S.[1], Salerno A.[3], Uliassi E.[3], Ilari A.[2], Bolognesi A.L.[3]

*[1]Istituto Superiore di Sanità, Department of Infectious Diseases, Rome, Italy; [2]Institute of Molecular Biology and Pathology (IBPM) of the National Research Council of Italy (CNR), c/o Department of Biochemical Sciences, Sapienza University Rome, Italy; [3]Department of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna, Bologna, Italy*

**Keywords:** PROTAC, Leishmaniasis, Trypanothione.

**INTRODUCTION:** Leishmaniasis is a neglected infectious tropical disease. The available limited therapeutic options for leishmaniasis are inadequate due to their poor pharmacokinetic profile, resistance, toxicity, and high cost. The identification of new targets and new approaches for leishmaniasis drug discovery are urgently needed (Field et al., 2017. Nat Rev Microbiol, 15:447). PROteolysis TArgeting Chimeras (PROTACs) have become an extremely appealing technology (Gu et al., 2018. BioEssays, 40:e1700247). PROTACs are heterobifunctional compounds comprising three elements: a ligand for a protein of interest (POI), an E3 ligase recruiter, and a linker. The formation of a ternary complex elicits POI degradation through the endogenous ubiquitin-proteasome system. As POI, we choose Trypanothione Reductase (TR), an enzyme involved in thiol homeostasis essential for *Leishmania* survival and validated as specific antileishmanial target. However, since TR is not effectively inhibited by currently available inhibitors due to some intrinsic limitations (Battista et al., 2020. Molecules, 25:1924), PROTACs can overcome the major drawbacks in TR inhibitors by acting catalytically to degrade super-stoichiometric amounts of the target protein.

**MATERIALS AND METHODS:** A set of PROTACs has been rationally designed and synthesized. Known TR inhibitors were selected as POI ligands. Thalidomide, known to bind human E3 ligase, was employed as E3 ligase binder. The POI and E3 ligase ligands were conjugated through different linkers. The PROTACs were tested on *Leishmania infantum* TR enzyme by X-ray crystallography and spectroscopic methods to evaluate inhibitory activity. In order to assess biological activity, the effect on cellular growth of axenic amastigotes and cytotoxicity effect on macrophages were evaluated by in vitro assay. Selectivity index (SI) showed the best PROTACs that were prioritized for *ex vivo* assay. To prove PROTACs degradation profile mediated by *Leishmania* proteasoma system, *in vitro* and *ex vivo* assays were performed with controls (kinetoplastid- and human-specific proteasoma inhibitors, negative control PROTACs, TR inhibitors). In parallel, Western blotting and proteomic analysis were performed to assess TR levels.

**RESULTS AND CONCLUSIONS:** Seven series of PROTACs were synthesized. Biochemical analysis showed a variable inhibitory activity on TR (range: 9-60%) for all of the series. However, considering not optimal SI values for all PROTACs, one PROTAC have been tested in *ex vivo* assay, showing a good growth inhibitory effect against intra-macrophagic amastigotes (IC50 1 μM). While preliminary quantitative proteomic results revealed a significant reduction in TR levels, the degradation profile of PROTAC needs to be confirmed by ongoing experiments. For the first time, this project has the potential to extend the application of PROTAC technology to infectious parasite infectious diseases. This work is supported by FISR2019_03796 PROLEISH (PROTACs to treat leishmaniasis).

# PARASITE DYNAMICS IN FERAL HORSES THROUGHOUT ONE CALENDAR YEAR

Genchi M.*, Piombo M., Cattabiani C., Kramer L., Vismarra A.

*Dept. Of Veterinary Medicine, Parasitology Unit, University of Parma, Parma, Italy*

**Keywords:** Feral horses, Gastrointestinal parasites, Epidemiology.

**INTRODUCTION:** Horses are host to a plethora of gastrointestinal parasites. Knowledge of the seasonality of parasite egg shedding and transmission dynamics are important for constructing sustainable parasite management programs, especially considering anthelmintic resistance problems that are increasingly worldwide. This study evaluated strongyle and ascarid egg shedding patterns and transmission dynamics in an untreated feral horse population.

**MATERIALS AND METHODS:** This study was conducted from October 2020 to November 2021 in the Aveto Regional Natural Park, Liguria, northern Italy. Twelve herds of 118 feral horses were followed throughout the year. Using an identification protocol applied during previous monitoring, it was possible to distinguish and recognize each horse. Horses were observed defecating and samples were collected from the fecal piles of individual animals. Fecal samples were immediately packaged, labeled, and stored at 4 °C for further laboratory processing. Fecal egg counts were performed for all samples using a Mini-FLOTAC technique (Bosco et al., 2018. BMC Vet Res, 14:7). To identify strongyle species in feral horses, we morphologically identified third stage larvae (L3) cultured from feces.

**RESULTS AND CONCLUSIONS:** A total of 314 fecal samples were collected. Strongyle eggs were detected in 99.7% of fecal samples and 30% for *Parascaris* spp. Total strongyle FEC ranged from 0-10000 EPG, with an overall mean of 889 EPG and *Parascaris* spp. from 0-30,000 EPG, with an overall mean of 223 EPG. Ascarid fecal egg counts were higher in young horses than adult horses; however, 9.6% of adult horses also had patent infections. Of 314 fecal samples, 11% were low shedders (< 200 EPG), 21% were moderate (200-500 EPG), and 69% were high (> 500 EPG), and in the summer season there was greater environmental contamination. Moreover, the dominant horses (male and female) in the herds were statistically more excretory than the non-dominant ones. The prevalence of *Anoplocephala* spp. across all populations was low (74/314, 23.6%). Egg shedding patterns in domestic horses have been described to almost always follow the 20/80 rule (Kaplan and Nielsen, 2010. Equine Vet Educ, 22:306-16; Nielsen and Reinemeyer, 2018. Handbook of Equine Parasite Control, Wiley- Blackwell, 1-229). Whether similar egg shedding patterns occur in feral horse populations is not clear. In the populations of horses in our study, the 20/80 rule was not followed. Moreover, although some horses showed high infections, throughout the study no horses showed externally observable clinical signs and no deaths were recorded. Our findings could help to better understand how to approach selective targeted treatments in a context of drug resistance.

# KILLER PEPTIDES INCAPSULATED IN HYALURONIC ACID NANOPARTICLES: A NEW STRATEGY FOR THE TREATMENT OF TOXOPLASMOSIS? PRELIMINARY *IN VITRO* STUDIES

Vismarra A.*[1], Giovati L.[2], Semeraro M.[1], Kramer L.[1], Genchi M.[1], Artesani L.[2], Zito M.[3], Bianchera A.[3]

*[1]Dept. of Veterinary Medicine, Parasitology Unit, University of Parma, Parma, Italy; [2]Dept. of Medicine and Surgery, Laboratory of Microbiology and Virology, University of Parma, Parma, Italy; [3]Food and Drug Department, ADDRes Lab, University of Parma, Parma, Italy*

**Keywords:** *Toxoplasma gondii*, Killer peptides, Hyaluronic acid nanoparticles.

**INTRODUCTION:** *Toxoplasma gondii* is a globally distributed, apicomplexan parasite with a high zoonotic potential, that causes toxoplasmosis in humans and animals. The most common drugs currently used for treatment are sulfadiazine and pyrimethamine that can be toxic for patients and not effective against tissue cysts. Therefore, novel and effective drugs are needed for the treatment of toxoplasmosis. In this context, basing on previous results obtained *in vitro* with killer peptide (KP) (Giovati et al., 2018. Front Microbiol, 9:753) we tried to improve the efficacy of this product incapsulating it into hyaluronic acid (HA) nanoparticles, with the purpose of targeting parasite-invaded cells that are reported to overexpress HA receptor CD44 (Hayashi et al., 2014. Parasitol Int, 63:479-84).

**MATERIALS AND METHODS:** Nanoparticles were prepared with an Elveflow (Elvesys) microfluidic system with two channels equipped with a Zeonor herringbone chip. A solution of sodium hyaluronate (MW 15-30 kDa) was prepared at 5 mg/mL in PBS 1.5X (pH 7.4) and mixed with a freshly prepared solution of KP at 1.5 mg/mL in water with a total flow rate of 300 µL/min and a flow rate ratio of 2:1 ([KP]final = 500 µg/mL). The size of the resulting nanoparticles was assessed by dynamic light scattering using a NanoZS. Loading efficiency was estimated by quantifying with BCA protein assay the amount of KP remaining in the supernatant of samples after centrifugation at 15,000 g for 30'. Both the KP alone and the encapsulated form (NP/KP) were then tested to assess their *in vitro* non-toxicity on cells and efficacy on *T. gondii*. The AlamarBlue assay was used to test the possible toxicity of KP/NP on HFF cells (human foreskin fibroblasts), while their possible efficacy on the parasite was assessed infecting HFF with transgenic *T. gondii* tachyzoites expressing beta-galactosidase. In both cases the IC50 value was extrapolated in order to define the KP/NP toxicity and NP/KP efficacy on the extracellular form of the parasite.

**RESULTS AND CONCLUSIONS:** DLS analysis revealed the presence of a main population of NPs with an average size of 10 ± 2 nm, and a second population of 260 ± 202 nm; encapsulation efficiency was 87 ± 2 %. The IC50 for KP alone on HFF was > 428 µg/mL, while on *T. gondii* beta-gal was 120 µg/mL. The IC50 for KP/NP on HFF was about 270 µg/mL. Experiments on *T. gondii* are still in progress. The preliminary results highlighted that the NP composition might be partially toxic for HFF, for this reason NP/KP will be tested at a lower concentration on *T. gondii* Beta-gal to establish their possible improved efficacy in killing the parasite. The ideal IC50 on *T. gondii* might be lower for NP/KP than KP alone.

# ANTI-*LEISHMANIA* ACTIVITY OF INHIBITORS OF THE HUMAN GTPASE RAC1

Sannella A.R.*, Orsini S., Scalone A., Fiorentino E., Paone S., Olivieri A., Di Muccio T.

*Istituto Superiore di Sanità, Department of Infectious Diseases, Rome, Italy*

**Keywords:** Rac1, Leishmaniasis, Anti-*Leishmania* drug.

**INTRODUCTION:** *Leishmania* are intracellular protozoan parasites responsible for various diseases in humans. Following their inoculation into a mammalian host by the sand fly, the *Leishmania* promastigotes are internalized by macrophages where they differentiate into amastigotes. Phagocytosis is accompanied by highly microbicidal reactive oxygen species producted by NADPH phagocyte oxidase complex. Previous studies suggested that *Leishmania* evade the ability of macrophages to produce superoxide during Rac1-dependent phagocytosis that occurs in the absence of significant NADPH oxidase activation. Moreover, *Leishmania* engages Rac1 to build up a protective coat of F-actin around its phagosome to prevent fusion with lysosomes, essential for its survival (Lerm et al., 2006. Inf Immun, 74:2613). The human GTPase Rac1 plays a role in infection of the human host cell by many intracellular pathogens, including intracellular parasites such as *Plasmodium falciparum* and *Toxoplasma gondii* (Paone and Olivieri, 2022. Microorganisms, 10:1370). It was recently shown that 12 Rac1 inhibitory compounds have antimalarial activity on *P. falciparum* cultures, three of them showing half inhibitory concentrations (IC50) in nanomoles, the most effective being EHop-016 (IC50 139 nM) (Parapini et al., 2022. Antimicrob Agents Chemother, 66(1): e0149821). These evidences suggest a novel host-targeted approach to treat leishmaniasis. The aim of this work is to test a panel of Rac1 inhibitors as potential anti-*Leishmania* drugs.

**MATERIALS AND METHODS:** Cytotoxic Concentration (CC50) of the compounds was evaluated against human and murine macrophages. The effect against *Leishmania* was evaluated by using *ex vivo* assays. Both human and murine macrophages were infected by *Leishmania infantum* in different conditions to evaluate their effect on: i) efficiency of host cell invasion; ii) inhibition of intra-macrophage amastigotes growth. Moreover, a possible toxic activity against the parasite itself was assessed on extracellular amastigotes of *L. infantum* by *in vitro* assay and expressed as IC50.

**RESULTS AND CONCLUSIONS:** Eight Rac1 inhibitors with different chemical structures and different inhibition mechanisms tested for their toxicity against macrophages showed a moderate toxicity (CC50 range: 8 to >50 μM). We started testing the anti-*Leishmania* effect of the Ehop-016 compound. Ehop-016 showed a moderate toxicity (CC50 10 μM), reduced macrophage invasion efficiency by 10% at 4 h, and it significantly affected intra-macrophage parasite growth (IC50 1 μM at 24 h). It also showed a moderate activity against extracellular amastigotes (IC50 8 μM). Tests on other compounds are ongoing. These preliminary results showed the potential of Rac1 inhibition as a strategy for the development of new drugs against leishmaniasis. Being Rac1 a studied subject for its involvement in cancer, several inhibitors are available and can be repurposed as antimalarial and anti-*Leishmania* drugs.
This work is supported by Project FESR LAZIO: MaLeRac A0375-2020-36527.

# IT FINALLY HAPPENED: FIRST CASE IN EUROPE OF MACROCYCLIC LACTONE-RESISTANT *DIROFILARIA IMMITIS*

Traversa D.*[1], Colombo M.[1], Diakou A.[2], Kumar S.[3], Chaintoutis S.[4], Venco L.[5], Di Cesare A.[1], Morelli S.[1], Betti Miller G.[6], Prichard R.[3]

*[1]University of Teramo, Department of Veterinary Medicine, Teramo, Italy; [2]Aristotle University of Thessaloniki, School of Veterinary Medicine, Thessaloniki, Greece; [3]McGill University, Institute of Parasitology, Sainte Anne-de-Bellevue, Canada; [4]Aristotle University of Thessaloniki, Faculty of Health Sciences, Thessaloniki, Greece; [5]Vet Practitioner, Ospedale Veterinario Città di Pavia, Pavia, Italy; [6]Vet Practitioner, Ambulatorio Veterinario Farnesina, Rome, Italy*

**Keywords:** Heartworm, Prevention, Anthelmintic resistance.

**INTRODUCTION:** Heartworm disease caused by *Dirofilaria immitis* is one of the most important parasitosis of dogs worldwide. This mosquito-transmitted nematode is potentially life-threatening, has zoonotic implications and has been increasingly reported around the world due to climate change and other drivers. Treating heartworm disease is a long, expensive and intricate process that requires a strict adherence in terms of dog management and therapeutic scheme or surgical procedures. Therefore, prevention is pivotal to safeguard the health of dogs living in enzootic areas. Despite the high efficacy of Macrocyclic Lactones (MLs) in the prevention of the disease, in recent years some *D. immitis* strains have been proven to be resistant to MLs in the US (Diakou and Prichard, 2021. Pathogens, 10:1323). These records have raised high concerns for possible emerging or spreading of resistant strains in other areas of the world.

**MATERIALS AND METHODS:** A ~ 2-year-old Australian Shepherd female born in Louisiana, USA, was transferred to Rome in June 2023 with a negative *D. immitis* antigenic test performed in April 2023. Despite the dog received a monthly treatment with a ML for 5 months after arriving in Rome, the animal tested positive for heartworm antigen and for *D. immitis* microfilariae in November 2023. The clinical examination was normal, and the owners did not report any clinical signs. A microfilariae suppression test (MFST) was performed and the microfilarial DNA was analyzed using droplet digital PCR-based duplex assays targeting four Single Nucleotide Polymorphisms (SNP1, SNP2, SNP3, SNP7) and able to distinguish between resistant and susceptible isolates (Kumar et al., 2023. Int J Parasitol Drugs Drug Resist, 23:10-18). After the results of the MFST, the dog was treated according to the guidelines of the American Heartworm Society and the European Society of Dirofilariosis and Angiostrongylosis.

**RESULTS AND CONCLUSIONS:** The MFST indicated a resistant strain and the genetic analysis confirmed that microfilariae had a ML-resistant genotype at SNP1 and SNP7 positions. These results are compatible with a resistant strain of *D. immitis* and represent the first unequivocal description in Europe. Given that with all likelihood the dog arrived in Italy already infected, the present report underlines the factual risk of importing drug-resistant heartworm in different areas of the world. Therefore, dogs travelling from areas enzootic for ML-resistant strains should be subjected to antigenic and Knott test before travelling and for at least 6-9 months following their arrival at the new destination. Such measures are pivotal to control the spreading of *D. immitis* and to avoid the dissemination of resistant strains.

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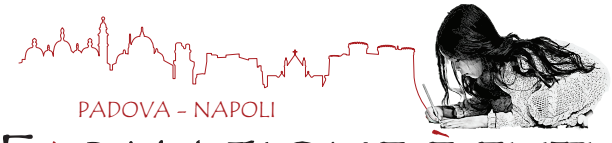
# PARASITES IN FISH AND OTHER AQUATIC ANIMALS



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# AN UPDATE ON *EUSTRONGYLIDES* SPP. IN CENTRAL ITALY: A SURVEY IN FISH SPECIES FROM SIX LAKES IN TUSCANY AND LATIUM REGIONS

Guardone L.*[1], Di Maggio M.[1], Coltraro M.[2], Tinacci L.[1], Ricci E.[2], Mecatti M.[4], Corradini C.[3], Susini F.[2], Armani A.[1]

*[1]Department of Veterinary Sciences, University of Pisa, Pisa, Italy; [2]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Pisa, Italy; [3]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Roma, Italy; [4]FIPSAS Comitato Regionale Toscana, Firenze, Italy*

**Keywords:** Fishborne parasites, Seafood products, Risk assessment.

**INTRODUCTION:** Nematode larvae of the genus *Eustrongylides* (Family: Dioctophymatidae) infect a variety of freshwater fish species, localizing in the viscera and in the muscle (Castiglione et al., 2023. Food Control, 153:109894). Although zoonotic cases are rarely reported, the presence of viable larvae may affect fishery products' safety. In addition, even when inactivated, the larvae make these products unfit for human consumption. An increased presence of this parasite has been reported recently, also in lakes in north and central Italy (Menconi et al., 2021. Water, 13:3581; Franceschini et al., 2022. Food Control, 136:108858; Rusconi et al., 2022. J Parasitol, 108:209-16). This study aimed to assess the occurrence of *Eustrongylides* spp. in fish species in four lakes in Tuscany (Bilancino, Chiusi, Montedoglio and San Cipriano) and in two lakes in Latium (Bolsena and Bracciano), water basins previously not investigated.

**MATERIALS AND METHODS:** Overall 1650 fish specimens belonging to the following 17 species were collected between April 2022 and May 2023: *Perca fluviatilis* (79), *Lepomis gibbosus* (92), *Sander lucioperca* (11), *Micropterus salmoides* (60), *Esox lucius* (1), *Atherina boyeri* (424), *Tinca tinca* (36), *Coregonus lavaretus* (32), *Ictalurus punctatus* (20), *Squalius cephalus* (63), *Scardinius erythrophthalmus* (157), *Alburnus alborella* (604), *Sarmarutilus rubilio* (26), *Protochondrostoma genei* (20), *Telestes muticellus* (11), *Carassius auratus* (5) and *Abramis brama* (9). All the specimens were analysed by visual inspection followed by a chloro-peptic digestion performed separately on viscera and muscle. Isolated nematodes were morphologically identified to genus level and submitted to molecular analysis (PCR and sequencing) targeting the ITS gene for species identification (Mazzone et al., 2019. J Parasitol, 641:882-89).

**RESULTS AND CONCLUSIONS:** *Eustrongylides* spp. larvae were not found in any of the fish species analyzed, with the exception of one larva found in the belly flap muscle of one *P. fluviatilis* (European perch) out of the 30 specimens caught in Bracciano lake (P%=0.3; MI:1; MA:0.03). The larva was molecularly identified as *E. excisus*. Infection in *P. fluviatilis* was already documented in other Italian lakes (Dezfuli et al., 2015. Parasit Vectors, 8:1-9; Menconi et al., 2020. IJRPH, 17:4171; Menconi et al., 2021. Water, 13:3581; Franceschini et al., 2022. Food Control, 136:108858; Rusconi et al., 2022. J Parasitol, 108:209-16). This finding adds Bracciano Lake to the list of the several Italian lakes in which *Eustrongylides* spp. nematodes occurr. Even though the observed prevalence is low, risk management measures to prevent contaminated products from reaching final consumers and to avoid negative effects on local fishery supply chains should always be implemented, especially considering that fishery products from Bracciano lakes are commercialized. Future investigations, ideally also including definitive hosts as the great cormorant, will show if the parasite expands in these areas.

# HISTOPATHOLOGY AND INNATE IMMUNE RESPONSE OF *ANGUILLA ANGUILLA* INFECTED WITH *ACANTHOCEPHALUS RHINENSIS* (ACANTHOCEPHALA)

Franchella E.*[1], Carosi A.[2], Lorenzoni M.[2], Bosi G.[3], Sayyaf Dezfuli B.[1]

*[1]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy; [2]Department of Chemistry, Biology, and Biotechnology, University of Perugia, Perugia, Italy; [3]Department of Veterinary Medicine and Animal Science, University of Milan, Lodi, Italy*

**Keywords:** *Anguilla anguilla*, Acanthocephala, Immune cells.

**INTRODUCTION:** During the past three decades, the *Anguilla anguilla* population has declined dramatically. Habitat loss, climatic and oceanic changes, pollution, mortality due to river obstacles, overexploitation, and parasites have been suggested as possible reasons for such decline (Dekker, 2003. Eel biology, Tokyo pp. 497).

**MATERIALS AND METHODS:** A total of 37 European eels, *A. anguilla*, sampled from Lake Piediluco, Central Italy, and measuring 35 to 75.5 cm in total length (mean±1 SD, 56.41±10.89 cm) were examined, and their acanthocephalan infections assessed. Thirty-two (86.49%) eels were infected with *Acanthocephalus rhinensis* (range, 1-350).

**RESULTS AND CONCLUSIONS:** *A. rhinensis* has a limited distribution in Europe, it has been reported in eels in only two cases: in 2008 in Germany and, in 2012 in Lake Piediluco (Italy). It displays a high specificity for its definitive host *A. anguilla*, in fact, in Lake Piediluco other species of fish feed on the same gammarid, *Echinogammarus tibaldii*, but are not infested by this parasite. Enteric helminths often cause inflammation of the digestive tract, inducing the recruitment of different types of immune cells at the site of infection. The most common cell types associated with enteric parasite infections in fish are neutrophils, mast cells (MC), and rodlet cells (RC). Neutrophils are recruited early to the point of parasite attachment where they bind, engulf, and kill pathogens. In fish infected with helminths, MCs tend to migrate and accumulate in large numbers at the site of infection, they react to parasites by releasing their contents through degranulation (Dezfuli et al., 2011. Parasite Immunol, 33:116-23). With references to the RCs, usually, they occur in the epithelial tissue of different teleost organs and are recruited and mobilized in response to viral, bacterial, and metazoan infections (Dezfuli et al., 2003. Dis Aquat Org, 53:257-62; Bosi et al., 2018. J Fish Diseas, 00:1-11). Mucous cells are also involved in the immune response, they show hyperplasia and increased release of mucins and AMP (antimicrobial peptides) in the digestive tract of infected eels, to protect the intestinal mucosa from mechanical and biochemical damage due to parasite invasion. The pathogenicity of acanthocephalans is attributed to two factors: the density of the parasite burden and the depth of worm penetration. The present study has documented the histological changes caused by *A. rhinensis* to the intestine of *A. anguilla*, the abundance of this species was high in eels and the penetration of the worm's proboscis induced severe damage to the intestinal wall. Herein an increase in the number of neutrophils, MCs, RCs and mucous cells near the site of acanthocephalan attachment was observed.

# MOLECULAR CHARACTERIZATION AND IMPACT OF *PHILOMETRA OBLADAE* ON *OBLADA MELANURA* JUVENILE SPECIMENS FROM THE TYRRHENIAN SEA

De Benedetto G.*[1], Riolo K.[2], Giannetto A.[2], Gaglio G.[1]

*[1]Department of Veterinary Sciences, University of Messina, Messina, Italy; [2]Department of Chemical, Biological, Pharmaceutical, and Environmental Sciences, University of Messina, Messina, Italy*

**Keywords:** Philometridae, Saddled seabream, Mediterranean Sea.

**INTRODUCTION:** *Oblada melanura* (*L.*) is a seawater species of the Sparidae family. It is a common opportunistic predator, present in the Central Mediterranean Sea, and in oceans. Nematodes belonging to the genus *Philometra* Costa, 1845 include species parasitizing freshwater, brackish and seawater teleost worldwide. Female specimens are bigger than males, from a few centimetres up to 1 m in length, while males are usually between 2 and 4 mm. To date, no data on abnormal fish behaviour caused by *Philometra* infection have been reported. Aim of the present study was to describe the abnormal swimming pattern due to the presence of *Ph. obladae* in juvenile *O. melanura*, and to provide a first molecular characterization of this species.

**MATERIALS AND METHODS:** In July 2023, during a dive off the coast of the Province of Messina, Tyrrhenian Sea, some *O. melanura* were observed showing abnormal swimming patterns, and two dead specimens were found on the sea bottom. Due to this observation, monthly monitoring from July to October was carried out. In August, another two dead fish of the same species were found. All samples were transferred to the laboratory for necropsy and parasitological examination. Fish coelomic cavities were inspected for parasites. All retrieved nematodes were isolated under a stereomicroscope, then stored in 70% ethanol and frozen at -80 °C. For morphological assessment, nematodes were diaphanized in glycerin and observed under a light microscope. Genomic DNA from four parasite specimens was extracted. Then, two molecular markers for phylogenetic analyses, the small ribosomal subunit (18S rDNA) and the mitochondrial cytochrome c oxidase 1 (COI), were amplified by polymerase chain reaction (PCR). The 18S and cox1 sequences obtained from the isolates were aligned with available nucleotide sequences of *Philometra* sp. using the MUSCLE algorithm and further used for phylogenetic analyses.

**RESULTS AND CONCLUSIONS:** The four dead *O. melanura* specimens were estimated as < 1 year. Examination of the coelomic cavity showed total dislocation of the internal organs, due to the presence of five gravid female nematodes of the family Philometridae in two of the four fish. The other fish showed the presence of fluid and degraded *Ph. obladae* females and larvae in the celomic cavity. Morphological evaluation allowed to identify the parasites as *Ph. obladae*. All the isolates showed positive amplification for 18S and cox1 genes. Partial sequences of (823 bp), and cox1 (292 bp) obtained for *Ph. obladae* were identical among isolates and the representative DNA sequences were submitted to GenBank.
The study reports new data on swimming impairment suggested by the presence of *Ph. obladae*. The atypical swimming behaviour of infected *O. melanura* observed suggests a significant impact on fish health status of *Ph. obladae*, also reporting molecular data on this species for the first time. Epidemiological and phylogenetic studies are in progress to improve knowledge on *Ph. obladae*.

# TRANSCRIPTOMIC PLASTICITY OF THE ANTARCTIC PARASITE *CONTRACAECUM OSCULATUM* SP. D (NEMATODA: ANISAKIDAE) IN RESPONSE TO THERMAL STRESS EXPOSURE

Palomba M.*[1], Liberati F.[1], Rodriguez Fernandez V.[2], Roca-Geronés X.[2], Macali A.[1], Castrignanò T.[1], Canestrelli D.[1], Mattiucci S.[2]

*[1]Dept. Ecological and Biological Sciences (DEB), Tuscia University, Viterbo, Italy; [2]Dept. of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, Rome, Italy*

**Keywords:** *Contracaecum osculatum* sp. D, Thermal stress, RNAseq.

**INTRODUCTION:** Understanding the genomic underpinnings of thermal adaptation is a hot topic in eco-evolutionary studies of parasites. Heteroxenous marine parasites, encompassing free-living larval stages, ectothermic intermediate hosts and homeothermic definitive hosts in their life cycles, offer compelling systems for the study of thermal adaptation (Wharton et al., 1999. Parasitology, 119:7-17). Transcriptomic modulation emerges as the primary mechanism driving phenotypic plasticity in response to thermal habitat variations (Pfenning et al., 2010. Trends Ecol Evol, 25:459-67). This process enables parasites to dynamically regulate the expression of specific genes, thus effectively adjusting physiological processes to cope with environmental challenges. However, a notable gap persists in understanding how heteroxenous parasites orchestrate the interplay between molecular responses and environmental thermal stimuli. *Contracaecum osculatum* sp. D is an antarctic marine parasite able to survive and thrive both at cold and warmer temperatures of the environment and its hosts. It has free-living larval stages in marine waters, L3 stages in ectothermic invertebrates and icefish hosts and adult stage in homeothermic pinniped hosts (*Leptonychotes weddellii*).
This study seeks to explore the transcriptomic profile of *C. osculatum* sp. D, aiming to discover molecular mechanisms driving its ability to thrive thermal fluctuations. Specifically, it aims to identify genes associated with parasite thermal tolerance and investigate their expression patterns according to different thermal conditions experienced by the parasite.

**MATERIALS AND METHODS:** L3 stages of *C. osculatum* D were obtained from the ice fish, *Chionodraco hamatus* caught in the Ross Sea, Antarctica, during the XXXV Italian expedition. Larvae were cultured *in vitro* under different thermal profiles (−2 °C, 1 °C, 37 °C), to simulate the temperature conditions experienced by the parasite during its life cycle. At designated time points (1 minute, 24 hours), L3 were preserved in RNAlater solution. RNA was extracted and cDNA library prepared. Products were paired-end sequenced on a NovaSeq 100PE. The obtained data were subjected to bioinformatic analyses.

**RESULTS AND CONCLUSIONS:** A de novo transcriptome was first generated for *C. osculatum* sp. D. Differential expression analysis identified a suite of genes associated with heat shock proteins, chaperones, and other stress response mechanisms, indicating the activation of conserved cellular defence pathways under thermal stress. Additionally, we observed differential expression patterns in genes involved in metabolism, suggesting a reprogramming of cellular processes to cope with temperature fluctuations. The achieved results provide new insights into the response mechanisms of the parasite to chilling and warmer stress and advance our understanding of the molecular adaptation that allow parasites to shift into different temperatures during their life cycle.

Granted by MUR-PNRA 2019

# PATTERNS OF HYBRIDIZATION BETWEEN THE TWO ZOONOTIC SPECIES *ANISAKIS PEGREFFII* AND *A. SIMPLEX* (*S. S.*) BY WIDE NUCLEAR MULTILOCUS GENOTYPING APPROACH: EVOLUTIONARY AND ECOLOGICAL IMPLICATIONS

Mattiucci S.*[1], Palomba M.[2], Belli B.[2], Aco-Alburqueque R.[2], Cipriani P.[1], Roca-Geronés X.[1], Santoro M.[3], Canestrelli D.[2], Nascetti G.[2]

*[1]Dept. of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, Rome, Italy; [2]Dept. Ecological and Biological Sciences (DEB), Tuscia University, Viterbo, Italy; [3]Dept. Integrative Marine Ecology, Stazione zoologica Anton Dohrn, Napoli, Italy*

**Keywords:** Multilocus approach, Hybridization, *Anisakis simplex* s.l.

**INTRODUCTION:** The evolutionary boundary between closely related parasite species represents an important window for investigating microevolutionary processes, speciation mechanisms, genetic architecture, and gene flow between interacting species. These boundaries often result permeable, leading to natural hybridization phenomena in the contact zones. Previous genetic, phylogenetic and ecological evidences support the view that the two anisakid species of the *A. simplex* (*s. l.*) complex, i.e. *A. pegreffii* and *A. simplex* (*s.s.*), constitute two well-differentiated sister taxa. These species have an allopatric zonation following a latitude gradient. However, their range of distribution overlaps in some regions, such as Spanish-Portuguese coast and the Western Pacific Ocean. The occurrence of 'putative hybrids' between the two species has been previously reported (reviewed in Mattiucci et al., 2018. Adv Parasitol, 99). However, many of these studies used a single nuclear marker. Given the assumption that a high number of diagnostic markers is necessary to identify categories of F1 and introgress individualsbetween closely related species, this study employs a wide multilocus genotyping approach along with Bayesian population structure analysis, to disclose: i) the genetic structure of both species in a contact zone, in comparison with their allopatric populations; ii) different patterns of hybridization between them.

**MATERIALS AND METHODS:** A total of N=400 specimens of *A. simplex* (*s.l.*) collected at adult and larval stages in allopatric and sympatric areas of the Atlantic Iberian coast and Alboran Sea waters, were genotyped by sequencing analysis of several nuclear loci, based on partially deposited genome of *A. simplex* (*s.l.*), and on 11 DNA microsatellite loci. Mitochondrial gene loci (mtDNA cox2) was also sequenced. Bayesian clustering algorithm was implemented in STRUCTURE v.2.3.3 to obtain an estimate of mixing proportions between the two species in the analysed samples. Additionally, NEWHybrids was used to evaluate the most parsimonious allocation of individuals showing mixed ancestry into distinct hybrid classes.

**RESULTS AND CONCLUSIONS:** Several diagnostic SNPs were discovered in nuclear genes, as well as, novel diagnostic SSRs loci were disclosed between the two species. Through STRUCTURE Bayesian analysis, specimens were assigned (100% probability) to either the "pure parental" species or identified as having mixed ancestry. NEWHYBRIDS analysis accurately categorized (100% probability) these specimens into their respective hybrid classes (i.e.F1 hybrids and introgressed). The multilocus genotyping approach provides a valuable tool for detecting ongoing hybridization and bi-directional introgression between the two interacting species in the sympatric area. Mitochondrial introgression was also found. Evolutionary and ecological significance of the observed hybridization patterns was attempted.

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# "GENDER REVEAL PARTY" IN LARVAL ANISAKIDS: UNVEILING THE SEX OF ANISAKIS PEGREFFII LARVAE BY DNA MICROSATELLITE LOCI

Aco-Alburqueque R.*[1], Palomba M.[2], Belli B.[2], Santoro M.[3], Nascetti G.[2], Mattiucci S.[1]

*[1]Dept. of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, Rome, Italy; [2]Dept. Ecological and Biological Sciences (DEB), Tuscia University, Viterbo, Italy; [3]Stazione zoologica, Anton Dohrn, Napoli, Italy*

**Keywords:** Sex-linked loci, Gender assignment, *Anisakis pegreffii*.

**INTRODUCTION:** In dioeciuos parasites, investment in offspring of both sexes is typically equal. However, deviations from this unity can occur over evolutionary time, driven by various factors such as mating systems (monogamy versus polygamy), mating probabilities, parasite populations density. These may lead to adaptive adjustments in the proportions of females versus males. In polygamous nematodes, female-biased sex ratios are more commonly observed than male-biased ones (Poulin, 1998, Evol Ecol Parasites Chapman & Hall Edit, London, 212 pp). Scanty data exists regarding the sex ratio in adult anisakids. This is completely absent for their larval stages due to the impossible gender assignment to L3 and L4 stages, based on traditional morphological approach. Microsatellite DNA (SSRs) loci have been developed (Mladineo et al., 2017. Int J Parasitol, 47:215-23; Mattiucci et al., 2019. Parasitology, 146:1387-1403) in the *A. simplex* (*s.l.*) species complex (Mattiucci et al., 2019). Among these loci, 5 have been found as sex-linked (Mattiucci et al., 2019). These are genes located on the X-chromosome. Aim of the study was to use SSRs linked loci for gender assignment of L3 specimens of *A. pegreffii* and to investigate sex distribution in different populations and infection sites.

**MATERIALS AND METHODS:** 5 SSRs loci (Anisl 0314, Anisl 07, Anisl22, Anisl15, Anisl4) were first analysed in adult (N=167) morphologically recognised as male and female specimens of *A. pegreffii* collected in various cetaceans. Larval specimens were collected from Mediterranean Sea fish species.

**RESULTS AND CONCLUSIONS:** In adult samples, significant deviations from Hardy-Weinberg Equilibrium (HWE) were observed at all loci, with positive FIS values, indicating an excess of homozygotes. However, when the genotypes at these loci were subdivided in adult male and female worms, it was seen that the males were homozygous at these loci (FIS=1), and the females samples turned to be in HWE at these loci. Given that that males are hemizygous (monoallelic), and females are heterozygous (biallelic), sex-linked loci allowed the identification of females at any life-history stage, when they showed a heterozygote genotype at one or more of those loci. The probability of the expected homozygous genotypes in females at all the loci, was also estimated to be very low. Sex determination was possible for a huge number of larval nematodes (N= 380). Female biased sex-ratio was observed as well as a possible differential gender distribution by site of infection.
This study provides a genetic tool for the sex determination of *A. pegreffii* at any developmental stage of its life cycle. It will allow to provide information about the frequency distribution of sex ratio in larval and adult parasite population, also in relation to population size. The tool will also permit to investigate the differential gene plasticity between larval males and females in the environment, as well as in natural and in accidental hosts (humans).

# WORMY FINDINGS ON THE SHORE - GASTROINTESTINAL TAPEWORMS OF THE SUNFISH *MOLA MOLA* STRANDED IN NORTHERN ADRIATIC SEA

Tedesco P.*[1], Caffara M.[1], Marcer F.[2], Marchiori E.[2], Zaccaroni A.[3], Fioravanti M.[1], Gustinelli A.[1]

*[1]Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Italy; [2]Department of Animal Medicine, Production and Health, University of Padova, Legnaro, Italy; [3]Department of Veterinary Medical Sciences, University of Bologna, Cesenatico, Italy*

**Keywords:** Triaenophoridae, *Mola mola*, Mediterranean Sea.

**INTRODUCTION:** The sunfish *Mola mola* is the largest teleost species, reaching over 3 m in length and weighing up to 2.3 t; it shows a cosmopolitan distribution, living in pelagic-oceanic waters from 30 to 1500 m depth. Despite being part of the charismatic marine megafauna, this species is still poorly understood (Pope et al., 2010. Rev Fish Biol Fish, 20:471-87). Parasites can provide useful insights on the distribution and life history of their fish hosts; *M. mola* is known to host a rich parasitic fauna with over 40 parasite species reported worldwide (de Figueiredo et al., 2018. Pubvet, 12:1-9). Nevertheless, parasitological data available in the literature are scattered (and, in some instances, outdated), partly due to the limited accessibility of wild specimens. In such context, stranded or by-catch individuals represent an invaluable source of information. Recently, 2 *M. mola* specimens were found stranded along the Italian coasts of northern Adriatic Sea; in this work we analyzed their gastrointestinal parasites with particular reference to cestodes.

**MATERIALS AND METHODS:** Stranded specimens were transported to the laboratory and subjected to necropsy and parasitological examination. Collected cestodes were washed in saline and preserved in 70% ethanol for morphological analyses in light microscopy and for molecular analysis based on amplification and sequencing of the 28S rDNA. A subsample was also preserved in 10% buffered formalin for morphological analysis in scanning electron microscopy.

**RESULTS AND CONCLUSIONS:** The necroscopic examination allowed to identify extremely high intensities of cestode parasites in the stomach and intestine of both stranded specimens. Based on morphological and molecular analyses, two cestode species were identified, both belonging to the family Triaenophoridae (order Bothriocephalidea): the species *Anchistrocephalus microcephalus*, already reported in *M. mola* from Adriatic Sea (Gustinelli et al., 2006. Biol Mar Med, 13:872-76) and from other Mediterranean and extra-Mediterranean areas, and another Triaenophorid, preliminarily referable to the species *Fistulicola plicatus*. Among Triaenophoridae, in addition to *A. microcephalus*, previous investigations on *M. mola* report Triaenophoridae Type I and Type II (Gustinelli et al., 2006. Biol Mar Med, 13:872-76), *Anchistrocephalus* sp. and *F. plicatus*. The latter is mainly described from *Xiphias gladius* but also recorded in *M. mola* based on a specimen in the collection of the University of Illinois (Linton, 1941. Proc U S Natl Mus, 90:417-42). Further analyses are ongoing to characterize also other helminth groups parasitizing the 2 *M. mola* under study, with the final aim to provide updated parasitological data on this charismatic yet elusive fish host.

# PARASITOLOGICAL FINDINGS IN TWO CETACEAN SPECIES STRANDED ALONG THE ITALIAN COASTLINES OF NORTH-WESTERN ADRIATIC SEA

Marcer F.*[1], Mattiucci S.[2], Tessarin C.[1], Centelleghe C.[1], Plault N.[3], Mercier A.[3], Marchiori E.[1]

*[1]University of Padova, Padova, Italy; [2]University of Rome Sapienza, Rome, Italy; [3]Université de Limoges, Limoges, France*

**Keywords:** Marine mammals, Parasites, Mediterranean Sea.

**INTRODUCTION:** In the North-Western Adriatic Sea, a semi-enclosed basin characterized by shallow waters, the common bottlenose dolphin (*Tursiops truncatus*; Tt) is regularly observed along the coastline, while the striped dolphin (*Stenella coeruleoalba*; Sc), a pelagic species, is far more rarely encountered. Being cetaceans apex predators, the study of their parasitic communities can give important information on the food chain stability and the health of the marine environment.

**MATERIALS AND METHODS:** We studied the parasitofauna of 44 Tt and 7 Sc stranded along the Italian coast of Northern Adriatic Sea in the period 2006-2023. During the necropsy, a complete parasitological exam was performed. Helminths were identified by morphological and, when opportune, molecular methods. To detect *Toxoplasma gondii* infections, a real-time PCR assay was used on tissue samples, including brain and muscle; analysis of 15 microsatellite was performed on positive samples for strain genotyping (Ajzenberg et al., 2010. J Clin Microbiol, 48:4641-45).

**RESULTS AND CONCLUSIONS:** Overall, nine different helminth taxa were identified in Tt, while 11 different parasite species were isolated from Sc. For both host species, the greatest diversity was found at gastrointestinal level. In bottlenose dolphins, the digeneans *Pholeter gastrophilus* and *Synthesium tursionis* had the highest prevalence (23/44 and 22/44 respectively), followed by *Braunina cordiformis* (17/44). *Pholeter gastrophilus* and *B. cordiformis* were also isolated from Sc, but with lower frequency (2/7 and 1/7 respectively). Conversely, the nematode *Anisakis pegreffi* was more frequent in Sc (2/44 vs 5/7). Adult cestodes of the species *Diphyllobothrium stemmacephalum* and *Tetrabothrium forsteri* were found in Tt and Sc respectively with different frequency (1/44 and 7/7). Finally, acantocephalans were rare in both host species: *Corynosoma* sp. and *Bolbosoma* sp. were recorded in one Tt and one Sc respectively. The nematodes *Halocercus* spp. were detected in the lungs of Tt and Sc with similar prevalence (22/42 and 3/7 respectively); in Tt also *Stenurus ovatus* was isolated at the level of the bronchi (8/42). *Crassicauda* sp. was isolated from the subcutaneous tissue of 1 Tt and 3 Sc. Merocercoid and plerocercoid larvae of *Clistobothirum delphini* (6/7), *C. grimaldii* (5/7) and *Clistobothrium* sp. (3/5), and the copepod *Pennella balaenopterae* were only recorded in internal organs and skin of Sc, suggesting that the life cycles of these parasites may be linked to oceanic ecosystems. Three *T. gondii* strains were found and successfully genotyped in Tt, revealing their correspondence with European Type II lineage and confirming predominance of this type in Mediterranean waters. The richness of the intestinal helminth fauna is lower than observed in the Western Mediterranean for both host species, hypothetically due to the lower diversity in hosts species in the study area, resulting in lower exchange rate of parasites.

# PARASITIC CASTRATION BY BUCEPHALID DIGENEANS: A FURTHER HURDLE TO OVERCOME FOR THE EUROPEAN FLAT OYSTER *OSTREA EDULIS*

Loi B.[1], Tedesco P.[2], Brundu G.[1], Caffara M.[2], Fioravanti M.[2], Carboni S.[1], Gustinelli A.*[2]

*[1]IMC-International Marine Centre, Torregrande, Oristano, Italy; [2]Department of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia (BO), Italy*

**Keywords:** *Ostrea edulis*, *Prosorhynchoides* sp., Parasitic castration.

**INTRODUCTION:** The European flat oyster *Ostrea edulis* has had a troubled history due to over-exploitation and climatic issues, as well as diseases that dramatically reduced its populations in the 70-80s, leading to shift to the use of the Pacific cupped oyster *Crassostrea gigas* to support European production. Hence, recently numerous initiatives have arisen with the aim of producing flat oyster seed to address European strategies focused on the conservation and restoration of *O. edulis* populations and aquaculture demands. Among the biotic factors that could negatively influence the reproductive capacity of oysters, digenean trematodes must certainly be included. In particular, when bivalves represent the first intermediate host, digenean larvae infection can lead to castration of the host. Here we describe the occurrence of an infection due to bucephalid digeneans in *O. edulis* broodstock in Sardinia.

**MATERIALS AND METHODS:** In March 2022, adults (90.3 +/- 23.8 g wet weight) of *O. edulis* were collected by scuba diving from a muddy sea bottom site covered by Posidonia oceanica meadow (3 m depth), located nearby a floating fish cage farming of sea bream, sea bass and meagre, in the gulf of Oristano, central western Sardinia (39.892775° N; 8.498230° E) and immediately taken to an experimental hatchery. During their housing, for 3 sampling times, 6 random individuals were sacrificed to monitor the reproductive status through histology. Soft tissues were removed, and samples of gills, digestive system, mantle and gonads were fixed in formalin. In addition, part of the tissues was observed under the stereomicroscope, and the parasitic elements found were fixed in 70% ethanol for morphological studies. A sample of soft tissue including a small portion of digestive gland and gonad was also preserved in RNAlater for molecular identification.

**RESULTS AND CONCLUSIONS:** In the 3 sampling times, independently from sex (hermaphrodites included) and reproductive stage, the 83% of the oysters were found with heavy parasitic infection by bucephalid digenean trematodes. Infection was widespread in both digestive gland and gonads, largely replacing their respective tissues. Sporocysts were numerous and had rounded or tubular shape with constrictions and contained a great number of cercariae at different developmental stages. Mature cercariae, also found spread in the tissues, were of the Gasterostome type showing a visible forked tail with long lateral arms. After amplification of the ITS rDNA and analysis by BLAST, the sequence showed 100% identity with *Prosorhyncoides* sp. (Bucephalidae). In the 50% of the samples, the infection caused severe gonad castration, making impossible the sex identification. The marked pathological effect on *O. edulis* by Bucephalidae represents a further limiting health factor for this species, also for the difficult application of prevention and control strategies.

This research was undertaken under the National Recovery and Resilience Plan (NRRP) - National Biodiversity Future Center (NBFC) - Spoke 2.

# SURVEY OF NEMATODES LARVAE IN CEPHALOPODS FROM NORTH EAST ATLANTIC AND MEDITERRANEAN SEA

Abdelfadel A.[1], Pirollo T.[1], Forzano R.[2], Gustinelli A.[1], Fioravanti M.L.[1], Caffara M.*[1]

*[1]Department of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia, Italy; [2]Fiorital spa, Venezia, Italy*

**Keywords:** Cephalopods, Anisakids, Raphidascarids.

**INTRODUCTION:** Squid plays a significant role in the global food market, particularly in countries like Spain, Italy, and Japan where it is highly consumed and imported. However, one critical aspect often overlooked is the presence of various parasites, including potentially zoonotic ones such as Anisakids, in cephalopods. Despite the economic importance of squid, there are a scarcity of epidemiological studies focusing on Anisakids in ommastrephids. This study aims to investigate the prevalence, tissue distribution and species composition of larval nematodes in commercially significant squid species of the Ommastrephidae family, caught in NE Atlantic Ocean and Mediterranean Sea.

**MATERIALS AND METHODS:** From November to December 2023, 238 squids (*Illex coindetii*, *I. illecebrosus* and *Todaropsis eblanae*) caught by bottom trawl have been sampled from 4 FAO areas. Mantel and internal organs were inspected by naked eye, to detect Anisakid nematodes following the "Hygiene Package" 853/2004 and following amendments, the EU Regulation 2074/2005 and EU Directive 1276/2011 but also by the UV-Press method. All the recovered nematodes were identified by PCR-RFLP of the ITS rDNA.

**RESULTS AND CONCLUSIONS:** Out of 238 examined squids, 53 (22.2%) were positive for third-stage larvae (L3) of nematodes from the families Anisakidae and Raphidascaridae. Among these larvae, 45.3% were exclusively found in the mantle, 39.6% solely in the viscera, and 15.1% were present in both tissues. Visual inspection detected nematodes in only 16 squids (6.7%), while 37 squids (15.5%) exhibited nematodes under UV-press examination. Interestingly, only 6 squids (2.5%) showed nematodes using both methods. Regarding sampling seasons, similar prevalence rates were observed in November (20.8%) and December (23.7%). In terms of fishing FAO areas, only 3 were positive, with prevalence values ranging from 43.1% to 18.3%. Furthermore, PCR analysis of 84 L3 larvae subjected to RFLP revealed distinct restriction patterns, enabling identification of *Anisakis simplex* (22.6%), *A. pegreffii* (11.3%), and 2 hybrid strains of *A. simplex/A. pegreffii*. Additionally, among the Raphidascaridae, larvae of *Lappetascaris* sp. (45.3%) and *Hysterothylacium* sp. (7.5%) were identified. The findings of our study revealed the presence of both well-known zoonotic (*A. simplex* and *A. pegreffii*) and non-zoonotic (*Lappetascaris* sp. and *Hysterothylacium* sp.) nematodes. It's noteworthy that the squid species examined in our study is frequently consumed raw as sushi or undercooked, raising concerns for public health risks. In conclusion, the preliminary results of our study contribute to the growing body of research on the presence of zoonotic Anisakids in cephalopods sold in the Italian market. Furthermore, there is a need to establish an official diagnostic method, as visual inspection alone proved to be insufficiently sensitive. This underscores the importance of further research and regulatory measures to ensure food safety in the consumption of squid and other cephalopods.

# EXPLORING ACTINOSPOREAN STAGES OF MYXOZOA PARASITES IN *BRANCHIURA SOWERBYI* (ANNELIDA: OLIGOCHAETA)

Passarini A., Devos M., Luci V., Cantori A., Gustinelli A., Fioravanti M.L., Caffara M.*

*Department of Veterinary Medical Sciences University of Bologna, Ozzano Emilia, Italy*

**Keywords:** Actinospores, *Branchiura sowerbyi*, Goldfish.

**INTRODUCTION:** Recently, in a pond's goldfish farms (*Carassius auratus auratus*) in Modena province, recurring episodes of infections of muscle and fin skin associated with Myxozoa infections have been observed, particularly referring to *Myxobolus lentisuturalis* and *Thelohanellus hoffmanni*. It is well known that Myxozoa use benthonic annelids as alternate hosts to complete their life cycle through development of actinosporean stages infective for fish hosts.

**MATERIALS AND METHODS:** During the spring 2022 and 2023, four mud samplings were carried out to verify the presence of infected alternate hosts of *M. lentisuturalis* and *T. hoffmanni*. In the lab, the mud was sieved, the oligochaetes collected, washed, placed in multi-well plates with deionized water and divided based on size into "large" and "small" worms. The plates were kept at RT and observed under a stereomicroscope every day for approximately 2 months; after each observation the water in each well was changed and if actinospores were present, filtered through a 20 µm mesh filter, measured by light microscope, placed in 2 ml tubes, and frozen at -20°C for molecular identification. All "large" oligochaetes were identified morphologically as *Branchiura sowerbyi*, while the "small" ones were identified as tubificids.

**RESULTS AND CONCLUSIONS:** Only branchiurids released different types of actinosporean stages, morphologically identified as belonging to 3 collective groups: Neoactinomyxum (3 types), Aurantiactinomyxon (2 types), and Raabeia (1 type). Molecular analysis allowed their identification as follow: Neoactinomyxum type 1 100% similarity with *Thelohanellus wangi*, Neoactinomyxum types 2 and 3, 99.5% similarity with *T. wuhanensis*, Aurantiactinomyxon types 1 and 2 high similarity with *Thelohanellus* sp., and Raabeia 100% identity with *M. lentisuturalis*. Some *B. sowerbyi* negative for actinospores were experimentally infected with a suspension of *M. lentisuturalis* spores from an infected goldfish. The oligochaetes were kept for about 2 months (estimated incubation time based on literature) in a small container equipped with an aerator and fed with spirulina once a week without ever changing the water. After the incubation period, the water was filtered and observed under a light microscope; Raabeia actinospores, the alternate stage of *M. lentisuturalis*, were detected and the identity confirmed by molecular analysis. Finally, some experimentally infected *B. sowerbyi* specimens underwent histological examination, showing pansporocysts of Raabeia at different developmental stages in intestinal epithelium.
This study confirmed that in Italian goldfish farms, *B. sowerbyi* is the oligochaetes that most frequently is involved as alternate host of several Myxozoa species infecting goldfish. Finally, the results of this study allowed the identification of the Raabeia actinosporean stage of *Myxobolus lentisuturalis*, but not the ones of *Thelohanellus hoffmanni* that need further investigations.

# TEMPERATURE AND SALINITY AS DRIVERS OF EGGS HATCHING SUCCESS OF *CONTRACAECUM RUDOLPHII* SP. A AND SP. B (NEMATODA: ANISAKIDAE): INSIGHTS FROM AN *IN VITRO* STUDY

Palomba M.*[1], Belli B.[1], Chiatante G.[1], Nascetti G.[1], Mattiucci S.[2]

*[1]Dept. Ecological and Biological Sciences (DEB), Tuscia University, Viterbo, Italy; [2]Dept. of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, Rome, Italy*

**Keywords:** *Contracaecum rudolphii (s.l.)*, Eggs hatching, Abiotic factors.

**INTRODUCTION:** Eggs hatching in parasitic nematodes is a complex and poorly understood process, despite its fundamental significance. Indeed, eggs serve various functions, acting as protective barriers and vehicles for the first larval stage to initiate infection (Mkandawire et al., 2022. Trends Parasit, 38:174-87). It has been found that abiotic factors, such as water salinity and temperature, are crucial cues for the hatching of anisakid eggs (Gomes et al., 2023. Parasit Int, 92:102684). To date, there is no knowledge about the hatching success of eggs of the anisakids *C. rudolphii* sp. A and *C. rudolphii* sp. B. Previous studies suggested that the two species have a life cycle adapted to brackish and freshwater environment, respectively (Mattiucci et al., 2020. Parasitol Res, 119:1-15). At adult stage, they occur in the great cormorant *Phalacrocorax carbo sinensis* with different relative proportions (Mattiucci et al., 2020).
The aim of this study is to investigate the influence of temperature and salinity on the hatching success of eggs of the two species, shedding light on how these factors contribute to maintain the distinctiveness of their life cycle in different aquatic ecosystems.

**MATERIALS AND METHODS:** Experiments were *in vitro* performed using eggs obtained from the uterus of each single female genetically identified as *C. rudolphii* sp. A or *C. rudolphii* sp. B. Approximately 500-1000 eggs from each female were placed in a 6-well cell culture plate, containing water at different increasing salinity values ranging from 0 to 70 psu and under thermal conditions of 5°C, 13°C, 20°C, to simulate conditions speculated in both freshwater and saline environments. The temperature of 32°C was also chosen to mimic a heatwave scenario. Six replicates for each combination of parameters were performed. Eggs hatching success and count were assessed daily.

**RESULTS AND CONCLUSIONS:** Eggs from both species successfully developed and hatched in freshwater at temperatures of 13°C, 20°C, 32°C, with the exception of 5°C, at which development was not observed. In both species the hatching duration varied, with the shortest observed at 32°C (3 days) and the longest at 13°C (14 days). Notably, significant differences between the species were observed in their salinity tolerance. *C. rudolphii* sp. A exhibited successful egg hatching across a salinity range of 0 to 70 psu, with optimal hatching occurring at 0-40 psu. In contrast, eggs of *C. rudolphii* sp. B showed narrower salinity tolerance, hatching only within the range of 0 to 20 psu. These findings suggest distinct adaptive strategies of *C. rudolphii* sp. A and *C. rudolphii* sp. B to saline and freshwater environments, respectively. These ecological adaptations may result from evolutionary selective pressures that have shaped the physiological characteristics of both species. This phenomenon likely underlies the differential distribution of the two species found in fish and cormorants from the two aquatic ecosystems (Mattiucci et al., 2020).

# *ANISAKIS* SPP. (NEMATODA: ANISAKIDAE) DIVERSITY IN PYGMY SPERM WHALE, *KOGIA BREVICEPS* (CETACEA: KOGIIDAE) STRANDED AT THE EDGE OF ITS DISTRIBUTION RANGE IN THE NORTHEAST ATLANTIC

Cipriani P.[1], Palomba M.[2], Aco-Alburqueque R.[2], Andolfi R.[1], Giulietti L.[3], Ten Doeschated M.[4], Brownlowd A.[4], Davison N.[4], Mattiucci S.*[1]

*[1]Dept. of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, Rome, Italy; [2]Dept. Ecological and Biological Sciences (DEB), Tuscia University, Viterbo, Italy; [3]Institute of Marine Research (IMR), Nordnes, Bergen, Norway; [4] Scottish Marine Animal Scheme, Institute of Biodiversity, Animal Health & Comparative Medicine University of Glasgow, Glasgow, United Kingdom*

**Keywords:** Anisakid species, *Kogia breviceps*, Northeast Atlantic.

**INTRODUCTION:** Anisakid nematodes are a globally distributed group of marine mammals parasites. Their life cycles are complex, involving adult stages that develop primarily in cetaceans, while planktonic or semi-planktonic crustaceans serve as first intermediate hosts, and fish and mollusks act as intermediate/paratenic hosts. Among the several cetacean species hosting adult stages of anisakid nematodes, Kogiid whales, including pygmy sperm whales, host a peculiar assemblage of specific anisakid species. So far, *Skrjabinisakis brevispiculata*, *S. paggiae*, and *Pseudoterranova ceticola* have all been originally described and found at adult stage only in kogiid whales (Mattiucci et al., 2018. Adv Parasitol, 99:93-263). The aim of this study was to investigate the anisakid species diversity in an individual pygmy sperm whale stranded at the northern boundary of its distribution range in the Northeast Atlantic, in the North of Scotland.

**MATERIALS AND METHODS:** Worms were collected from the cardiac section of the whale's stomach by Scottish Marine Animal Scheme (SMASS) and stored in 70% ethanol. A subsample of 95 nematodes was sent to the Sapienza University of Rome, Italy for identification. Nematodes were assigned to genus based on morphology and identified by sequence analysis of the mtDNA cox2 and the rDNA ITS genes.

**RESULTS AND CONCLUSIONS:** *S. brevispiculata* was the most prevalent species, accounting for 55% of the identified nematodes, predominantly adult females and males. All four Pseudoterranova specimens were females, exhibiting adult features but without fertilized eggs, presumably representing immature stages. The four *S. paggiae* specimens were female, with two of them showing mature developed eggs. All the worms assigned to the *A. simplex* complex morphotype were in a pre-adult developmental stage, with a mean length below 25 mm. This report marks the first observation of syntopic infection with adult stages of *Skrjabinisakis brevispiculata*, *S. paggiae*, and *Pseudoterranova ceticola* nematodes in a dwarf sperm whale in the NE Atlantic Ocean and represent the northernmost record of most of these species in this area. These species definitive host specificity for kogiid whales indicates a long co-evolutionary relationship, wherein the host-parasite species ecology has been shaped adapting to the meso- and bathypelagic zone. The pygmy sperm whale is rarely documented in Scottish waters, and its occurrence in the area could suggest a potential expansion of its range (Santos et al., 2003. Mar Mamm Sci, 22:600-16; Plön et al., 2023. Adv Mar Biol, 96:85-114). The presence of *S. brevispiculata*, *S. paggiae*, and *P. ceticola* in this whale species in this region may indicate that the whale was feeding in more southern regions before stranding, or either that the whole host community involved in the life cycle of these parasites is shifting into northern waters, most likely as a consequences of climate changes.

# XXXIII

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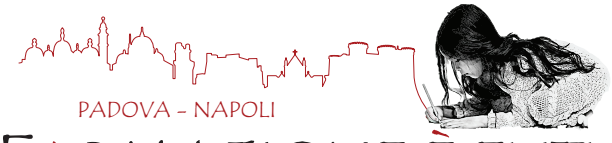
# PARASITIC INFECTIONS IN COMPANION ANIMALS



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# PREVALENCE OF ZOONOTIC HELMINTHS PARASITES IN HUNTING DOGS FROM SOUTHERN ITALY: A POTENTIAL PUBLIC HEALTH CONCERN

Humak F.*[1], Blazejak K.[2], Buono F.[1], Rivolta A.[3], Castaldo E.[1], Locantore F.[5], Piantedosi D.[1], Sgroi G.[4], Scarcelli S.[1], Mencke N.[2], Veneziano V.[1]

*[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy; [2]Vetoquinol S.A., Paris, France; [3]Vetoquinol Italia Srl, Forlì, Italy; [4]Experimental Zooprophylactic Institute of southern Italy, Department of Animal Health, Portici, Italy; [5]Department of Veterinary Medicine, University of Bari, Bari, Italy*

**Keywords:** Hunting dogs, Helminths, Zoonoses.

**INTRODUCTION:** Hunting dogs are involved in several hunting practices and activities linked to human in rural and sylvatic environments in close contact with wildlife (i.e. red foxes, wolves), increasing the spread of several zoonotic parasites to domestic animals and humans (Otranto et al., 2015. Vet Parasitol, 213:24-37). Thus, in the context of high prevalence and diffusion of parasitic infections, the risk of transmission of potentially zoonotic parasites to humans should not be neglected (Sgroi et al., 2022. Acta Trop,106502). This study aims to assess the prevalence of helminth infections and investigate associated risk factors in hunting dogs in southern Italy.

**MATERIALS AND METHODS:** The study was performed from October 2023 to January 2024 on 387 hunting dogs during one hunting season in Campania and Basilicata regions (southern Italy). Individual faecal samples were collected from each dog and individual Faecal Egg Counts (FECs) were performed using Mini-FLOTAC technique with a detection limit of 5 eggs per gram (EPG) of faeces. The floatation medium was saturated ZnSO4 (specific gravity 1.350) (Cringoli et al., 2017. Nat Protoc,12:1723-32). Dog owners were interviewed using a questionnaire to obtain further dog data on sex, age class (<2, 2-7, >7), hunting district and to increase knowledge on helminth control practices.

**RESULTS AND CONCLUSIONS:** On 387 dogs, 208 (53.7%) were males and 179 (46.3%) females; 72 (18.6%), 204 (52.7%), 40 (10.3%) belonging to the following age class <2, 2-7, >7, respectively. In 71 (18.3%) age was not reported. In total, 51.9% (201/387) of dogs were helminth positive in single or mixed infection. *Ancylostoma caninum* (51.2%) was the commonest helminth, followed by *Trichuris vulpis* (44.8%), *Capillaria* spp. (27.9%: *C. aerophila* 57.1%, *C. boehmi* 26.8%, and coinfection 16.1%), *Toxocara canis* (23.4%), *Dipylidium caninum* (12.4%), *Taenia* spp. (2.5%) and, *Toxascaris leonina* (0.5%). Age was a significant factor ($p<0.001$) for all species except *Capillaria* ($p=0.16$). Hunting district was significant ($p<0.001$) for *A. caninum*, *T. canis* and *D. caninum*. Sex was not significant for any parasite ($p>0.05$). Most of the dogs (68.3%) were treated 1 or 2 time/years, while 31.7% were treated more than 2 time/year. This parasite control practice suggests a low frequency of anthelmintic treatment in this dog population. Considering the high exposure to helminth infection in hunting dogs the treatment frequency needs to be increase as recommended in high risk dogs (ESCCAP GL1,2021,1-42). The most prevalent zoonotic parasite detected was *A. caninum*, followed by *T. canis*, an important parasite causing human toxocariosis affecting several organ systems including the central nervous system. The high helminth prevalence and the risk of zoonotic transmission suggest the development of a Piano Assistenza Parassitologica Cani Caccia (PAPCC) to preserve the health and welfare of both hunting dogs and humans in One Health perspective.

# CANINE LEISHMANIOSIS CAUSED BY *LEISHMANIA INFANTUM* IN THE BOLIVIAN CHACO

Napoli E.[1], Macchioni F.[2], Cosmi F.[3], De Benedetto G.[1], Medina W.U.[4], Moreno M.M.[4], Brianti E.[1], Gabrielli S.*[5]

*[1]University of Messina, Department of Veterinary Sciences, Messina, Italy; [2]University of Pisa, Department of Veterinary Sciences, Pisa, Italy; [3]Convenio de Salud, Camiri, Bolivia; [4]Universidad Autónoma Gabriel René Moreno, Facultad Integral del Chaco, Camiri, Bolivia; [5]Sapienza University of Rome, Department of Public Health and Infectious Diseases, Rome, Italy*

**Keywords:** *Leishmania*, Low-income country, One-health.

**INTRODUCTION:** Canine leishmaniosis (CanL) is widespread in South America (Dantas-Torres, 2009. Parasit Vectors, Suppl 1:S1) but epidemiological data on CanL from Bolivia are limited (Mollinedo et al., 2020. Rev Soc Bras Med Trop, 53:e20190421). We conducted the present study in the Bolivian Chaco, where, despite the improvements in the last 15 years, poverty remains high in rural areas. People live in remote settlements characterized by a low hygiene level and by a high sympatry between humans and domestic animals.

**MATERIALS AND METHODS:** Sampling was carried out in two rural communities, Camiri and Villamontes, with a total of 46 and 143 blood samples collected from dogs, respectively. Sera were tested by a rapid diagnostic test (Apacor) and by a commercial ELISA (IDVet). Furthermore, 83 and 68 dried blood spots (DBSs) were collected from healthy school-age children (SAC), 8-9 y.o., in Camiri and Villamontes, respectively. Human sera were extracted from DBSs (Formenti et al., 2016. Front Microbiol, 7:1778) and tested by ELISA (Cypress Diagnostics). Genomic DNA was extracted from both dog blood samples and human DBSs and tested by qPCR (Genesig®). Additionally, a partial region of the ITS1 (~300bp) from qPCR-positive samples were successively amplified (Schönian et al., 2003. Diagn Microbiol Infect Dis, 47(1):349-58) and sequenced. Sequences were compared with those available in GenBank using the BLASTn tool.

**RESULTS AND CONCLUSIONS:** Eighty-seven dog sera (46.03%) tested positive for *Leishmania* spp. in at least one serological assay, with 29.88% of the sera positive to both tests (k= 69%). Significant difference in prevalence was observed between Villamontes (seroprevalence=60%) and Camiri (seroprevalence=26%) ($p<0.05$). This different prevalence reflects the clinical status of the tested animals as most dogs from Villamontes showed clinical signs of CanL while those from Camiri were clinically healthy. *Leishmania* DNA was found in the blood of 7.2% seropositive dogs and identified by sequencing like *L. infantum*. Furthermore, anti-*Leishmania* antibodies were found in 13.24% of the children from Villamontes while none of the SAC from Camiri scored seropositive. qPCR yielded negative results for all human DBSs. According to a One Health approach, surveys to define the epidemiological scenario of zoonotic pathogens are of great importance, especially in low-income countries where humans and animals live in close contact. The results of this survey confirmed the role of dogs as reservoir of leishmaniosis in the domestic environment in Bolivia. Despite no parasite circulation was identified in SAC, 13% of the healthy children showed antibodies against *Leishmania*. Considering the poor hygiene conditions in rural communities and the lack of systematic vector control and prevention measures for CanL, further studies are needed to assess the prevalence of human leishmaniosis in such close and low-income environment where many interactions between humans and infected/diseased dogs occur.

# *CAPILLARIA BOEHMI* (TRICHOCEPHALIDA: CAPILLARIIDAE) AND OTHER HELMINTH INFECTIONS IN DOGS FROM SOUTHERN ITALY

Lia R.P.*, Bezerra-Santos M.A., Locantore F., Mendoza-Roldan J.A., Otranto D.

*Department of Veterinary Medicine, University of Bari, Valenzano, Italy*

**Keywords:** Dog hunting, Truffle hunting, *Eucoleus boehmi*.

**INTRODUCTION:** *Capillaria boehmi* (syn. *Eucoleus boehmi*) and *Capillaria aerophila* (syn. *Eucoleus aerophilus*) are trichurids of the upper respiratory tract (i.e., nasal turbinates and the frontal and paranasal sinuses) of domestic carnivores as well as foxes. Clinical signs in infected dogs include nasal discharge, sneezing, and epistaxis. Despite the morphological differences in egg morphology, *C. boehmi* infections are occasionally misdiagnosed with *C. aerophila* infections. In Italy, data on the occurrence of *C. boehmi* in dogs is scarcely reported. In this study, we provide data of a coprological survey in hunting dogs, reporting the presence of *C. boehmi* in Basilicata region.

**MATERIALS AND METHODS:** Fresh fecal samples were collected from 197 hunting dogs living in 10 different rural areas of the Basilicata region (southern Italy). Samples were evaluated by Mini-FLOTAC using zinc chloride flotation solution for detecting helminth eggs and by modified Baermann technique to investigate the presence of lungworms.

**RESULTS AND CONCLUSIONS:** Out of 197 dog fecal samples, 88 (44.6%) scored positive for one or more helminth species. The total number of dogs infected by respiratory nematodes (i.e., *C. aerophila* and *C. boehmi*) was 20 out of 88 (22.72%) of which 12 dogs were positive for *C. boehmi*, three for *C. aerophila*, five co-infected by both parasites, and eight co-infected with other intestinal nematodes (see below). Sixty-eight out of 88 (77.27%) dogs scored positive for intestinal nematodes (i.e., hookworms, *Trichuris vulpis*, *Toxocara canis* and *Toxascaris leonina*), with Ancylostomatidae being the most frequent parasite species retrieved (61.5%), followed by *T. vulpis* (17.7%), *T. canis* (13.2%) and *T. leonina* (4.4%). In the samples examined at the Baermann technique; first stage larvae of Angiostrongylus vasorum (2.5%) and *Crenosoma vulpis* (0.5%) were detected. Data herein reported provides information on the occurrence of respiratory and intestinal helminths in hunting dogs from Southern Italy. Noteworthy, the presence of C. bohemi in some areas of the Basilicata region is higher than what was found in a previous report in Italy (i.e., 2% in Sicily; Brianti et al., 2018. XXX Congresso SoIPa, Milano). Veterinarians must carry out periodic copro-microscopic examinations to identify *C. bohemi* positive hunting and truffle-hunting dogs so that olfactory ability is not impaired. Diagnostic experience is therefore required to morphologically identify the eggs of *C. aerophila* and *C. bohemi* to provide a more accurate diagnosis, as the morphological differences in the eggs between both parasites are minimal.

# ASSESSING THE CANINE LEISHMANIOSIS IN CENTRAL ITALY: A TWO-YEAR SEROLOGICAL STUDY IN OWNED DOGS

Gabrielli S.*[1], Pombi M.[1], Trotta M.[2], Leto V.[2], Trichei S.[1], Augello A.[1], Fanì C.[2]

*[1]Sapienza University of Rome, Department of Public Health and Infectious diseases, Rome, Italy; [2]CDVet, Laboratorio Analisi Veterinarie, Rome, Italy*

**Keywords:** Leishmaniosis, Epidemiology, Dogs.

**INTRODUCTION:** Canine leishmaniosis (CanL) caused by *Leishmania infantum* is an important zoonosis in Mediterranean basin where domestic dogs are considered the main reservoir hosts. CanL is endemic in Italy, with highest prevalence in southern and insular regions, according to the distribution of the sand fly vector species and of the dog reservoirs (Otranto et al., 2010. Parasit Vectors, 3:2; Mendoza-Roldan et al., 2020. Parasit Vectors, 13(1):193). In Central Italy, CanL has been scantly investigated with a recent prevalence of 2.5% reported in kennel dogs (Sauda et al., 2018. Parasite, 25:2) and of 74.3% in owned dogs (Rombolà et al., 2021. PLoS One, 16(1): e0244923).

**MATERIALS AND METHODS:** A retrospective study was conducted from January 2021 to December 2022 to evaluate the seroprevalence for *L. infantum* in owned dogs admitted at the CDVet Research laboratory (Rome, Italy) for routine screening or with clinical suspicion of leishmaniasis. 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 the cut-off dilution of 1:80, as recommended by LeishVet guidelines (Solano-Gallego et al., 2011. Parasit Vectors, 4:86).

**RESULTS AND CONCLUSIONS:** A total of 14,322 serum samples (5,205 in 2021, 9,117 in 2022) were collected for the diagnosis of *Leishmania*. Although samples were collected from 28 provinces across 13 Italian regions, 94% of these were from Rome province (Latium region). The overall rate of CanL infection was 38.5%, confirming the high circulation of the parasite in the country. The seroprevalence in the three most heavily sampled regions was as follows: Latium 38.3% (N=13,693), Umbria 35.8% (N=232), and Marche 54.4% (N=202). For the mostly represented region, Latium, 2021 and 2022 the rate of positive samples was 29.7% and 43.1%, respectively.
This study unveils an high prevalence of *Leishmania* infection in Central Italy, confirming a widespread presence of the parasite within the dog reservoir. The elevated infection rates observed in the Marche, Latium, and Umbria regions further underscore the geographic disparity and potential regional hotspots of *Leishmania* transmission. The upward trend in rates detected in Latium suggests an intensification of *L. infantum* circulation, raising significant public health concerns.

# INTESTINAL PARASITIC INFECTIONS IN KENNELS OF SARDINIA, ITALY

Zeinoun P., Nonnis F.*, Cavallo L., Carta C., Arshad F., Sini M.F., Sechi S., Caggiu S., Pentcheva P., Pinna Parpaglia M.L., Tedde F., Tamponi C., Scala A., Varcasia A.

*University of Sassari, Department of Veterinary Medicine, Sassari, Italy*

**Keywords:** Gastrointestinal parasites, Shelter dogs, Endoparasites.

**INTRODUCTION:** Gastrointestinal parasites are a continuous challenge for animal kennels. Appropriate measures require much time and the groups of animals in shelters are constantly changing because of continuous migration of the animals, therefore parasitic infections are considered an inherent factor of the environment of animal shelters. Contamination of the environment with eggs, larvae and cysts of parasites facilitates re-infection of the animals and poses a risk for humans since zoonotic parasites are commonly found in animal shelters. This is particularly for those infections transmitted by direct contact or ingestion of contaminated food, water or soil, and from licking contaminated surfaces (Oliveira-Sequeira et al., 2002. Vet Parasitol, 103:19-27). In Sardinia, no recent studies show the prevalence of intestinal parasites in kennels, for this reason we found it appropriate to conduct this study.

**MATERIALS AND METHODS:** In total, 264 fecal samples were collected from the boxes of dogs from 3 kennels in Sardinia: 217 from Sassari, 27 from Cardedu (NU) and 20 from Tortolì (NU). The research was performed using a copromicroscopic examination with a centrifugation flotation test using a zinc sulphate solution (ZnSO4, specific gravity 1200) (Zajak et al., 2002. J Am Anim Hosp Assoc, 38:221-24).

**RESULTS AND CONCLUSIONS:** Parasite eggs and cysts were found in 100% (3/3) of monitored kennels and in 56% (148/264) of the animals examined. Prevalence rates varied between kennels, with the highest being in Sassari 58.1% (126/217), followed by Tortolì 55% (11/20) and finally Cardedu with the lowest percentage 40.7% (11/27). The difference in prevalence values between the kennels was not significant ($\chi 2=2.935$; df=2; P= 0.230). The most common findings were respectively Hookworms 26.9% (71/264) and *Trichuris vulpis* 25.4% (67/264), amongst other findings were *Giardia duodenalis* 14% (37/264), Ascarids 5.7% (15/264), *Cystoisospora* sp. 2.7% (7/264) and finally *Sarcocystis* sp. 0.8% (2/264). The prevalence rates of parasites causing gastrointestinal infections indicates the need of efficient veterinary and biosecurity measures in dog shelters. Our results highlight the need for strategies to control parasites in stray and shelter dogs in Sardinia, particularly considering that many of the identified parasite species are also potential zoonotic diseases. These results will be useful for establishing health care programs in dog shelters and implementing effective strategies for the control of intestinal parasites. Therefore, a combination of routine screening of fecal samples for parasites and a strategic anthelmintic regime is recommended.

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# EPIDEMIOLOGICAL UPDATES ON INTESTINAL PARASITES IN DOGS AND CATS FROM SARDINIA, ITALY

Tamponi C.*, Nonnis F., Dessì G., Tosciri G., Cavallo L., Zeinoun P., Sini M.F., Carta C., Arshad F., Varcasia A., Scala A.

Department of Veterinary Medicine, University of Sassari, Sassari, Italy

**Keywords:** Endoparasites, Pets, Zoonosis.

**INTRODUCTION:** The constant monitoring of intestinal parasites (IP) in pets is of primary importance as they can lead to severe clinical signs and also can be a cause of concern for human health, as pets can be infected by zoonotic parasites. The last study on pets endoparasites carried out in Sardinia referred to the years 2011-2015 (Tamponi et al., 2017. Vet Parasitol Reg Stud Reports, 10:13-17). The aim of this study is therefore to update the epidemiological data after almost 10 years to see how the parasitological scenario have been changed.

**MATERIALS AND METHODS:** Faecal samples of 735 owned dogs and 1390 owned cats referred between April 2014 and March 2024 were examined for IP. For each sampled animal, data on sex and age were recorded. Copro-microscopic examination was performed using the Wisconsin technique, with a zinc sulphate (ZnSO4) flotation solution (s.g. 1.2) as previously described (Tamponi et al., 2017. Vet Parasitol Reg Stud Reports, 10:13-17).

**RESULTS AND CONCLUSIONS:** Overall, 39.2% (288/735) of examined dogs and 41.2% (573/1390) of cats tested positive for IP, showing no significant differences between prevalence ($\chi2 = 0.829$; $P = 0.362$). In dogs, protozoan infections were more frequent than helminth infections (28.2% vs 17 %; $\chi2 = 24.439$; $P < 0.001$). In details, the detected parasites were *Giardia duodenalis* (18.4%), *Cystoisospora* sp. (11.7%), ascarids (10.9%), hookworms (3.5%), *Trichuris vulpis* (3.0%), *Sarcocystis* sp. (1.5%), and Taeniid cestodes (1.0%). Among cats, protozoan infections were more prevalent than helminth infections (28.8% vs 17 %; $\chi2 = 54.834$; $P < 0.001$) and the recovered parasites were *Cystoisospora* sp. (21.2%), ascarids (14.0%), *G. duodenalis* (9.4%), hookworms (2.9%), Taeniid cestodes (1.9%), *Eucoleus aerophilus* (0.5%) and *Sarcocystis* sp. (0.1%). The mean age of the sampled dogs was 20.5 months (SD ± 33.2), while that of cats was 13 months (SD ± 22.7). Animals younger than 6 months were found to be more frequently infected by IP than older animals both in dogs (48.7% vs 31.5 %; $\chi2 = 19.769$; $P < 0.001$) and cats (49.02% vs 29.6%; $\chi2 = 44.2$; $P < 0.001$). Prevalence found in male dogs (39.8%) did not statistically differ from those found in females (33.2%; $\chi2= 2.99$; $P = 0.083$), whereas the male cats showed significantly higher prevalence (44.5%) than females (38.7; $x2 = 4.692$; $P = 0.03$). The statistical comparison between this study and the previous one carried out 10 years ago showed a similar prevalence to the for IP in dogs (39.2% vs 34.6%; $\chi2= 3.063$; $P = 0.08$), but a significantly higher prevalence in cats (41.2% vs 34.4%; $\chi2= 5.338$; $P = 0.02$). Interestingly, the distribution of helminth/protozoan had an inversion with the protozoan parasites being more prevalent in this last survey. This could be due to the increased attention of owners to the parasite prevention, that are mainly achieved by the administration of antiparasitic formulation that are effective against helminths but not against protozoan. Acknowledgements: Funded by Bando Fondazione Sardegna 2018-2021 FDS1821VARCASIA

# CLINICAL EFFICACY OF NEXGARD® COMBO (EPRINOMECTIN, ESAFOXOLANER AND PRAZIQUANTEL) IN THE TREATMENT OF NATURAL AELUROSTRONGYLOSIS AND TROGLOSTRONGYLOSIS

Di Cesare A.[1], Veronesi F.[2], Crisi P.E.[1], Vignoli M.[1], Morganti G.[2], Colombo M.[1], Morelli S.[1], Iorio R.[1], Tielemans E.[3], Beugnet F.[3], Traversa D.*[1]

*[1]Department of Veterinary Medicine, University of Teramo, Teramo, Italy; [2]Department of Veterinary Medicine, University of Perugia, Perugia, Italy; [3]Boehringer Ingelheim Animal Health, Lyon, France*

**Keywords:** Aelurostrongylosis, Troglostrongylosis, Clinical efficacy.

**INTRODUCTION:** Aelurostrongylosis and troglostrongylosis caused by *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* are among the most common feline respiratory diseases. Adult parasites inhabit the respiratory tract and cats become infected by ingesting infectious third-stage larvae (L3) in intermediate hosts, i.e. terrestrial gastropods, or more commonly by preying on paratenic hosts, i.e. birds, rodents, small reptiles. *Troglostrongylus brevior* can be also transmitted vertically from the queen to the litter (Morelli et al., 2021. Pathogens, 10:454). Usually, the efficacy of parasiticides vs. lungworms is assessed by evaluating the reduction of adult worms in the lungs of treated cats and the interruption of first-stage larvae (L1) faecal shedding. Conversely, data on the efficacy of parasiticides in terms of clinical recovery of cats from lungworm disease are rare. This study evaluated the clinical efficacy of a combination of eprinomectin, esafoxolaner and praziquantel (NexGard® COMBO) in cats with natural aelurostrongylosis and/or troglostrongylosis by assessing the resolution of clinical, radiographic and laboratory abnormalities in treated animals, (partly) in comparison to untreated animals.

**MATERIALS AND METHODS:** Overall, 36 naturally infected cats were randomly assigned to one of two Study Groups (G), which included 18 cats each, i.e. 6 cats infected with *A. abstrusus*, 6 with *T. brevior*, and 6 with mixed infection. Cats of G1 were treated on Study Days (SDs) 0 and 28±2, while those of G2 remained untreated until SD 56±2 (acting as a control group), then received the treatment on SDs 56±2 and 84±2. Clinical and parasitological evaluations were conducted at different timepoints (SDs 28±2 and 56±2 for G1 cats, while G2 cats were further evaluated on SDs 84±2 and 112±2) *via* clinical examination, chest radiography and quali-quantitative Baermann examination to evaluate the number of larvae per gram (LPG) in faeces. The efficacy criteria were the reduction of larval shedding in faeces and the clinical response in terms of radiographic and pathological scores.

**RESULTS AND CONCLUSIONS:** Two administrations of NexGard® COMBO 28 days apart were 100% effective in the interruption of larval shedding in cats with single and mixed infection. Significant recovery from clinical signs and radiographic alterations was observed in 34 cats within 8 weeks after the first administration. Two cats with monospecific infection by *A. abstrusus* and *T. brevior*, respectively, required 16 and 12 weeks for complete radiographic healing. These results indicate that a proper follow-up of cats with aelurostrongylosis and/or troglotrongylosis should combine clinical visit, thoracic x-ray and hematobiochemical analyses.

# XXXIII

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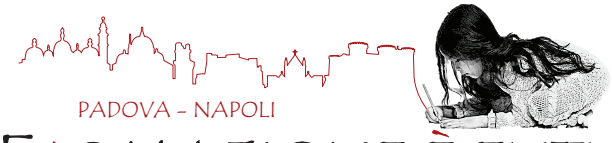
# PARASITIC INFECTIONS IN FARM ANIMALS



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# UNRAVELING IMPLICATIONS OF *NEOSPORA CANINUM* IN DAIRY CATTLE FARMS IN ITALY: SEROPREVALENCE AND EFFECT ON HERD REPRODUCTIVE AND PRODUCTIVE PARAMETERS

Villa L.*[1], Allievi C.[1], Gelati G.[2], Zanchetta R.[3], Gazzonis A.[1], Mortarino M.[1], Manfredi M.T.[1]

*[1]Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Lodi, Italy; [2]Bovine veterinarian, Crema, Italy; [3]Bovine veterinarian, Milano, Italy*

**Keywords:** Neosporosis, Cows, Serology.

**INTRODUCTION:** *Neospora caninum*, a protozoan parasite, is a major cause of bovine abortion worldwide. An epidemiological study on tank bulk milk reported a prevalence of 30.7% in farms located in the Po Valley (Villa et al., 2024. Acta Trop, 254:107194); a preliminary study on two dairy herds in Lombardy suggested an adverse effect of the parasite in early pregnancy and on milk yield (Villa et al., 2022. Animals, 12:786). This epidemiological study aimed to evaluate the seroprevalence (P) of *N. caninum* at the herd level and the relationship of bovine serostatus on herd reproductive and productive parameters in dairy cattle farms in Italy.

**MATERIALS AND METHODS:** In 13 selected herds with a positive result during the screening on tank bulk milk, 2,576 blood samples from all cows above 24 months of age were analyzed by an immunofluorescence antibody test (Megacor). These were medium-large farms with herd consistency between 120 and 860 animals raised in intensive production system based on Holstein Friesian cows. The herds were located in the provinces of Bergamo (n=1), Brescia (n=1), Cremona (n=8), and Milano (n=3). The abortion rate in these farms varied between 0.9 and 15.1%. Information on herd reproductive and productive performances and individual data of cattle were collected. Generalized linear models (GLMs) were developed.

**RESULTS AND CONCLUSIONS:** 834 individual samples showed *N. caninum* antibodies (P=32.4%); the intra-herd seroprevalence varied between 8.9 and 61.6%. Medium age and number of lactations of seropositive animals were slightly lower than negative ones (47.1 vs 47.3 and 2.17 and 2.23). Overall, the number of inseminations for conception (2.4 vs 2.2) and days in milking (209.6 vs 198.6) were higher in seropositive than seronegative cows; on the contrary, daily milk production (34.4 kg vs 35.7 kg) and 305 mature equivalent milk yield (305ME) (11730.8 vs 12184.4) were lower in seropositive than seronegative animals. According to the number of insemination classes, the cows with 3 or more inseminations showed a higher seroprevalence (P= 37.5 %) if compared to those with less than 3 inseminations (P=29.2%) (OR=1.4, $p$=0.000). Moreover, a higher seroprevalence of *N. caninum* was detected in cows with a daily milk production lower than or equal to 20 kg (P=40.6%) and between 21 and 25 kg (P =34.8%) (OR=1.8 and 1.4, $p$=0.027 and 0.049, respectively), whereas animals producing between 26 kg and 30 kg (P=29.2%) and more than 30 kg (P =27.3%) of milk were less often positive. Besides, a higher seroprevalence was detected in cows with 305ME lower than 10000 if compared to those with this value equal to or higher than 10000 (P=39.3 and 29.7%, respectively) (OR=1.5, $p$=0.011). Antibodies to *N. caninum* resulted widely spread in Italian dairy herds. A relationship between the serostatus and reproductive and productive parameters of the dairy cows was demonstrated. However, even if in most of the surveyed herds the number of abortions was limited, data seem to support an effect of *N. caninum* in early pregnancy.

# ADVANCEMENTS IN DIAGNOSING *BESNOITIA BESNOITI* IN CATTLE: EVALUATING THE DIAGNOSTIC ACCURACY NASAL AND SCLEROCONJUNCTIVAL SWABS AS NEW DIAGNOSTIC MATRICES

Dini F.M.*[1], Galuppi R.[1], Graziosi G.[1], Militerno G.[1], Poluzzi A.[1], Ogundipe T.G.[1], Gentile A.[1], Goncalves Pontes Jacinto J.[2]

*[1]Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy; [2]Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Bern, Switzerland*

**Keywords:** Besnoitiosis, Diagnosis, Swabs.

**INTRODUCTION:** Bovine besnoitiosis, caused by the parasite *Besnoitia besnoiti*, is re-emerging globally, affecting cattle health and causing economic losses (Gentile et al., 2012. Vet Parasitol, 184:108-15). The disease involves acute and chronic phases, with tachyzoites causing clinical symptoms in the former and bradyzoite-containing tissue cysts leading to chronic manifestations. The definitive host remains unidentified, though domestic cats are suspected. Transmission occurs through close contact or via biting flies (Álvarez-García et al., 2013. Trends Parasitol, 29:407-15). Early detection is crucial, with PCR as a reference technique, though invasive skin biopsies are currently used. The aim of our study was to describe the diagnostic accuracy of PCR to identify *B. besnoiti* in naturally exposed cattle using innovative non-invasive diagnostic matrices as nasal and conjunctival swabs, in comparison to the same PCR protocol applied to skin biopsies and to the histological examination of skin biopsies.

**MATERIALS AND METHODS:** The study involved 57 Limousine cattle from various farms in the Apennines mountains in Italy. Clinical examinations, focusing on mucosae and tegmentum inspection, were conducted. Skin biopsies were obtained from the neck, nasal and scleroconjunctival swabs were sampled. Histopathology was performed on one 8mm biopsy for each animal, while mucosal swabs and one 5mm skin biopsy for each animal underwent DNA extraction and were tested by end-point PCR targeting the ITS gene of *B. besnoiti*. Diagnostic sensitivity (Se) and specificity (Sp) of histological examination of skin biopsies (HIS-SK), end-point PCR of skin biopsy (PCR-SK), nasal swabs (PCR-NS) and scleroconjunctival swabs (PCR- SC) were assessed using a Bayesian Latent Class Model analysis.

**RESULTS AND CONCLUSIONS:** In the selected model, histological examination of skin biopsies (HIS-SK) exhibited the highest Sp (99.1%), compared to the PCR-SK (71.6%) and PCR-NS-SC (47.6%). Regarding Se, PCR-SK and PCR-NS-SC showed similar results (PCR-SK = 91.0%), compared with 85.0% of PCR-NS-SC, while the Se of HIS-SK resulted lower (33.0%). Overall, the sensitivity of the PCR-NS-SC was adequate to diagnose *B. besnoiti*, however, it lacked in specificity. Consequently, the PCR-NS-SC could serve as a valuable screening test for detecting bovine besnoitiosis. This new diagnostic approach can be of valuable importance in clinical routine, as the non-invasive matrices used for diagnosis do not require strict containment of the animal, thereby also promoting animal welfare.

# EQUINE BESNOITIOSIS IN DONKEYS: CLINICAL AND LABORATORY FINDINGS IN A CASE-STUDY STABLE

Gazzonis A.L.*[1], Morganti G.[2], Falomo M.E.[3], Frangipane Di Regalbono A.[3], Ciuca L.[4], Buono F.[4], Buffa E.[1], Calgaro V.[2], Giordano A.[1], Sironi G.[1], Veneziano V.[4]

*[1]Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Lodi, Italy; [2]Department of Veterinary Medicine, University of Perugia, Perugia, Italy; [3]Department of Animal Medicine, Production and Health, University of Padova, Legnaro, Italy; [4]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Napoli, Italy*

**Keywords:** Equine besnoitiosis, Donkey, *Besnoitia* sp.

**INTRODUCTION:** Besnoitiosis in donkeys is an emerging disease in Europe, with clinical cases reported also in Italy, in parallel with the (re-)emergence of besnoitiosis in cattle. Equine besnoitiosis (EB) is caused by *Besnoitia bennetti*, and the clinical course of the infection is similar to that observed in cattle: the majority of animals remains asymptomatic, and only a small number develops cysts on the skin and in other areas (e.g., scleral cysts pathognomonic of *Besnoitia* sp. infection) and other skin lesions (alopecia, thickening, and crusting), until general health conditions deteriorate. The present work aims to describe clinical features and laboratory findings of EB detected in a donkey stable in northern Italy.

**MATERIALS AND METHODS:** EB was suspected in one (Donkey.6) of the ten animals of the stable for alopecic and crustose lesions in the facial region, involving the muzzle and the nasal plane up to the periorbital region. To confirm the suspected diagnosis, a skin punch was performed from the muzzle for histological and molecular analysis (ITS-1 Real-Time PCR). Blood sample was taken from Donkey.6 and the other nine animals which were healthy and non-symptomatic for EB: EDTA-blood samples were submitted to haematological analysis and the detection of circulating parasitic DNA, while serum samples were used for biochemistry, including serum amyloid A (SAA) and electrophoresis, and immunological analyses for specific antibody detection. Particularly, serological analysis was carried with ELISA and indirect immunofluorescence test using in parallel two commercial kits: ID Screen® *Besnoitia* Indirect 2.0 (IDvet, France) with a secondary non-species-specific conjugate, and MegaFLUO® *BESNOITIA besnoiti* (Megacor, Austria) using a 1:50 cut-off (Elsheikha et al., 2020. Parasit Vectors, 13:279) with a secondary anti-horse IgG.

**RESULTS AND CONCLUSIONS:** Histology showed the presence of *Besnoitia* sp. cysts in the skin biopsy, confirmed by the molecular detection of parasitic DNA. Molecular characterization of the isolate is ongoing to confirm the identification of *Besnoitia* species involved. Specific antibodies were found in Donkey.6 and in other four animals, while parasitic DNA was not found in blood samples. Haematological analyses highlighted a shift in the neutrophil/lymphocyte ratio, with a higher lymphocytes percentage in seropositive than in seronegative animals (mean Neu%-Lymph%: 37.9-46.8 and 45.1-37.4, respectively), with the exception of Donkey.4, the only seropositive animal with markers suggestive of inflammation (e.g., SAA =315 ug/mL, normality range 0-45 ug/mL). The investigations confirmed besnoitiosis in the symptomatic donkey and the spread of the infection within the animals belonging to the stable. Further longitudinal investigations will allow to verify the spread of the infection and the possible appearance of new clinical cases. The study-case here described suggests how EB is possibly widespread in Italy, highlighting the need to plan epidemiology studies at a large-scale.

# LONG TERM STUDY ON ANOPLOCEPHALIDAE INFECTIONS IN HORSES IN ITALY

Buono F.*[1], Veronesi F.[2], Castaldo E.[1], Mazzeo G.[3], Cimini S.[3], Roncoroni C.[4], Veneziano V.[1]

*[1]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Napoli, Italy; [2]Department of Veterinary Medicine, University of Perugia, Perugia, Italy; [3]MSD Animal Health, Milano, Italy; [4]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri, Roma, Italy*

**Keywords:** Equids, Risk factor, Tapeworms.

**INTRODUCTION:** Equids can be infected by three species of tapeworms: *Anoplocephala perfoliata*, *Anoplocephala magna* and *Paranoplocephala mamillana*, of which the most pathogenic is *A. perforliata* that can cause hyperemia, mucosal thickening, diptheric membrane and necrotic ulcers at the site of attachment. *Anoplocephala* spp. infections can reach a prevalence up to 60% (Nielsen, 2016. Equine Vet Educ, 28:388-95) and generally horses with a low infection rate do not show clinical signs. To date, in Italy there are only few local reports on the prevalence and distribution of *Anoplocephala* spp. infections. The aim of this study was to determine the prevalence and distribution of *Anoplocephala* spp. in horses throughout Italyand to investigate the associated risk factors.

**MATERIALS AND METHODS:** The study was performed for nine years (November 2014 - November 2023) and included 12,056 horses. Individual faecal samples were collected by veterinary in practice involved in a national project “Guida al Programma di Controllo degli endoparassiti del cavallo e dell’antielmintico resistenza (AHR)”. For each horse sex (intact male, female, and gelding), age, body condition score (BCS scale 1-5), presence of pasture (yes or no), living area (northern, central, southern Italy and islands) and, sampling season period (spring, summer, autumn, and winter) were recorded. Individual faecal egg counts (FECs) were performed using the Mini-Flotac technique (Barda et al., 2013. PLoS Negl Trop Dis, 7:e2344) and a centrifugation/floatation technique (Proudman and Edwards, 1992). The floatation medium used was the Sheather’s sugar solution with a specific gravity of 1.250. Statistical analysis was performed using Chi-square test.

**RESULTS AND CONCLUSIONS:** An overall prevalence of 3.7% (452/12,056, 95%CI: 3.4 - 4.1) of *Anoplocephala* spp. eggs was found. Most of positive horses (173/452 - 38.3%) aged between 1-4 years, followed by horses <1 year (121/452, 26.8%), between 5-15 years (120/452, 26.5%), and >15 years (38/452, 8.4%). Of 452 positive samples, 402 (88.9%) belonging to horses that had access to pasture whereas 50 (11.1%) belonging to animals that lived in stall box with or without access to a small, annexed paddock. Regarding living area, 252/452 (55.8%) come from central Italy, followed by 126/452 (27.9%), 72/452, (15.9%) and 2/452 (0.4%) from northern Italy, southern Italy, and islands, respectively. The majority of positive samples (141/452 - 31.2%) were recorded in autumn, followed by 129/452 (28.5%) in winter, 111/452 (24.6%) in spring, and 71/452 (15.7%) in summer. Sex, age class, access to pasture, living area were significantly associated with the infection. The prevalence reported in the present study and the observation that tapeworms cannot infect all horses present in the same premises highlight the need to develop a “Parasitological Assistance Plan in Equids” (PAPE) specific for each horse.

# SURVEY ON INTESTINAL STRONGYLES IN STABLED HORSES IN NORTHERN ITALY, WITH A FOCUS ON THE PRESENCE OF *STRONGYLUS VULGARIS*

Gazzonis A.L.*, Cafiso A., Dalla Costa E., Dolia A., Sobrero L., Riva M.G., Villa C., Molteni S., Stocchero C., Zanzani S., Bazzocchi C., Mortarino M., Manfredi M.T.

*Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Lodi, Italy*

**Keywords:** Intestinal strongyle, *Strongylus vulgaris*, Horse.

**INTRODUCTION:** The presence of intestinal parasites, especially intestinal strongyles, is normal in horses. Over the years, however, the use of anthelmintic drugs, while on the one hand significantly reduced the prevalence of parasites, especially of migratory compared to non-migratory strongyles, on the other hand also led to the rise of resistance phenomena to most available anthelmintic drugs (Matthews, 2014. Int J Parasitol Drugs Drug Resist, 4:310-315). To mitigate the further spread of drug resistance, international consensus suggests the use of pharmacological protocols based on selective or strategic treatments. It is therefore important to evaluate the reappearance of highly pathogenic species. The present study aims to evaluate the parasitological status of horses stabled in Lombardy and to investigate the presence of *Strongylus* spp. using both morphological and molecular diagnostic techniques.

**MATERIALS AND METHODS:** Fecal samples from 440 horses from 25 stables were collected and analyzed with FLOTAC Dual-Technique for fecal egg count. For samples testing positive for the presence of Strongyle eggs, individual coprocultures were prepared to obtain third-stage larvae (L3) to be subjected to morphological identification and molecular analysis for the detection of *Strongylus* spp. DNA through real-time PCRs (Jürgenschellert et al., 2022. Front Vet Sci, 10:892920), followed by sequencing for species confirmation. Individual and management risk factors influencing the spread of Strongyle and particularly of *S. vulgaris* were statistically analyzed through Generalized Mixed Models (GLMM).

**RESULTS AND CONCLUSIONS:** An overall prevalence of 51.4% was obtained (mean EPG±s.d.=135.98±418.037). The morphological identification of 4,699 L3 revealed Cyathostominae Type A and Type C as the most widespread (mean%: 85 and 9.3, respectively), with a variability in richness among animals and stables. Migratory strongyles were also detected in 20 positive horses (4.5%) from 12 different stables: morphological and molecular analyses revealed a total of 4 and 19 *S. vulgaris* positive samples, respectively, while no other species of the *Strongylus* genus were detected. The age of *S. vulgaris* positive animals was between 3 and 21 years (mean±s.d.=9.05±5.115), with 11 horses showing less than 200 EPG. Interestingly, three horses had been only recently introduced into the stables. Statistical analysis highlighted the young age of the animals (B±s.e. =0.069±0.032; *p*-value=0.035) and the possibility of access to the paddock (B±s.e.=-2.206±0.592; *p*-value=0.0001) as predictive variables for the presence of *S. vulgaris*.
The study findings emphasize that *S. vulgaris*, nowadays deemed uncommon, persists within the equine population in Northern Italy. Given its detection in horses exhibiting low Strongyle EPG levels, it's advisable including an approach coupling morphological and molecular methods, preferably on individual samples, in standard coprological diagnosis protocols.

# UPDATES ON MORPHOLOGY AND MOLECULAR IDENTIFICATION OF *FASCIOLA* SPP. ISOLATED FROM SHEEP IN NORTHWEST TUNISIA

Hammami I.*[1], Ciuca L.[2], Maurelli M.P.[2], Romdhane R.[1], Limam S.[1], Rjeibi M.R.[3], Farhat N.[4], Simo A.K.[5], Rinaldi L.[2], Rekik M.[6], Gharbi M.[1]

*[1]University of Manouba, École Nationale de Médecine Vétérinaire de Sidi Thabet, Sidi Thabet, Manouba, Tunisia; [2]University of Naples Federico II, Department of Veterinary Medicine and Animal Production, CREMOPAR, Napoli, Italy; [3]Institut de La Recherche Vétérinaire de Tunisie, Tunis, Tunisia; [4]Circonscription de la production animale, Bizerte, Tunisia; [5]Université des Montagnes, Faculté des sciences de la santé, Bangangté, Cameroun; [6]International Center for Agricultural Research in the Dry Areas (ICARDA), Amman, Jordan*

**Keywords:** *Fasciola hepatica*, Morphometry, PCR-RFLP.

**INTRODUCTION:** Fasciolosis is a hepatobiliary helminthic disease caused by trematodes of the *Fasciola* genus; the two species *Fasciola hepatica* and *Fasciola gigantica* leads to major economic losses in domestic ruminants (Itagaki et al., 2022. Infect Genet Evol, 99:105248). Despite its significant impact, few studies have highlighted the prevalence of fasciolosis in Tunisian sheep, while the occurrence and morphology of the flukes have never been investigated. Therefore, the present study aimed to characterize the Tunisian liver flukes by morphometric and molecular analyses.

**MATERIALS AND METHODS:** A total of 335 flukes were collected from 66 sheep livers in Sejnane slaughterhouse (Bizerte governorate, Northwest Tunisia) between January and March 2021. Five morphometric parameters were determined for all the liver flukes, as follows: total body length (BL), distance between ventral sucker and the tail (VS-T), distance between oral sucker and ventral sucker (OS-VS), abdomen diameter (AD), tail diameter (TD) and the body length to width ratio (BL/BW) (Valero et al., 2001. Vet Parasitol, 102:85-100; Akhlaghi et al., 2017. Turkiye parazitolojii Derg, 41:192-97; Diyana et al., 2020. J Parasitol Res, 2020:9-14; Sgroi et al., 2021. Comp Immunol Microbiol Infect Dis, 77). The molecular and phylogenetic analysis of the fluke specimens identification was carried out by targeting a 680 bp sequence of the internal transcribes spacer 1 (ITS1) gene (Itakagi et al., 2005. J Vet Med Sci, 67:1115-18) and a 500 bp sequence of the ITS2 gene (Itakagi et al., 2005. Parasitology, 131:679-85).

**RESULTS AND CONCLUSIONS:** The morphometric measurements showed that the mean of the total body length of the adult flukes was 21.1 ± 2.7 mm with minimum and maximum lengths of 13 and 31 mm, respectively. The PCR-RFLP analysis revealed a single profile consisting of three bands of approximately 370, 100, and 60 bp. *Fasciola* sequences described in the present study (GenBank numbers: OQ457027 and OQ457028) showed 99.58-100% identity to *F. hepatica* isolated from different hosts and different regions throughout the world. Molecular and phylogenetic analyses confirmed the presence of a single liver fluke species (*F. hepatica*) in the Sejnane region of northwest of Tunisia. However, further studies are needed to determine the occurrence of *Fasciola* species in other Tunisian regions.

# SURVEY ON TICK-BORNE HAEMOPARASITES AND ASSOCIATED TICKS IN CATTLE FROM BENIN, WEST AFRICA

Grillini M.*[1], Yessinou E.R.[2], Dotto G.[1], Koumassou A.[2], Zhang T.[1], Simonato G.[1], Cassini R.[1]

*[1]Department of Animal Medicine, Production and Health, University of Padova, Legnaro, Italy; [2]Communicable Disease Research Unit (URMaT), University of Abomey-Calavi, Cotonou, Benin*

**Keywords:** Bovine piroplasms, Ticks, Benin.

**INTRODUCTION:** In Africa, ticks represent a concern to livestock production due to direct/indirect effects (Heylen et al., 2023. Parasit Vectors, 16(1):423). Among indirect effects, ticks can act as vectors of *Babesia* and *Theileria* species, causative agents of piroplasmoses in cattle (Adjou Moumouni et al., 2018. Vet Parasitol Reg Stud Reports, 214:137-43). In West Africa, Benin is an agricultural country where livestock plays a predominant role (Yessinou et al., 2018. J Parasitol Res, 2018:2570940). Tick species such as *Rhipicephalus microplus* and *Amblyomma variegatum* were widely found in the country (Heylen et al., 2023. Parasit Vectors, 16(1):423), whereas their tick-borne diseases (TBDs) are reported in some areas (Yessinou et al., 2023. Vet Med Sci, 9(1): 345-52), but knowledge on their distribution is still insufficient. Thus, this study aims to investigate TBDs in cattle and associated ticks to update the epidemiological data in Benin.

**MATERIALS AND METHODS:** Whole blood was collected from cattle in 2 areas in Northern and 3 in Southern Benin. Ticks have been collected on animals and morphologically identified. Individual ticks and blood were analysed using real-time and end-point PCR targeting the LSU-rRNA gene and the SSU-rRNA gene, respectively, to detect protozoal pathogens. The obtained nucleotide sequences were edited by ChromasPro v.2.1.8 (Technelysium Pty Ltd., Brisbane, Australia) and compared to those present in GenBank®. Prevalence values and their 95% confidence intervals (CI) were calculated.

**RESULTS AND CONCLUSIONS:** Overall, 81 blood samples and 246 ticks were collected and analysed. *Rhipicephalus microplus* (n=75, 30.5%) and *A. variegatus* (n=74, 30.1%) prevail as species, followed by *Hyalomma rufipes* (n=45, 18.3%), *Hyalomma truncatum* (n=37, 15.0%), and *Rhipicephalus sanguineus* (n=15, 6.1%). Based on the molecular results, *Theileria velifera* (n=31, 38.3%; 95%CI: 28.5-49.2), *Theileria mutans* (n=6, 7.4%; 95%CI:3.4-15.2), *Theileria* spp. (n=7, 8.6%; 95%CI:4.3-16.8), and *Babesia* spp. (n=2, 2.5%; 95%CI:0.7-8.6) were detected in blood samples at least in 2 sites. In addition, *T. velifera* was isolated in 1 *R. microplus* and *B. bigemina* in 1 *R. microplus* e 1 *H. rufipes*. Tick identification confirmed *R. microplus* and *A. variegatus* to be the prevalent species as published in previous reports. All five species were found in two or more of the considered sites, demonstrating a wide geographical distribution. The isolated piroplasms species are in line with the literature (Adjou Moumouni et al., 2021. Pathogens, 11(1):31), although both ticks and blood samples showed a very low positivity to *Babesia* species. Our findings added new molecular information on piroplasm species circulating in the country, although the sensitivity of this approach may be affected in case of co-infections.

# MOLECULAR CHARACTERIZATION OF HYDATID CYSTS RETRIEVED FROM CATTLE IN URUGUAY

Armúa Fernández M.T.*, Ferre C., Azambuja M., Salazar Ojeda M., Olhagaray Torres E.

*University of the Republic, Faculty of Veterinary, Department of Pathobiology, Veterinary Parasitology Unit, Montevideo, Uruguay*

**Keywords:** *Echinococcus* spp, Cattle, Uruguay.

**INTRODUCTION:** Cystic echinococcosis (CE) is a parasitic zoonosis disease caused by the metacestode of *Echinococcus granulosus sensu lato*. Although taxonomy of *Echinococcus granulosus sl* is still very controversial, to date, the following species had been recognized; *Echinococcus granulosus sensu stricto* (G1, G3), *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5), *Echinococcus intermedius* (G6 and G7), *Echinococcus canadensis* (G8 and G10) and *Echinococcus felidis* (lion strain) (Manterola, 2021. Acta Parasitol, 1-25). This disease is widely distributed throughout the world. In South America, is considered endemic and hyperendemic in some regions. Based on sequencing data, *E. granulosus s.s.* has the widest distribution, followed by *E. ortleppi* (Cucher et al., 2016. Trop Med Int Health, 21:166-175). Information about the CE situation in Uruguay is very scarce, which highlights the importance of determining the species present in our territory. This study aims to report the first preliminary results of the molecular characterization of hydatic cysts obtained from cattle in slaughterhouses in Uruguay.

**MATERIALS AND METHODS:** Nine hydatic cysts (HC) were retrieved from lungs during cattle slaughtering, refrigerated and sent to Molecular Biology lab at Unidad de Parasitología Veterinaria, Facultad de Veterinaria, Montevideo. Germinal layers of each sample were used for DNA extraction. A fragment of cytochrome c oxidase subunit 1 was amplified by PCR (Bart et al., 2006. Parasitol Res, 98:130-37). Amplicons of expected sizes were sent for sequencing.

**RESULTS AND CONCLUSIONS:** All HC samples yielded amplicons after the PCR. Five samples were confirmed as *E. granulosus* ss (G1-G3), and the remaining four as *E. ortleppi* (G5). These preliminary results of *E. ortleppi* in cattle indicate the existence of a dog cattle cycle in Uruguay. More samples are currently under molecular characterization in order to establish the *E. ortleppi* distribution in Uruguay.

# *EIMERIA* SPP. IDENTIFICATION IN ITALIAN NATIVE CHICKEN BREEDS

Raffaelli M.*, Perrucci S.

*University of Pisa, Department of Veterinary Sciences, Pisa, Italy*

**Keywords:** *Eimeria* spp., Identification, Italian native chickens.

**INTRODUCTION:** Coccidia (*Eimeria* spp.) are included among the most common and important parasites of poultry, causing serious health problem in bird and production losses to the chicken industry worldwide (Blake et al., 2020. Vet Res, 51:1-14). Genetic resistance towards *Eimeria* spp. infections of some native chicken breeds is known (Pinard-Van Der Laan et al., 1998. Poult Sci, 77:185-191). Data on *Eimeria* spp. infections in Italian native chicken breeds are lacking. This study was aimed to identify *Eimeria* species infecting four native Italian chicken breeds (Livorno bianca-LB, Bianca di Saluzzo-BS, Valdarnese bianca-VB, Mugellese-M) reared in an alternative farming system.

**MATERIALS AND METHODS:** In May, June and July 2022, faecal samples (2 pools/breed/sampling) were taken from 68 Italian native chickens (23 LB, 19 BS, 18 M, 8 VB) of the same age and reared in the same farm in outdoor pens. No control measures based on anticoccidial drugs or vaccines were used in the farm. Samples were examined by copro-microscopical analysis for the detection and counting of coccidian oocysts. For *Eimeria* spp. identification, positive samples/breed/sampling were dissolved in 2% potassium dichromate solution (K2Cr2O7) and placed into Petri dishes for seven days at 25 °C for sporulation of the oocysts. Identification was based on morphology performed by microscopical analysis, a morphometric computational tool (COCCIMORPH), and by molecular analysis (Pellerdy, 1974, Coccidia and Coccidiosis 2nd edition, Paul Parey, berlin and hamburg, pp. 959; Castanon et al., 2007. Pattern Recognition, 40: 1899-1910; Haug et al., 2008. Avian Pathol, 37: 161-70; Carvalho et al., 2011. Vet Parasitol, 176: 95-100; Kumar et al., 2014. Vet Parasitol, 199: 24-31).

**RESULTS AND CONCLUSIONS:** *Eimeria* spp. infection was detected in all breeds. At microscopical analysis, *Eimeria tenella* was identified in all breeds, *Eimeria necatrix* in M and VB, *Eimeria mitis* in M and LB, *Eimeria brunetti* in BS. Coccimorph evaluation confirmed data from microscopical analysis, except for *E. necatrix* in M that was identified with *E. tenella*, and *E. mitis* in LB that was identified with *Eimeria acervulina*. Molecular analysis showed *E. tenella* infection in all breeds, *E. brunetti* in BS, *E. necatrix* in BS, VB and LB, and *E. acervulina* in LB and M. *E. mitis* was not identified at molecular analysis. Differences in the field diagnosis of *Eimeria* species using different methods were confirmed (Haug et al., 2008. Avian Pathol, 37:161-70; Carvalho et al., 2011. Vet Parasitol, 176:95-100). Nonetheless, differences in *Eimeria* spp. composition among the examined Italian native chicken breeds were evidenced by all methods used. Further investigations are needed to assess the significance of results obtained and differences, if any, in resistance/tolerance/susceptibility to *Eimeria* species among the different breeds examined.

# COPROMICROSCOPIC AND MOLECULAR SURVEY ON MAJOR ENDOPARASITES IN PIGS IN ITALY

Allievi C.*, Valleri M., Zanzani S.A., Zanon A., Mortarino M., Manfredi M.T.

*Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Lodi, Italy*

**Keywords:** Pigs, Endoparasites, Fattening unit.

**INTRODUCTION:** In Italy pig farming is concentrated in northern regions, where most of the national pig herd is reared in intensive systems, which have fostered significant improvements in hygiene and biosecurity measures. In this context, parasitic infections often show a subclinical course and are rarely included as causative agents in the differential diagnosis of gastrointestinal disorders. However, parasites can predispose to other diseases and cause a reduction in growth rate and feed conversion; some of them may also pose a risk of infection for professionals involved in the food chain for their zoonotic potential (Kipper et al., 2011. Vet Parasitol, 181:316-20). The main purpose of this study was to provide current data on the main endoparasites in pigs and to investigate the risk factors that could predispose to parasitic infections.

**MATERIALS AND METHODS:** The study included 22 fattening pig farms located in northern Italy and a total of 880 faecal samples were collected in two different sessions: at time 1, at the beginning of the fattening period, and at time 2, just before slaughter. From each herd, data on farm management were collected by interviewing the farmer. For each sample, the FLOTAC® dual technique was used with two different flotation solutions, FS2 (sodium chloride, NaCl) and FS7 (zinc sulphate, ZnSO4). For coccidia, the identification of sporulated oocysts was done using Sheather's solution, while to identify cestode eggs, an end-point PCR was performed. The prevalence values of each parasite were associated with the farm management characteristics and introduced into generalized linear mixed models (GLMMs).

**RESULTS AND CONCLUSIONS:** A total of 95 samples out of 880 tested positives for at least one parasite taxon (10.8%), and the most detected parasite was *Ascaris suum* (7.6%), followed by *Trichuris suis* (1.7%) and *Cystoisospora suis* (0.9%). In addition, eggs with morphometric characteristics compatible with those of *Hymenolepis diminuta* were detected in 16 samples (1.8%) and the comparison of sequences with those in the GenBank database confirmed the identification of the cestode eggs. Statistical analysis showed that two variables were associated with a lower risk of *A. suum* infection; in particular, large farms and those applying the all-in/all-out system had a lower risk. The farm size would play a central role as hygienic conditions might be less adequate and biosecurity systems less organized in small than in large farms (Pettersson et al., 2021. Porcine Health Manag, 7:12). Further, the application of the all-in/all-out system would allow systematic washing and decontamination between batches, reducing parasite pressure and environmental resistance of eggs (Martínez-Pérez et al., 2017. Vet Parasitol, 248:33-8).
This study evidenced that parasites are persistent, albeit with low prevalences, throughout the fattening cycle and would need specific measures to reduce their effects on both animal health and productivity.

# EVIDENCE OF *BESNOITIA BESNOITI* DNA IN HARD TICKS FROM LIVESTOCK AND VEGETATION IN ITALY (APULIA REGION)

Raele D.A.*[1], Cavaliere N.[1], Scaltrito D.[1], Sordillo M.[1], Lauriola S.[2], Cafiero M.A.[1]

*[1]Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy; [2]Azienda Sanitaria Locale, Foggia, Italy*

**Keywords:** Bovine besnoitiosis, *Besnoitia besnoiti*, Ticks.

**INTRODUCTION:** Bovine beisnoitiosis (Bb), caused by the tissue cyst-forming apicomplexan parasite *Besnoitia (B.) besnoiti*, is considered an emerging disease in the European countries (Alvarez García et al., 2013. Trends Parasitol, 29(8):407-15). The infection can cause a chronic and weakening disease characterized by skin lesions and systemic clinical signs in infected cattle and it is responsible for severe economic losses for the breeder. Many aspects of the epidemiology of Bb persist undefined, including the role of blood-sucking arthropods in the transmission of the parasite (Hornok et al., 2015. Parasit Vectors, 8:450). The aim of the present study was to investigate the presence of *B. besnoiti* DNA in hard ticks collected in cattle where previous serological screenings tested positive for Bb.

**MATERIALS AND METHODS:** In December 2022, ticks were collected from the vegetation by flagging and directly removed from podolic breed cattle. All specimens were morphologically identified, DNA extracted individually and molecularly analyzed for the presence of *B. besnoiti* using a qPCR assay (Schares et al., 2011. Vet Parasitol, 178(3-4):208-16). The positive samples were analyzed by an end-point PCR targeting the ITS1 gene and gathered amplicons were sequenced.

**RESULTS AND CONCLUSIONS:** Out of 23 collected ticks (18 from livestock and 5 from vegetation), 3 species were identified: *Rhipicephalus turanicus* (5 females and 2 males), *Hyalomma marginatum* (11 females) and *Ixodes ricinus* (5 nymphs). Out of 23 extracted DNA, 5 tested positive to molecular assays (2 *H. marginatum* and 3 *I. ricinus*). Consequent sequence analysis of the amplicons identified DNAs as *B. besnoiti*. Bb is considered a summer disease with a slow and sneaky spread within herds without effective treatments and licensed vaccines (Gutiérrez-Expósito et al., 2017. Intern J Parasitol, 12:737-51). Consequently, biosecurity measures, including the prevention of bloodsucking parasites are keys to control it. This study provides the first evidence of *B. besnoiti* in ticks in Southern Italy and it suggests that ticks could play an epidemiological role in the spread of the disease.

# ASSOCIATIONS BETWEEN PRODUCTIVE PARAMETERS AND BULK TANK MILK ANTIBODY LEVELS AGAINST *OSTERTAGIA OSTERTAGI* AND *FASCIOLA HEPATICA* IN DAIRY CATTLE FARMS IN ITALY

Villa L.*[1], Allievi C.[1], Di Cerbo A.R.[1], Zanon A.[1], Sommariva F.[2], Zanini L.[2], Manfredi M.T.[1]

*[1]Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Lodi, Italy; [2]Associazione Regionale Allevatori della Lombardia, Crema, Italy*

**Keywords:** Gastrointestinal nematodes, Fasciolosis, Bulk tank milk serology.

**INTRODUCTION:** Among the available diagnostic techniques, antibody detection in bulk tank milk represents a useful tool to screen the presence of parasitic agents in dairy herds. *Ostertagia ostertagi* is one of the most important gastrointestinal nematodes for cattle worldwide. Fasciolosis, caused by the liver fluke *Fasciola hepatica*, can have a major economic impact on the livestock industry. Both parasitic diseases mostly occur in subclinical forms but may lead to production losses in affected herds. Therefore, the study aimed to evaluate the spread of *O. ostertagi* and *F. hepatica* and their impact on herd milk production parameters in dairy cattle in Italy.

**MATERIALS AND METHODS:** Bulk tank milk (BTM) samples from 350 dairy herds of the largest dairy production area in Italy (Lombardy) were analyzed by indirect ELISA for the detection of *O. ostertagi* and *F. hepatica* antibodies (Svanova). BTM samples were classified according to ODR values (Charlier et al., 2005. Vet Parasitol, 129:67-75; Charlier et al., 2007. Prev Vet Med, 78:57-66). Data on 5 milk production parameters were collected; generalized linear models (GLMs) were developed.

**RESULTS AND CONCLUSIONS:** The overall mean ODR for *O. ostertagi* revealed a value of 0.59. 107 herds resulted positive (ODR>0.6) with a prevalence of 30.6%. Besides, 138 farms showed ODR between 0.3 and 0.6 considered as a “grey zone” including animals within the pre-patency or weeks after treatment, when antibody titers are decreased. A higher prevalence was detected in the provinces of Mantova (P=62%), Bergamo and Brescia (P=50%) and Pavia (P=28%). Lower prevalence values between 6 and 10% were evidenced in Cremona, Lodi and Milano. An association was evidenced between the ODR values and the productive parameters: in particular, in herds with ODR>0.5, both daily milk production (24.8 vs 24.1) ($p$>0.05) and mature equivalent milk yield (9388.9 vs 10229.3) ($p$=0.001) were reduced. Somatic cell count was higher in positive herds (253,554 vs 238,289) ($p$=0.05). Fat (3.85 vs 3.81) and protein (3.40 vs 3.38) content in milk were similar in positive and negative herds. In 24 herds the infection with liver fluke was evidenced (P=6.9%). Positive farms were located in the provinces of Milano, Mantova, Bergamo, Pavia and Cremona with prevalence values between 4 and 18%. An effect of *F. hepatica* on productive parameters was demonstrated: daily milk production (20.7 vs 24.8) ($p$=0.026) and mature equivalent milk yield (8895.6 vs 9992.6) ($p$=0.046) were lower in positive versus negative farms. Somatic cell count was slightly higher in positive herds (253,307 vs 243,204) ($p$>0.05). Fat and protein content in milk were similar in positive and negative herds (3.84 vs 3.82 and 3.46 vs 3.38, respectively).
This study provides an assessment of the exposure to *O. ostertagi* and *F. hepatica* evidencing the impact of these parasites on herd performances in Italian dairy herds. The screening for antibodies, assessing the infection level, is an instrument to determine the need for anthelmintic control in the herds.

# GASTROINTESTINAL PARASITES INFECTING CALVES FROM DIFFERENT FARMING SYSTEMS IN NORTHERN ITALY: PRELIMINARY RESULTS

Dini F.M.*, Bordoni T., Massmann A.J., Galuppi R.

*Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy*

**Keywords:** Endoparasites, Cattle, Coprology.

**INTRODUCTION:** Various intestinal protozoan parasites, such as *Cryptosporidium* spp., *Eimeria* spp., and *Giardia* spp., are known to cause outbreaks of intestinal diseases in both humans and animals (Thomson et al., 2017. Vet Res, 48:42; Ryan et al., 2021. Int J Parasitol, 51:1099-1119). Protozoan parasites are frequently found in the digestive tract of cattle, particularly in the rumen and intestines, and they can significantly impact the health of these animals. Their presence in the calves are commonly associated with intestinal diseases and potentially contribute to increased mortality in calves affected by gastrointestinal diseases, often alongside other non-eukaryotic enteropathogens (McGuirk, 2008. Vet Clin North Am Food Anim Pract, 24:139-53). In the frame of a study on the effect of feeding and management activities on the spread of these parasites, an epidemiological investigation was conducted to assess the presence of primary intestinal parasites in calves.

**MATERIALS AND METHODS:** Fecal samples were collected from calves on nine farms with different production systems (milk or beef) located in the provinces of Bologna and Cremona. Whenever possible, samples were obtained from all calves present in each barn. A check list about management and biosecurity data was filled out. The fecal samples underwent parasitological microscopic examination, which included sediment analysis using Lugol and Ziehl-Neelsen staining, as well as a flotation technique. Samples that tested positive using the flotation technique were further analyzed using Mc Master quantitative analysis to quantify the parasitic burden. For fecal samples that tested positive for *Giardia* sp., DNA extraction was performed to conduct a nested PCR targeting the TPI gene in order to genotype the strains.

**RESULTS AND CONCLUSIONS:** A total of 155 calves, both with and without diarrhea, were sampled, ranging in age from one week to six months. The prevalence of parasitic protozoa observed was: 28% for *Eimeria* spp., 28% for amoebae cysts; 15% for *Cryptosporidium* spp.; 10% for *Giardia duodenalis*, 1.29% for *Buxtonella* sp., 0.65% for *Blastocystis* sp. In some calves also *Strongyloides papillosus* (1.29%), and *Toxocara vitulorum* (0.65%) were found. Preliminary molecular results indicated that the genotype of *G. duodenalis* in tested calves was Assemblage E. Quantitative coprological analysis revealed that *Eimeria* oocyst counts ranged from <20 to 50120 oocysts per gram, while all detected helminths showed egg counts of less than 20 eggs per gram. *Eimeria* spp. was detected in all sampled farms, *Cryptosporidium* in 89% of the barns, and *Giardia* sp. in 67%. Amoebae cysts, rarely described in cattle, were recorded in 56% of the investigated farms; in particular, in two dairy barns of the province of Bologna, they were found in 41.17% and 59.45% of the calves.

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# EPIDEMIOLOGICAL UPDATES ON CYSTIC ECHINOCOCCOSIS IN SARDINIA, ITALY

Cavallo L.*[1], Tarot M.[2], Carta C.[1], Arshad M.F.[1], Marras A.[3], Rossi P.[1], Nonnis F.[1], Sini M.F.[1], Zeinoun P.[1], Tamponi C.[1], Pinna Parpaglia M.L.[1], Burrai G.P.[1], Lai G.[1], Scala A.[1], Jacquiet P.[2], Varcasia A.[1]

*[1]Università Degli Studi di Sassari, Department of Veterinary medicine, Parasitology and parasitic diseases, Sassari, Italy; [2]Department of Parasitology, Ecole Nationale Vétérinaire de Toulouse, Toulouse, France; [3]ASL N.3 Nuoro, Servizio Veterinario di Macomer, Nuoro, Italy*

**Keywords:** Cystic echinococcosis, Sheep, Sardinia.

**INTRODUCTION:** Cystic echinococcosis (CE) is a zoonotic disease caused by the larval stage of the tapeworm *Echinococcus granulosus*. CE is endemic in the Mediterranean region, where sheep are bred in an extensive farming system, like Sardinia, which is among the most affected countries (Varcasia et al., 2020. Parasitol Res, 119:2207-15). Prevalence of human CE is estimated at 9.3/100,000 inhabitants in Sardinia (Mastrandrea et al., 2012. Acta Trop, 123:184-89) and the costs could amount to several million of euros (Piseddu et al., 2017. PLOS Negl Trop Dis, 11:e0005771). Several epidemiological surveys have already been carried out in Sardinia, so the present study is part of a continuous monitoring for the disease in order to have up-to-date data available and possibly reinforce control plans.

**MATERIALS AND METHODS:** From April 2021 to March 2024 a total of 837 sheep were examined in 7 different slaughterhouses of Sardinia, Italy (Bortigali, Buddusò, Macomer, Lula, Ottana, Thiesi, Tula). A *post-mortem* examination was carried out in animal slaughtered to highlight the presence of hydatid cysts. The cysts in the livers and the lungs collected were counted and sorted according their stages (fertile, sterile, caseous and calcified). The fertility rate was assessed on the liquid cysts and strain identification was performed on some samples.

**RESULTS AND CONCLUSIONS:** An overall prevalence of 65.7% (550/837) for CE was recorded and the fertility rate was 9.3% (34/364). The prevalence of CE in lungs was 13.9% (116/837); 17.8% (149/837) sheep were infected only in the liver and 34.0% (285/837) sheep had cysts in both lungs and liver. Among the 1745 cysts studied, 47.0% (821/1745) were liquid; 43.3% (755/1745) were calcified and 9.7% (169/1745) were caseous. Of all the sheep examined, 37.3% (312/837) had at least one liquid cyst; 44.9% (376/837) had at least one calcified cyst and 16.6% (139/837) had at least one caseous cyst. CE prevalence found was very similar to our previous study in 2020 (Varcasia et al., 2020. Parasitol Res, 119:2207-15) which was 65.3% ($\chi2$= 0.048; $p$ = 0.825). Although not statistically significant, the fertility rate slightly decreased from 11.7% to 9.3% ($\chi2$= 1.648; $p$ = 0.199).

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# UPDATES ON BRONCHO-PULMONARY NEMATODES OF SHEEP IN SARDINIA (ITALY)

Arshad M.F.[1], Cavallo L.*[1], Rekik S.[2], Zeinoun P.[1], Tarot M.[3], Ahmed F.[4], Carta C.[1], Tamponi C.[1], Sini M.F.[1], Nonnis F.[1], Lai G.[1], Burrai G.P.[1], Pinna Parpaglia M.L.[1], Scala A.[1], Varcasia A.[1]

*[1]Department of Veterinary Medicine, University of Sassari, Sassari, Italy; [2]Ecole Nationale de Médecine Vétérinaire, Sidi Thabet, Tunisia; [3]Department of Parasitology, Ecole Nationale Vétérinaire de Toulouse, Toulouse, France; [4]Ulster University, Northern Ireland, Ulster, United Kingdom*

**Keywords:** Sheep, Broncho-pulmonary nematodes, Sardinia.

**INTRODUCTION:** The production and welfare of sheep can be severely impacted by widespread broncho-pulmonary nematodes (BPN) as they compromise sheep's respiratory functions. The presence of BPN in live sheep can be clinically suspected from respiratory symptoms such as coughing and dyspnea. Given the detrimental impact of BPN on sheep farms, vigilant surveillance of infectious burden is of paramount importance. Moreover, discerning the individual prevalence of BPN species is essential for tailored treatment and prevention measures. This study aims to investigate epidemiology of BPNs in Sardinia, Italy.

**MATERIALS AND METHODS:** From July 2021 to March 2024 a total of 460 adult Sarda sheep from 38 farms have been examined in 4 slaughterhouses (Buddusò, Lula, Thiesi, Tula) from Sardinia, Italy. Trachea was opened and inspected up to the bronchial bifurcation to look for the adult specimens of *Dictyocaulus filaria*. Baermann technique was performed on tissue samples collected from the dorsal lung parenchyma of each lung in order to isolate BPNs. First stage larvae were classified and counted according to the morphometric keys (van Wyk et al., 2004. Vet Parasitol, 119(4): 277-306).

**RESULTS AND CONCLUSIONS:** An overall prevalence of 45% (207/460) for BPNs was found in examined animals. Detailed prevalences were as following: *Dictyocaulus filaria* 2.1% (n.9); *Muellerius capillaris* 37.1% (n.171); *Neostrongylus linearis* 23% (n.106); *Cystocaulus ocreatus* 6.9% (n.32). The differences in prevalence of the detected species were highly significant ($\chi 2=226.2568$; $P< 0.00001$). It is evident from their prevalence rates that BPNs are a potential health problem for local Sardinian sheep with a prevalence rate of 45%. *Muellerius capillaris* and *Neostrongylus linearis* are found to be dominant BPNs in Sardinian sheep. The modest prevalence of *Cystocaulus ocreatus* and *Dictyocaulus filaria* still represent a potential problem as they can compromise the overall well-being of sheep. No sample was found to be positive for *Protostrongylus rufescens*. Overall, the significant economic loss for sheep farmers could be attributed to parasitosis with BPNs as they reduce growth rates, wool quality, and lead to potential mortality in affected animals. Therefore, veterinarians and breeders should not underestimate the importance of timely copromicroscopic investigations, deworming strategies, and pasture management.

**Acknowledgements**: This work was partially (Years 2023/24) developed within the framework of the project e.INS- Ecosystem of Innovation for Next Generation Sardinia (cod. ECS00000038) funded by the Italian Ministry for Research and Education (MUR) under the National Recovery and Resilience Plan (NRRP) - MISSION 4 COMPONENT 2, "From research to business" INVESTMENT 1.5, "Creation and strengthening of Ecosystems of innovation" and construction of "Territorial R&D Leaders". Authors kindly thanks all the personnel of hygiene of products of animal origin service (SIAOA) for their helpful collaboration in sampling.

# PRELIMINARY RESULTS OF A LONGITUDINAL STUDY ON ENDOPARASITES INFECTIONS IN DAIRY SHEEP FARMS FROM SARDINIA, ITALY

Cavallo L.*[1], Tamponi C.[1], Nonnis F.[1], Zeinoun P.[1], Sini M.F.[1], Carta C.[1], Arshad M.F.[1], Scarano C.[1], Piras F.[1], Meloni M.P.[1], Siddi G.[1], Cannas A.[2], Porcu M.A.[2], Dettori G.[3], Piras A.[3], Madau G.[4], Argiolas G.[5], Deiana C.[5], Scala A.[1], Varcasia A.[1]

*[1]Department of Veterinary Medicine, University of Sassari, Sassari, Italy; [2]Department of Agriculture, University of Sassari, Sassari, Italy; [3]Cargill s.r.l Milano, Milano, Italy; [4]Veterinary Practitioner, Sassari, Italy; [5]Sementusa Tech srl, Cagliari, Italy*

**Keywords:** Endoparasites, Breeding, Lactation.

**INTRODUCTION:** Endoparasites could represent a major health and production concern when not monitored and can impact on milk and meat productions. The aim of this study was the total screening of animals present in five dairy sheep farms in Sardinia to establish the parasitological status of the herds and to define the dynamics, prevalence, impact of parasitic infections during lactation.

**MATERIALS AND METHODS:** Five dairy sheep farms have been enrolled: Nule (farm1), Orune (farm2), Oschiri (farm3), Villamassargia (farm4) and Iglesias (farm5). Thirty sheep were sampled from each farm (for a total of 150), carried out once a month from January to March 2024. Coprological examinations were carried out with the McMaster technique on single individual samples for the detection of Gastrointestinal nematodes and *Eimeria* spp.

**RESULTS AND CONCLUSIONS:** An average prevalence of 82.7% for Gastrointestinal Strongyles (GIS) was recovered after the first sampling, showing from medium to low epg values(<300 epg), with the only exception for farm4, where values over 300 epg have been found. All farms were positive for *Eimeria* spp. (100%). In farm1, 83.3% (25/30) of the animals tested positive for GIS in January, with an average of 227 epg, in February 86.7% (26/30), with an average of 236 epg excreted and 92.6% (25/27) with 168.3 epg in March, showing no statistical difference between prevalence ($\chi2$= 1.123; df=2; P=0.57) and epg levels (Kruskal-Wallis test, H= 1.08; P=0.583). In farm2, 86.7% (26/30) of the animals tested positive for GIS in January, with an average of 151 epg, 93.1% (27/29) in February, with an average of 294.8 epg and 85.7% (24/28) with 268.4 epg in March ($\chi2$=0.917; df=2; P=0.632 - H=1.71; P=0.425). In farm3, 56.7% (17/30) of the animals tested positive for GIS in January, with an average of 63.5 epg, in February 95.40% (21/22), with an average of 130.2 epg excreted and 86.7% (26/30) with 122 epg in March, showing a significant variation between prevalence ($\chi2$=13.196; df=2; P=0.001) and epg levels (H=11.3; P=0.004). In farm4, 100% (30/30) of the animals tested positive for GIS in January, with an average of 455.8 epg, in February 96.7% (29/30), with 1229 epg excreted and 96.7% (29/30) with 1645 epg in March, with significant variation between epg levels (H=14.28; P=0.001), but none between prevalence ($\chi2$=0.341; df=2; P=0.842). In farm5, 86.7% (26/30) of the animals tested positive for GIS in January, with an average of 82 epg, 85.2% (23/27) in February, with 142.2 epg and 78.6% (22/28) with 110.9 epg in March, showing significant variation between epg levels (H=11.98; P=0.003), but not between prevalence ($\chi2$=0.768; df=2; P=0.68). The in-progress study showed interesting trends that deserve a more complete examination, including microbiological and zootechnical management examinations, as planned by operative unit of Sassari Task 5.3.6 of AGRITECH_PNRR “National Research Centre for Agricultural Technologies” Progetto: CN00000022-CUP UNISS:J83C21000300006. Authors thanks all personnel of farms for their kindly collaboration.

# A TEN-YEAR RETROSPECTIVE STUDY OF GASTROINTESTINAL NEMATODE INFECTIONS IN SHEEP FARMS IN SOUTHERN ITALY

Martone G.*[1], Bosco A.[2], Santaniello M.[2], Rinaldi L.[2], Cringoli G.[2], Maturo F.[1], Mannocci A.[3]

*[1]Universitas Mercatorum, Faculty of Economics, Rome, Italy; [2]University of Naples Federico II, Department of Veterinary Medicine and Animal Production, Naples, Italy; [3]Telematic University "San Raffaele", Department for the promotion of Human Sciences and Quality of Life, Rome, Italy*

**Keywords:** Gastrointestinal nematodes (GINs), Parasitological monitoring, Sheep.

**INTRODUCTION:** Gastrointestinal nematodes (GINs) are ubiquitous in grazing ruminant production systems and are responsible for significant production losses especially on sheep farms (Vineer et al., 2020. Parasite, 27:69). Control programmes of these parasites in ruminants are based on the use of anthelmintic drugs. The emergence of anthelmintic resistance (Maurizio et al., 2023. Parasitology, 150:1105-18), together with global warming, is responsible for the changing epidemiology of these helminths in many geographical areas (Tong et al., 2021. Parasit Vectors, 14(1):604). Parasitological diagnosis with reliable techniques and regular monitoring of GIN infections are of paramount importance in defining control strategies. The aim of the study was to identify predictors of variation in the GIN intensity (eggs per gram of faeces - EPG) in sheep farms located in the Campania region (southern Italy) in the last ten years (2013-2023).

**MATERIALS AND METHODS:** The study was conducted through the results of the diagnostic activity of the Regional Center for Monitoring Parasitic infections (CREMOPAR, Campania region, southern Italy). Parasitological data were obtained from a ten-year monitoring program (2013-2023) of CREMOPAR on 548 sheep farms sampled in the Campania region. For each farm pooled fecal samples were analyzed using the FLOTAC technique (Cringoli et al., 2010. Nat Protoc, 5:503-15) with a detection limit of 2 EPG. A retrospective observational study according to the STROBE statement was performed (Erik von Elm et al., 2008. J Clin Epidemiol, 61:344). Data were analysed using the SPSS 27 software. Descriptive statistics was conducted using mean (SD), median, minimum and maximum, for quantitative variables; frequencies and percentages were used to describe qualitative ones. The comparison of the mean parasite burden of between farms with two versus more than two follow-ups was conducted.

**RESULTS AND CONCLUSIONS:** A total of 169 farms were selected, representing 12.06 % of the sheep farms with more than 50 heads raised in the Campania region and with two or more follow-ups during the studied years. Regarding monitoring, the following characteristics are observed: on average, farms carried out 3.76 monitoring activities during the period 2013-2023 (SD=2.97; min=2; Max=23; median=3). Specifically, 44.3% conducted 2 monitoring activities, and 24.6% conducted 3. RFU (Follow-ups rate) averages every 12.86 months (SD=11.6). MFU GIN (Mean of follow-ups GINs) of the monitoring for each farm was inversely correlated with the RFU r=-0.157 ($p$=0.042); likewise, the mean value of parasite burden of the monitoring estimated for each farm was inversely correlated with the NFU (Number of follow-ups) r=-0.160 ($p$=0.039). The results of this retrospective study over ten years showed that as the averages and medians of the number of follow-ups increase, a decrease in the average parasitic burden was observed for each follow-up. Therefore, monitoring is the basis for setting up a correct control strategy for GINs.

# NEW INSIGHT IN LYMNAEID SNAILS (MOLLUSCA, GASTROPODA) AS INTERMEDIATE HOSTS OF *FASCIOLA HEPATICA* AND *CALICOPHORON DAUBNEYI* IN SOUTHERN ITALY

Ciuca L.*, Hammami I., Maurelli M.P., Vitiello P., Bosco A., Rinaldi L.

University of Naples Federico II, Department of Veterinary Medicine and Animal Production, Naples, Italy

**Keywords:** Lymnaeid snails, *Fasciola hepatica*, *Calicophoron daubneyi*.

**INTRODUCTION:** Fasciolosis caused by *Fasciola hepatica* (FH) and pharamphistomosis caused by *Calicophoron daubneyi* (CD) are among the most common parasitic infections of livestock in Italy and other countries in Europe. However, there is a large gap regarding the intermediate host (i.e. Lymnaeidae species) responsible for the transmission of FH and CD in cattle in Italy. The only study conducted in Italy, was on the presence of *Fascioloides magna* and its snail intermediate host (Varzandi et al., 2024. Sci Total Environ, 916:170338). Therefore, the present study aimed to assess: (i) the snail species found on pastures grazed by cattle infected by liver and/or rumen flukes in southern Italy; (ii) the presence of *F. hepatica* and *C. daubneyi* in host snails.

**MATERIALS AND METHODS:** The present study was carried out from May 2022 to August 2023 on freshwater snails (N=190) collected from 11 cattle farms with known positivity for rumen and liver flukes and located in Campania, Basilicata and Molise regions in southern Italy. Ten snail samples from each farm included in the study (N=110) were selected and submitted to molecular testing for snail species identification using a 400 bp species-specific region of ITS-2 gene (Bargues et al., 2001. Infect Genet Evol, 1:85-107). All the pooled snails (N=10 snails for each pool) were tested for *F. hepatica* targeting a 112 bp species specific region of ITS-2 gene (Králová-Hromadová et al., 2008. J Parasitol, 94:58-67) and for *C. daubneyi* targeting a 167 bp species specific region of COX-1 gene (Jones et al., 2017. Vet Parasitol, 240:68-74).

**RESULTS AND CONCLUSIONS:** The morphological and molecular analysis of the snails revealed two species, i.e. *Galba truncatula* (62/110; 56.4%) and *Physella acuta* (48/110; 43.6%). Overall, both *G. truncatula* and *P. acuta* snail samples were infected with *C. daubneyi*, whereas *G. truncatula* was only infected with *F. hepatica*. Specifically, only one of 11 snail pools from the Basilicata region was positive for *F. hepatica* infection with a positive single snail in the pool. In addition, 6 out of 11 snail pools were positive for infection with *C. daubneyi*. Of these, four were from the Campania region and two from the Basilicata region. Our findings emphasize the need for further malacological surveys in the different Italian regions to improve the diagnosis of infection and to develop integrated strategies for snail control to prevent the spread of liver and rumen flukes in cattle farms.

# XXXIII

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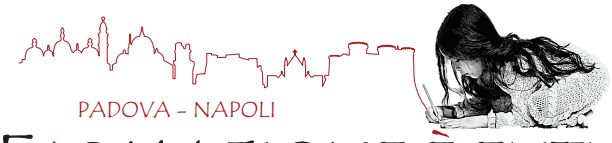
# VECTORS AND VECTOR-BORNE DISEASES



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# DEVELOPMENT OF COMPUTER VISION APPROACHES TO CHARACTERIZE ECOLOGY AND BEHAVIOR OF MOSQUITO VECTORS IN ITALY

Sarleti N.*[1], Ciardiello A.[2], Tubito A.[3], Silvestrini F.[1], Severini F.[1], Di Luca M.[1], Gigante G.[4], Alano P.[1]

*[1]Department of Infectious diseases, Istituto Superiore di Sanità, Rome, Italy; [2]National Institute for Nuclear Physics, Rome, Italy; [3]Department of Mathematics "Guido Castelnuovo", University of Rome "La Sapienza", Rome, Italy; [4]National Centre for Radiation Protection and Computational Physics, Istituto Superiore di Sanità, Rome, Italy*

**Keywords:** Vectors, Artificial Intelligence, INF-ACT project

**INTRODUCTION:** Artificial Intelligence technology is a promising tool to address problems in the surveillance activities. In particular, advances in deep learning and computer vision methods have shown great promise in adult mosquito identification (Brey et al., 2022. Front Trop Dis, 2:810062). Deep learning methods have been used in several mosquito classification studies but most of these have the limitation that tools are based on single-object detection in a laboratory environment. In agreement with the argument that "developing a tool to allow the classification of adult mosquitoes in the field, considering the environmental issues, would be very important" (Motta et al., 2019. PLoS One, 14:e0210829), the «MOSquito Artificial Intelligence COntrol» (MOSAICO) project of the Istituto Superiore di Sanità, supported by the INF-ACT project, aims to develop an automatic mosquito-detection device to support local health authorities and research institutes in the national mosquito surveillance. The device aims to optimize surveillance campaigns, generate homogeneity in the procedures and reduce the time between mosquito sampling and identification.

**MATERIALS AND METHODS:** Samples of 4 mosquito species (*Aedes albopictus*, *Aedes aegypti*, *Culex pipiens*, *Anopheles stephensi*), from the colonies reared by MOSAICO partners in 12 research institutions, were used to obtain high-resolution multi-object images using a commercial camera under standardized conditions able to capture each specimen at the size of 512 × 512 pixels, similar to the resolution used for published deep learning image analysis based on single-mosquito image acquisition (Goodwin et al., 2021. Sci Rep, 11:13656). The gallery of images was split into three independent subsets to perform different phases of the development of the deep learning model, i.e. training, validation and test phase. Convolutional neural networks were used to analyse a total of 3,766 images and to obtain a classification of the mosquito specimens at species level.

**RESULTS AND CONCLUSIONS:** The confusion matrix of the results of the testing phase indicated that each species was classified appropriately with the following scores: *Ae. albopictus* 91%, *Ae. aegypti* 71%, *Cx. pipiens* 99% and *An. stephensi* 99%. Results obtained with specimens from insectary colonies indicated that the multi-object detection system coupled with the image analysis by the deep learning model have achieved a high accuracy of identification of the mosquito species analysed. According to these promising results, the next steps of this project are 1- to increase the image dataset with samples derived from traps across multiple sites nationwide, including additional species of health relevance or/and particularly abundant; 2-to develop a prototype of the MOSAICO device (image acquisition equipment, AI engine, software for database communication) to be tested in the field.

# DETERMINATION OF THE IMMUNOSTIMULATORY ROLE OF *ASAIA* IN *AEDES AEGYPTI*: A POTENTIAL SYMBIONT-BASED CONTROL APPROACH?

Sorana S.*[1], Cappelli A.[2], Damiani C.[1, 2], Ricci I.[2], Favia G.[2]

*[1]School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy; [2]School of Biosciences and Veterinary Medicine, University of Camerino, CIRM Italian Malaria Network, Camerino, Italy*

**Keywords:** Mosquito-borne diseases, Immunity, Symbiotic control.

**INTRODUCTION:** Recurring outbreaks of arboviruses such as dengue on a global scale, it is imperative to adopt multifaceted strategies to limit the transmission of mosquito-borne diseases (MBDs). Insecticide and drug resistance, coupled with the absence of effective vaccines, presents a significant obstacle in combating MBDs. Recent years have seen a deeper understanding of mosquito microbiota, revealing its role in different traits of the mosquito biology such as sexual reproduction, development, nutrition, and resistance to pathogens. As a reflection of this new knowledge, exploitation of symbiotic bacteria of vector mosquitoes has emerged as a potential control-strategy of MBDs. For instance, the symbiotic bacterium *Asaia* has been shown to activate immune genes in different insect hosts including *Anopheles* where it elicits an anti-plasmodium response (Cappelli et al., 2019. Front Genet, 10:836; Gonnella et al., 2019. Front Physiol, 10:795). The present study investigates the effect of *Asaia* on the immune system of a main vector of arboviruses such as *Ae. aegypti*.

**MATERIALS AND METHODS:** A laboratory strain of *Ae. aegypti* (New Orleans 2011) was reared at standard conditions. The experimental set up included three groups of female mosquitoes (a, b and c). Groups a and b received different dietary boosts of *Asaia*, respectively 105 cell/ml and 108 cells/ml while the control group c received a normal diet. Half of the mosquitoes per each group were feed with blood meal and collected daily for 5 days post feeding. The *Asaia* amount was monitored in all the tested samples by qPCR. The expression of transcription factors (Rel 1, Rel 2) and effectors genes of IMD and Toll (Cecropin A, Defensin C, Gambicin and C-type Lectin) cascades together with two genes (Heme peroxidase 7, Superoxide dismutase) codifying enzymes involved in the degradation of Reactive oxygen Species (ROS) were evaluated by qPCR. Moreover, the effect of *Asaia* supplementation on the microbiota composition was assessed through 16S MiSeq analysis.

**RESULTS AND CONCLUSIONS:** Outcomes suggest that the analysed antimicrobial peptide genes and transcription factors are not affected by *Asaia* overloads, nonetheless the expression of two ROS genes increased concurrently with the proliferation of the bacterium on the second day post-blood meal. These observations need to be corroborated by further analysis for quantifying specific metabolites associated with oxidative stress.
Microbiota analysis indicates a marked proliferation of *Asaia* following blood-feeding, emerging as the predominant bacterium. Other symbiont such as *Pantoea* exhibited a modest increase in abundance and *Pseudomonas* experienced a sharp decrease.
Exploring the *Asaia-Ae.aegypti* system to investigate the influence of symbiotic bacteria on stimulating the mosquito immune response against arboviruses, holds potential. This could pave the way for the development of symbiotic-based interventions that can complement existing approaches in the field.

# AN INDICATOR FRAMEWORK FOR THE MONITORING OF VECTOR-BORNE DISEASE: A STUDY IN ITALY AND GREECE

Falcinelli M.*[1], Damiani C.[2], Cappelli A.[2], Gavaudan S.[3], Canonico C.[3], Currà C.[4], Mavridis K.[5], Vontas J.[5], Ricci I.[2], Favia G.[2]

*[1]School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy; [2]School of Biosciences and Veterinary Medicine, University of Camerino, CIRM Italian Malaria Network, Camerino, Italy; [3]Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Perugia, Italy; [4]Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; [5]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Greece*

**Keywords:** Mosquito, Vector-borne diseases, Arbovirosis

**INTRODUCTION:** In the last decades, we are experiencing a rapid warming of the Earth and a deterioration of natural environments that are leading to disequilibrium and biodiversity loss of unprecedented proportions (Rocklöv et al., 2020. Nat Immuno, 21:479-83). This scenario is impacting human and animal health, as vector-borne diseases (VBDs) are (re-)emerging in several countries as shown by recent outbreaks in Italy (De Carli et al., 2023. Euro Surveill, 28(44): 2300552). Thus, a multifaceted surveillance approach is crucial to understand the levels of risk that countries face and to define the necessary counteractions. This study aims to understand which biotic and abiotic factors affect the (re)-emergence of VBDs transmitted in selected Italian and Greek areas and their related ecological contexts through a coordinated approach of vector surveillance, pathogens screening, and environmental monitoring.

**MATERIALS AND METHODS:** Entomological surveillance was conducted in 67 sampling sites in the Marche and Umbria regions (Italy), and in 14 sampling sites in central Crete (Greece). Adult mosquitoes were collected over two years (2022-2023) from May to October. Each site was georeferenced. Date and onsite meteorological variables were recorded. Human population density, vegetation covering, and human land use were retrieved by remote sensing data. Specimens were morphologically and molecularly identified. Screening of USUTU (USUV) and West Nile viruses (WNV) was performed by RT-PCR on pooled *Culex pipiens*. *Aedes albopictus* and *Cx. pipiens* were analysed to detect filariae and both V1016G and L1014F kdr mutations by PCR.

**RESULTS AND CONCLUSIONS:** In the three studied areas several mosquito species have been identified, most of them were *Cx. pipiens* and *Ae. albopictus*. In Italy, we detected USUV and *Setaria* spp in *Cx. pipiens*. Studying the abundance of the USUV reservoir (wild birds), we observed a correlation between host frequency and the positivity of *Cx. pipiens* across months. Additionally, we identified two land cover variables (rural area and urbanization) that can affect the distribution of *Cx. pipiens* and *Ae. albopictus* in the Marche region. In Crete, for the first time, we found pools of *Cx. pipiens* positive for the WNV. Moreover, we observed an increasing frequency of hybrids of *Cx. p. pipiens/Cx. p. molestus* that could be considered a risk factor for the perpetuation of viral transmission among animals and humans since they are opportunistic feeders. The pathogen screening also revealed a pool of *Ae. albopictus* positive for *Dirofilaria repens*. Regarding the L1014F mutation implied in the insecticide resistance, we registered an increasing frequency of the resistant genotype in *Cx. pipiens* in both Italy and Greece. This approach repeated over time will provide a detailed map of the risks associated with mosquitoes and a large amount of data accessible to all the scientific community, offering an opportunity to optimize control strategies.

# FIRST CHARACTERIZATION OF PYRETHROID RESISTANCE MECHANISMS IN EUROPEAN POPULATIONS OF THE MAIN WEST NILE VECTOR, *CULEX PIPIENS*

Pichler V.*[1], Itokawa K.[2], Salvemini M.[3], Caputo B.[1], De Marco C.M.[1], Serini P.[1], Bellini R.[4], Veronesi R.[4], De Liberato C.[5], Romiti F.[5], Arnoldi D.[6], Rizzoli A.[6], Lia R.P.[7], Otranto D.[7], Michaelakis A.[8], Bisia M.[8], Minakawa N.[9], Kasai S.[2], della Torre A.[1]

*[1]Università Sapienza, Rome, Italy; [2]Department of Medical Entomology, National Institute of Infectious Diseases, Tokyo, Japan; [3]Dipartimento di Biologia, Università degli Studi di Napoli Federico II, Napoli, Italy; [4]Centro Agricoltura Ambiente "G. Nicoli", Crevalcore, Italy; [5]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy; [6]Fondazione Edmund Mach, San Michele all'Adige, Italy; [7]Dipartimento di Medicina Veterinaria, Università degli Studi di Bari, Valenzano, Italy; [8]Laboratory of Insects & Parasites of Medical Importance, Benaki Phytopathological Institute, Athens, Greece; [9]Department of Vector Ecology and Environment, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan*

**Keywords:** *Culex pipiens*, Pyrethroid resistance, Resistance mechanisms.

**INTRODUCTION:** The only insecticides allowed in European Union for adult mosquito control are pyrethroids, targeting the voltage-gated-sodium-channel (VGSC). The concerning rise in resistance to pyrethroids (PR) is anyway threatening their effectiveness. For *Culex pipiens*, the most abundant nuisance mosquito species and main West Nile virus vector in Europe, phenotypic resistance has been detected in Greece, Spain, Belgium and Italy (ECDC 2023, doi: 10.2900/05537), but little is known on the mechanisms underlying PR. The aim of the present study was to provide an overview on target-site and metabolic resistance mechanisms in Italian and Greek *Cx. pipiens* populations.

**MATERIALS AND METHODS:** Target-site resistance was investigated by performing an oligo-hybridization capture approach allowing high-coverage sequencing of the coding region of the whole vgsc gene for 82 *Cx. pipiens* specimens from 4 Italian and 1 Greek region. Metabolic resistance mechanisms were investigated by an RNAseq approach aiming at the detection of differentially expressed genes between pools of resistant and susceptible mosquitoes obtained from 3 regions.

**RESULTS AND CONCLUSIONS:** Overall, 218 polymorphic sites were identified across the coding region of the whole vgsc gene, 26 of which were missense mutations. Mutations with known or suspected impact on PR were detected for five aminoacidic positions: i) Locus 1014: allele 1014F was found in all sampled regions with an allelic frequency varying from 25 to 87%, while allele 1014C was found only in Greece (Freq=50%) and 1014S was found in a single north-Italian specimen in heterozygosis; ii) mutation F1534L was present in all Italian regions at an overall frequency of 8% but absent in Greece; iii) mutations at low frequencies (<5%) were detected in positions 253, 918 and 1879, with mutation M918T -known for conferring a strongly enhanced resistance phenotype, when in association with allele 1014F- being detected for the first time in mosquitoes. Illumina paired end sequencing was successful for 18 pools of specimens with a mean throughput of 33.5 million of fragments per pool. Data analysis aiming at the identification of genes overexpressed in resistant specimens and potentially involved in metabolic resistance is currently ongoing. Results suggest that target-site-resistance is spreading in European *Cx pipiens* and raise awareness about the need to develop further genotyping tools able to detect the most important variants associated with resistance. The resistance phenotype of novel mutations needs to be investigated along with possible synergisms between different target-site and metabolic resistance mechanisms as well as the operational impact of these resistance mechanisms. Such information is crucial for the choice of the most appropriate vector control and insecticide resistance management strategies, aiming at maintaining the effectiveness of the only chemical tool nowadays available for the reduction of adult mosquito abundances.

# PREVALENCE OF *D. IMMITIS* AND *D. REPENS* DNA IN MOSQUITOES CAPTURED IN EMILIA-ROMAGNA REGION

Vismarra A.*[1], Calzolari M.[2], Bianco G.[1], Semeraro M.[1], Dalmonte G.[2], Grisendi A.[2], Dottori M.[2], Cattabiani C.[1], Kramer L.[1], Genchi M.[1]

*[1]Dept. of Veterinary Medicine, Parasitology Unit, University of Parma, Parma, Italy; [2]Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Sede Territoriale di Reggio-Emilia e Brescia, Reggio Emilia, Italy*

**Keywords:** Mosquitos, Regional monitoring, Dirofilariosis.

**INTRODUCTION:** *Dirofilaria immitis* and *Dirofilaria repens* are mosquito-borne filarial nematodes that primarily affect dogs, causing heartworm disease and subcutaneous dirofilariosis (also in humans). Apart from a few seroepidemiological studies on dogs and cats (Genchi et al., 2024. Vector Borne Zoonotic Dis, 24(3): 151), studies about the vector mosquito species are limited in our area. Taking advantage of the entomological surveillance carried out by Istituto Zooprofilattico della Lombardia e dell'Emilia-Romagna (IZSLER), we evaluated the presence of *D. immitis* and *D. repens* DNA in mosquitos captured in our region. The purpose of this study was to identify the predominant vectors of *D. immitis* and *D. repens* by using a multiplex PCR with species-specific oligonucleotides.

**MATERIALS AND METHODS:** Mosquitos were capture by IZSLER in 2019 (between July 25 and October 2) in four sites in the provinces of Reggio Emilia and Modena (Mirandola, Novellara, San Polo d'Enza, Castelnovo Monti) using different model of attractive traps. These were separated by sex and species and frozen till the transportation to Parma University lab. *Culex pipiens* was the most abundant species, followed by *Aedes caspius* and *Ae. albopictus*. DNA and RNA from monospecific pools with a maximum of 20 female mosquitos were extracted using an automated system (Maxwell RSC, Promega), after modifications of the protocol, and analysed through a multiplex-Real-time PCR for *D. immitis* and *D. repens* specific genetic markers (Latrofa et al., 2012. Parasit Vectors, 5:76). Moreover, positive pools were also tested for West Nile Virus (WNV) by IZSLER (Eiden et al., 2010. J Vet Diagn Invest, 22(5): 748-53).

**RESULTS AND CONCLUSIONS:** A total of 94 DNA pools were prepared and analyzed. Thirty resulted positive for *D. immitis*. Eight positive pools were from the group of *Ae. albopictus*, nine from *Ae. caspius*, seven from *Cx. pipiens*. From a geographical point, ten and eight positive pools, respectively, derived from mosquitos trapped in Mirandola and Novellara municipalities (Modena province). Moreover, two pools of *Cx. pipiens* from Reggio Emilia province also resulted positive for WNV. The present study is the preliminary step for a more in-depth analyses of the risk associated with mosquitos in an area where the prophylaxis for dirofilariosis is diffused and practiced by most veterinarians (Genchi et al., 2023. Vet Parasitol Reg Stud Reports, 46:100934), however information about the parasite circulation through its own vectors is limited. The next steps of our project will include a wider area of sampling and could exploit the samples collected in the frame of the Regional plan for surveillance of Arboviruses, in particular for WNV surveillance. This is essential for verifying if the infection of mosquitos with *D. immitis* (or *D. repens*) will facilitate the infection of vectors with other pathogens, as WNV, after the blood-feeding on infected hosts (Vaughan et al., 2012. J Med Entomol, 49(6): 1430-41).

# A METAGENOMIC APPROACH TO INVESTIGATE THE MOSQUITO MYCOBIOTA FOR THE DEVELOPMENT OF FUNGAL BASED INNOVATIVE BIOPRODUCTS FOR ECO-FRIENDLY CONTROL OF MOSQUITOES

Cappelli A.*, Damiani C., Favia G., Ricci I.

*School of Biosciences and Veterinary Medicine, University of Camerino, CIRM Italian Malaria Network, Camerino, Italy*

**Keywords:** Mycobiota, Mosquitoes, Vector control.

**INTRODUCTION:** Mosquito control is a crucial aspect of public health and environmental management, especially in regions where mosquitoes pose a threat due to disease transmission. Traditional vector control methods, such as insecticides, have proven effective but are often associated with environmental concerns and the development of insecticide resistance. In recent years, there has been growing interest in alternative and environmentally sustainable approaches to be used, in conjunction with traditional methods, to control the risks associated with mosquitoes. The characterization of the microorganisms inhabiting the mosquitoes has emerged as a significant area of research due to its implications in various aspects of mosquito biology, ecology, and vector competence. Understanding the composition and function of the microbial communities could have profound implications for vector control strategies. While the bacterial community of mosquitoes has been deeply studied, the fungal component is still little appreciated (Malassigné et al., 2020. Pathogens, 9:564). This study represents an in-depth investigation fungal community associated with Italian mosquito larvae aimed to identify suitable candidates for 'lure and kill' products leveraging attractant and entomopathogenic microbial properties.

**MATERIALS AND METHODS:** In 2022 and 2023, around a thousand mosquito larvae were collected in the Marche (*Aedes albopictus* and *Culex pipiens*) and in the Veneto regions (*Aedes koreicus*). Metagenomic analysis was performed on pools of ten L4 larvae utilizing an Illumina sequencing strategy, targeting the fungal ITS1-4 region. Furthermore, fungal strains were isolated from the same larval samples using culture-dependent methods.

**RESULTS AND CONCLUSIONS:** The analysis unveiled a diverse fungal community including Ascomycota (budding yeasts) and Basidiomycota. Some fungi are specifically associated with mosquito species, such as *Hyaloraphidium* and *Microidium* in *Cx. pipiens* and *Ae. koreicus*, respectively. Whereas other fungi, such as *Wickerhamomyces anomalus*, *Metschnikowia pulcherrima*, and *Candida parapsilosis* were detected across all analysed species. Metagenomic outcomes were confirmed using culture-dependent methods. Isolated fungi are under characterisation for both entomopathogenic activity against larvae and attractant properties mediated by volatile organic compounds towards gravid mosquitoes.
To our knowledge, this represents the first in depth comparative description of the larval mycobiota in *Ae. albopictus*, *Ae. koreicus* and *Cx. pipiens*. Selected attractant and entomopathogenic fungi would provide a microbial repository available for functional tests. Fungal blends might be used for the implementation of 'lure and kill' formulations to be released in artificial or natural breeding sites or to be added in gravid mosquito traps. Such innovative fungal-based bio-products might contribute to the mosquito control following a sustainable 'ready to use' technology.

# *WOLBACHIA* IN *AEDES AFRICANUS*: A SOURCE TO STUDY MICROBIAL COMPETITION IN MOSQUITO

Damiani C.*[1], Cappelli A.[1], Catapano P.L.[2], Mayi M.P.A.[2], Commandatore F.[3], Ricci I.[1], Favia G.[1]

*[1]School of Biosciences & Veterinary Medicine, University of Camerino, CIRM Italian Malaria Network, Camerino, Italy; [2]School of Biosciences & Veterinary Medicine, University of Camerino, Camerino, Italy; [3]Pediatric CRC Romeo ed Enrica Invernizzi, Department of Biosciences, Università di Milano, Milano, Italy*

**Keywords:** *Wolbachia*, *Aedes africanus*, Vector control.

**INTRODUCTION:** *Wolbachia* is an obligate intracellular bacterium naturally found in about 60% of all arthropod's species (Hilgenboecker et al., 2008. FEMS Microbiology Letters, 281(2): 215-20). Its significance lies in its influence on host fitness (ability to survive and mate) and its ability, when specific strains are introduced into mosquitoes lacking *Wolbachia*, to decrease disease transmission by mosquitoes. These attributes of *Wolbachia* present a prospective vector control approach for diseases like dengue and other vector-borne illnesses. In either scenario, the outcome is a decline in viral transmission. Recently we have identified *Wolbachia* in the sylvatic African vector *Aedes africanus*, a mosquito vector widely distributed throughout sub-Saharan Africa, except Madagascar, where it acts as one of the important vectors of yellow fever (Smithburn et al., 1949. Ann Trop Med Parasitol, 43(1): 74-89), zika (Macnamara, 1954. Trans R Soc Trop Med Hyg, 48(2): 139-45) and chikungunya (Saluzzo et al., 1980. Bull Soc Pathol Exot Filiales, 73(4): 390-99). Here, for the first time, we have investigated the microbiota of *Ae. africanus* and the potential relationships and competition dynamics between *Wolbachia*, *Asaia*, and *Pantoea* symbionts.

**MATERIALS AND METHODS:** Microbiota analysis was performed on *Ae. africanus* mosquitoes collected in Cameroon in 2023. These specimens underwent morphological and molecular identification. The prevalence and quantity of *Wolbachia* were assessed using qPCR. Additionally, a cohort comprising both female and male mosquitoes was sacrificed to detect *Wolbachia* via fluorescence in situ hybridization (FISH). Microbiota composition was explored through 16S NGS analysis, focusing on the V3-V4 regions. Furthermore, a phylogenetic examination of the *Wolbachia* strain was conducted using multilocus sequence typing (MLST) analysis.

**RESULTS AND CONCLUSIONS:** *Wolbachia* was found in nearly all the specimens examined, displaying varied quantities. The phylogenetic analysis via MLST revealed that this *Wolbachia* strain belonged to Supergroup B yet exhibited closer resemblance to *Wolbachia* strains observed in Lepidoptera and Hymenoptera rather than mosquitoes. FISH analysis demonstrated *Wolbachia* localization in both male and female reproductive organs. Microbiota analysis suggested a potential competition among *Wolbachia*, *Asaia*, and *Pantoea* symbionts, findings corroborated by qPCR. Genome sequencing of the three bacteria was conducted via shotgun analysis to facilitate a phylogenomic investigation and we are exploring metabolic pathways potentially influencing the dynamics of competition. Further comprehensive studies are underway to deepen our understanding of this competition phenomena. This kind of studies are relevant to determine phenomenon of microbial competition that may affect *Wolbachia*-based mosquitoes borne disease control.

# MODELLING THE ROE DEER'S SUPERPOWER: OPPOSITE TRENDS IN THE PRESENCE OF *BORRELIA* AND *ANAPLASMA PHAGOCYTOPHILUM* IN ENGORGED ADULT TICKS ACCORDING TO THEIR ESTIMATED FEEDING TIME

Cialini C.*[1], Fesce E.[1], Cafiso A.[1], Waldeck M.[2], Lindgren P.[2], Stocchero C.[3], Ferrari N.[1], Grandi G.[4], Bazzocchi C.[1]

*[1]University of Milan, Lodi, Italy; [2]Linköping University, Linköping, Sweden; [3]University of Pavia, Pavia, Italy; [4]Swedish University of Agricultural Sciences, Uppsala, Sweden*

**Keywords:** Feeding time, TBPs, Roe deer.

**INTRODUCTION:** Roe deer are recognized as one of the most important maintenance hosts for *Ixodes ricinus* populations which are relevant for tick-borne pathogens (TBPs) transmission (Jaenson et al., 2018. Parasit Vectors, 11:477). However, roe deer, as other deer species, is considered an incompetent reservoir for *Borrelia burgdorferi sensu latu*, due to an indiscriminatory borreliacidal activity of their sera (Jaenson & Tälleklint, 1992. J Med Entomol, 29:813-17; Kurtenbach et al., 1998. Appl Environ Microbiol, 64:1169-74). The complement system of roe deer is capable of reducing the *Borrelia* load in the host and lyse spirochaetes in the midgut of the feeding tick (Kurtenbach et al., 2006. Nat Rev Microbiol, 4:660-69). The longer the blood meal is, the less the chances for attached ticks to carry *Borrelia* spp. are (Rosef et al., 2009. Acta Vet Scand, 51:47). Therefore, while infectious agents are generally acquired by ticks during blood-meal and can subsequently be transmitted upon their next feeding, in roe deer, *Borrelia* undergoes a reduction in the bacterial load. On the other hand, no effects of roe deer immunity on *Anaplasma* spp. have been described so far. The aim of this study was thus to model the relation between the occurrence in ticks of *Borrelia* spp. and a bacterium not negatively influenced by deer serum (as the case of *A. phagocytophilum*) and the relative estimated feeding duration of these ticks.

**MATERIALS AND METHODS:** Female ticks were collected from roe deer in Skåne (the southernmost county of Sweden) in 2014. Ticks were morphologically identified, photographed, measured, and the estimated feeding duration was calculated based on the scutal index (SI) and/or the coxal index (CI), using regressing equations (Gray et al., 2005. Int J Med Microbiol, 295:567-72). Total nucleic acids extraction was performed, and specific Real Time PCR assays were carried out to test the samples for the presence of *Borrelia* spp. and *A. phagocytophilum*. To assess the effects of the duration of feeding on the probability of a tick to test positive for *Borrelia* spp. or *A. phagocytophilum*, generalized linear mixed models (GLMMs) with binomial distribution were used. Animal host ID and area of sampling were included as random factors.

**RESULTS AND CONCLUSIONS:** All the collected ticks (n=362) were identified as *I. ricinus*. Among these, 14.6% and 65.5% were positive to *Borrelia* spp. and *A. phagocytophilum*, respectively. The results of the GLMMs highlighted that as the feeding duration on roe deer increases, ticks' likelihood of testing positive for *Borrelia* spp. decreases. Conversely, an extended feeding duration is associated with a higher likelihood of ticks to test positive for *A. phagocytophilum*. These findings endorse the borreliacidal effect of roe deer serum and the consequent incompetence of roe deer to serve as reservoir hosts for *Borrelia* spp. (Ratti et al., 2021. Ticks Tick Borne Dis, 12:101724). Further analyses are required to extend these results to other cervid species.

# *WOLBACHIA* ADVANCEMENTS: STRENGTHENING DENGUE CONTROL IN AFRICA

Mancini M.V.*[1], Murdochy S.[2], Bilgo E.[3], Diabate A.[3], Failloux A.[4], Sinkins S.[2]

*[1]University of Pavia, Pavia, Italy; [2]MRC- Centre for Virus Research, University of Glasgow, Glasgow, United Kingdom; [3]Institut de Recherche en Sciences de la Santé, Direction Régionale de l'Ouest, Burkina Faso, Bobo Dioulasso, Burkina Faso; [4]Institut Pasteur, Université Paris Cité, Arboviruses and Insect Vectors Unit, Paris, France*

**Keywords:** *Wolbachia*, Dengue, Vector control.

**INTRODUCTION:** Dengue represents an increasing public health burden worldwide. In Africa, under-reporting and misdiagnosis often mask its true epidemiology, and dengue is likely to be both more widespread than reported data suggest and increasing in incidence and distribution. *Wolbachia*-based dengue control is underway in Asia and the Americas, but has not to date been deployed in Africa. Due to the genetic heterogeneity of African *Aedes aegypti* populations and the complexity of the host-symbiont interactions, characterization of key parameters of *Wolbachia*-carrying mosquitoes is paramount for determining the potential of the system as a control tool for dengue in Africa.

**MATERIALS AND METHODS:** *Wolbachia* strains were stably introduced into a field-collected African population of *Ae. aegypti* by introgression. *Wolbachia* density and phenotypic stability were assessed under laboratory setting and stressing conditions, such as high larval rearing temperatures. Finally, the ability of *Wolbachia* to interfere with dengue virus (DENV) was also explored.

**RESULTS AND CONCLUSIONS:** *Wolbachia* showed high intracellular density in whole bodies and in different mosquito tissues; high intracellular density was also maintained following larval rearing at high temperatures. No effect on adult lifespan induced by *Wolbachia* presence was detected. Moreover, the ability of this strain to strongly inhibit DENV-2 dissemination and transmission in the host was also demonstrated in the African background. Our findings suggest the potential of harnessing *Wolbachia* for dengue control for African populations of *Ae. aegypti*.

# SAND FLIES SAMPLING AND PATHOGENS DETECTION IN VENETO REGION (2022-2023)

Gobbo F.[1], Danca L.[1], Toniolo F.*[1], Danesi P.[1], Marsili G.[2], Mangiapelo C.[2], Fortuna C.[2], Russo F.[3], Montarsi F.[1]

*[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy; [2]Istituto Superiore di Sanità, Roma, Italy; [3]Department of Prevention, food safety and veterinary, Veneto Region, Venezia, Italy*

**Keywords:** Phlebotominae sand flies, Toscana Virus, Punique Virus.

**INTRODUCTION:** Sand flies are vectors of pathogens for animals and humans as parasites (*Leishmania* spp.) and several viral agents (*Phlebovirus* spp.). The Phleboviruses have been described as causative agents of summer fevers and among these, the Toscana Virus (TOSV) is associated with neurological disorders. Despite high seroprevalences in humans and domestic animals in endemic areas, there is still a lack of knowledge on TOSV epidemiology, and no specific surveillance plans are applied in human and veterinary entomology fields. In the present study, we report a survey for sand flies presence/abundance and pathogens (*Leishmania infantum* and *Phlebovirus* spp.) carried out in Veneto region.

**MATERIALS AND METHODS:** In 2022, eight sites were selected in Veneto region (Vicenza, Verona, Treviso and Padova). Sand flies were trapped using one-night active CD-CO2-light trap and ten sticky traps per site every two weeks. Thus, the specimens were sent to the laboratory for identification, count, sexing and pooling for the molecular detection of *Leishmania infantum* and *Phlebovirus* spp. In 2023, basing on the results obtained in the previous season, only two sites in Padova province were selected for the sampling (Baone and Este) located in rural and urban environment, respectively.

**RESULTS AND CONCLUSIONS:** In 2022, a total of 4122 sand flies were collected in Veneto Region and identified as 3287 *P. perniciosus* (79.7%), 74 *P. neglectus* (1.8%), 8 *P. perfiliewi* (0.2%), 53 *Sergentomya minuta* (1.3%) and 700 as *Phlebotomus* spp. (16.9%). Pathogens investigation pointed out 22 pools positive for *L. infantum* (18 in *P. perniciosus*, 3 in *S. minuta* and 1 in *P. neglectus*). Interestingly, one pool of 35 males of *P. perniciosus* resulted positive for TOSV in the municipality of Baone. In 2023, a total of 1619 sand flies were collected and identified as 779 *P. perniciosus* (48.1%), 21 *P. neglectus* (1.3%), 41 *P. perfiliewi* (2.5%), 1 *S. minuta* (0.1%) and 777 as *Phlebotomus* spp. (47.9%). One pool of *P. perniciosus* was positive for *L. infantum* and 3 pools of *P. pernicious* for Phleboviruses; further sequencing analysis of these positive samples attributed these viral strains to Punique virus, TOSV and *Phlebovirus* spp. None examined pool demonstrated coinfection of *L. infantum* and phleboviruses. The results obtained in this study demonstrated the presence of several phlebotominae species and *P. perniciosus* resulted in the predominant species. Moreover, molecular investigation demonstrated the first detection of TOSV in *P. pernicious* in Veneto and the first evidence of Punique virus in Italy and Europe. Finally, this study provided evidence of circulation, maintenance and heterogenicity of phleboviruses in the vector *P. perniciosus* in two consecutive summer seasons in province of Padova. These findings should promote specific monitoring activities on sandflies as a preventing factor for public health.

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# DECIPHERING THE *ANOPHELES COLUZZII* RESPONSES AGAINST *PLASMODIUM FALCIPARUM* DURING MIDGUT INVASION

Bevivino G.*, Dipaola M.G., Arcà B., Lombardo F.

*Sapienza University, Department of Public Health and Infectious Diseases, Rome, Italy*

**Keywords:** Mosquito innate immunity, Malaria transmission, Mosquito-parasite interplay.

**INTRODUCTION:** Malaria is one of the deadliest infectious disease and a significant public health problem concern. According to the World Health Organization, malaria is responsible of about 249 million cases and 608,000 human deaths, with a higher burden in sub-Saharan Africa where highest mortality and morbidity is registered every year (WHO, 2022). It is well known that the survival and development of *Plasmodium falciparum* within the *Anopheles* mosquito vector, as well as the transmission efficiency to the human host, are heavily influenced by the mosquito responses. Between 12-36 hours after the ingestion of an infected blood meal, a significant reduction in *Plasmodium* numbers occurs within the mosquito's gut during the formation and maturation of ookinetes. Many of these losses are attributed to the activation of humoral and/or cellular mosquito innate immune responses (Smith et al., 2014. Mem Inst Oswaldo Cruz, 109:644-61; Graumans et al., 2020. Trends Parasitol, 36(8): 705-16). We recently performed RNA-seq transcriptomic study aimed to compare the responses of *Anopheles coluzzii* to high and low *P. falciparum* infection intensities before (12 hpi), during (24 hpi) and after (36 hpi) ookinete invasion of mosquito midgut. The aim of this work is to shed light on parasite-mosquito molecular interaction and to investigate mosquito immune responses against *Plasmodium* during midgut-early stage of parasite invasion.

**MATERIALS AND METHODS:** *An. coluzzii* mosquitoes were experimentally infected with *P. falciparum* and collected at 12, 24 and 36 hours post infection. The parasite infection level (high and low) was assessed in single mosquito by quantifying the expression of ookinete-specific markers (ctrp, warp, soap and cht1) through RT-qPCR analysis. RNA sequencing was performed on mosquito pools bearing high and low infection intensity for a total of 3 biological replicates per condition. Differential gene expression analysis (DE) was employed to evaluate differences in transcripts abundance between mosquitoes with high and low ookinete parasite load.

**RESULTS AND CONCLUSIONS:** Bioinformatic analysis revealed that mosquitoes with low infection intensities exhibited a higher abundance of transcript corresponding to immune factors. These data suggested that early and persistent innate immune reactions may be responsible for removing parasites before they infect mosquito midgut tissue, leading to low parasite infection intensities. Interestingly, genes involved in arginine metabolism, whose role in mosquito immunity has not been investigated so far, were also up-regulated in conditions of low infection intensities. As future step, in order to clarify their role in the immune defense against *Plasmodium*, we aim to functionally characterized these gene using a gene silencing approach. Overall, this study contributes to a better understanding of *Anopheles-Plasmodium* interplay and to the identification of novel potential target for blocking malaria transmission in the mosquito vector.

# DEVELOPMENT AND VALIDATION OF AN INNOVATIVE APPROACH TO ASSESS THE AGE OF *AEDES ALBOPICTUS* ADULTS

Foti M.[1], Micocci M.[1], Pazmino Betancourth M.[2], Caputo B.[1], Casas I.[2], Baldini F.[2], della Torre A.*[1]

*[1]Sapienza University, Rome, Italy; [2]University of Glasgow, Glasgow, United Kingdom*

**Keywords:** *Aedes albopictus*, Age-grading, Mid-Infrared spectroscopy.

**INTRODUCTION:** An accurate and reliable assessment of mosquito age structure is crucial not only to evaluate risks of pathogen transmission and the impact of vector control interventions, but also to establish a threshold for planning adulticidal interventions in critical situations (e.g., the catholic Jubilee to be held in Rome in 2025, during which >30 million tourists are expected to arrive from all over the world). Nonetheless, gold standard methods to estimate mosquito age are extremely labour-intensive, inaccurate, and imprecise. In 2022, a new technique based on Mid-Infrared spectroscopy (MIRS) coupled with a Supervised Machine Learning Approach (SML) was developed to accurately predict age, sex, and species of *Anopheles* malaria vectors (Siria et al., 2022. Nat Commun, 13:1501). Here, we report the encouraging results of a first attempt to exploit MIRS-SML to estimate the age of invasive *Aedes albopictus* populations in temperate regions where the species is increasingly responsible of autochthonous transmission of exotic arboviruses.

**MATERIALS AND METHODS:** *Aedes albopictus* eggs were reared to adulthood either under laboratory or semi-field conditions. Males and females at different physiological states were collected every 3 days until day-36 under laboratory conditions, and every 3 consecutive days (from 1-3 to 31-33 day old) in case of semi-field adults. Spectra from individual mosquitoes were acquired by Attenuated Total Reflection FT-IR spectroscopy using a Bruker ALPHA II spectrometer between 4,000 and 400 cm−1 with 4 cm−1 resolution. The dataset was split into training (80%) and test (20%) sets. Several machine-learning algorithms were tested, and Support Vector Classifier (SVC) was selected for optimisation based on the accuracy of the predictions.

**RESULTS AND CONCLUSIONS:** These results demonstrate the potential of this innovative approach as a tool for epidemiological investigations and for the evaluation of control interventions for this important vector. Results from laboratory reared adults encouraged to progress towards the analyses of semi-field samples. In this case, when age-groups included females and males emerged in 3 consecutive days, the SML model's estimation accuracies ranged between 52% and 91% and 68-91%, respectively. When age-groups included females ≤9 and >9 days old (corresponding to 100% non-infectious females and potentially infectious females, respectively) the accuracy raised up to 97%. These results support the high potential of MIRS-SML as the first non-morphological approach to accurately and cost-effectively assess the age structure of *Ae. albopictus*. Further studies are needed to confirm its reliability on predicting field-collected specimens.

# OLD BUT GOLD: DIVERSITY, HOST-ASSOCIATION AND VECTOR-BORNE PATHOGENS OF TICKS FROM WILDLIFE IN NORTHWEST ITALY

Moroni B.[1], Garcia-Vozmediano A.[2], Accorsi A.[3], Nogarol C.[4], Robetto S.[5], Wolfsgruber L.[3], Orusa R.[5], Zoppi S.[1], Razzuoli E.[3], Listorti V.[3], Mandola M.L.[4], Guardone L.*[3]

*[1]S.C. Diagnostica Generale, Istituto zooprofilattico sperimentale Piemonte, Liguria, Valle d'Aosta, Torino, Italy; [2]BEAR - Biostatistica, Epidemiologia e Analisi del Rischio, S.S. REA - Rischi alimentari ed Epidemiologia degli Alimenti, Istituto Zooprofilattico Sperimentale Piemonte Liguria e Valle D'Aosta, Torino, Italy; [3]S.S. Sezione di Genova - Portualità, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle D'Aosta, Genova, Italy; [4]S.S. Virologia Specialistica, Istituto zooprofilattico sperimentale Piemonte, Liguria, Valle d'Aosta, Torino, Italy; [5]S.S. Patologie della Fauna Selvatica, Istituto Zooprofilattico Sperimentale Piemonte, Liguria e Valle d'Aosta, Centro di Referenza Nazionale Malattie Animali Selvatici (CeRMAS), Aosta, Italy*

**Keywords:** Vectors, Tick-borne diseases, One Health.

**INTRODUCTION:** Wildlife vertebrate hosts are essential components of the life cycles of ticks, which represent important vectors of pathogens (TBPs) of public health concern (Tsao et al., 2021. J Med Entomol, 58:1565-87). This survey investigated the tick fauna, tick-host association and TBPs in a range of wildlife species, in the frame of an ongoing research project (Ricerca finalizzata GR-2021-12374932).

**MATERIALS AND METHODS:** Ticks were collected from animals found dead or from portions (ear/skin) of hunted animals submitted to IZSPLV sections in Piemonte, Liguria and Valle d'Aosta from October 2023 to April 2024. Ticks were morphologically identified using taxonomical keys (Estrada-Peña et al., 2017. Ticks of Europe and North Africa: a guide to species identification, Springer Nature, Switzerland, 75-342), counted and grouped into pools homogeneous for species, sex and life stage. DNA was extracted from each pool and molecularly screened for spotted fever group (SFG) rickettsiae, *Coxiella* spp., *Anaplasma* spp., and *Borrelia burgdorferi sensu lato* (OIE Manual for Terrestrial Animals, 2018; Regnery et al., 1991. J Bacteriol, 173:1576-89; Skotarczak et al., 2002. AAEM, 9(1); Stuen et al., 2003. CVI, 10:692-95).

**RESULTS AND CONCLUSIONS:** Overall, 848 ticks were collected from 182 wild animals, including canids (n=68), wild ruminants (n=67), wild boars (n=26), mustelids (n=17), and small rodents (n=4). *Ixodes ricinus* (n=623, 73.5% of the total ticks) was the most common tick species identified, in a wide range of animal hosts. *I. hexagonus* (n=112) was mainly collected from red foxes and mustelids, while *Dermacentor marginatus* (n=27) from wild boars. Other less frequent tick species and associated hosts included *Haemaphysalis erinacei* and *I. canisuga* in the red fox (n=2), *I. trianguliceps* in a badger, and *Ha. concinna* in roe deer from Piemonte; *Rhipicephalus sanguineus s.l.* and *R. turanicus* in wolves (n= 2) and *Ha. punctata* in a wild boar and two roe deer from Liguria. Interestingly, *Ha. sulcata* was found in an Alpine chamois found dead above 1,500m asl, despite this tick species is typically associated with a Mediterranean climate (Estrada-Peña et al., 2017). Additionally, *D. reticulatus* (n=6) was found in wild boars from Piemonte and Liguria, confirming its further expansion in North-west Italy (Garcia-Vozmediano et al., 2020. Vet Sci, 7:157). Preliminary results on pathogens based on 50 pools (n= 103 ticks from Piemonte) revealed the occurrence of SFG rickettsiae in all life stages of *I. ricinus* (27.6% of the examined pools; 95% CI=12.7-47.2) collected from two grey wolves, two roe deer and a red squirrel and in one pool of *I. hexagonus* nymphs (7.7%; 0.2-36.0) collected from a red fox. *Anaplasma* spp. and *Coxiella* spp. were also detected in two pools of *I. ricinus* females from a roe deer and a wolf, respectively, while *B. burgdorferi s.l.* was not found, so far. The results contribute to update ticks and TBPs epidemiology in Northwest Italy, showing the presence of a wide range of tick species and TBPs.

# INVESTIGATION ON THE PRESENCE OF SAND FLIES (DIPTERA: PSYCHODIDAE) IN PERI-URBAN HABITATS IN THE BERGAMO DISTRICT AND ON THE PREVALENCE OF *LEISHMANIA* SPP. IN VECTORS AND SYNANTHROPIC REPTILE HOSTS

Alvaro A.*[1], Cattaneo G.M.[1], Varotto-Boccazzi I.[1], Sanchez-Ruiz L.[1], Molteni R.[1], Gabrieli P.[1], Otranto D.[2], Mendoza-Roldan J.A.[2], Bandi C.[1], Epis S.[1]

*[1]EntoPar UniMi, Department of Biosciences, University of Milan, Milan, Italy; [2]Department of Veterinary Medicine, University of Bari, Valenzano, Italy*

**Keywords:** Sand flies, *Leishmania*, Reptiles.

**INTRODUCTION:** Leishmaniases are parasitic diseases endemic to the tropics, subtropics, and southern Europe. In Italy, the infection is mainly caused by the pathogen *Leishmania infantum*, which is transmitted by phlebotomine sand flies of the genus *Phlebotomus*. In northern Italy, endemic foci of leishmaniasis have been reported since the early 1990s, due to the northward expansion of the vector range and the arrival of infected dogs from the south, after traveling or relocation (Gradoni et al., 2022. Vet Parasitol Reg Stud Reports, 27:100676). In our country, other than *L. infantum*, the reptile-associated *Leishmania tarentolae* has been detected and isolated in the southern regions (Mendoza-Roldan et al., 2021. Parasit Vectors,14:461). The sand fly *Sergentomyia minuta* is the herpetophilic vector of *L. tarentolae*, common in the south and recently observed also in northern Italy (Gradoni et al., 2022. Vet Parasitol Reg Stud Reports, 27:100676). Nevertheless, no data have been published on the occurrence of *L. tarentolae* in sand flies and reptiles in northern Italy.

**MATERIALS AND METHODS:** The sampling of sand flies was carried out in 12 sites in the Bergamo district, during the years 2022 and 2023. Both light and sticky traps were employed. Collected sand flies were brought to the EntoPar lab of the University of Milan. Males were identified following a morphological key (Dantas-Torres et al., 2014. Parasit Vectors, 7:479), while females were identified through PCR-RFLP (Latrofa et al., 2012. Vet Parasitol, 184:267-70). In engorged females, the blood meal was analyzed in order to identify the host on which the sand fly fed (Kasičová et al., 2021. Parasite, 28:58). The screening for *Leishmania* spp. was performed with PCR using pan-*Leishmania* primers targeting the ITS-1 gene (el Tai et al., 2000. Trans R Soc Trop Med Hyg, 94:575-9). The resulting amplicons were sequenced on both strands with Sanger technology. Synanthropic reptiles were captured around the sand fly sampling sites. Blood, feces, and tissue samples were also collected, as well as salivar and cloacal swabs. DNA was extracted from the collected samples and screened for *Leishmania*, as described for sand flies.

**RESULTS AND CONCLUSIONS:** Sand flies were collected in 7 out of the 12 investigated sites, and were identified as belonging to the *S. minuta*, *Phlebotomus perniciosus* and *Phlebotomus neglectus* species. *Leishmania* DNA sequences were obtained from engorged females of all these species, and clustered with homologous sequences from *L. tarentolae*. Blood meal analysis showed that *S. minuta* fed primarily on reptiles while *P. perniciosus* and *P. neglectus* primarily on domestic mammals. In reptiles, *L. tarentolae*-related sequences were obtained from blood and tissue samples, feces and swabs. Our study reports the first molecular evidence for the existence of an autochthonous cycle of a reptile-associated *Leishmania* in sand flies and synanthropic reptiles in northern Italy.

# LONGITUDINAL SURVEY OF INSECTICIDE RESISTANCE IN A VILLAGE OF CENTRAL REGION OF BURKINA FASO REVEALS CO-OCCURRENCE OF 1014F, 1014S AND 402L MUTATIONS IN *ANOPHELES COLUZZII* AND *ANOPHELES ARABIENSIS*

Perugini E.*[1], Pichler V.[1], Guelbeogo W.M.[2], Micocci M.[1], Poggi C.[1], Di Maio F.[1], Manzi S.[3], Ranson H.[4], della Torre A.[1], Mancini E.[5], Pombi M.[1]

*[1]Sapienza University of Rome, Department of Public Health and Infectious Diseases, Rome, Italy; [2]Centre National de Recherche et Formation sur le Paludisme, Ouagadougou, Burkina Faso; [3]Istituto Zooprofilattico delle Venezie, Parasitology Unit, Legnaro, Italy; [4]Liverpool School of Tropical Medicine, Department of Vector Biology, Liverpool, United Kingdom; [5]Sapienza University of Rome, Department of Biology and Biotechnology Charles Darwin, Rome, Italy*

**Keywords:** *Anopheles gambiae s.l.*, Pyrethroid, Malaria vector control.

**INTRODUCTION:** Pyrethroid resistance of *Anopheles* mosquitoes is seriously threatening the effectiveness of insecticide threated bed nets (ITNs), a pyrethroid based-tool recommended by WHO in all endemic countries for malaria vector control and prevention. Pyrethroid insecticides target the voltage-gated sodium channel (VGSC) causing aberrant nervous signalling and death in mosquitoes which, in contrast, can limit their effects mainly mutating in the vgsc gene or increasing the metabolic activity through the over-expression of detoxifying enzymes. Genotyping of mutations in the voltage gated sodium channel (VGSC) gene is widely used to easily assess the evolution and spread of pyrethroid target-site resistance among malaria vectors. L1014F and L101S substitutions are the commonest and best characterized VGSC mutations in major African malaria vectors of the *Anopheles gambiae* complex. Recently, a substitution involved in pyrethroid resistance, i.e. the V402L, has been detected in specimens of *Anopheles coluzzii* from west Africa lacking of any other resistance alleles at locus 1014 (Clarkson et al., 2021. Mol Ecol, 30:5303-17; Williams et al., 2021. Pest Manag Sci, 78:1155-63). We here monitored the temporal dynamic of target-site resistance mutations 1014F/S and 402L, in *An. coluzzii* and *An. arabiensis* specimens from a Burkina Faso village over 10 years after the massive ITN scale-up started in 2010.

**MATERIALS AND METHODS:** *Anopheles coluzzii* (N= 300) and *An. arabiensis* (N=362) specimens, collected in 2011, 2015 and 2020 in Goden village, were genotyped for the three target site resistance mutations by TaqMan assays (Bass et al., 2007. Malar J, 6:111) and sequencing (Fan et al., 2020. PLoS Negl Trop Dis, 14:e0008154). Allele frequencies were statistically investigated over the years.

**RESULTS AND CONCLUSIONS:** A divergent trend in resistant allele frequencies was observed in the two species from 2011 to 2020: 1014F decreased in *An. coluzzii* (from 0.76 to 0.52; $\chi2=29.39$, $p<0.0001$) but increased in *An. arabiensis* (from 0.18 to 0.70; $\chi2=183.09$, $p<0.0001$); 1014S was detected only in *An. arabiensis*, slightly decreasing over time (from 0.33 to 0.23); 402L increased in *An. coluzzii* (from 0.15 to 0.48; ($\chi2=42.2$, $p<0.0001$) and was found for the first time in one *An. arabiensis* specimen. In 2020 the co-occurrence of different resistance alleles was 43% in *An. coluzzii* (alleles 410L and 1014F) and 32% in *An. arabiensis* (alleles 1014F and 1014S). Overall, after 10 years from ITNs first implementation in the village, an increasing level of target-site resistance was observed among the vector population, with only 1% of specimens being wild type at both loci. This, together with the evidence of existing co-occurrence of different mutations in the same specimens, calls for future investigations on the possible synergism between resistance alleles and their phenotypic effects in order to implement local tailored vector control strategies.

# REDISCOVERY OF *ANOPHELES SACHAROVI* IN ITALY: DETECTION OF THE FORMER MALARIA VECTOR AFTER MORE THAN 50 YEARS

Di Luca M.*[1], Raele D.A.[2], Severini F.[1], Toma L.[1], Menegon M.[1], Boccolini D.[1], Tortorella G.[3], Cafiero M.A.[2]

*[1]Dipartimento di Malattie Infettive, Reparto di Malattie Trasmesse da Vettori, Istituto Superiore di Sanità, Rome, Italy; [2]Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy; [3]Azienda Sanitaria Nazionale (ASL), Servizio Veterinario Sanità Animale, Lecce, Italy*

**Keywords:** *Anopheles maculipennis* complex, Residual anophelism, qPCR.

**INTRODUCTION:** Malaria was endemic in Italy until mid-twentieth century and among the species belonging to *Anopheles maculipennis* complex, *Anopheles labranchiae* and *Anopheles sacharovi* were historically recognized as the most competent malaria vectors. Once widely distributed throughout the country, *An. sacharovi* gradually disappeared likely due to the progressive modification of its larval habitats and it has not been found since the end of 1960s. In September 2022, as a part of a Surveillance Project, funded by the Italian Ministry of Health to monitor the presence and distribution of *An. maculipennis* complex in Basilicata and Apulia regions, a single specimen was collected in Lecce municipality and molecularly identified as *An. sacharovi*. This record prompted the implementation of a targeted entomological investigation.

**MATERIALS AND METHODS:** In September 2023, a survey was carried out in the areas around the first record, focusing on livestock farms, riding stables and potential breeding sites. Adult and immature specimens were collected using active search or traps in several natural and rural sites. All mosquitoes morphologically identified as *An. maculipennis sensu lato*, were molecularly screened by a home-made quantitative PCR-assay, developed specifically for the rapid identification of *An. labranchiae*, the member of the complex common and abundant in the region. Those *An. maculipennis sl* specimens negative for the test were processed by amplifying and sequencing the nuclear ribosomal internal transcribed spacer 2 (ITS-2) gene.

**RESULTS AND CONCLUSIONS:** Among the 11 investigated sites, 6 were positive for *Anopheles* presence. All 20 *An. maculipennis sl* (7 adults, 10 larvae and 3 pupae) collected were subsequently identified as *An. sacharovi* by the ITS-2 sequence. The evaluation of the morphological characters reported in the ancient literature to identify the adult specimens of *An. sacharovi* from the other species of the *An. maculipennis* complex is underway using microscopic observations and digital photo acquisitions. The rediscovery of *An. sacharovi*, considered disappeared from Italy for over 50 years, has a significant public health implications, highlighting an increase in the receptivity of the Southern areas. Since imported malaria cases are reported in Italy every year, the possibility of introducing *Plasmodium* gametocyte carriers from malaria-endemic areas, potentially capable of infecting vectors circulating in an area at risk, should be taken into consideration. Our findings indicate the need for reevaluation and formulation of new predictive models for the expansion of introduced malaria. Moreover, strengthening residual anophelism surveillance in southern regions is necessary to reduce the risk of disease reintroduction.

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# EXPLORING CRYOPRESERVATION ALTERNATIVES FOR *DIROFILARIA IMMITIS* MICROFILARIAE

Sturiale E.*[1], Napoli E.[1], Nonnis F.[2], Tamponi C.[2], Varcasia A.[2], Venco L.[3], Cavallero S.[4], Bellini I.[4], Gabrielli S.[4], Pombi M.[4], De Benedetto G.[1], Gaglio G.[1], Brianti E.[1]

[1]Department of Veterinary Sciences, University of Messina, Messina, Italy; [2]Department of Veterinary Medicine, University of Sassari, Sassari, Italy; [3]Ospedale Veterinario Città di Pavia, Pavia, Italy; [4]Department of Public Health and Infectious Diseases, Roma, Italy

**Keywords**: Cryopreservation, *Dirofilaria immitis*, Microfilariae.

**INTRODUCTION:** Canine Heartworm Disease is a vector-borne disease caused by *Dirofilaria immitis* that affects canids and felids and poses zoonotic hazard. Since 1929, pioneering studies by Filleborn have laid the groundwork for cryopreservation research. The earliest publications employed temperatures around -70°C, achieving acceptable survival rates, but microfilariae (mf) showed alterations in morphology and motility (Weinman & McAllister, 1947. Am J Hyg, 45:102-21; Taylor, 1960. J Helminthol, 34:13-26; Bemrick et al., 1965. J Parasitol, 51:954-7). Later liquid nitrogen was used, reaching a final temperature of -196°C, reporting a duration of 30 days for mf of *D. immitis* and up to 540 days for mf of other species (Lowrie, 1983. Am J Trop Med Hyg, 32:138-45; Bartholomay et al., 2001. Am J Trop Med Hyg, 65:162-3). The liquid nitrogen represents an excellent tool for long-term preservation, albeit the higher costs and the requirement for specialized equipment. For this reason, the aim of this study was to devise a protocol for the use of a cryopreservation medium allowing a storage at -80°C, without liquid nitrogen, in order to enhance reproducibility, particularly in laboratories with limited ultra-freezing equipment.

**MATERIALS AND METHODS:** The medium of cryoprotectants used in this study were prepared with 5% DMSO, 20% of newborn calf serum and 75% of saline solution. Blood from a *D. immitis* naturally infected dog was diluted into 1.8 ml cryovials with the medium at a ratio of 1:1, and stored at -80°C using a freezing container (1°C/min cooling rate) for the first 24h. On the following Study Days (SD) 1, 7, 30, 60, 90, 120, 150 one cryovial was thawed and examined for survival, motility, length and morphology of mf.

**RESULTS AND CONCLUSIONS:** On SD 1, the mf exhibited a survival rate of 99%. By SD 7, despite the same survival rate, a notable shift in motility patterns was observed, with complete and normal movement, albeit progressively slower as the study progresses. After 5 months of freezing (SD150) the survival rate was > 50% and the mf did not exhibit morphological alterations nor signs of vacuolar degeneration. Regarding the measurements during the 150 days of study there was noticeable trend of shrinking, suggesting a progressive reduction in total length of mf. This study marks the first protocol since 1965 where the -80°C freezer has been employed for cryopreservation, integrating the application of contemporary cryoprotectants and novel techniques for gradual temperature transition. The findings revealed that cryopreserved mf of *D. immitis* can endure up to 150 days at -80°C with a survival rate exceeding 50%, with no morphological alterations, thereby ensuring a satisfactory post-thaw survival. The results of this study provided a simple and reproducible protocol applicable in a wide range of laboratories.

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# SPATIAL ANALYSIS OF ENTOMOLOGICAL FACTORS AFFECTING MALARIA TRANSMISSION IN THE VILLAGE OF GODEN (BURKINA FASO)

Poggi C.*[1], Perugini E.[1], Guelbeogo W.M.[2], Bevivino G.[1], Pichler V.[1], Ranson H.[3], della Torre A.[1], Pombi M.[1]

*[1]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy; [2]Centre National de Recherche et Formation sur le Paludisme, Ouagadougou, Burkina Faso; [3]Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

**Keywords:** *Anopheles*, Malaria transmission, Spatial analysis.

**INTRODUCTION:** Burkina Faso faces significant challenges with malaria, ranking among the top ten countries globally affected by malaria-related morbidity and mortality. Despite extensive efforts such as the distribution of Long-Lasting Insecticide-treated bed Nets (LLINs) programs, which achieved 80% coverage in Burkina Faso between 2010 and 2022, malaria remains a significant health burden, constituting about 7% of malaria cases in West Africa (World Malaria Report, 2023). Studies have identified various mosquito resistance mechanisms, both behavioural and physiological, against LLINs (Ranson et al., 2011.Trends Parasitol, 27:91-8). In our study area, the rural village of Goden (central Burkina Faso), a high transmission intensity persists primarily driven by *Anopheles coluzzii* and *Anopheles arabiensis* (Pombi et al., 2018. Sci Rep, 8:12806; Perugini et al., 2020. Parasit Vectors, 13:277). This study aims to investigate the spatial patterns of entomological factors related to malaria transmission in the village to identify potential hotspots within the village.

**MATERIALS AND METHODS:** Entomological surveys were conducted in 2019 across 31 compounds in Goden by pyrethrum spray collections (PSC), over two nights in October. Data on compound coordinates, number of inhabitants in each house, human bed net protection and number of animal hosts, were recorded. Mosquitoes were identified for the species, analysed for the blood meals, and tested for *Plasmodium* positivity in the abdomens. A multiple regression model was estimated to analyse the relation between the log-transformed *Plasmodium* positivity and geographical factors, human blood index (HBI) and mosquitoes' log-abundance. Additionally, linear interpolation techniques, Inverse Distance Weighting (IDW) was used to visualize the spatial distribution of HBI and *Plasmodium* sp. positivity.

**RESULTS AND CONCLUSIONS:** In total 1,492 *An. gambiae s.l.* females were collected: 78% were identified as *An. coluzzii*, 19% as *An. arabiensis* and the remaining 3% as hybrids. Overall, blood meal analysis revealed a HBI of 45% (N=670; 53% *An. coluzzii*; 13% *An. arabiensis*). An abdomen infection rate of 49% was observed for human fed mosquitoes while animal fed mosquitoes (N=370) showed a 6% of positivity. Taking this value as a proxy of oocyst rate (OR), our data suggests that almost half of blood meals taken on humans were *Plasmodium* infected. The regression model on the log-transformed *Plasmodium* positivity corrected for the OR (Adj. R-sq: 0.69) revealed a significant effect of the compound coordinates ($P$ <0.05) and a positive relation with HBI and mosquitoes' abundance ($P$ <0.05). Moreover, interpolation techniques revealed a heterogeneous pattern of entomological parameters in the village, suggesting potential hotspots associated with malaria transmission, useful to identify potential targets for control strategies complementary to current ones in this hyper-endemic context.

# UNDERSTANDING THE ROLE OF MOSQUITO COMMUNITY COMPOSITION IN WEST NILE VIRUS TRANSMISSION

Fesce E.*[1], Ferraguti M.[2], Ferrari N.[1]

*[1]Wildlife Health Lab, Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Lodi, Italy; [2]Department of Conservation Biology and Global Change, Estación Biológica de Doñana (EBD), CSIC, Seville, Spain*

**Keywords:** Vector-borne infection, Epidemiology, Modelling.

**INTRODUCTION:** The composition of mosquito communities plays a pivotal role in the transmission of vector-borne pathogens, including West Nile virus (WNV). WNV is a zoonotic emerging pathogen (arbovirus) that is increasing in both incidence and distribution worldwide. It is maintained in an enzootic cycle involving birds as primary host and mosquitoes as vectors. While humans can become infected through mosquito bites, they act as dead-end hosts and cannot transmit the infection further. Surveillance efforts, aimed at detecting WNV circulation in hosts and vectors, are ongoing in endemic countries, but the complexity of WNV epidemiological cycle, coupled with the involvement of numerous species in its maintenance, poses challenges for accurate prediction of WNV outbreaks. *Culex pipiens* and *Cx. perexiguus* have emerged as key vectors responsible for the transmission of WNV in both the United States and Europe, although their role in the transmission of WNV is still unclear. These mosquito species differ in their habitat requirements and feeding preferences, and this divergence may impact WNV spread.

**MATERIALS AND METHODS:** We developed a set of mathematical models, based on the SEIR (Susceptible-Exposed-Infectious-Recovered) framework, to investigate the impact on WNV dynamics of the differences in habitat requirements and feeding preferences of mosquito species. The models incorporate two vector species (*Cx. pipiens* and *Cx. perexiguus*), as well as two vertebrate hosts (birds, as amplifying hosts, and humans, as dead-end hosts), and account for the influence of climatic variables on mosquito abundance and epidemiological parameters. We generated different scenarios of WNV transmission to test the effects of different abundances and of different feeding preferences of the two mosquito species. For each scenario, we predicted the daily number of infected mosquitoes as a proxy of human infection risk.

**RESULTS AND CONCLUSIONS:** Our analyses revealed that a feeding preference of mosquitoes on birds increases the number of infected mosquitoes. Additionally, when the two mosquito species have same preference for birds, they contribute equally to the spread of the infection. If mosquito species differ in feeding preference instead, they contribute differently to the spread of the infection and their relative abundance plays a central role in driving WNV dynamics. Our findings enhance the need to improve current vector surveillance and control programs by identifying specific vector species in particular environments, particularly those most susceptible to environmental shifts. The proposed model can be developed to fit data collected on field to test the hypotheses produced, furthermore, given the generalizability of the framework, it can be adapted to investigate other mosquito-borne infections.

# TICKS ON DOGS AND CAT: WHATS NEW?

Ferroglio E.*[1], Zanet S.[1], Reza Varzandi A.[1], Trisciuoglio A.[1], Occhibove F.[1], Vada R.[1], Colombo L.[2]

*[1]University of Turin, Grugliasco, Italy; [2]MSD Animal Health, Milano, Italy*

**Keywords:** Ticks, tick borne pathgens, Global changes

**INTRODUCTION:** Recently a national survey carried out in Italy reported that ticks can be found on dogs even in winter (Maurelli et al., 2018. Parasit Vectors, 11:420) and that *Rhipicephalus sanguineus* group and *Ixodes* spp represent almost 99% of the species found on dogs (respectively two-thirds the first and one the second). In those ticks *Babesia* spp (27%) is the most frequent pathogen found on those ticks (Zanet et al., 2020. BMC Vet Res, 16:46). To confirm if those data are true also in continental climate areas, we deemed it important to survey mountain areas of the central/northern part of the country.

**MATERIALS AND METHODS:** The survey has been done in 5 veterinary cabinets that monthly have to check at least 10 dogs and cats for ticks' presence from spring 2021 to summer 2022. Collect ticks have been classified and DNA has been extracted and tested for tick-borne pathogens according to Zanet et al., 2020 (BMC Vet Res, 16:46).

**RESULTS AND CONCLUSIONS:** A total of 221 dogs and 67 cats have been found infected with at least one tick. Ticks have been found in dogs during the whole year, with a peak from March to July. On cats, the trend is similar only no tick has been found in August and September. Only *R. sanguineus* group and *Ixodes* spp (*I. ricinus* and *I. hexagonous*) have been found. In dogs *R. sanguineus* accounts for 37% of the tick, *I. ricinus* represented 57% and the rest was *I. hexagonous*, while in the cats *R. sanguineus* wast 23%, *I. ricinus* 72% and the remaining were *I. hexagonous*. The vast majority (95%) of ticks are adult females in both hosts and tick's genera. Living in a garden for both dogs and cats is the higher factor risk (67%) and the head, neck, and shoulders are the places where it is easier to find ticks. Among pathogens *Rickettsia* spotted fever group was the most preeminent with a prevalence of 51.7%, followed by *Babesia* spp 26.1%, Anaplasmataceae 5,3%, and *Borrelia burgdorferi* 2.2%. Data obtained evidence of tick infestation in both dogs and cats during the whole year with a high prevalence of several pathogens, some of them with a potential zoonotic risk. The highest prevalence of *Ixodes* spp to *Rhipicephalus* spp. is in line with previous data and the evidence of a greater presence of the first in the Northern part of Italy reflects also the predilection of this tick for wooded areas and big game, especially deer, presence. Our results highlight the risk of tick exposure, with the related during the whole year even in continental and alpine climate areas, and support the adoption of preventive treatment for ticks for twelve months even in those areas.

# FROM THE LABORATORY TO THE FIELD: MOLECULAR STUDIES ON MALARIA TRANSMISSION

Lombardo F.*[1], Bevivino G.[1], Dipaola M.G.[1], Perugini E.[1], Guelbeogo W.M.[2], Pombi M.[1], Arcà B.[1], Modiano D.[1]

*[1]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy; [2]Centre National de Recherche et de Formation Sur le Paludisme, Ouagadougou, Burkina Faso*

**Keywords:** Malaria, Mosquito, Transmission.

**INTRODUCTION:** Malaria is one of the deadliest infectious diseases worldwide and a significant global public health concern, with approximately 249 million cases and 608,000 human deaths, mostly occurring in sub-Saharan Africa, reported by World Health Organization (WHO) in 2022. The quantification of *Plasmodium falciparum* parasites inside both the human host and the mosquito vector is crucial to understand the dynamics of malaria transmission. Currently, microscopy and PCR approaches are employed to quantify: i) mature gametocytes in the peripheral human blood, ii) oocysts and sporozoites located, respectively, in the midgut basal lamina and in the salivary glands of experimentally infected mosquitoes. However, these approaches are troubled by logistical, technical and ethical issues related to human blood collection and laboratory mosquito infection. The aim of this study is to develop a new molecular method based on RT-qPCR approach to detect and quantify *P. falciparum* ookinete markers inside experimentally and naturally infected *Anopheles gambiae s.l.* mosquitoes.

**MATERIALS AND METHODS:** *Anopheles coluzzii* mosquitoes were experimentally infected with *P. falciparum* NF54 strain and singularly collected at different time points (TP) post-infection (TP12, TP18, TP24 and TP36 hours post infection, hpi) spanning the development of the ookinete stage. Total RNA and genomic DNA were obtained from 50 females/TP using both phenol-chloroform and spin-column chromatography extraction methods. A SYBR green qPCR assay was developed to define the transcriptional profiles of selected ookinete genes (CTRP, SOAP, WARP and CHT1) by absolute quantification. Pilot field mosquitoes' indoor collection campaigns were performed in 2022 and 2023 rainy seasons in Goden, Burkina Faso, to obtain around 2000 half gravid *An. gambiae s.l.* mosquitoes singularly stored in RNA later. To date, gDNA and total RNA from around 200 samples were extracted, the quality and quantity evaluated, and employed in RT-qPCR assays. Also, blood meal sources were identified by end point PCR.

**RESULTS AND CONCLUSIONS:** We have developed a molecular protocol to reveal nucleic acids from parasite, human host, and mosquito vector in single mosquitoes infected in laboratory conditions. RT-qPCR results confirmed that *P. falciparum* ookinete's markers CTRP, WARP, SOAP and CHT1 show a progressive transcriptional increase up to 24 hpi, corresponding to the ookinete invasion of the midgut. A strong reproducibility and coherence of markers' quantification was observed. End-point PCR from a pilot set of field mosquitoes (N=200) showed that around 40% were human fed. *Plasmodium* ookinetes were identified and quantified in around 20% of human-fed mosquitoes. This data is in agreement with previous observations. The molecular analysis of human gDNA in infected mosquitoes is ongoing. The application of the molecular protocol on the entire set of field-collected mosquito might provide novel insight in the study and epidemiology of malaria transmission.

# ENTOMOLOGICAL SURVEILLANCE AT POINTS OF ENTRY: A NEW MOLECULAR TOOL FOR MONITORING THE INTRODUCTION OF *AEDES AEGYPTI*

Menegon M.*, Severini F., Toma L., Di Luca M.

*Istituto Superiore di Sanità, Department of Infectious Diseases, Rome, Italy*

**Keywords:** *Aedes aegypti*, Multiplex PCR, Entomological surveillance

**INTRODUCTION:** *Aedes* genus include some extremely invasive mosquito species, vectors of numerous arboviruses including dengue, Chikungunya and Zika viruses. In recent decades, Italy has experienced the establishment of three exotic *Aedes* species, relevant to human health: *Aedes albopictus*, *Aedes koreicus* and *Aedes japonicus*. *Aedes aegypti* Linnaeus, 1762, the main vector of dengue and yellow fever, occurs in Africa, where it originates, and in many subtropical regions of America, Asia and Australian continent. Although historically present in all Mediterranean countries, *Ae. aegypti* distribution is currently limited to some countries bordering the Black Sea, Cyprus, Madeira (Portugal), Canary Islands (Spain) and Egypt, even if the risk for its re-establishment in southern Europe, where the climatic conditions are more suitable for the species, is very high.
Even if the adults present distinctive morphological characteristics, these *Aedes* species could be difficult to identify if observed by a non-expert eye, when the specimens are damaged or at the egg stage. In 2021, Bang et al. (Parasit Vectors, 14(1):380) developed a multiplex PCR for the identification of *Aedini* species, not including *Ae. aegypti*. In this study, a species-specific primer was designed for the simultaneous identification of *Ae. aegypti* for use in the above mentioned diagnostic assay.

**MATERIALS AND METHODS:** A reverse primer specific for *Ae. aegypti* was designed by aligning the ITS2 sequences of *Ae. aegypti* (KY382418; MF142281), *Ae. albopictus* (MN062758), *Ae. koreicus* (KF471622) and *Ae. japonicus* (KF471614) species. DNA was extracted from adults and eggs of the four species by using the PureLink Genomic DNA Kit (Thermo Fisher Scientific). The original *Ae. aegypti* specific primer was validated to be used in the diagnostic protocol described by Bank et al. (2021).

**RESULTS AND CONCLUSIONS:** In this study, a reverse primer was developed for the diagnosis of *Ae. aegypti*. This original primer worked perfectly in combining with the diagnostic assay developed by Bank et al. (2021). The amplified fragment from *Ae. aegypti* (254 bp) was clearly distinguished by gel electrophoresis from the amplicons from *Ae. albopictus* (438 bp), *Ae. koreicus* (361 bp) and *Ae. japonicus* (160 bp). Due to the increasing number of imported human cases of arboviruses and the widespread presence of *Ae. albopictus*, two epidemics of Chikungunya and two of dengue have occurred in Italy. To address this health risk, the National Plan for the Prevention, Surveillance and Response to Arboviruses (2020-2025) is active, which aims to detect imported and autochthonous human cases and the introduction of new mosquitoes at the Points of Entry, sensitive sites as ports and airports. Although the presence of *Ae. aegypti* has not yet been recorded in Italy, it is now available a rapid and cost-effective molecular test for the identification of all invasive *Aedes* species, to use as a tool for local surveillance and control activities.

# INVESTIGATING THE LARVAL MYCOBIOTA IN ITALIAN MOSQUITOES

Zubair M.S.*[1], Cappelli A.[2], Damiani C.[2], Favia G.[2], Ricci I.[2]

*[1]School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy; [2] School of Biosciences and Veterinary Medicine, University of Camerino, CIRM Italian Malaria Network, Camerino, Italy*

**Keywords:** Mosquito, Larvae, Fungi

**INTRODUCTION:** Every year Mosquito-borne diseases (MBDs) cause almost 1.2 million fatalities worldwide (Weaver et al., 2010. Antivir Res, 85:328-45). The major MBDs include Zika, Yellow fever, Dengue, West Nile, Chikungunya, Rift Valley fever, and Malaria (Sanfelice et al., 2022. Health Econ, 31:73-93). Due to increasing pesticide resistance and safety concerns related to the use of synthetic chemicals, it is essential to develop alternative eco-friendly mosquito control methods. Mosquito larvae interact with many microorganisms, such as bacteria and fungi, in both water and land environments. These microbial communities can be recovered from larvae. In particular, research on fungus-mosquito interactions might lead to the identification of attractive or entomopathogenic agents to be used as bioinsecticides for treatment of breeding sites (Tawidian et al., 2021. Msphere, 6:101-28). In the present study the larval mycobiota of Italian mosquitoes has been characterized to investigate potential entomopathogenic or symbiotic fungi.

**MATERIALS AND METHODS:** Mosquito larvae of different species were collected from different regions of Italy. Larvae were identified by morphological characters. After identification, the larval samples from each collection sites and species were pooled for the fungal isolation. Larval homogenates were plated on YM agar medium and incubated overnight at 30°C. Fungal colonies were categorized and selected based on colony morphology and grown in YM medium overnight at 30°C. For the DNA extraction fungal samples were inoculated in YPD medium and incubated overnight at 30°C. To identify the yeast species, the DNAs were amplified with universal primers for ribosomal target ITS1/4 (Wasinpiyamongkol et al., 2019. bioRxiv, 518241). Amplicons were sequenced using the Sanger method by Microsynth company and the sequences were analyzed using ClustalW and BLAST software.

**RESULTS AND CONCLUSIONS:** About a thousand *Aedes koreicus*, *Aedes albopictus* and *Culex pipiens* larvae from the Veneto and the Marche regions have been analysed for the associated mycobiota. Both Ascomycota and Basidiomycota have been identified. Those belonging to Ascomycota included: *Schwanniomyces vanrijiae, Candida parapsilosis, Barnettozyma californica, Wickerhamomyces anomalus,* and *Metschnikowia pulcherrima*. Fungi belonging to Basidiomycota included: *Sporobolomyces* sp. and *Rhodothorula paludigena*.
Here we provided a list of fungi associated with mosquito larvae that are proposed for next functional studies to investigate their attractive and entomopathogenic properties. Knowledge coming from this research is essential for the development of fungal product to lure and kill mosquitoes. Findings will disclose innovative bioproducts as larvicidal tools for the MBDscontrol of MBDs.

# EXTRACELLULAR VESICLES FROM ZOONOTIC VECTOR-BORNE *DIROFILARIA* SPP.: HOST-PARASITE INTERACTION AND CLUES FOR CONTROL STRATEGIES

Gabrielli S.[1], Napoli E.[2], Varcasia A.[3], Brianti E.[2], Pombi M.[1], Venco L.[4], Bellini I.[1], Tamponi C.[3], Rondón S.[1], Chiovoloni C.[1], Nonnis F.[3], Sini M.F.[3], Sturiale E.[2], D'Amelio S.[1], Cavallero S.*[1]

*[1]Department of Public health and infectious diseases, Sapienza University of Rome, Rome, Italy; [2]Department Veterinary Sciences, University of Messina, Messina, Italy; [3]Department of Veterinary Medicine, University of Sassari, Sassari, Italy; [4]Ospedale Veterinario Città di Pavia, Pavia, Italy*

**Keywords:** *Dirofilaria*, Extracellular vesicles, miRNAs

**INTRODUCTION:** According to the WHO, filariae are disease agents included among Neglected Tropical Diseases, for which global efforts are urgently needed to reduce their impact. Nemexit project aims to explore the role of extracellular vesicles (EVs) in heartworm disease and subcutaneous filariasis caused by the zoonotic nematodes *Dirofilaria immitis* and *D. repens* (Genchi and Kramer, 2020. Vet Parasitol, 280:108995), as a model. EVs are a new paradigm in host-parasite communication, as they transport proteins and smallRNAs in a protected state. Given helminths' ability to cause long-lived infections through their masterful ability to manipulate the host immune system, the Nemexit project aims to explore *Dirofilaria* spp. EVs cargo and to understand if EVs can be used to "control" the parasitic burden in hosts and to use serum circulating miRNAs as biomarkers for diagnosis.

**MATERIALS AND METHODS:** A laboratory infection system with the mosquito *Aedes albopictus* is employed for the collection of both *D. immitis* and *D. repens* third stage larvae (L3s) for EVs production, using blood of naturally infected dogs. Concurrently, *D. immitis* adults were extracted alive from naturally infected dogs and incubated to produce EVs. Once EVs are released by the parasite stages (i.e., L3s and adults), the EVs enriched fraction is used to estimate size and concentration, membrane proteins, and cargo (microRNAs), according to the MISEV2023 (Welsh et al., 2024. J Extracell Vesicles, 13:e12404).

**RESULTS AND CONCLUSIONS:** So far, five dogs positive for *D. immitis*, two for *D. repens* and one with mixed infection from Sardinia and Sicily regions were enrolled in the study. Recruited dogs showed a parasitemia of at least 3000mf/ml and received no anthelmintic treatment. Two infection tests with *A. albopictus* and microfilariae from *D. immitis* and *D. repens* were performed. Moreover, two *D. immitis* adults (i.e., one female and one male) were directly collected from a dog and used for EVs production. According to Nanoparticle Tracking Analysis, 3.93 x 10^10 particles/ml with an average diameter of 149.6nm were obtained, confirming the size and concentration of parasitic EVs. Once biological triplicates for each *Dirofilaria* spp. will be obtained, we aim to explore aspects of interest for basic research in biology, as the role of microRNAs in different developmental stages (larvae and adults) and those specifically packaged into EVs as potential messengers of pathogenicity and immunomodulation. Moreover, some applicative aspects will be explored, as the sensitivity of EVs release to different drugs and the potential use of EVs as vaccination tool in the vector host. In fact, pretreatment with EVs from protozoans and helminths has revealed their protective role, resulting in a decrease of parasites burden (Drurey et al., 2020. Int J Parasitol, 9:623).

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# PRELIMINARY DATA ON THE PYRETHROID RESISTANCE STATUS OF *AEDES CASPIUS* POPULATIONS SYMPATRIC WITH RESISTANT *AEDES ALBOPICTUS* AND *CULEX PIPIENS*

Badieinia F.*[1], Pichler V.[1], Bellini R.[2], Veronesi R.[2], della Torre A.[1], Caputo B.[1]

*[1]Sapienza University, Department of Public Health and Infectious Diseases, Rome, Italy; [2]Centro Agricultura Ambiente "G. Nicoli", Department of Medical and Veterinary Entomology, Crevalcore, Italy*

**Keywords:** Mosquito, Pyrethroid resistance, *Aedes caspius*.

**INTRODUCTION:** Pyrethroid insecticides - targeting the voltage-gated-sodium-channel (VGSC) in insect nervous system - are the main pesticides allowed in Europe to control adult mosquitoes outdoors. While for mosquito control, they are only recommended in case of ongoing arbovirus transmission, they are also widely used to reduce biting nuisance (as well as for insect agricultural pest control). This has caused development and spreading of pyrethroid resistance (PR) in both the most widespread and public health relevant mosquito species, *Aedes albopictus* and *Culex pipiens* (Moyes, et al., 2017. PLoS Negl Trop Dis, 11(7): e0009084). Herein, we focus on *Aedes caspius*, a floodwater species whose marked anthropophily and aggressive biting behavior strongly affects outdoor activities and represents a major target of mosquito control programs in Italian coastal touristic areas (Veronesi et al., 2012. Jour Vect of Entoml, 37(1): 49-61). Despite this no data are available concerning its PR status in Italy.

**MATERIALS AND METHODS:** *Aedes caspius* specimens were sampled in 2020 in three coastal sites from Ferrara province in Italy were previous studies highlighted high phenotypic PR and high frequencies of knock-down-resistance (kdr) mutations within the vgsc gene associated with PR in sympatric *Ae. albopictus* and *Cx. pipiens*. Sequencing of domains I, II and III (Kasai et al., 2011. Jpn J Infect Dis, 64:217-21; Fan et al., 2020. PloS Negl Trop Dis, 14:1-22) of the vgsc was carried out to investigate the presence of mutations with known impact on PR in mosquitos. Phenotypic PR was evaluated by exposing specimens from one coastal site for one hour to permethrin 0.75% according to WHO guidelines (WHO, 2016).

**RESULTS AND CONCLUSIONS:** DNA was extracted from 121 specimens and sequencing was successful for 43, 101 and 61 specimens for domain I, II and III respectively. No known kdr mutations were found in the examined sequences. Exposure of *Ae. caspius* specimens to permethrin 0.75% resulted in a 99% mortality, suggesting complete susceptibility. Despite the reported high insecticidal usage in the sampling sites and the high presence of phenotypic and genotypic PR in sympatric *Cx. pipiens* and *Ae. albopictus* (where frequencies of kdr alleles were above 90% and 25%, respectively), (Pichler et al., 2018. Pest Manag Sci, 74:1319-27; Pichler et al., 2022. Med Vet Entomol, 36:390-95) the present study did not detect any signs of genotypic or phenotypic PR. The absence of diagnostic dosages for this species limits interpretation of bioassay results and further studies will be needed to confirm the susceptibility status of *Ae. caspius* and to understand the possible differential selective pressure of pyrethroid usage on the different mosquito species.

# *HEPATOZOON CANIS* INFECTION IN DOMESTIC DOGS FROM SARDINIA, ITALY

Chisu V.[1], Bianco P.[1], Giua L.[1], Muroni G.[1], Foxi C.*[2], Masala G.[1], Piredda I.[1]

*[1]Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy; [2]Istituto Zooprofilattico Sperimentale della Sardegna, Italy*

**Keywords:** Ticks, Dogs, *Hepatozoon*.

**INTRODUCTION:** Protozoa belonging to *Hepatozoon* genus comprises about 340 species that cause diseases of veterinary importance in a broad range of vertebrate hosts. Hematophagous arthropods act as hosts and/or vectors as they can transmit these apicomplexan parasites. Although the brown dog tick *Rhipicephalus sanguineus sensu lato* is the main vector of these pathogens, the vectorial role of other tick species belonging to the *Amblyomma*, *Haemaphysalis*, and *Rhipicephalus* genera has been experimentally proved, and are now considered potential vectors of *Hepatozoon* species. Since pet dogs can potentially be exposed to an increased risk of acquiring *H. canis* during the period when *R. sanguineus* ticks are most active, the aim of this study was to determine the presence of DNA of *Hepatozoon* species in a subset of Sardinian domestic dogs.

**MATERIALS AND METHODS:** The survey was conducted from March 2020 to May 2021 by collecting blood samples from dogs presented at veterinary clinics for routine medical checks. Blood samples from each dog were collected and tested molecularly for the presence of *Hepatozoon* DNA by targeting a 672-base pair (bp) portion of the 18S rRNA gene. All amplicons were then purified and directly sequenced. DNA sequences were analyzed using the Chromas Pro software package and their identity was confirmed by comparison to sequences available in the GenBank database using the NCBI Basic Local Alignment Search Tool (BLAST). Genetic and phylogenetic analyses were performed in all positive samples.

**RESULTS AND CONCLUSIONS:** The results of the PCR assay targeting the 18S rRNA fragment gene showed that nine samples were positive for *Hepatozoon* spp. (18%, 9/51; 95%CI: 7-29) and that all generated sequences shared a nucleotide sequence that showed 100% identity with *H. canis* sequences available in GenBank. These sequences grouped with 18S rRNA strains of *H. canis* within a monophyletic-clade strongly supported that includes all previously described genotypes from different hosts species and geographic regions (Spain, Taiwan, Italy, India, Thailand, and Israel). The resulting data are interesting since they provide the valuable information on the presence of *H. canis* infection in dogs from Sardinia, Italy. In this study, although *H. canis* infection was confirmed by PCR in nine dogs (18%; 95%CI: 8-28), only six (12%; 95%CI: 12-76) showed clinical unspecific symptoms; the others did not show any clinical signs. Infected dogs frequently show unspecific symptoms, estimating the real burden of the disease is a challenge, and it could lead to a probable significant lack of prevention of long-term complications for at-risk dogs. Therefore, improving knowledge about hepatozoonosis will involve the establishment of control programs, which will be fundamental to adequately manage and control the spread of the disease.

# FIRST RECORD OF A NASAL MYIASIS BY *MEGASELIA SCALARIS* (DIPTERA: PHORIDAE) IN ITALY

Barlaam A.*[1], Colonna R.[2], Zani G.[3], Terzitta M.[4], Giangaspero A.[1]

*[1]University of Foggia, Department of Science of Agriculture, Food and Environment, Foggia, Italy; [2]Centro Agricoltura Ambiente, Crevalcore (Bologna), Italy; [3]UO Anestesia e Rianimazione Ausl Romagna, Lugo di Romagna, Italy; [4]UO Anestesia e Rianimazione Ausl Romagna, Ravenna, Italy*

**Keywords:** Nosocomial myiasis, Human, Phorids.

**INTRODUCTION:** In the family Phoridae, the cosmopolitan genus *Megaselia* includes over 1,400 species showing a wide variety of eco-biological characteristics including diet. Often involved in corpse decay, *Megaselia* species have been reported worldwide as responsible of accidental myiasis in humans and animals (Disney, 2008. Annu Rev Entomol, 53:39-60). In Italy, *Megaselia rufipes* has recently identified as responsible of human nasal myiasis (Giangaspero et al., 2021. J Med Entomol, 58:121-24) while *Megaselia scalaris* has been described as agent of myiasis only in an Indian caged python (Vanin et al., 2013. J Med Entomol, 50:209-11).

**MATERIALS AND METHODS:** In September 2023, a 70-year-old female patient was admitted to the intensive care Unit at Ravenna hospital and ventilated by a tracheal gold tube for a severe comatose state. Four days after the admission, during the objective examination, a dozen of whitish 1 mm-long live larvae from the left nasal coana, along the nasogastric tube, were noticed. The larvae were individually collected, sent to the Centro Agricoltura Ambiente (Crevalcore, Bologna) where the larvae were reared into adults and sent for identification to the Parasitology Unit of the University of Foggia. Here they were first observed under a stereomicroscope and then subjected to confirmatory molecular identification. Genomic DNA was extracted from 5 adults and 5 puparia using the Nucleospin Tissue kit (Macherey-Nagel). A conventional PCR assay was performed to amplify a 710-bp gene fragment of the cox1 gene-based DNA barcode using the primers LCO1490 and HCO2198 (Folmer et al., 2004. Mol Mar Biol Biotechnol, 3:294-99). Purification and sequencing of the PCR products were performed. The sequences generated were compared with the ones available in GenBank using Nucleotide BLAST.

**RESULTS AND CONCLUSIONS:** The specimens were identified morphologically as *Megaselia scalaris* (Diptera: Phoridae) and the sequences matched with *M. scalaris* with a 100% homology. The cox1 gene is a robust diagnostic marker for the identification of Phoridae flies in the field of forensic entomology (Boehme et al., 2010. Int J Legal Med, 124:577-81). *Megaselia scalaris* has been recorded in cases of urogenital, intestinal, nasopharingeal and ocular myiasis (Giangaspero et al., 2021. cited above). This is the first report of accidental myiasis in humans due to *Megaselia scalaris* in Italy. The flies were attracted to foul-smelling nasal discharge and crawled along the nasogastric tube.
Correct identification of the myiasis agent provides more detailed information on the responsibility that health units (hospitals, geriatric homes, etc.) must have towards patients. In this view, hygiene, protection from flies by physical barriers, efficient waste disposal measures to reduce the smell of decomposition, and insecticide sprays are basic prevention measures that hospitals should take.

# SEROPREVALENCE AND MOLECULAR DETECTION OF *LEISHMANIA INFANTUM* IN CATS FROM CENTRAL ITALY

Gabrielli S.*[1], Fanì C.[2], Villanueva-Saz S.[3], Marteles D.[3], Messina F.[2], Fiorillo C.[2], Trichei S.[1], Bruno F.[4], Castelli G.[4], Vitale F.[4], Trotta M.[2]

*[1]Sapienza University of Rome, Department of Public Health and Infectious diseases, Rome, Italy; [2]CDVet Research, Veterinary Laboratory, Rome, Italy; [3]University of Zaragoza, Animal Pathology Department, Zaragoza, Spain; [4]C.Re.Na.L. Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy*

**Keywords:** Leishmaniosis, Diagnosis, Cat.

**INTRODUCTION:** In Italy, since canine leishmaniosis is endemic, cats are often exposed to *Leishmania infantum*. An overall cumulative *L. infantum* prevalence of 3.9% was recorded in cats by serology and by qPCR, 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(7): 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(3):181-91; Morelli et al., 2020. Front Vet Sci, 7:616566). The aim of this study was to assess the presence of *L. infantum* in a feline population from Central Italy by the means of serological and molecular methods.

**MATERIALS AND METHODS:** A total of 215 serum and blood samples were collected from cats admitted at the CDVet Research Laboratory (Rome, Italy) for routine controls. Detailed information about sex, age, and lifestyle was recorded for each animal. Sera were examined by means of i) an indirect home-made immunofluorescence antibody test (IFAT); ii) a commercial IFAT (Megacor Diagnostik GmbH); iii) an home-made enzyme immunoassay (Alcover et al., 2021. Parasit Vectors, 14(1):178), for the detection of specific anti-*Leishmania* IgG antibodies using an anti-cat IgG conjugate. The cut-off dilution of 1:80 was applied for both IFAT tests, following the LeishVet guidelines. Samples were classified as positive or negative for *Leishmania* when at least two of the tests yielded a positive or negative result. Genomic DNA was extracted from blood samples and tested by qPCR (Genesig® standard kit, UK). Additionally, a partial region of the ITS1 (~300bp) from qPCR positive samples were successively amplified by convPCR (Schönian et al., 2003. Diagn Microbiol Infect Dis, 47(1):349-58) and sequenced. Sequences were compared with those available in GenBank using the BLASTn tool.

**RESULTS AND CONCLUSIONS:** Overall, antibodies against *L. infantum* were found in 11 of the 215 (5.1%) examined cats. No significant association was found with age, sex, lifestyle or other co-infections. *Leishmania* DNA was found in the blood of one animal, which showed antibody titres of 1:640. The infected cat was under immunosuppressive therapy for previous intestinal lymphoma. The BLAST analysis of the amplicons revealed a high identity (98-100%) to *L. infantum* sequences deposited in GenBank. Our study highlights the presence of *L. infantum* in 5% of the examined cats, in complete absence of historical features and physical signs compatible with the disease. The employment of different tests is an important critical issue influencing the different results in serodiagnosis. Thus, a standardization of procedures for a prompt diagnosis of *L. infantum* in cats is crucial for a better understanding of the epidemiology and of the potential role of these animals as reservoirs of leishmaniosis.

# FIRST REPORT OF *ACANTHOCHEILONEMA RECONDITUM* IN DOGS FROM URUGUAY

Olhagaray Torres E.*, Gonzalez E., Azambuja M., Ferré C., Salazar Ojeda M., Armúa Fernández M.T.

*University of the Republic, Faculty of Veterinary, Department of Pathobiology, Veterinary Parasitology Unit Montevideo, Uruguay*

**Keywords:** *Acanthocheilonema reconditum*, Molecular diagnosis, Uruguay.

**INTRODUCTION:** Adult stages of *Acanthocheilonema reconditum* (Nematoda: Onchocercidae) parasitize the subcutaneous connective tissue of canids. Meanwhile, its larvae (microfilariae) inhabit the bloodstream. It was first differentiated from *Dirofilaria immitis*, in 1892 by Grassi and Calandruccio (Pennington, 1971. DissAbstrInt, 2:772). The life cycle involves fleas and lice as its intermediate hosts/vectors and canids as definitive hosts (Jovanović et al., 2023. VetGlas, 77:1-15). It is a parasite of low pathogenicity causing mild clinical signs (Jovanović et al., 2023. VetGlas, 77:1-15). Moreover, it is cited as a minor zoonotic agent (Huynh et al., 2001. BJO, 85:1384-84). However, its significance lies in the high possibility of misdiagnosis, since its microfilariae can be easily confused with those from *D. immitis* and *Dirofilaria repens*. Confusing microfilariae of *A. reconditum* with *D. immitis*, can directly impact the treatment and prognosis of the patient since the previous is a non-pathogenic agent, meanwhile, *D. immitis* is a life-threatening parasite (Knight, 1987. Vet Clin North Am Small Anim Pract, 17:1463-1518). Filariases are routinely diagnosed by Knott test. Nonetheless, this test is based on microfilaria´s morphology differentiation and, as mentioned before, it could easily lead to a misdiagnose. Nevertheless, molecular methods such as PCR, can solve this problem. Despite it being found in some countries of Europe, Africa, Oceania, and the Americas, there have been no reported cases of *A. reconditum* in Uruguay until now.

**MATERIALS AND METHODS:** In a dog hemoparasite survey, 404 samples were analyzed using the Knott test, in 3 samples, filaroid larvae were observed. However, their identity could not be confirmed based on morphological characteristics. DNA was extracted from these samples using a commercial kit (Qiagen, Germany). A PCR targeting a fragment of 16S rRNA gene was carried out and the amplicons were sent for sequencing (Vezzani, 2011. Parasitol Res, 108:985-89).

**RESULTS AND CONCLUSIONS:** The three obtained sequences were identified as *A. reconditum*. To our knowledge, this is the first molecular confirmation of *A. reconditum* in dogs in Uruguay.

# DISTRIBUTION AND SPREADING OF INVASIVE MOSQUITO *AEDES JAPONICUS JAPONICUS* AND *AEDES KOREICUS* IN ITALY

Gradoni F.[1], Negri A.[2], Soresinetti L.[3], Arnoldi D.[4], Corona C.[5], Berrone E.[5], Tessarolo C.[5], Accorsi A.[5], Listorti V.[5], Sgubin S.[1], Manzi S.*[1], Visentin P.[6], Martini S.[6], Rizzoli A.[4], Mosca A.[7], Gobbo F.[1], Gabrieli P.[2], Epis S.[2], Montarsi F.[1]

*[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy; [2]Department of Biosciences and Pediatric Clinical Research Center "Romeo ed Enrica Invernizzi", University of Milan, Milan, Italy; [3]Department of Biology and Biotechnology, University of Pavia, Pavia, Italy; [4]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy; [5]Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, Torino, Italy; [6]Entostudio s.r.l, Ponte San Nicolò (PD), Italy; [7]Istituto per le Piante da Legno e l'Ambiente, I.P.L.A. S.p.A., Turin, Italy*

**Keywords:** *Aedes koreicus*, *Aedes japonicus*, Invasive mosquito species.

**INTRODUCTION:** *Aedes j. japonicus* and *Aedes koreicus* are two invasive mosquitoes native to East Asia that are quickly establishing in temperate regions of Europe (Zadra et al., 2023. Insects, 14:904). Their diffusion is particularly rapid in Italy, where they have been detected all over northern Italy within a few years (Gradoni et al., 2021. Data Brief, 36:107047). Knowledge of invasive mosquito presence permits to assess the risk for potential spread of vector-borne diseases. In this study, we report data on the occurrence and spread of *Ae. j. japonicus* and *Ae. koreicus* in Italy.

**MATERIALS AND METHODS:** Mosquitoes were collected in the frame of different projects by larval search, traps for adult mosquito and ovitraps from 2011 to 2022 in the period March-November. Species identification was performed morphologically and molecularly by PCR and sequencing. Sites and municipalities were considered positive if larvae, adults or eggs (larval identification after hatching) were found.

**RESULTS AND CONCLUSIONS:** During the last 12 years of entomological surveillance, 1703 municipalities of 7 Italian Regions (all in northern Italy) were monitored. *Aedes koreicus* occurs in 456 municipalities (26.8%). After its first detection in 2011 in Veneto Region, *Ae. koreicus* spread throughout northeast over the next five years; it was also found in Lombardy at Italian Swiss border. It reached northwest nine years later. A probably new introduction was recorded in Liguria region (northwest Italy) in 2015. *Aedes j. japonicus* occurs in 210 municipalities (12.3%). It was found for the first time in Italy in 2015 in a municipality bordering Austria and since then has spread throughout the northern part of Italy reaching the Northwest in 2019. To date, *Ae. j. japonicus* spread seems slower compared to *Ae. koreicus*. The expansion of both species southwards seems to be limited by the high mean summer temperatures and by the high density of the competitor species *Ae. albopictus* in the plain area. The overlapping of *Ae. koreicus*, *Ae. j. japonicus* and *Ae. albopictus* distribution is complicating the entomological monitoring system, due to their similar biology and morphology. Therefore, long-term surveillance for early detection and plan control actions against these invasive mosquitoes are needed to limit their further spread.

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# COMPARISON OF BG-PRO VERSUS CDC LIGHT TRAP PERFORMANCE FOR *PHLEBOTOMUS PERFILIEWI* COLLECTION

Bernardini I.*[1], Mangiapelo C.[1], Bianchi R.[1], Minelli G.[2], Manno V.[2], Di Luca M.[1], Pombi M.[3], Bongiorno G.[1]

*[1]Italian National Institute of Health, Department of Infectious Diseases, Vector-Borne Diseases Unit, Rome, Italy; [2]Italian National Institute of Health, Bureau of Statistics, National Centre of Epidemiology, Surveillance and Promotion of Health, Rome, Italy; [3]Sapienza University of Rome, Department of Public Health and Infectious Diseases, Parasitology Unit, Rome, Italy*

**Keywords:** Sand fly, Trapping methods, Monitoring.

**INTRODUCTION:** The choice of a sand fly collection method depends on the objectives of the study, the knowledge of the local fauna, and the ecology of the study site. If the intent is to collect alive sand flies for colonization or experimentation, specific techniques are required, limiting the available tools. One of the most widely used is the CDC light trap (CDC) equipped with incandescent lamp, which is considered the gold standard (Alexander et al., 2000. Med Vet, 14:109-22). In this study, a CDC was compared with a novel trap developed for mosquito collection, the Biogents BG-Pro trap (BG), baited with light-emitting diodes (LEDs) to evaluate their efficacy in sand fly collection and the survival rates according with environmental parameters (temperature and relative humidity levels).

**MATERIALS AND METHODS:** Field tests were performed from August to October 2022 in a farmhouse of Magliano in Toscana (Grosseto municipality) following a Latin square sampling design. The traps were alternatively placed in two different positions for 12 consecutive hours from sunset to sunrise recording environmental parameters (temperature and relative humidity). Morphological identification was conducted on all collected specimens for samples within 1,000 individuals, above this threshold value, only subsamples of 500 specimens was considered. The high number of total collected sand flies required an indirect count analysis based on the estimation formula: “299.7 of sand flies = 0.5 ml” adopted to the volume of each falcon/pot occupied by sand flies (dead and alive) in each replicate.

**RESULTS AND CONCLUSIONS:** An estimated total of 1,629,024 *P. perfiliewi* sand flies was obtained by 19 replicates. Concerning trap comparison, BG (n= 1,514,152) is performer than CDC (n= 114,872), but the survival rate seems to be significantly higher in CDC (42.2%) than in BG (9.6%) (RR= 4.37; p= 0.0045). On the contrary, the sex ratio is in favour of females in both methods, reaching 0.22 in CDC and 0.62 in BG, as well as overall value (M/F= 0.42). The reported results suggest that *P. perfiliewi* population is widespread in the study area, with a peak abundance during late summer, according with temperature levels. Although the survival rate in this trapping system could be affected by overcrowding, BG resulted at least twice time more efficient than CDC, also thanks to the lower power consumption and the major attractiveness of LED light. For these reasons, BG is a good alternative device for capturing sand fly and it may be successful used for entomological surveys, in view of the advantages compared to the standard CDC.

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# PRELIMINARY RESULTS OF AN EPIDEMIOLOGICAL STUDY OF THE MOSQUITO FAUNA IN CAMPANIA AND BASILICATA REGIONS

Montella M.O.*, Ciuca L., Buono F., Lattero N., Rinaldi L., Maurelli M.P.

*University of Naples Federico II, Department of Veterinary Medicine and Animal Production, CREMOPAR, Naples, Italy*

**Keywords:** Mosquitoes, Campania, Basilicata.

**INTRODUCTION:** The rise in environmental temperature due to climate change is altering the spread of mosquitoes and mosquito-borne diseases across Europe (Bezzera-Santos et al., 2023. Acta Trop, 238:106746). Information on the mosquito fauna in southern Italy is scarce and fragmented (Busani et al., 2012. Rapporti ISTISAN 12/22, 5-9). The lack of entomological surveys in this area is an important gap in knowledge about the occurrence of native and invasive mosquito species, as well as about the risks of emerging mosquito-borne pathogens. The aim of this study was to conduct an epidemiological study of mosquito fauna to better understand the population dynamics in the Campania and Basilicata regions of southern Italy.

**MATERIALS AND METHODS:** From May to October 2023, mosquitoes were collected using BG-traps Sentinel (Biogents AG, Germany) placed in seven sample sites (two urban, three peri-urban and two rural sites) in the Campania and Basilicata regions. The urban collection sites were located into two points in the centre of Naples. The peri-urban sites included a horse farm located in Lago Patria (Naples), a dog kennel located in Castel Volturno (Caserta) and one sampling point located in Palinuro (Salerno). The rural sites were in Tolve (Potenza) and Eboli (Salerno). All entomological samples were preserved at -20°C before identification. Mosquitoes were identified using morphological keys as described in the literature (Becker et al., 2020, Mosquitoes: identification, ecology and control. 3rd Edition. Springer Nature Switzerland AG, Cham, 193 - 390; Severini et al., 2009. Fragm Entomol, 41(2), 213-72). Specimens that were difficult to recognise morphologically underwent molecular analysis using the protocol described by Folmer et al., 1994 (Mol Mar Biol Biotechnol, 3(5): 294-9) to amplify a 710 bp fragment of the mitochondrial cytochrome C oxidase subunit 1 gene (COI). Subsequently, the PCR products were purified, and sequenced. Sequences were analysed using the Chromas version 2.6.6 software and compared with those present in GenBank using BLASTn.

**RESULTS AND CONCLUSIONS:** Preliminary results showed that in Palinuro, 55.6% and 44.4% of the specimens collected belonged to the species *Culex pipiens* and *Aedes albopictus*, respectively, while in Tolve the species *Cx. pipiens*, *Ae. albopictus*, *Cx. hortensis* and *Anopheles maculipennis s. l.* were detected, with a prevalence of 79.2%, 15.1%, 3.8% and 1.9%, respectively. However, the morphological and molecular analyses of the mosquitoes collected in the other sites have not yet been completed, and further sampling will be carried out to provide a comprehensive understanding of the distribution of mosquito species in these regions.

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# EXPERIMENTAL INFECTIONS OF *ANOPHELES STEPHENSI* WITH *PLASMODIUM BERGHEI*: *IN VIVO* STUDIES FOR MALARIA TRANSMISSION BLOCKING STRATEGIES

Boccolini D.*[1], Grasso F.[1], Mochi S.[1], Preira C.M.F.[2], Fratini F.[1], Piselli E.[3], Di Luca M.[1], Favia G.[3], Damiani C.[3], Siden-Kiamos I.[2], Pizzi E.[1], Ponzi M.[1], Currà C.[1]

*[1]Istituto Superiore di Sanità, Rome, Italy; [2]Foundation for Research and Technology Hellas, Institute of Molecular biology and Biotechnology, Heraklion, Greece; [3]School of Biosciences & Veterinary Medicine, University of Camerino, Camerino (Macerata), Italy*

**Keywords:** Mosquito mid-gut dissection, Oocyst proteins, Vector-parasite interactions.

**INTRODUCTION:** *Anopheles stephensi* is a major Asian vector of human malaria. However, it is also highly competent for Plasmodia of small rodents. In particular, *An. stephensi* supports the complete development of *Plasmodium berghei*, thus becoming a model for *in vivo* infection that has provided important basic knowledge on vector-parasite studies. The oocyst, protected by the capsule, is the longest *Plasmodium* stage and occurs in the mosquito midgut after a blood meal. It takes about two weeks to grow and, upon rupture, the infectious sporozoites are released targeting to the salivary glands. Little is known about oocyst biology and composition of its protective capsule. In this work, protein analyses of isolated early, mid, and late oocysts were reported with the aim of identifying relevant processes in their development.

**MATERIALS AND METHODS:** For the experiments, the long-established colony of *An. stephensi* Sind-Kasur was used, reared both in the insectarium of the University of Camerino and in that of the Istituto Superiore di Sanità. Four biological replicate infections were carried out. Mosquitoes of 6-8 day old were fed for 30 minutes on anesthetized mouse (BALB/c or CD1) infected with wild type *P. berghei* ANKA strain. After the infected meal, engorged mosquitoes were maintained in controlled conditions (21 °C temperature, 80% relative humidity, under 12 hours night/day regime). At days 5, 8 (young oocysts), and 12 (mature oocysts with sporozoites) post-feeding, potentially infected mosquitoes were dissected. The midguts were concentrated and the oocysts were isolated according to the protocol (Siden-Kiamos et al., 2020. Sci Rep, 130 10:7262). As control, microscopic observations of some fresh midguts and the oocyst count were carried out. Proteomic analyses were performed by comparative quantitative mass spectrometry; immunofluorescence and Western blot assays confirmed the timing of expression of selected proteins.

**RESULTS AND CONCLUSIONS:** A high percentage of mosquitoes were fully engorged (80%). In total over 2,000 mosquitoes were processed. In the control specimens, the number of oocysts/mosquito ranged from a dozen to hundreds, as reported in the literature. The study showed molecules/pathways involved in *Plasmodium* oocyst development. Nearly 600 oocyst-specific candidate proteins were found, varying expression and abundance during maturation process. In young oocysts a strong activity of protein and DNA synthesis were reported. In mature oocysts, proteins involved in oocyst and sporozoite development (particularly regarding gliding, motility, and invasion) were mostly abundant. Western blot and immunofluorescence analyses of selected candidates confirmed the expression profile obtained by proteomic analyses. Future perspectives aim at identifying new targets to block malaria transmission. Investigations on the oocyst protein pathways occurring in mutant parasites lacking genes, important for the mechanism of oocyst rupture, are ongoing.

# EQUINE PIROPLASMOSIS: DIAGNOSTIC CHALLENGES AND FIRST EVIDENCE OF *THEILERIA HANEYI* IN ITALY

Dini F.M.*, Facile V., Galuppi R., Urbani L., Imposimato I., Mariella J., Magliocca M., Battilani M., Balboni A.

*Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy*

**Keywords:** Piroplasmosis, Horse, *Theileria haneyi*.

**INTRODUCTION:** Equine piroplasmosis (EP) is a disease that affects equids endemic to tropical and subtropical areas, including the Mediterranean region, such as Italy. The disease can manifest in hyperacute, acute, subacute, and chronic forms. It is primarily caused by the Apicomplexan parasites *Theileria equi* and *Babesia caballi*. However, other species such as *Babesia canis*, *B. capreoli* (Zanet et al., 2017. Vet Parasitol, 236:38-41), *Theileria annae/sergenti/buffeli*, and *Babesia microti*-like (now *B. vulpes*) have also been reported in Italy (Moretti et al., 2010. Vet J, 184:346-50). Recently, a new *Theileria* species, *Th. haneyi*, was discovered in horses at the United States-Mexico border (Knowles et al., 2018. Int J Parasitol, 48:679-90) and subsequently described in Africa (Bhoora et al., 2019. Ticks Tick Borne Dis, 11:101358; Coultous et al., 2019. Transbound Emerg Dis, 67:1213-21). Here, we report the first evidence of this species in Italy.

**MATERIALS AND METHODS:** Following the observation of an equine blood sample that tested negative at endpoint qualitative PCR for *B. caballi* and *Th. equi* but was positive on smear examination, a re-evaluation was carried out on 68 equine blood samples tested for piroplasms at the Veterinary Teaching Hospital of Bologna University from 2016 to 2022. Each sample underwent comprehensive testing, including endpoint qualitative PCR (Alhassan et al., 2005. Vet Parasitol, 129:43-9), a real-time PCR specific for *Babesia* species (Qurollo et al., 2017. Parasit Vectors, 10:1-13), nested PCR (Jefferies et al., 2007. Vet Parasitol, 144:20-7), and sequencing of the 18S rDNA.

**RESULTS AND CONCLUSIONS:** Twenty-four horses tested positive for piroplasms: 4 (5.9%) were confirmed as *B. caballi* at sequencing; twenty-two horses (32.3%) tested positive for *Theileria* spp., with sequencing identifying 9 (13.2%) as *Th. equi*, 6 (8.8%) as *Th. haneyi*, and 7 (10.3%) as comparable with *Theileria* sp. Africa—an unclassified species in the genus *Theileria* with numerous distinctive nucleotide mutations compared to *Th. equi*. Coinfection with *B. caballi* and *Th. haneyi* was found in 2 cases. The comparison of the different diagnostic assays showed advantages and limitations and no single assay can be used to diagnose and discriminate piroplasm species quickly and at low cost. These results highlight several important aspects: a) The presence of *Th. haneyi* in horses was firstly described in Italy. b) Routine diagnosis may not always detect piroplasms with a single test. c) Single tests may not identify coinfections. d) Identification of these species could be crucial due to potential loss of susceptibility to imidocarb dipropionate (Sears et al., 2020. Pathogens, 9:1035). These issues are significant for timely diagnosis and therapy, especially considering that some countries (e.g., USA) are free of EP, and movements of horses without accurate diagnosis pose a high risk for spreading the disease into these areas.

# VECTOR COMPETENCE OF *CULICOIDES* BITING MIDGES FOR EPIZOOTIC HAEMORRHAGIC DISEASE IN ITALY: FIELD SURVEY AND LABORATORY ORAL INFECTION

Quaglia M.*[1], Foxi C.[2], D'Alessio S.G.[1], Florio S.[1], De Ascentis M.[1], Spedicato M.[1], Cappai S.[2], Puggioni G.[2], Bechere R.[2], Leone A.[1], Pisciella M.[1], Portanti O.[1], Di Gialleonardo L.[1], Savini G.[1], Satta G.[1], Goffredo M.[1]

*[1]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy; [2]Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy*

**Keywords:** Epizootic Hemorrhagic Disease, *Culicoides*, Italy.

**INTRODUCTION:** Epizootic Hemorrhagic Disease (EHD) is an infectious viral disease transmitted by *Culicoides* that affects wild and domestic ruminants. In November 2022 the first incursion of EHDV occurred in Italy and Europe. Herein we report the detection of EHDV-8 in European species of Culicoides, as a result of a field entomological investigation during the Sardinian outbreaks. In addition, to further investigate the role as vectors of European *Culicoides* species, vector competence studies were performed under laboratory conditions on Italian populations of *Culicoides imicola* and *Culicoides obsoletus/scoticus* with EHDV-8.

**MATERIALS AND METHODS:** An intensive entomological field activity was performed in four EHDV outbreaks located in the south-western part of Sardinia, using blacklight suction traps. All *Culicoides* collected were identified, age graded, and sorted in pools of maximum 25 midges. Each pool was tested by realtime RT-PCR. Artificial oral infections were performed on wild caught *C. obsoletus/scoticus* and *C. imicola* collected in Abruzzo and Sardinia regions, respectively. Alive *Culicoides* of the two taxa were fed on defibrinated sheep blood, infected with EHDV-8, through cotton wool pledgets. Engorged *Culicoides* females were incubated in cardboard boxes at 25 ◦C and 80% > HR > 40% for at least 10 days with ad libitum access to 10% sucrose solution. The midges survived to the incubation period were individually identified and tested by real time RT-PCR. A multiplex PCR based on internal transcribed spacer 2 ribosomal DNA sequences (ITS2) was used to separate *C. obsoletus* and *C. scoticus*.

**RESULTS AND CONCLUSIONS:** Overall, during the field survey, 18 pools resulted positive to EHDV-8 out of 411 tested pools (5,721 midges). Of these, ten pools were composed of *C. imicola* (MIR 1.1%), four of *C. obsoletus/scoticus* (MIR 0.1%), two of *C. pulicaris s.s.* (MIR 0.7%), one of *C. newsteadi* (MIR 0.5%), and one of *C. bysta* (MIR 1.3%). During the oral infection trials, a total of 122 midges of *C. obsoletus/scoticus* and 61 *C. imicola* survived to the extrinsic incubation period. Among these, five midges of *C. obsoletus/scoticus* (four identified as *C. scoticus* and one *C. obsoletus*) and six midges of *C. imicola* resulted positive to EHDV-8, showing Ct values ranging between 35.4 - 36.2 and 36 - 41 respectively. EHDV-8 was detected in five *Culicoides* species caught on affected farms, belonging to the subgenera Avaritia (*C. imicola* and *C. obsoletus/scoticus*) and *Culicoides* (*C. newsteadi*, *C. pulicaris s.s.*, and *C. bysta*). In addition to the field investigation, our findings under laboratory conditions confirm that *C. imicola* and *C. obsoletus/scoticus* are competent vectors of EHDV-8. According to our results, EHDV seems to use the same transmission patterns of BTV: *C. imicola* may play a pivotal role in the Mediterranean Basin, while *C. obsoletus/scoticus* may act as main vector in Europe.

# NOVEL APPROACH BASED ON MODIFIED BLACKLIGHT TRAPS WITH HONEY-SOAKED FTA® CARDS FOR BLUETONGUE SURVEILLANCE

Foxi C.*[1], Marcacci M.[2], Mincarelli L.F.[2], Curini V.[2], Puggioni G.[1], Bechere R.[1], Vento L.[1], Quaglia M.[2], Goffredo M.[2], Satta G.[1]

*[1]Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy; [2]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy*

**Keywords:** *Culicoides*, Bluetongue, FTA® Card.

**INTRODUCTION:** The Bluetongue virus (BTV) detection from *Culicoides* biting midges is based on collections with blacklight suction traps placed in livestock farms, morphological species identification, and screening of midges' pools by real time RT-PCR test. Here we propose a novel approach based on modified traps equipped with FTA® card sugar feeding system that aims at simplifying the laboratory activities by investigating the presence of BTV directly from the *Culicoides* saliva on the FTA® card.

**MATERIALS AND METHODS:** On November 2023, three modified blacklight suction traps operated overnight on a sheep farm located in Bari Sardo (Sardinia, Italy), during a BTV-4 outbreak. The collection sac was placed in a BugDorm and a cylindrical container with honey-soaked FTA® cards was added to allow the *Culicoides* feeding. This system allows the alive *Culicoides* to feed and release saliva on the cards. The collected *Culicoides* midges were morphologically identified at species level and the parous females were divided in pools. Total RNA was purified from both *Culicoides* pools and FTA® cards and then tested for BTV detection by real time RT-PCR. The FTA® card samples positive for BTV were sequenced by a metagenomic approach based on a combination of SISPA protocol and NGS technology.

**RESULTS AND CONCLUSIONS:** Ten pools of 25 *C. imicola* (dominant species, >97% of total *Culicoides*), and 13 FTA® cards were obtained and tested. BTV-4 was detected in six pools, with Ct range 27.1-39.2. The Minimum Infection Rate (number of positive pools/number of tested midges) was 2.4%. Four RNA samples purified from FTA® cards turned out positive by real time RT-PCRBTV with Ct range 25-32. Deep sequencing produced reads assigned to BTV species from all four samples. In details, we obtained complete or nearly complete consensus sequences for all BTV segments. Consensus sequences of Seg-2 (VP2) were obtained from two cards that allowed us to perform serotype identification as BTV-4. BLAST analysis revealed the highest nucleotide identity (98.7-99.6%) with homologous sequences of BTV-4 Kosovo 2020, BTV-4 France 2017 and BTV-4 Macedonia 2020. This study described the first application of modified blacklight traps with FTA® cards feeding systems in the field for BTV surveillance. The detection by real time PCR and characterization by NGS of BTV strains in the FTA® cards confirmed the reliability of this novel approach to detect viral circulation in *Culicoides*. The FTA® card ensures environmental preservation of nucleic acids, allowing continuous collection and feeding activity of specimens, and reducing the laboratory activities for virus detection and characterization. Further applications of this novel approach in different epidemiological scenarios (e.g. in contexts with different vector species and lower infection rates) are needed to corroborate these findings.

# A MULTI-ACCESS KEY FOR THE IDENTIFICATION OF ITALIAN TICKS (IXODIDA)

Casale F.*[1], Toma L.[2], Di Giulio A.[1]

*[1]University of Roma Tre, Rome, Italy; [2]Istituto Superiore di Sanità, Rome, Italy*

**Keywords:** Vectors, Ixodida, identification.

**INTRODUCTION:** The identification of an organism is the first step for any scientific research, both basic and applied. However, the identification of ticks can be arduous as they display a great intraspecific morphological variability depending on sex, nutritional state, and developmental life-stage. Only a few identification keys for Italian ticks are available, however they are inadequately illustrated and mostly aimed to identify adults (especially females) rather than immature stages; most importantly these keys are all dichotomous, hence the fixed sequence of identification is based on characters of the mouthparts that are often difficult to detect, since they can easily be altered while collecting the specimen from the host. Thus, the first aim of this project is to create a multi-access key to identify ticks using a wide range of morphological characters.

**MATERIALS AND METHODS:** The multi-access key was made using the software Lucid4 Builder (<www.lucidcentral.org>). The creation of a multi-access key requires the building of a data matrix of species x characters, in which states of characters are attributed to the respective taxa. After the matrix is created, the program allows marking the presence, or absence, of a character or a certain character state. The characters state for the key were reevaluated and rearranged on the base of the dichotomous keys by Manilla (Manilla, 1998, Fauna d'italia, Calderini, Bologna, 280 pag) and Iori et al. (Iori, A., Di Giulio, A., De Felici, S., 2005, Zecche d'Italia. In: Cringoli, Iori, Rinaldi, Veneziano, Genchi: Mappe parassitologiche 6 (parte III), Rolandi editore, Napoli, 263 pag). Furthermore, the key is illustrated with pictures of specimens acquired by using the Zeiss Axio zoom V16 stereoscope and simplified drawings of the characters aimed at facilitating the user. The photographed specimens were partially collected during a two-year sampling (2022/23), carried out in Latium region, Italy, which involved both field activities and collaborations with wildlife rescue centres.

**RESULTS AND CONCLUSIONS:** In the case of ticks, every species of hard tick includes four "entities" (larva, nymph, adult female and adult male) while soft ticks only include three as males and females cannot be morphologically distinguished. This led to the creation of four, for the former, and three, for the latter, different matrices of characters and character state for the Italian species. Ultimately, the matrix includes 69 features with 174 character states for 102 entities. During the sampling 400 ticks were collected, belonging to 6 genera and 10 species, which were used to illustrate the key, while other specimens were from museums and private collections. Currently the key is undergoing trials to ensure its functionality before publication.

# UNVEILING THE UNEXPLORED: MOLECULAR DETECTION OF TICK-BORNE PATHOGENS IN IXODIDAE TICKS SAMPLED FROM WILD DONKEYS IN THE ASINARA NATIONAL PARK

Cialini C.*[1], Villa L.[2], Cafiso A.[1], Montella M.O.[3], Bazzocchi C.[2], Pintore E.[4], Careddu G.[5], Garippa G.[4], Manfredi M.T.[2]

*[1]University of Milan, Department of Veterinary Medicine and Animal Sciences, Lodi, Italy; [2]University of Milan, Department of Veterinary Medicine and Animal Sciences, Research Laboratory of Animal Parasitic Diseases and Zoonoses (ParVetLab), Lodi, Italy; [3]University of Naples Federico II, Department of Veterinary Medicine and Animal Production CREMOPAR, Naples, Italy; [4]University of Sassari, Department of Veterinary Medicine (DIMEVET), Sassari, Italy; [5]Parco Nazionale dell'Asinara, Porto Torres, Italy*

**Keywords:** TBPs, One Health, Wild donkeys.

**INTRODUCTION:** The Mediterranean islands provide favourable habitats for ticks and the transmission of tick-borne pathogens (TBPs). Within the Asinara National Park (ANP) in the Sardinian Archipelago (Italy), wild donkeys (*Equus asinus*) serve as hosts to several tick species (Zanzani et al., 2019. Med Vet Entomol, 33(2): 238-46). Ticks parasitize a wide range of vertebrate species, including humans, and transmit numerous microorganisms of medical and veterinary importance (Otranto et al., 2014. Parasit Vectors, 7:328). Therefore, understanding the ecology and transmission dynamics of TBPs is crucial for safeguarding public health and advancing veterinary medicine. Despite the potential implications of ticks for public health in the ANP, the circulation of TBPs within this unique habitat remain unexplored. Hence, the aim of this study was to investigate TBPs presence in ticks collected form donkeys in the ANP and consequently the risks for the health of animals and tourists on the island.

**MATERIALS AND METHODS:** Adult ticks were collected in 2015 from wild donkeys in the ANP and identified using morphological keys. DNA extraction was performed, followed by specific Real Time PCR assays to test the samples for the presence of TBPs. In detail, *Babesia caballi* and *Theileria equi*, the agents of piroplasmosis, and *Rickettsia* spp. and *Anaplasma* spp., microorganisms potentially transmitted to animals and humans, were investigated. A novel species-specific Real Time PCR assay targeting *Rickettsia aeschlimannii* was subsequently performed on *Rickettsia*-positive samples.

**RESULTS AND CONCLUSIONS:** A total of 197 adult ticks were collected from 110 wild donkeys and identified as *Rhipicephalus bursa* (n=114), *Haemaphysalis punctata* (n=76) and *Hyalomma marginatum* (n=7). Overall, 23.3% of the ticks tested positive to at least one of the examined pathogens, and only 2% presented coinfections. In particular:

- *Th. equi* was detected in *R. bursa* (16.6%), *H. punctata* (9.2%) and *H. marginatum* (14.2%) ticks (confirmed by sequencing);
- *B. caballi* was detected in *R. bursa* (3.5%), *H. punctata* (2.6%) and *H. marginatum* (14.2%) ticks (later identified by sequencing as *Babesia ovis* in three samples);
- no ticks were infected with *Anaplasma* spp.;
- *Rickettsia* spp. was detected in *R. bursa* (7%), *H. punctata* (3.9%) and *H. marginatum* (71.4%) ticks (later identified by PCR in 7 samples as *R. aeschlimannii* and confirmed by sequencing).

This study unveils, for the first time, the occurrence of TBPs, including zoonotic strains, within the ANP. Since the island represents a tourist destination, these findings underscore the need for a comprehensive monitoring strategy for ticks and their related pathogens.

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# FIRST REPORT OF AN AUTOCHTHONOUS CASE OF *BABESIA VOGELI* IN A DOG FROM URUGUAY

Armúa Fernández M.T.*, Olhagaray Torres E., Salazar Ojeda M., Cabrera S., Montero V., Montiel N.

*University of the Republic, Faculty of Veterinary, Department of Pathobiology, Veterinary Parasitology Unit, Montevideo, Uruguay*

**Keywords***: Babesia vogeli*, Molecular confirmation, Uruguay.

**INTRODUCTION:** *Babesia* (Piroplasmida:Babesiidae) is a genus of tick-borne apicomplexan protozoa that parasitizes erythrocytes in vertebrate hosts (Penzhorn, 2020. Parasit Vectors, 13:1-9). Several *Babesia* species parasitize dogs, causing the disease known as canine babesiosis. *Babesia* spp. multiplies into the erythrocytes, causing their lysis and the subsequent symptoms of hemolytic anemia such as pale mucous membranes and petechiae, apathy and lethargy, fever, anorexia, low blood pressure, hemoglobinuria and uremia, tachycardia, ischemia, central nervous signs, and coma. In some cases, could lead to the animal's death (Panti-May, et al., 2020. Vet Parasitol Reg Stud Reports, 21:100417). Variation in the severity of the cases relies on the animal´s immune status as well as the *Babesia* species and/or strains involved. *Babesia vogeli*, whose biological vector is the brown dog tick, *Rhipicephalus sanguineus*, can cause mild symptoms ranging from anemia and weakness in puppies to asymptomatic infections in adults. Despite *R. sanguineus* is widely distributed in Uruguay, there has been no *B. vogeli* confirmation until now. This study aims to report the first molecular confirmation of an autochthonous case of *B. vogeli* in a dog from Uruguay.

**MATERIALS AND METHODS:** In October 2023, a female dog, mixed breed, of about 4 months old was found wandering in a peripheral area in the city of Montevideo. The dog was in very poor condition, slightly depressed, and heavily parasitized by ticks. After the dog´s clinical examination, ticks and blood samples were taken for blood biochemistry and molecular diagnosis. Blood and Ticks DNA were extracted using a commercial kit. A PCR for the detection of a fragment of the small subunit ribosomal RNA gene of Family Babesiidae/Theileriidae DNA was performed (Soares et al., 2011. Vet Parasitol 18:203-8). Along with the samples, positive and negative controls of *Theileria equi* and DNA-free water, respectively, were included. The amplicons of the expected size were sent to MacroGen, Korea, for sequencing.

**RESULTS AND CONCLUSIONS:** *Babesia* spp DNA was confirmed by PCR only in the blood sample. However, the remaining tick samples were negative. The obtained sequence was revealed to be 100% identical to a sequence of *Babesia vogeli* from Buenos Aires, Argentina (accession number KY290977). The sequence was registered in GenBank under the accession number PP373120. This is the first molecular confirmation of an autochthonous case of canine babesiosis caused by *Babesia vogeli* in a dog in Uruguay.

# TICK-BORNE PATHOGENS IN ALPINE MARMOTS *MARMOTA MARMOTA*

Zanet S.*[1], Mochettaz G.[1], Ferrari C.[2], Bassano B.[2], Varzandi A.R.[1], Ferroglio E.[1]

*[1]Università degli Studi di Torino, Dip. Scienze veterinarie, Grugliasco, Italy; [2]Parco Nazionale Gran Paradiso, Noasca, Italy*

**Keywords:** *Marmota marmota*, *Babesia divergens*, Ixodidae.

**INTRODUCTION:** Ticks and tick-borne pathogens (TBPs) pose a growing concern globally as both veterinary and human health threats. The incidence of numerous tick-borne diseases has surged across Europe and temperate regions, with their spread into previously unaffected areas and increased prevalence primarily attributed to anthropogenic and ecological factors. The investigation of TBP dynamics within Alpine marmots (*Marmota marmota*) presents a unique opportunity to examine how a species interacts with and adapts to emerging pathogens of various origins (protozoa, bacteria, and rickettsiae). This study also explores the potential overlap between TBPs found in marmots and those affecting domestic livestock sharing grazing pastures.

**MATERIALS AND METHODS:** As part of a comprehensive ecological study within Italy's Gran Paradiso National Park, blood samples were collected from 47 Alpine marmots alongside samples from a herd of 21 cattle and 9 goats grazing in the same vicinity during summer months. All samples underwent end-point PCR analysis to determine the prevalence of *Babesia/Theileria* spp. (ITS hypervariable region V4) (Zanet et al., 2014. Parasites Vectors, 7:70). *Anaplasma phagocytophilum* (16SrDNA), *Rickettsia* of the Spotted Fever Group (OmpA), and *Borrelia burgdorferi s.l.* (23S rDNA) (Battisti et al., 2020, Front Vet Sci, 7:1). Positive samples were sequenced and compared to existing data in Genebank using MegaX. Environmental surveillance for questing Ixodidae ticks was conducted monthly along an altitudinal gradient ranging from 1,500 to 2,500 meters above sea level between April and October 2019.

**RESULTS AND CONCLUSIONS:** Among the marmots, a prevalence (P) of 6.38% (95% CI 2.19-17.16%) for *Babesia* spp. was detected, with positive individuals predominantly associated with family groups inhabiting forested and shrub-rich areas conducive to Ixodidae presence. Sequencing revealed the presence of *B. divergens* in one marmot, a significant agent of bovine and human babesiosis in Europe. Other targeted TBPs were not detected in marmots, contrasting with higher prevalence rates observed in livestock. Specifically, *Babesia/Theileria* spp. DNA was detected in 5 cattle and 1 goat (P=23.81%; 95%CI: 10.63-45.09 and P=11.11%; 95%CI: 1.99-43.50, respectively), while *Anaplasma* spp. exhibited an overall prevalence of 50.0% (95%CI: 33.15-66.85) in the tested domestic animals. None of the tested animals were positive for SFG *Rickettsia* (P=0.00%; 95%CI: 0.0-11.35), and one goat tested positive for *Borrelia burgdorferi s.l.* (P=11.11%; 95%CI: 1.99-43.50). This study represents the first documentation of tick-borne parasites in *M. marmota*. While the prevalence of TBPs remains relatively low in these mountain-dwelling rodents, ongoing monitoring of Ixodidae presence is essential to understand how environmental shifts may impact disease dynamics in a species historically with limited or no exposure to TBPs.

# XXXIII

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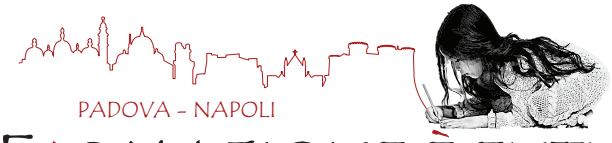
# ZOONOSES AND ONE HEALTH



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# FIRST DETECTION OF *ECHINOCOCCUS CANADENSIS* (G7) IN CYPRUS

Santoro A.[1], Santolamazza F.*[1], Constantinou P.[2], Casulli A.[1]

*[1]WHO Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis, Department of Infectious Diseases, Istituto Superiore di Sanità, European Union Reference Laboratory for Parasites (EURLP); [2]Veterinary Pathology and Parasitology Laboratory, Cyprus Veterinary Services, Ministry of Agriculture, Natural Resources and Environment, Nicosia, Cyprus*

**Keywords:** *Echinococcus granulosus s.l*, Genotypes G1 and G7, Cyprus.

**INTRODUCTION:** Cystic echinococcosis (CE) is a zoonotic disease caused by the larval stages of *Echinococcus granulosus sensu lato* (*s.l.*). The life cycle of the parasite involves dog and other canids as definitive hosts and ungulates, such as sheep and goat, as intermediate hosts. The island of Cyprus was an historical endemic area for CE in the Mediterranean. During last decades, Cyprus has been a model for testing and implementing control measures aiming to eliminate CE as a public health problem. Despite control and surveillance measures implemented during last 50 years, molecular characterization of *E. granulosus s.l.* specimens has been never provided (Ruh and Taylan Özkan, 2018. Cyprus J Med Sci, 3:193-96).

**MATERIALS AND METHODS:** In February 2023, the carcass of a stray dog collected in the Nicosia district was examined by the Veterinary Services and found infected with *Echinococcus* spp. worms. The worms were sent to the European Union Reference Laboratory (EURLP; https://www.iss.it/en/eurlp-chi-siamo) for species/genotype identification. In November 2023, a parasitic liver cyst was observed during the *post-mortem* examination of a mouflon from the same area of the dog's finding. The cyst sample was also referred to EURLP for identification and comparison with tapeworms previously collected from the dog. Genomic DNA was extracted from worms and from the membrane and protoscoleces of the mouflon's cyst. A method based on PCR-RFLP and Multiplex PCR was applied for *E. granulosus s.l.* species identification. Fragments of the mitochondrial NAD dehydrogenase subunit 2 were amplified by PCR and sequencing, to distinguish genotype G6 from G7, as well as distinct haplogroups within genotype G7 (Laurimäe et al., 2019. Parasitol Res, 118:2193-2201).

**RESULTS AND CONCLUSIONS:** The sequences analysis of cox1 gene allowed to identify the cyst as *E. granulosus sensu stricto*, genotype G1. While the sequences analyses of nad2 and nad5 genes allowed to identify the tapeworms as *Echinococcus canadensis*, genotype G7b. this finding represents the first documented molecular characterization of *E. granulosus s.l.* in Cyprus and the first detection of *E. canadensis* in this country. The finding of two different species of *E. granulosus s.l.* in a limited area raises epidemiological questions on the origin of the samples: whether distinct transmission cycles are present or a recent introduction event have occurred. From a public health perspective, it will be essential to conduct further molecular epidemiology studies to clarify the recent transmission dynamics of *Echinococcus* species in Cyprus.

# ENVIRONMENTAL-BORNE CYSTIC ECHINOCOCCOSIS: LESSONS LEARNT FROM PERITAS PROJECT IN SOUTH AMERICA

Casulli A.*

*WHO Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis, European Union Reference Laboratory for Parasites, Department of Infectious Diseases, Istituto Superiore Di Sanita', Rome, Italy*

**Keywords:** *Echinococcus granulosus sensu lato*, Cystic echinococcosis, Pathways of transmission.

**INTRODUCTION:** PERITAS (Molecular epidemiological studies on pathways of transmission and long lasting capacity building to prevent cystic echinococcosis infection) was an international collaborative project, aiming to elucidate the pathways of transmission of *Echinococcus granulosus sensu lato* (*s.l.*), which are poorly understood and have never been systematically investigated (Tamarozzi et al., 2020. Trends Parasitol, 36:427-34).

**MATERIALS AND METHODS:** PERITAS was designed as a two-stage project conducted in selected areas of Argentina, Chile and Peru. STAGE-1 was a cross-sectional ultrasound-based prevalence study to identify highly endemic clusters of human abdominal cystic echinococcosis (CE) with active cyst stages, where the STAGE-2 was implemented. STAGE-2 was a village-based case-control study that involved sampling of environmental matrices (soil, lettuces, dog's feces, dog's fur, shoe sole, flies) for molecular identification (Stefanić et al., 2004. Parasitol Res, 92:347-51; Guggisberg et al., 2020. Pathogens, 9:624) of *E. granulosus s.l.* Such village-based sampling was conducted in the: a) households/backyards of human positive CE cases (owners with active CE stages on STAGE-1; positive control), b) households/backyards of human negative CE cases (owners that were CE negative on STAGE-1; negative control), and c) village public areas (outgroup) such as squares and parks.

**RESULTS AND CONCLUSIONS:** A total of 4,512 people were screened during 2019, in the regions of Coquimbo (Chile; N=2,439), Rio Negro (Argentina; N=892) and Junin (Peru; N=1,181) (Uchiumi et al., 2021. Parasit Vectors, 14:262; Acosta-Jamett et al., 2022. PloS Negl Trop Dis, 16:e0010280). The mean prevalence of abdominal CE in these three areas was 1.6%, 4.7% and 3.7%, respectively. Despite COVID-19 pandemic, sampling of matrices were successfully conducted in Chile and Peru in 2020-2021 from high CE endemic villages. Considering all matrices analysed (soil, lettuces, dog's feces, dog's fur, shoe sole, flies), 21% (117/557) of samples tested positive to *E. granulosus s.l.* DNA. Neither vegetables (0/25) nor flies (0/6), tested positive. Shoe sole, soil, dog's fur and dog's faeces tested positive in 21% (25/118), 24% (34/141), 22% (33/150), 21% (25/118), respectively. Considering dog faeces, which we assumed as a direct measure of human risk infection, the percentage of samples contaminated by E. granulosus s.l. DNA was found to significantly increase from CE-negative (3%; 2/61) to CE-positive (17%; 2/13) households/backyards up to public areas (91%; 39/43). Such results documenting high environmental contamination suggest a paradigm shift: i) from CE individual risk to community risk in endemic areas, ii) from mainly "food-borne" to more broadly "environmental-borne" disease (transmitted by food, water, but also hand-to-mouth).

This research was funded by PERITAS project by EU-LAC Health, G.A. EULACH16/T02-0067. https://www.iss.it/web/iss-en/who-cc-peritas.

# MANAGEMENT-RELATED RISK FACTORS FOR *ECHINOCOCCUS GRANULOSUS* TRANSMISSION IN SHEEP FARMS SITUATED IN THE ALTO MACERATESE (CENTRAL ITALY), AN AREA POPULATED BY WOLVES

Habluetzel A.*[1], Pacifici L.[2], Propoggia G.[3], Crotti S.[4], Manciola G.[2], Pennesi S.[2], Morandi F.[5], Morandi B.[6]

*[1]University of Camerino, School of Pharmacy, Camerino, Italy; [2]"Aziende sanitarie territoriali AST, Macerata", Department for Prevention, Macerata, Italy; [3]University of Camerino, School of Biosciences and Veterinary Medicine, Camerino, Italy; [4]Experimental Zooprophylactic Institute of Umbria and Marche "Togo Rosati", Perugia, Italy; [5]Monti Sibillini National Park, Visso, Italy; [6]Experimental Zooprophylactic Institute of Umbria and Marche "Togo Rosati", Tolentino. Italy*

**Keywords:** Echinococcosis, Risk factors, Wolves.

**INTRODUCTION:** In the Alto Maceratese area of the Marche Region, *Echinococcus granulosus sensu lato (s.l.)* is circulating in both the domestic and sylvatic cycle, as confirmed by official veterinary inspection of cattle (5 and 9 positive cases in 2022 and 23 respectively) and wild boars (0.12% and 0.41% positives). Wolves, definitive sylvatic hosts, are present at a density of about 10 animals/100 km$^2$. Concerns have been raised that, as a result of the threat posed by wolves, but also due to socio-economic challenges, sheep husbandry practices have changed, possibly entailing increased transmission risks in sheep farms and risk of cystic echinococcosis for breeders.

**MATERIALS AND METHODS:** A questionnaire study was conducted during 2023 in the Alto Maceratese (Apennine mountains, Marche Region), an area characterized by small-scale, family-based farming, with sheep mostly raised for meat production and often kept together with cattle. The questionnaire, structured in open and closed questions, was administered to 40 sheep breeders of ten municipalities, after due introduction by a local veterinary practitioner. A descriptive analysis of the data is provided.

**RESULTS AND CONCLUSIONS:** The 40 sheep farmers keep their animals (median of 150 sheep/farm) on natural pastures and forage cultivated fields (after harvest) during summer and in stables during winter. Breeders usually have both, shepherd dogs (1.2 dogs/100 sheep) and livestock guardian dogs (2.9 dogs/100 sheep) to protect their flocks against wolf attacks. Almost all (31/39) farmers with dogs, report to administer anti-parasitic drugs, however only about half of them (17/31) use drugs effective against cestodes. More often, treatments are given only once (15/31) or twice (12/31) a year. When not with grazing flocks, dogs are frequently left free roaming (31/39), passing feces on pastures around farms and inside hay barns and stables. Several breeders (11/39) keep guardian dogs deliberately in stables, to protect sheep from wolf attacks. Most breeders (34/40) have been hit by wolf attacks. During the last 3 years, 26 breeders reported at least one attack and lost in total 514 sheep in 47 attacks, with a median of 4 per attack. According to interviewees, some predation episodes remain undetected. About half of the breeders (22/39) observe occasionally dogs with pieces of sheep carcasses. In 2023, most of them (35/40) declared loss of sheep (in total, 363 animals). Thus, it can be estimated that about 3.6% of raised sheep (n=10,121) may end up furnishing viscera to wolves and dogs. Scarce treatment of dogs with appropriate drugs and access of wild and domestic carnivores to viscera of intermediate hosts rank high among the risk factors for echinococcosis transmission in the study area. Ongoing studies on dog feces as well as sheep inspected at slaughterhouses will allow to get some insight into the epidemiological dynamics of *E. granulosus* genotypes in the Alto Maceratese.

# RESULTS OF INTEGRATED SURVEILLANCE SYSTEM FOR WEST NILE VIRUS AND USUTU VIRUS IN VENETO REGION, IN 2022 AND 2023

Chiarello G.[1], Gradoni F.[1], Sgubin S.[1], Danca L.[1], Carlin S.[1], Toniolo F.[1], Poletto E.[1], Porcellato E.[1], Mazzucato M.[1], Bortolami A.[1], Monne I.[1], Danesi P.[1], Montarsi F.[1], Favero L.[2], Russo F.[2], Sinigaglia A.[3], Pacenti M.[3], Barzon L.[3], Gobbo F.*[1]

*[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy; [2]Department of Prevention, food safety and veterinary, Veneto Region, Venice (VE), Italy; [3]Department of Molecular Medicine, University of Padova, Italy, Padua. Italy*

**Keywords:** Arbovirus, Flavivirus, One Health.

**INTRODUCTION:** West Nile Virus (WNV) and Usutu virus (USUV) are Flaviviruses transmitted by mosquito vectors; avifauna is the reservoir of viruses, while equids and humans are end-hosts. In Italy, the surveillance system of WNV and USUV consists of entomological, veterinary and human surveillances. This integrated approach allows an early detection of these viruses in mosquitoes and birds for the purpose of human health prevention. This work reports the results of the surveillance in Veneto region in 2022 and 2023.

**MATERIALS AND METHODS:** Entomological surveillance was carried out from May to October of each year using 57 CDC-like traps baited with CO2 and 7 Gravid Trap. The traps were activated for one night every two weeks. All captures were morphologically identified, then pooled female specimens of *Culex* spp., *Aedes albopictus* and *Ochlerotatus caspius* were tested by rRT-PCR screening for the genus Flavivirus followed by sequencing. Veterinary surveillance provided sampling organs and blood of avifauna and clinically suspected equines. Samples were tested for WNV by duplex one-step rRT-PCR and for USUV by one-step qualitative rRT-PCR. Annual human surveillance was enhanced during vector transmission seasons from May to November. Samples of human cases with neurological symptoms or febrile illness were tested by one-step real-time RT-PCR. All positive sequence to Flavivirus were sequenced.

**RESULTS AND CONCLUSIONS:** In 2022, entomological surveillance sampled 93,213 mosquitoes (85,897 females tested). First positive mosquito pool to WNV was on 7th June and 89 positivity were registered during season (48 WNV-1 and 55 WNV-2). In veterinary surveillance, 166 birds out of 2,176 were infected (7 co-infected) and 12 outbreaks in equines were notified. 337 human cases of WNV were confirmed, including 142 WNND, 161 WNF and 26 asymptomatic infections in blood donors. USUV was found in 14 mosquito pools and 17 birds. During 2023 season, 133,648 mosquitoes (126,063 females) and 1,823 birds were sampled. The first positivity for WNV was on 13th July 2023 in vectors and the surveillance activities confirmed positivity in 25 mosquito pools, 61 birds, 1 horse and 64 human cases (22 WNND, 38 WNF and 4 blood donors). USUV was recorded in 19 mosquito pools and 37 birds. The results of integrated surveillance showed early and high viral circulation of WNV in the 2022, probably due to peculiar climatic condition during the winter season and to the re-introduction of WNV lineage 1 in late 2021, which then spread in 2022. In both years, WNV was initially detected in mosquitoes and avifauna, before the occurrence of equines and human outbreaks, confirming that they are the best target for the early detection of WNV. The application of the integrated Arboviruses surveillance supports the efficacy and the potential of One Health approach.

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# OPISTHORCHIASIS IN CENTRAL ITALY, STILL A NEGLECTED PROBLEM

Papalini C.[1], Ludovisi A.*[2], Francisci D.[1], Marucci G.[2], Mercuri A.[1], Stolaj E.[1], Gomez Morales M.A.[2]

*[1]Infectious Diseases Clinic, Santa Maria della Misericordia Hospital, University of Perugia, Perugia, Italy; [2]Istituto Superiore di Sanità, Roma, Italy*

**Keywords:** *Opisthorchis felineus*, Human outbreak, Epidemiology.

**INTRODUCTION:** In Central Italy, *Opisthorchis felineus* is present (De Liberato et al., 2011. Vet Parasitol, 177:67-71) and outbreaks took place for the consumption of tenches fished in two lakes (Bolsena and Bracciano) from 2003 to 2011. This study aims to report epidemiological and clinical characteristics of the last human opisthorchiasis outbreak occurred in Central Italy in 2022 and to compare it with previous events.

**MATERIALS AND METHODS:** The study involved symptomatic and not symptomatic patients, who ate marinated filets in the restaurant of Sant'Arcangelo in May 2022 and were diagnosed with an *O. felineus* infection from June to December 2022. A case of opisthorchiasis was defined as the presence of Opisthorchidae eggs or a positive PCR in a faecal sample and/or anti-*O. felineus* antibodies in the serum of persons with epidemiological link.

**RESULTS AND CONCLUSIONS:** Sixty-seven individuals were traced back by epidemiological investigation. Forty-seven received a diagnosis of opisthorchiasis, of which 45 confirmed and two probable cases. The median of the signs and symptoms onset was 16.5 days (IQR 13.75-21.25) after fish consumption. The median between signs and symptoms onset and positive parasite examination of stool was of 14 days (IQR 7-22.5). All but 20 presented symptoms, mostly fever (44.7%). Sixteen (34%) cases (15 confirmed and 1 probable) required hospitalization. Feces examination revealed Opisthorchidae eggs in 35/45 (78%) confirmed cases. Thirty individuals underwent to serology and molecular stool test: 5 (16.7%) results positive to the former, 1 (3.3%) to the latter, while 4 (13.3%) to both. Laboratory tests, available in 28 patients, showed eosinophilia in 82.1%, increase of alanine aminotransferase, gamma-glutamyl transferase and alkaline phosphatase in 64.3%, 75% and 67.9% of cases, respectively. All the confirmed cases, except for an asymptomatic, were treated: 22 (48.9%) with PZQ and 22 (48.9%) with ABZ, of which 13 failed clearing the parasite and received a second treatment with PZQ. No therapeutic failure was noticed in the 22 confirmed cases firstly treated with PZQ, neither in the 13 individuals treatedwith this drug after the treatment with ABZ. Opisthorchiasis still represents a challenging in the diagnosis, in particular for asymptomatic patients, reaching 40% of the confirmed cases in the present outbreak. This increases the risk of underdiagnoses, which can lead to chronic infections. In this study, five asymptomatic cases that had tested negative for Opisthorchidae eggs were detected only by serology. Serology resulted a good tool also for the follow up of the patients after pharmacological therapy. Symptomatic people (60%), presenting mainly fever (77.8%) and eosinophilia (82.2%), were the most frequent signs in opisthorchiasis infections. In this outbreak, epidemiological and clinical findings were similar to those of previous Italian events but with higher incidence of symptomatic, hospitalized and ABZ unsuccessful treatment of cases.

# OVINE CYSTIC ECHINOCOCCOSIS (*ECHINOCOCCUS GRANULOSUS S.L.*) IN NORTHERN ITALY: FROM THE SLAUGHTERHOUSE TO THE LABORATORY IN A ONE HEALTH PERSPECTIVE

Rega M.*, Vismarra A., Semeraro M., Cattabiani C., Kramer L.H., Muresu Ibba G.M., Genchi M.

*University of Parma, Parma, Italy*

**Keywords:** Cystic echinococcosis, Ovine, Epidemiology.

**INTRODUCTION:** Human cystic echinococcosis (CE; *Echinococcus* spp.) is among the 20 disease groups prioritized for control by the World Health Organization as part of Neglected Tropical Diseases due to its impact on patients' and global public health. The disease is chronic and often asymptomatic, which renders evaluation of the actual prevalence difficult (Carvelli et al., 2020. PLoS One, 15:e0240551; Casulli et al., 2023. PLoS Negl Trop Dis, 17:e001161). Furthermore, the occurrence of the disease is likely underestimated due to the lack of standardized surveillance programs (Loi et al., 2019. PLoS ONE, 14:e0214224). CE is widespread in many domestic animal species in Italy, with the G1-G3 genotype predominating. The aim of the study was to determine the prevalence of ovine CE in North Italy through *post-mortem* inspection at slaughterhouses.

**MATERIALS AND METHODS:** Sample collection was performed in an ovine slaughterhouse in the Emilia-Romagna region. Lungs and livers with suspected cystic lesions were conferred to the Parasitology Unit, University of Parma. Lesions were first evaluated macroscopically. Molecular confirmation was then performed by end-point Multiplex PCR and positive samples were submitted for genotyping. Infected animals' gender, age, geographical origin and eventual movements were recorded.

**RESULTS AND CONCLUSIONS:** A total of 421 slaughtered sheep were evaluated and suspected CE was detected in 101/421 (24%) at the slaughterhouse. Macroscopic evaluation of lung and liver lesions in the laboratory, however, resulted in only 35/421 (8.3%) slaughtered animals as being affected by CE. Finally, PCR was positive for *Echinococcus granulosus* in 24/421 animals (5.7%). Further analysis for genome sequencing and genotype identification is in progress. The diagnosis of CE in the intermediate host is fundamental for epidemiological studies and disease risk analyses. Results here suggest that visual *post-mortem* inspection in the slaughterhouse lacks specificity and likely overestimates true prevalence. Especially in endemic areas, the registration of cases of cystic echinococcosis in sheep is essential to systematically study the disease and assess the spread of different genotypes (Loi et al., 2019. PLoS ONE, 14:e0214224).

# INVESTIGATION OF PARASITIC CONTAMINATION OF FROZEN BERRIES SOLD IN SOUTHERN ITALY

Barlaam A.*[1], Puccini A.[2], Datteo M.[1], Perdonò S.[1], Giangaspero A.[1]

[1]*University of Foggia, Department of Science of Agriculture, Food and Environment, Foggia, Italy;* [2]*Azienda Sanitaria Locale, Foggia, Italy*

**Keywords:** Foodborne parasites, Frozen berries, Real-time PCR.

**INTRODUCTION:** Over the past few decades, due to the rising demand for healthy dietary options, berry fruits have become increasingly popular in industrialized countries. Berries represent healthy food choices and contain bioactive compounds associated with decreased risk of diseases. Despite their health benefits, these products can be contaminated by pathogenic microorganisms, including parasites. Among foodborne parasites, *Cryptosporidium parvum*, *Giardia duodenalis*, *Toxoplasma gondii*, *Cyclospora cayetanensis* and *Echinococcus multilocularis* are of significant public health importance and have been recently detected in Europe in fresh berries, including Italy (Barlaam et al., 2021. Food Microbiol, 98:103792; Barlaam et al., 2022. Int J Food Microbiol, 370:109634). Berries can be purchased fresh or frozen, and it is worrying that even frozen berries could represent a risk for the consumer, in fact, several parasites can resist freezing temperatures (Temesgen et al., 2021. Microorganisms, 9:1463) and have been responsible of outbreaks of infection (Ho et al., 2002. Emerg Infect Dis, 8:783-88). The aim of this study was to investigate the presence of *C. parvum*, *G. duodenalis*, *T. gondii*, *C. cayetanensis* and *E. multilocularis* in frozen berries with simplex and multiplex real-time PCR protocols.

**MATERIALS AND METHODS:** A total of 108 packages of mixed frozen berries belonging to four different industrial brands were bought from supermarkets located in Foggia and Barletta-Andria-Trani (Apulia region, Italy). The packages contained raspberries, blackberries, blueberries, strawberries, redcurrants and blackcurrants in different proportions and the origin of the fruits was not indicated. Fifty grams of frozen berries from each package were processed according to the U.S. Food and Drugs Administration Bacteriological Analytical Manual (BAM) chapter 19b and the DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen). The samples were tested using two simplex real-time PCR protocols targeting *C. parvum* (Temesgen et al., 2020. Food Microbiol, 89:103447) and *G. duodenalis* (Klotz et al., 2021. Microorganisms, 9:1610), respectively, and a multiplex real-time PCR targeting *T. gondii*, *C. cayetanensis* and *E. multilocularis* (Temesgen et al., 2019. Int Food Res, 125:108636).

**RESULTS AND CONCLUSIONS:** None of the investigated parasites were detected in the frozen berries' samples tested. The samples may have been negative in the first place or not contaminated along the food chain or contaminated by such a low number of oo/cysts or eggs that they would be undetectable by the employed methods. This research topic is still unexplored and, due to its repercussions on human health, it is the subject of intense debate among researchers and encouraged by the international scientific community. These results represent a first attempt to investigate parasitic contamination of frozen berries sold on the market, however, more extensive investigations and larger sample sizes are needed to shed light on this matter.

# SPATIAL AND GENETIC DIVERSITY OF CLINICAL ISOLATES OF *BLASTOCYSTIS* SP. IN ITALY: A NETWORK ANALYSIS

Guadano Procesi I.*, Berrilli F., De Vito M., Di Cave D.

*Department of Clinical Sciences and Translational Medicine, Faculty of Medicine, University of "Tor Vergata", Rome, Italy*

**Keywords:** *Blastocystis*, One Health, Haplotypes.

**INTRODUCTION:** The ubiquitous protist *Blastocystis* sp. can colonize the gastrointestinal tracts of humans and non-human hosts (Tan, 2008. Clin Microbiol Rev, 21:639-65). Despite being one of the most common intestinal protists found in humans worldwide (Andersen and Stensvold, 2016. J Clin Microbiol, 54:542-48), the clinical importance of *Blastocystis* infection is still debatable. Significant genetic diversity exists across isolates from people and animals, with humans capable of hosting many zoonotic genotypes. Due to the apparent variable host specificity in different *Blastocystis* subtypes (STs), assessments of incidence and STs diversity in specific geographic areas are significant for understanding their epidemiology and zoonotic potential. In this study we reported the pattern analysis of genetic relationship among different haplotypes of *Blastocystis* STs to investigate spatial and genetic diversity of this protist in Italy.

**MATERIALS AND METHODS:** From July 2021 to October 2022 (Azienda Ospedaliera Universitaria Policlinico Tor Vergata, Rome), 37 stool samples from symptomatic patients, resulted positive to *Blastocystis* through the AllplexTM Gastrointestinal Parasite Panel Assay, were collected, preserved at +4°C and processed within 24 hours. Thirty-one samples (Ct <30) were amplified via end-point PCR (Scicluna et al., 2006. Protist, 157:77-85). To this database, further 24 *Blastocystis* isolates previously sequenced in the period October 2014 - March 2017 have been added. The consensus sequences were compared with those available in GenBank, checked in PubMLST for allele attribution, and aligned through AliView with representative sequences as reference. A Neighbour joining (NJ) phylogenetic tree was generated with MEGA 11. Fifty-three sequences out of 55 were used for a haplotype analysis on polymorphic sites (DnaSP v.6), together with all available clinical sequences from Italy (107 sequences in total). PoPART genetic software was used to perform the Minimum-Spanning network calculation.

**RESULTS AND CONCLUSIONS:** Through the phylogenetic analysis the 55 new isolates were assigned as follows: 9 to ST1 (16.4%), 7 to ST2 (12.7%), 11 to ST3 (20%), 26 to ST4 (47.3%) 1 to ST6 (1.8%) and 1 to ST7 (1.8%). From the alleles analysis eight different variants were detected within the different subtypes: allele 4 (ST1), alleles 9 and 12 (ST2), allele 34 and 36 (ST3), allele 42 (ST4), allele 123 (ST6) and allele 137 (ST7). Forty-six haplotypes (hp) were identified across the 107 isolates. The most represented haplotypes were hp 41 (29.9%) and hp 34 (15.9%). The ST4, in Italy represented in its entirety by allele 42, matches to a single hp (hp 41). No spatial segregation has been observed, probably due to the reduced number of sequencies available from Italy. Further studies are therefore necessary to define the *Blastocystis* variability in Italy and all over Europe; indeed, this data will be shared within the Cost ACTION CA21105 to map and compare the practices and criteria used by diagnostic and research laboratories across Europe for isolation, identification, and subtyping of *Blastocystis*.

# REAL TIME PCR PROTOCOL FOR THE DIAGNOSIS OF *ECHINOCOCCUS MULTILOCULARIS* AND *ECHINOCOCCUS GRANULOSUS*

Da Rold G.*[1], Obber F.[1], Celva R.[1], Dalla Libera E.[1], Bonelli P.[2], Citterio C.V.[1]

*[1]Istituto Zooprofilattico Sperimentale delle Venezie-Centro specialistico fauna selvatica-SCT2 Treviso, Belluno e San Donà di Piave, Belluno, Italy; [2]Centro Nazionale di Referenza per l'Echinococcosi/Idatidosi (CeNRE), Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy*

**Keywords:** *Echinococcus*, qPCR, Faeces.

**INTRODUCTION:** *Echinococcus* spp. (Cestoda, Cyclophyllidea, Taeniidae) are small intestinal tapeworms causing zoonoses of public health importance worldwide. In Italy, *E. granulosus* is widespread, although with variable prevalence, being linked to the domestic dog (definitive host) - sheep (intermediate host) cycle. On the other hand *E. multilocularis*, whose cycle is wild and includes the red fox (definitive host) and small rodents (intermediate hosts), seems currently limited to the Alps, where other areas have recently added to the historical endemic area in South Tyrol (Massolo et al., 2018. Wildl, 7:309-16; Obber et al., 2022. PLoS One, 17:e0268045). The presence of both these parasites highlights the need for a reliable and cost-effective diagnostic method for their surveillance and zoonotic risk assessment. This work aimed to develop and validate, through sensitivity, specificity and repeatability tests, a Real-Time PCR protocol using specific probes for the differential diagnosis of *E. multilocularis* and *E. granulosus* directly from the faeces of definitive hosts.

**MATERIALS AND METHODS:** The material used in this study came from red foxes collected by IZSVe during 2012-2021 in Veneto, Trentino - Alto Adige and Friuli-Venezia Giulia regions (NE Italy), while dog samples were provided by the National Reference Centre for Echinococcosis (Ce.N.R.E.). Total DNA extraction was performed from 0.10 g of stool, using a multiplex TaqMan probes qPCR to detect *E. multilocularis* and *E. granulosus* DNA (Knapp et al., 2016. Appl Environ Microbiol, 82:2950-58; Maksimov et al., 2020. Pathogens, 9:791). To check the sensitivity of the test, we diluted *E. multilocularis* eggs extracted from adult worms in nine scalar dilutions of 1:2 PBS-D: this dilution was then used to spike negative fox feces. The values at cycle threshold (Ct) obtained from the extracted DNA were used to build a calibration curve linking the Ct with the presumed number of parasite's eggs. For *E. granulosus*, due to the absence of adult specimens, analytical sensitivity was determined by diluting (1:2) the DNA extracted from dog samples, in DNA extracted from negative fox faeces, in order to avoid biases due to different faecal matrix.

**RESULTS AND CONCLUSIONS:** The procedure validated in this research made it possible to achieve remarkably high performance analysis, showing a sensitivity of 100% and a specificity of 100% for both *Echinococcus multilocularis* and *Echinococcus granulosus*. Furthermore this procedure, relying directly on DNA extraction from the faecal material and on specific probes, and not requiring sequencing, greatly reduces the number of steps to be performed in the laboratory, as well as the relative costs.

# SEROLOGICAL AND MOLECULAR INVESTIGATION ON *TOXOPLASMA GONDII* IN GOAT MILK AND ABORTED TISSUES IN LAYYAH DISTRICT (PAKISTAN)

Barlaam A.*[1], Khan M.Y.[1,2], Gazzonis A.L.[3], Ferrari N.[3], Jimenéz-Meléndez A.[4], Robertson L.J.[4], Giangaspero A.[1]

*[1]University of Foggia, Department of Science of Agriculture, Food and Environment, Foggia, Italy; [2]University of Veterinary & Animal Sciences, Lahore, Pakistan; [3]Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy; [4]Norwegian University of Life Sciences (NMBU), Ås, Norway*

**Keywords:** *Toxoplasma gondii*, Goats, Epidemiology.

**INTRODUCTION:** *Toxoplasma gondii* is a parasitic protozoan infecting warm-blooded animals, including humans. Infection can be associated with fetal mortality, and a spectrum of other symptoms. Infection mainly occurs through ingesting undercooked or raw animal products (meat, but also milk) from infected animals, or water or raw fresh produce contaminated with oocysts, the transmission stage shed in the feces of the feline definitive hosts (Dubey, 1991. Southeast Asian J Trop Med Public Health, 22:89-92). Our study investigated the prevalence of *T. gondii* in goats in Layyah District (Pakistan) via analysis of blood for anti-*T. gondii* antibodies, and the presence of *T. gondii* DNA in milk and goats' aborted tissues. Our aim was evaluating the associated potential risk for public health.

**MATERIALS AND METHODS:** Goat farms in the study area were stratified by size, and 110 were randomly selected. From each farm, sera samples were collected from 12 goats (>1 year old) (1320 samples in total) and tested for anti-*T. gondii* antibodies by a commercial ELISA kit (ID Screen, ID-VET). From three highly seropositive farms, milk samples were collected from 40 goats at three different lactation intervals: 15-30, 90 and > 120 days after the parturition (360 samples in total). Additionally, both fetal brain and placenta tissue samples were collected from three aborted goats. DNA was extracted from all the samples using the Nucleospin tissue kit (Macherey-Nagel) and tested for *Toxoplasma* by real-time PCR targeting a 162 bp section of the 529-bp repeat element (Temesgen et al., 2019. Food Res Int, 125:108636). To confirm the presence of *T. gondii* and determine the clonal type, samples that tested positive were analyzed using a nested PCR protocol targeting four different markers: GRA6, SAG1, SAG2, CS3 (Ostevik et al., 2019. J Vet Diagn Invest, 31:875-78). Sequencing is ongoing.

**RESULTS AND CONCLUSIONS:** Overall, 31.5% (95%CI: 28.3-34.8) sera samples were positive for anti-*T. gondii* antibodies and 89.1% of the flocks had at least one seropositive goat. The proportion of seropositive goats within each flock ranged from 8.3% to 83.3%, with goat age, cat presence, floor, abortion history and owner education contributing to this heterogeneity. Of 360 milk samples, in 14 (3.9%), one collected during the first lactation interval, and five and eight during the second and third intervals, respectively, *T. gondii* DNA was detected. *Toxoplasma* DNA was also detected in all aborted tissues, except one fetal brain tissue. Results were confirmed by nested PCR in 13 of 14 milk samples and in two fetal brain and two placenta samples. This is the first study to determine the prevalence of anti-*T. gondii* antibodies in goat sera in Layyah district and the largest performed in Pakistan. The presence of *T. gondii* amongst goats in areas where goat farming has a crucial economic role may pose a production threat to the small-ruminant industry. Given that consumption of raw milk in this region is common these results are of concern to public health.

# PREVALENCE OF SOIL-TRANSMITTED HELMINTHS AND INTESTINAL PROTOZOA IN DOGS AND CHILDREN OF RURAL COMMUNITY IN THE BOLIVIAN CHACO

Napoli E.*[1], Macchioni F.[2], Gabrielli S.[3]

*[1]Department of Veterinary Sciences, University of Messina, Messina, Italy; [2]University of Pisa, Department of Veterinary Sciences, Pisa, Italy; [3]Sapienza University of Rome, Department of Public Health and Infectious Diseases, Roma, Italy*

**Keywords:** Soil-transmitted helminths, Zoonosis, Bolivia.

**INTRODUCTION:** Intestinal parasitic infections (IPIs) are widespread worldwide, and more than half of the world's population is at risk of infection, particularly in poor and developing nations in tropical and subtropical regions, where they are often termed "the cancer of developing nations". These infections, due to tapeworms, soil-transmitted helminths and protozoa, are often of zoonotic concerns. Studies have demonstrated that the close interaction between humans and feral or owned dogs without a proper antiparasitic treatment and in poor hygienic condition should enhance the prevalence of humans IPIs. In the rural communities of the Bolivian Chaco, the poorest region of Bolivia, the lack of access to safe water, sanitation, and hygiene and the presence of close contact with wild and domestic animals represent the key factors for the spread of IPIs. Aim of the present study was to assess the prevalence of IPIs in dogs and school-age children (SAC) living in six rural communities in semi-urban areas of the Bolivian Chaco.

**MATERIALS AND METHODS:** Sampling was carried out in six rural communities in the municipalities of Camiri (i.e., Ivamirapinta) and Villamontes (i.e., Tarairi, Palmarcico, Villamontes, Capirendita and Chimeo). Fecal samples were collected in the proportion of 1:2 from dogs and SAC in the same communities and analyzed by flotation, Baermann and sedimentation techniques. The observed parasites were identified according to morphological keys.

**RESULTS AND CONCLUSIONS:** A total of 105 and 330 fecal samples were collected from dogs and SAC, respectively. Briefly, 70.2% of dogs tested positive for gastrointestinal nematodes, while the prevalence of protozoa was 30.8%. In particular, STHs such as *Toxocara canis* (18.3%), *Ancylostoma* spp. (42.3%) and *Trichuris vulpis* (4.8%) were identified along with protozoa such as *Giardia* spp. (28.8%), *Isospora* spp. (9.6%), *Entamoeba* complex (4.8%), *Blastocystis* spp. (1.9%), *Dientamoeba* spp. (2.9%) and *Entamoeba coli* (1.9%). A total of 3.6% of the dogs tested positive for *Strongyloides* spp. using the Baermann technique. The only metazoan species found in humans was *Hymenolepis nana*, detected in 4.5% of SAC samples. Conversely, gastrointestinal protozoa were identified in 30.3% of the children. In detail, *Blastocystis* spp. (13.6%) was the most common species, followed by *E. coli* (12.1%), *Giardia* (8.2%), *Entamoeba* complex (3.6%) and *Entamoeba hartmanni* (0.9%). In humans, no STHs infections have been documented as a result of preventive deworming programme conducted in the area since 1987 (Cancrini et al., 1989. Ann Trop Med Parasitol, 83:591-94). Furthermore, in order to prevent COVID-19, Ivermectin treatments were proposed for Bolivian citizens in 2021 and 2022. However, poor general hygienic and sanitary conditions, coupled with the close contact with dogs (largely infected by *Giardia*), still represent important drivers of protozoan infection.

# SURVEILLANCE OF TICK-BORNE DISEASES IN OUTDOOR WORKERS AND TICK MONITORING IN THEIR WORKING PLACES

Morea A.*[1], Indraccolo F.[1], Lia R.P.[2], Lovreglio P.[1], Stufano A.[1], Schino V.[1], Carbonara M.[2], Castilletti C.[3], Di Leone G.[4], Otranto D.[2], Iatta R.[1]

*[1]Interdisciplinary Department of Medicine, University of Bari, Bari, Italy; [2]Department of Veterinary Medicine, University of Bari, Valenzano, Italy; [3]Department of Infectious-Tropical Diseases and Microbiology, IRCCS Sacro Cuore-Don Calabria Hospital, Negrar di Valpolicella, Verona, Italy; [4]Prevention and Safety of Workplaces of the Department of Prevention, ASL/BA, Bari, Italy*

**Keywords:** Tick-borne pathogens, Seroprevalence, Diagnostic tests.

**INTRODUCTION:** Tick-borne diseases (TBDs) represent an emerging threat to animal and human health (Madison-Antenucci et al., 2020. Clin Microbiol Rev, 33:e00083-18). People working outdoor and/or in contact with livestock animals, as well as those practicing recreational activities are more exposed to tick bites (De Keukeleire et al., 2015. Ticks Tick Borne Dis, 6:636-44). This study aims to assess the exposure of outdoor workers (i.e., breeders and farmers) to tick-borne pathogens (TBPs) by serological tests, along with tick distribution in their working places, providing a risk map of these arthropod vectors. In addition, the performance of serological tests routinely employed for TBDs diagnosis is evaluated.

**MATERIALS AND METHODS:** From January to March 2024, 140 sera were collected from outdoor workers from the Alta Murgia park (Apulia region) and tested for IgG against *Borrelia burgdorferi* complex, *Coxiella burnetii*, *Rickettsia conorii* and *Francisella tularensis* by chemiluminescent immunoassay (CLIA). Immunoblot was performed as confirmatory test for *B. burgdorferi* complex and immunofluorescence antibodies test (IFAT) for *C. burnetii* seropositive samples, as recommended by the CDC guidelines. For the latter, CLIA detects IgG anti-C. burnetii phase II antigen, whereas IFAT discriminates between IgG anti-*C. burnetii* phase I and phase II antigens (i.e., Q fever chronic and acute forms, respectively). From June 2023 to March 2024, ticks were collected by dragging and flagging and identified using morphological keys.

**RESULTS AND CONCLUSIONS:** Overall, 64.3% (90/140) workers tested positive for at least one TBP by CLIA, with *C. burnetii* being the most prevalent (52.1%, 73/140), followed by *R. conorii* (19.3%, 27/140), *B. burgdorferi* complex (9.3%, 13/140) and *F. tularensis* (2.8%, 4/140). *Coxiella burnetii* seropositivity to IgG against phase II antigen was confirmed by IFAT in 38.4% (28/73). Specifically, 17 sera were positive for phase II IgG, 10 for both phase I and II IgG, and 1 for phase II IgG and both phase I and II IgM (acute form). Moreover, phase I IgG were detected in 7 sera, including one with 1:1024 titer (chronic form). Out of 13 sera positive for *B. burgdorferi* complex by CLIA, two were confirmed by immunoblot. To date, 97 ticks were collected from areas nearby 7 farms and identified as *Riphicephalus sanguineus* group (n=60) and *Haemaphysalis* spp. (n=37). In conclusion, these findings indicate that outdoor workers are exposed to TBPs highlighting the importance of TBDs surveillance programs for these job categories, tick monitoring in their working places, and the use of confirmatory and/or gold standard tests for an accurate diagnosis. The diagnostic test performance, the prevalence of TBPs in ticks along with their map distribution and predictive models is currently ongoing.
The study is supported by EU funding within the Next Generation EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

# A ONE HEALTH SURVEY ON TICK-BORNE ZOONOSES INVOLVING HUMANS, DOGS, WILDLIFE AND ENVIRONMENTAL TICKS

Vada R.*[1], Calcagno A.[2], Benatti F.[1], Carpignano M.[3], Fantini E.[1], Garro E.[2], Occhibove F.[1], Pepe A.[2], Varzandi A.R.[1], Zanet S.[1], Ferroglio E.[1]

*[1]Department of Veterinary Sciences, University of Turin, Torino, Italy; [2]Department of Medical Sciences, University of Turin, Torino, Italy; [3]Comprensorio Alpino CACN3, Dronero, Italy*

**Keywords:** Acarological risk, One Health surveillance, Tick-borne zoonoses.

**INTRODUCTION:** Tick-borne zoonoses (TBZ) are emerging pathogens that often circulate from wildlife reservoirs to humans and domestic animals. Shifts in climate patterns and changes in land use practices, which also impact the population dynamics of wildlife, have contributed to the increase of ticks' abundance and expanded their geographical distribution (Medlock et al., 2013. Parasit Vectors, 6:1-11). The interface between wildlife, domestic animals and humans has widened (Gortázar et al., 2007. Eur J Wildl Res, 53:241-56), fostering the transmission of TBZ as well. This study aimed to set up, in a rural area, an integrated TBZ survey involving different target hosts (environmental ticks, wildlife, domestic animals and humans), to identify pathogen prevalence in the different groups.

**MATERIALS AND METHODS:** The study was performed in a hunting district covering an alpine valley. 124 pools of questing ticks were collected with dragging transects from 38 points across the valley. 133 spleen samples from culled wildlife (wild boar, red deer, chamois) were collected from the game control centre. Finally, 67 blood samples were taken from volunteer hunters associated with the district and their relatives, as well as from 38 of their dogs. All samples were tested by end-point PCR for *Babesia divergens/B. capreoli*, *Babesia microti*-like, *Borrelia burgdorferi sensu latu*, Rickettsia Spotted Fever Group, *Anaplasma phagocytophilum*. In humans, we also tested the serological prevalence of the same pathogens. An additional questionnaire was submitted to the participants to explore outdoor habits which may influence exposure to tick bites.

**RESULTS AND CONCLUSIONS:** The most prevalent pathogen in all groups was *Babesia* spp, reaching up to 45% (95%CI: 37-54) in wildlife. *A. phagocytophilum* in wildlife and environmental ticks reached a lower prevalence (1%, 95%CI: 0.8-2) than in humans and dogs (up to 11%, 95%CI: 3-18). *B. burgdorferi* and Rickettsia SFG prevalences were consistent between humans and questing ticks (2%, 95%CI: 0-4), but much higher in dogs (up to 16%, 95%CI: 4-27). Serological analysis in humans did return a similar pattern showing higher prevalences (up to 28%, 95%CI: 18-39, for *B. divergens*). Coinfections were recorded by end-point PCR in one person (Rickettsia SFG - Piroplasmids) and two dogs (Rickettsia SFG - *A. phagocytophilum*), while in ticks it was identified in 47/126 pools, although mostly among *Babesia* species. Serology returned positivity to more than one pathogen (and up to all tested pathogens) in 17 people. Results from the survey suggested that awareness is rising in people spending long hours in the natural environment. Our results remark on the close link between different tick hosts for the circulation of tick-borne zoonoses and the importance of broadening the view and implementing a holistic approach when it comes to disease surveillance and control strategies. The positive attitude of the hunting sector to participate in the study highlights its potential for data collection.

# OVERVIEW OF CANINE LEISHMANIOSIS FROM VETERINARIANS AND DOG OWNERS PERSPECTIVE

Nascimento Da Costa T.*, Grillini M., Marchiori E., Frangipane Di Regalbono A., Simonato G.

*Department of Animal Medicine, Production and Health, University of Padova, Legnaro, Italy*

**Keywords:** Canine Leishmaniosis, Zoonoses, Public Health.

**INTRODUCTION:** Canine Leishmaniosis (CanL) is a zoonotic disease of increasing interest in Italy for its recent spreading to Northern regions (Gradoni et al., 2022. Vet Parasitol Reg Stud Reports, 27:100676), associated with new foci of human infections (Todeschini et al., 2024. Euro Surveill, 29:2300190). Research is continuously ongoing but how its outcomes arrive to the stakeholders is not completely known. This study aims to collect information from veterinarians and pet owners to evaluate their knowledge and awareness of CanL and associated health risks.

**MATERIALS AND METHODS:** Two different questionnaires were prepared and submitted to vets and dog owners from Italy through an online survey software (QualtricsXM) from Aug 2022 to Apr 2023. Data collected from vets included provenance, management (prevention, therapeutic and diagnostic protocols) of CanL cases and their evaluation of pet owner awareness about CanL. Data collected from dog owners were mostly oriented to understanding their awareness of CanL and related pets and human health risks. Data were analysed through descriptive statistics.

**RESULTS AND CONCLUSIONS:** Questionnaires were filled out by 364 vets and 199 dog owners. More than 50% of vets noticed an increase in CanL cases coming mostly (63%) from Central and Southern Italy in the last 5 years. Among preventive measures, 43.6% (126/289) of vets preferably suggest an association of mechanical, chemical, and immunological prophylaxis, mostly recommending effective collars and spot-on against sandflies. Quantitative serological tests (59.1%,162/274) and electrophoresis (53.3%,146/274) are chosen as the first step for laboratory diagnostics and for the follow-up, respectively. Allopurinol and meglumine antimoniate are the elective therapeutic protocol for 73% (198/271) of vets. Most respondent vets (94.8%, 255/269) state dog owners are partially or not informed about CanL, adopt partially appropriate preventive measures, and are fairly or not aware of zoonotic risks. Respondent dog owners (100%,181/181) heard about CanL from their vet (44.8%), internet/mass media (38.1%), and direct experience (11.0%); most of them (80.9%) know the opportunity of CanL prophylaxis. Only 13.8% are highly worried about their health but 41.5% about their pets' health. Numerous owners declare their vets often/always inform them about pet health risks (78.3%,112/143) and preventive measures to adopt (81.8%,117/143); roughly half of them (49.7%) report vets inform them of zoonotic risks deriving from close contact with pets, the other half (50.3%) do it occasionally, rarely or never. To the authors' knowledge, this is the first data collection from Italian stakeholders about CanL. Most vets recommend efficient preventive approaches and adopt appropriate therapeutic and diagnostic protocols, suggesting ongoing high-level updating. Data collected from dog owners highlight the need to increase scientific dissemination to improve their knowledge about CanL and awareness of public health risks.

# EPIDEMIOLOGICAL UPDATE OF CYSTIC ECHINOCOCCOSIS IN LIVESTOCK AND ASSESSMENT OF PRACTICES RELATED TO ITS CONTROL IN THE MEDITERRANEAN AREA

Pepe P.*[1], Nocerino M.[1], Ciccone E.[1,2], Bosco A.[1,2], Boué F.[3], Umhang G.[3], Lahmar S.[4], Said Y.[4], Sotiraki S.[5], Laatamna A.[6], Bambacaro E.[1,2], Lattero N.[1,2], Leone A.[1,2], Mangieri G.[1,2], Quaranta P.[1,2], D'Orilia F.[2], Cringoli G.[1,2], Rinaldi L.[1,2]

*[1]University of Naples Federico II, Department of Veterinary Medicine and Animal Production, CREMOPAR, Naples, Italy; [2]Regional Reference Centre for Animal Health (CRESAN), Naples, Italy; [3]ANSES, Nancy Laboratory for Rabies and Wildlife Diseases, Technopôle agricole et vétérinaire, Malzéville, France; [4]University of Manouba, École Nationale de Médecine Vétérinaire, Sidi Thabet, Tunisia; [5]Veterinary Research Institute, Hellenic Agricultural Organisation-Demeter, Thessaloniki, Greece; [6]University of Djelfa, Faculty of Nature and Life Sciences, Djelfa, Algeria*

**Keywords:** *Echinococcus granulosus*, Epidemiology, One Health.

**INTRODUCTION:** Cystic echinococcosis (CE), caused by the tapeworm *Echinococcus granulosus*, is a zoonotic parasitic disease that still represents a serious threat to human and animal health worldwide (Casulli et al., 2023. Lancet Infect Dis, 23:e95-e107). The Mediterranean basin is recognized as one of the main hotspots of CE due to several factors, including the presence of diverse intermediate host species as well as socio-economic and cultural conditions of local communities (Borhani et al., 2020. PLoS Negl Trop Dis, 14(5): e0008114). This study aims to take a closer look at epidemiological data on CE in the Mediterranean area and assess the knowledge attitudes and practices of shepherds towards this disease in four countries (Algeria, Greece, Italy and Tunisia), highly endemic for CE, with the final goal of identifying highly endemic risk areas and practices in use which might potentially allow the persistence of *E. granulosus* infection in these areas.

**MATERIALS AND METHODS:** A comprehensive review of peer-reviewed literature on CE prevalence data published during the 2017-2023 period was carried out and, through a geographical information system (GIS), a map displaying the current CE distribution in the Mediterranean area was generated. In addition, a questionnaire survey was conducted through in-depth interviews of the farmers to collect information on their management system as well as knowledge attitudes and practices towards CE.

**RESULTS AND CONCLUSIONS:** The data obtained showed a prominent presence of *E. granulosus* infections in the Mediterranean region with high prevalence rates, although a decreasing trend in the number of CE cases was observed in ruminants in southern Europe, especially in southern Italy (Campania region) and Greece (Central Greece, Macedonia, Thessaly and Thrace). In particular, recent updated reports on the endemic status in Campania region showed a prevalence value of 9.7% in sheep, which is lower than that observed in prior epidemiologic investigation in the same study areas, 52.5% (Cringoli et al., 2021. Vet Parasitol, 290:109347), resulting in a noteworthy reduction of the parasite infection rates (e.g. up to 80%). From the farmer-participatory survey some risky practices emerged including the non-regular deworming of dogs (54.8%) or the use of ineffective drugs or dosing, as well as the provision of uncooked animal viscera to dogs (53.0%). Finally, lower levels of knowledge and awareness of the disease was observed among farmers from North Africa compared with those of European countries. In conclusion, the results obtained highlight that CE is still a very serious problem in Mediterranean areas and increased efforts are needed to promote awareness among farmers and to turn research results into policy in order to reduce the spread of this disease, according to the One Health perspective.

# *ECHINOCOCCUS GRANULOSUS SENSU STRICTO* IN ALGERIA: ASSESSING GENETIC DIVERSITY THROUGH NAD5 AND COX1 MITOCHONDRIAL DNA REGIONS

Chiovoloni C.*[1], Kheninef A.[2], Rondón S.[1], Bellini I.[1], Cavallero S.[1], D'Amelio S.[1]

*[1]Department of public health and infectious diseases, Sapienza University of Rome, Rome, Italy; [2]Laboratory of amelioration and development of plant and animal production, University of Setif, Setif, Algeria*

**Keywords:** *Echinococcus granulosus*, Algeria, Mithocondrial markers.

**INTRODUCTION:** Cystic echinococcosis (CE), caused by *Echinococcus granulosus*, is a widespread parasitic zoonosis, endemic worldwide, posing a significant public health threat particularly in rural communities (Deplazes et al., 2017. Adv Parasitol, 95:315-493). Algeria exhibits a high endemicity of CE, however epidemiological and molecular data is fragmented and geographically segregated. Previous literature based on mitochondrial cytochrome c oxidase subunit 1 (cox1) gene analysis highlights the predominance of G1 genotype in all intermediate hosts, including humans (Moussa et al., 2021. Parasitol Res, 120:3195-3202; Laatamna et al., 2019. Parasitol Res, 118:89-96). Aiming at investigating genotypic diversity, hydatid cyst sampling was conducted in rural areas of the Setif region in Algeria and analyzed using two mitochondrial genetic markers, cox1 and nad5.

**MATERIALS AND METHODS:** A total of 47 cysts were collected from ovine, bovine, goat and from human intermediate hosts. PCR was used to amplify partial fragments of the mitochondrial cox1 and nad5 genes, useful to identify strains (Bart et al., 2006. Parasitology, 133:571-79; Kinkar et al., 2018. Infect Genet Evol, 64:178-84). Cyst fertility was determined based on the presence of protoscoleces. Cox1 and nad5 sequences analyses and median-joining networks were performed to explore strain identity, and datasets from GenBank were used for comparison with available homologous sequences of *E. granulosus s.s.* circulating in Algeria.

**RESULTS AND CONCLUSIONS:** Readable nucleotide sequence alignments were obtained for 42 and 27 samples for cox1 and nad5, respectively. Haplotype G1 predominated, constituting 59.52% (cox1) and 51.85% (nad5) of the population. Microvariants accounted for 38.09% (cox1) and 44.44% (nad5) of the population, while the G3 genotype was observed in one human sample alone, as confirmed by both markers. Human and ovine cysts exhibited 100% fertility, while cattle cysts showed a lower fertility rate of 62.50%. The obtained data confirmed the dominance of the G1 strain of *E. granulosus s.s.* in intermediate hosts in Algeria, consistently with previous reports (Moussa et al., 2021. Parasitol Res, 120:3195-3202). The detection of the G3 haplotype in humans, previously limited to the central-desertic region (Kinkar et al., 2018. Parasitology, 145:1613-22), expands its distribution to the northern rural area of Algeria as well. The network showed a close vicinity of the G3 haplotype to those found in camels, suggesting a potentially greater role of this host on the parasite life cycle. A lower fertility of the cysts found in cattle aligns with their minor role in CE transmission among the examined livestock (Romig et al., 2017. Adv Parasitol, 95:213-314).

# ENTOMOLOGICAL AND EPIDEMIOLOGICAL ONE HEALTH APPROACHES SURVEY IN TWO ITALIAN *LEISHMANIA INFANTUM* ENDEMIC FOCI IN THE CONTEXT OF CLIMOS EUROPEAN PROJECT

Bongiorno G.*[1], La Russa F.[2], Bernardini I.[1], Bianchi R.[1], Mangiapelo C.[1], Scalone A.[1], Orsini S.[1], Gizzarelli M.[3], Perroni M.[4], Sanfilippo A.[5], Vitale F.[6], Oliva G.[3], Foglia Manzillo V.[3]

*[1]Department of Infectious Diseases, Vector-Borne Diseases Unit, National Institute of Health, Rome, Italy; [2]Laboratory of Entomology, Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy; [3]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy; [4]Department of Prevention, U.O.C. Veterinary Service, Animal Health And Farm Igiene Area, ASL of Viterbo, Tarquinia, Italy; [5]Veterinary Medical Director Municipal kennels of Sciacca, Agrigento, Italy; [6]National Reference Center for Leishmaniasis (C.Re.Na.L.), Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy*

**Keywords:** Sand fly, Canine leishmaniasis, One Health.

**INTRODUCTION:** In the context of the European Project CLIMOS "Climate Monitoring and Decision Support Framework for Sand Fly-borne Diseases Detection and Mitigation with COst-benefit and Climate-policy MeasureS", the aim is to assist mitigation of climate and vector-borne and zoonotic diseases emergences, transmission and spread, based on Eco-health and One Health approaches by quantifying climate and environmental-related drivers of sand fly vector populations and sand fly-borne diseases across Europe (Wint et al., 2023. Euro Surveill, 28:2200666).

**MATERIALS AND METHODS:** In 2023 entomological and canine serological surveys were conducted in Tarquinia and Sciacca (in Latium and Sicily regions respectively), to confirm *Leishmania infantum* endemicity (Maroli et al., 2008. Trop Med Int Health, 13:256-64). Sand fly collections were performed monthly by CDC light traps while dogs blood samplings twice in early June and late October.

**RESULTS AND CONCLUSIONS:** Preliminary results reported a total of 765 sand fly specimens in both sampled sites, belonging to two genus: *Phlebotomus perniciosus* showing higher prevalences (33%) followed by *Ph. perfiliewi* (27%), *Sergentomyia minuta* (25%) and *Ph. papatasi* (1%). In Tarquinia, *Ph. perfiliewi* was the prevalent species (63%) followed by *Ph. perniciosus* (30%) and *Se. minuta* (6%); while in Sciacca *Ph. perniciosus* registered higher prevalence (38%) followed by *Se. minuta*, *Ph. perfiliewi* and *Ph. papatasi* (31%, 26% and 1%, respectively). Blood samples were obtained from kenneled and owned dogs living in the same areas in which sand fly traps had been placed. Antibodies against *Leishmania infantum* were detected by IFAT (cut off: 1:80). Canine seroprevalence was higher in Sciacca than in Tarquinia ($p < 0.0001$), starting from 40% of positive dogs and reaching 74% at the end of sand flies season ($p < 0.0001$). In June sampling antibody titers ranged from 1:80 to 1:160 except for 1 symptomatic dog (1:1280). Seven new positive dogs out of 31 (17.5%) were detected in October and 9 (56.2%) increased their antibody titers by 2-3-fold dilution. The Tarquinia site showed five out of 36 dogs (13.8%) positive during June sampling, one symptomatic (IFAT result: 1:1280). In October, 3 new positives were detected (9.6%). One previously positive dog seroconverted negative. Although geographically distant, the two sites are confirmed as endemic areas, Sciacca could be identified as a hyper endemic focus for *L. infantum* due to both *Ph. perniciosus* higher prevalence and higher seroprevalence reported in dogs. The work was co-funded: the CLIMOS consortium is funded by the European Commission grant 101057690 and UKRI grants 10038150 and 10039289, and the NextGeneration EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases is funded by the EU (Project no. PE00000007, INF-ACT). We further acknowledge the six Horizon Europe projects, BlueAdapt, CATALYSE, CLIMOS, HIGH Horizons, IDAlert, and TRIGGER, which form the Climate Change and Health Cluster funded by the EU.

# ENHANCED TRANSCRIPTOMIC RESOURCES FOR *TRICHINELLA PSEUDOSPIRALIS* AND *T. SPIRALIS* UNDERPIN AN EXPLORATION OF MOLECULAR DIFFERENCES BETWEEN STAGES AND SPECIES

Currà C.*[1], Korhonen P.[2], Chang B.[2], Gomez Morales M.A.[1], La Rosa G.[1], Ludovisi A.[1], Pozio E.[1], Sumanam S.[2], Tonanzi D.[1], Tosini F.[1], Young N.[2], Gasser R.[2]

*[1]European Union Reference Laboratory for Parasites. Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; [2]Department of Veterinary Biosciences, Melbourne Veterinary School, School of Veterinary Science, University of Melbourne, Melbourne, Australia*

**Keywords:** Transcriptomics, *Trichinella spiralis*, *Trichinella pseudospiralis*.

**INTRODUCTION:** High-quality draft genomes exist for selected species/genotypes of *Trichinella* genus (Thompson et al., 2017. Parasitology, 144:1302-15). However, there have been limited detailed studies of transcriptomic and proteomic data sets, representing distinct developmental stages of these taxa, to understand molecular alterations/variations occurring between such life cycle stages within individual species. The goal of this study is to (i) markedly enhance transcriptomic resources for selected representatives - *T. pseudospiralis* (non-encapsulated) and *T. spiralis* (encapsulated); (ii) characterize and compare the composition of the transcriptomes of these two representatives; (iii) study transcriptional variation and/or differences among selected developmental stages for each of these species and link these differences to respective stage-specific biological pathways or processes.

**MATERIALS AND METHODS:** Muscle larvae (L1), adults and new borne larvae (NBLs) of *T. spiralis* and *T. pseudospiralis* were produced at the International Trichinella Reference Center (Rome, Italy). Total RNA was isolated from 3/4 biological replicates of each stage and species. cDNA libraries were built according to the manufacturer's instructions and then sequenced on an Illumina HiSeq™ 4000 instrument. Full-length genes were then annotated using InterPro v5.51-85.0 and BLAST database UniProt/SwissProt (Magrane and Consortium 2011), eukaryotes in KEGG and NCBI NR, and proteomes of both species in WormBase (v. WBP18). Differential transcription analysis was performed.

**RESULTS AND CONCLUSIONS:** A list of predicted genes encoding for proteins specific to both *T. spiralis* and *T. pseudospiralis* was generated for each stage of development. Functional analysis of predicted transcripts showed common features in the excretory/secretory (ES) products of the 3 stages but there was a tendency for less ES genes transcribed in NBL stage when compared to L1 and adult stages. Interestingly, 130 in *T. spiralis* versus 40 transcripts in *T. pseudospiralis* were associated to secretion, increasing our understanding in the modulation of the interaction host-pathogen. In agreement to both species, the subcellular localization of predicted transcripts was mainly extracellular or in cell membrane. Comparing different stages in both species, while in L1 genes related to genetic processes and peptidases were found, in adults we identified transcripts associated to RNA processing and lipid metabolism. In NBLs, genes associated with cellular processes were mainly found. Our study produced an extremely detailed dataset of transcripts with prediction for stages and species-specific proteins involved in biological processes of *Trichinella*, providing the basis for further studies on the host-pathogen interaction and development of new strategies for the control of trichinellosis.

# EPIDEMIOLOGICAL STUDY ON THE PRESENCE OF *SARCOCYSTIS* SPP. IN FATTENING PIGS FROM NORTHERN ITALY: A MOLECULAR APPROACH

Gazzonis A.L.*[1], Rubiola S.[2], Villa L.[1], Pasquariello L.[2], Civera T.[2], Chiesa F.[2], Manfredi M.T.[1]

*[1]Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Lodi, Italy; [2]Department of Veterinary Sciences, University of Turin, Grugliasco, Italy*

**Keywords:** *Sarcocystis miescheriana*, Pigs, Diagnostic.

**INTRODUCTION:** Sarcocystosis, caused by protozoan parasites of the *Sarcocystis* genus, often receives scarce attention as a potential food borne disease, yet it may pose a significant threat to food safety due to the potential for human infection through the consumption of raw/undercook contaminated meat. Of the over 200 species identified, *Sarcocystis miescheriana* and *Sarcocystis suihominis* utilize pigs as intermediate hosts, with canids and human/nonhuman primates as their definitive hosts, respectively. While epidemiological data concerning the spread of *Sarcocystis* spp. in wild suids have indicated the presence of the zoonotic species *S. suihominis* in Italy (Gazzonis et al., 2019. Parasitol Res, 118:1271-87), information regarding the presence of *Sarcocystis* sp. in Italian pigs is limited. To address this gap, a molecular epidemiology study has been planned to update the epidemiological data on the presence of *Sarcocystis* sp. and to identify the risk factors influencing its spread among pigs from intensive farming in Lombardy, one of Italian primary regions for pig industry.

**MATERIALS AND METHODS:** Cardiac muscle samples from 201 apparently healthy fattening pigs raised in 16 different farms in Lombardy were collected during slaughtering operations, submitted to DNA extraction and screened for the presence of *Sarcocystis* spp. by PCR targeting the 18S rRNA gene. Amplification products were sequenced for species identification. Samples revealing the presence of *S. miescheriana* DNA were further molecularly characterized amplifying and sequencing the cox1 mtDNA gene. Data on farm management and sanitary procedures applied were collected by interviewing the farmer and statistically analyzed through Generalized Linear Mixed Models (GLMM) to verify their influence on *Sarcocystis* infection.

**RESULTS AND CONCLUSIONS:** Out of 201 cardiac muscle samples of fattening pigs, 31 tested positive for *Sarcocystis* spp. DNA through the partial amplification of the 18S rRNA gene (15.42%, CI95%: 10.73-21.17); 14 out 16 farms scored at least one positive animals, with an intra-herd prevalence ranging from 0 to 30.8%. Sanger sequencing results confirmed the identification of *S. miescheriana* in 7 out of 31 samples. The amplification and sequencing of the cox1 gene resulted in 1043-1044 bp sequences showing 98.8-98.9% identity with *S. miescheriana* GenBank entries. Any of the considered variables showed a statistically significant association with the infection. The present study presents the first molecular examination of *Sarcocystis* spp. occurrence in Italian fattening pigs, demonstrating how *Sarcocystis* infection is ubiquitously widespread in intensive pig farming at both individual and farm level. Although the zoonotic *S. suihominis* was absent, suggesting a low risk for consumers, obtained data confirmed the pivotal function of slaughterhouses as epidemiological monitoring centers and emphasize the necessity for enhanced data collection on *Sarcocystis* spp. prevalence in domestic pigs.

# *TOXOPLASMA GONDII* AND *SARCOCYSTIS* SPP.: EXPLORING HIDDEN DANGERS IN LARGE-SCALE RETAIL HORSE MEAT

Gazzonis A.L.*, Cafiso A., Buffa E., Manfredi M.T.

*Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Lodi, Italy*

**Keywords:** *Sarcocystis fayeri*, *Toxoplasma gondii*, Horse meat.

**INTRODUCTION:** Foodborne diseases are receiving increasing attention in recent decades due to shifts in food processing and dietary habits, including the popularity of “ready-to-eat” food and the consumption of raw or undercooked meat. While regulations and standardized inspections of horse meat exist for some pathogens as *Trichinella* spp., protocols for the detection during slaughter are totally lacking for *Toxoplasma gondii*, or limited to the species forming macroscopic cysts for *Sarcocystis*. This gap persists despite documented human infections linked to the consumption of raw or undercooked horse meat (Kamata et al., 2014. J Food Prot, 77:814-9), and the scarcity of epidemiological data on *T. gondii* and *Sarcocystis* spp. infections in horses further complicates risk assessment. Therefore, this study aims to investigate the presence of these parasites in horse meat distributed through large-scale retail channels and estimate the associated risk for consumers.

**MATERIALS AND METHODS:** 110 pre-packaged cuts of meat samples from 5 commercial brands were collected in 12 supermarkets in Lombardy (Northern Italy). Meat juice samples were tested for the presence of anti-*T. gondii* antibodies using a commercial ELISA. DNA was extracted from muscle samples and used for the detection of the presence of the two parasites. Two real-time PCRs protocols targeting the B1 and 529 bp-RE genetic markers were performed for *T. gondii*, while qualitative PCRs targeting 18S rDNA and COX1 genes were carried out for *Sarcocystis* spp. A subset of positives was subjected to Sanger sequencing. Potential risk factors associated with the presence of the two pathogens (e.g., supermarket, commercial brand, and cut of meat) were evaluated using the $\chi^2$ test.

**RESULTS AND CONCLUSIONS:** For *T. gondii*, a seroprevalence of 11.8% (12/102) was observed, while no positives were detected through molecular analyses. *Sarcocystis* spp. DNA was highlighted in 63.6% (70/110) of the samples; COX1 sequences of 20 randomly selected samples confirmed 99-100% identity with *S. fayeri*. Statistical analysis revealed a significant difference among the different cuts of meat, indicating that those intended for raw or undercooked consumption are more at risk of testing positive for anti-*T. gondii* antibodies (*p*-value=0.019). The study highlights the presence of *T. gondii* and *Sarcocystis* sp. in horsemeat intended for large scale retail in Italy. As previously reported in horses, there was a limited concordance between serological and molecular data for *T. gondii*. Statistical analysis suggested a potential suitability of certain muscles in detecting anti-*T. gondii* antibodies. The presence of *S. fayeri* in over 60% of samples suggests a potential risk of food poisoning for consumers, especially considering recent trends favoring raw or undercooked horse meat consumption. These results underscore the necessity of establishing surveillance plans to detect and identify these pathogens, particularly in ready-to-eat large-scale retail products intended for raw consumption.

# TOXOPLASMOSIS: RISK FACTORS, CLINICAL SIGNS AND SEROLOGICAL STATUS IN CATS LIVING WITH WOMEN OF CHILDBEARING AGE

Traversa D.[1], Morelli S.[1], Astuti C.*[1], Di Cesare A.[1], Colombo M.[1], Iorio R.[1], Pagliaccia A.[1], Catalano C.[1], Paoletti B.[1], Brueckmann R.[2]

*[1]University of Teramo, Department of Veterinary Medicine, Teramo, Italy; [2]Institute for Experimental Immunology, affiliated to EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany*

**Keywords:** Toxoplasmosis, Serology, One Health.

**INTRODUCTION:** Toxoplasmosis is an important zoonosis caused by the apicomplexan protozoan *Toxoplasma gondii*, which is a health threat especially for of immunocompromised people and pregnant women (Jones et al., 2018. Am J Trop Med Hyg, 98:551-57). Domestic cats become infected when preying on intermediate hosts, and/or with the ingestion of raw or undercooked meat or sporulated oocysts from the environment. The present study aimed to investigate the frequency of acute toxoplasmosis and potential risk factors associated with the anti-*T. gondii* seropositivity in domestic cats with compatible clinical signs and living with women of childbearing age.

**MATERIALS AND METHODS:** Individual sera samples of 150 cats from Italy were tested to detect IgM and IgG antibodies against *T. gondii* using the Anti-*Toxoplasma gondii* IIFT Cat (IgG/IgM) (EUROIMMUN, Germany). The presence of significant associations between seropositivity and potential risk factors was statistically evaluated using the Fisher's exact test and the binomial logistic regression.

**RESULTS AND CONCLUSIONS:** A total of 31 (20.7%) cats were seropositive for antibodies against *T. gondii*. Out of them, 9 (6%) were positive for IgM only, 17 (11.3%) for IgG only, and 5 (3.3%) for both IgM and IgG. Overall, 14 cats (9.3%) had clinical signs and serological status compatible with acute toxoplasmosis. Significant statistical associations were positivity for IgM and/or IgG and male sex (p=0.0248), positivity for IgM and presence of 2 or more compatible clinical signs (p=0.0003), positivity for IgM and presence of neurological (p= 0.0025) or ocular (p= 0.0228) signs, and positivity for IgG and presence of gastrointestinal signs (p=0.0083). These results demonstrate that cats living with women of childbearing age may have acute toxoplasmosis and potentially shed oocysts in the environment, which pose a major risk for humans. In fact, infected cats seroconvert after they have shed the oocysts or during the last days of oocyst shedding. The diagnosis of clinical toxoplasmosis in infected cats is difficult as the clinical signs are non-specific, if present at all (Calero-Bernal et al., 2019. Front Vet Sci, 6:54). However, serological tests in cats with compatible clinical signs can be useful to identify recent and/or current infections by *T. gondii* and to obtain information on possible oocyst shedding timing. Further studies should integrate molecular techniques and copromicroscopy to confirm the factual occurrence of active infections and oocyst shedding. It is important for owners and veterinarians to maintain a high level of vigilance and awareness of toxoplasmosis to put in place preventive and therapeutic strategies to protect both animal and human health.

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# INSIGHT INTO PHYSICAL AND IMMUNOMODULATORY PROPERTIES OF HELMINTHS EXTRACELLULAR VESICLES: THE MODEL OF *ANISAKIS* SPP. AND HUMAN INTESTINAL ORGANOIDS

Bellini I.*[1], Scribano D.[1], Ambrosi C.[2], Chiovoloni C.[1], Rondòn S.[1], Pietrantoni A.[3], Kashkanova A.[4], D'Amelio S.[1], Cavallero S.[1]

*[1]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy; [2]Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Open University, IRCCS, Rome, Italy; [3]Core facilities, Istituto Superiore di Sanità, Rome, Italy; [4]Max Planck Institute for the Science of Light, Erlangen, Germany*

**Keywords:** *Anisakis*, Extracellular vesicles, Human intestinal organoids.

**INTRODUCTION:** The role of helminths derived extracellular vesicles (EVs) in manipulating host's immune response is well established (Drurey et al., 2021. Mol Immunol, 137:124-33). However, hurdles remain to describe their physical properties and precise biological effects due to challenges in parasitic EVs isolation, characterization and in the selection of suitable biological models for functional studies (White et al., 2023. J Extracell Vesicles, 12(1): 12298). Here, EVs released by the zoonotic marine nematode *Anisakis* spp. were isolated and characterized in terms of size, concentration, inner water content and protein amount. Thus, to test their immunomodulatory properties in the host, for the first time, *Anisakis* EVs were incubated with human intestinal organoids (HIO), an in vitro 2D-3D multicellular structure that mimic the corresponding *in-vivo* organ at a morphological and functional level (Clevers et al., 2016. Cell, 165:1586-97). The aims of this study were to add new information about physical properties of *Anisakis* EVs, exploiting different and innovative approaches and to investigate their immunomodulatory activity through the detection of cytokine response in a biological model that faithfully recreate the real niche of infection.

**MATERIALS AND METHODS:** A total of 140 *Anisakis* spp. L3 were collected from fish visceral cavities and cultured in RPMI 1640 in 1x P/S at 37°C with 5% CO2 for 24h. EVs were isolated using Exoquick kit and characterized through Nanoparticle Tracking Analyses (NTA) and Interferometric Nanoparticle Analysis (iNTA), an innovative technique never used on helminths EVs. Morphology was evaluated by Scanning and Transmission Electron Microscopy (SEM, TEM). EVs were incubated with HIO for 48h, and qRT-PCR and Luminex assay were used to estimate inflammatory cytokines amounts.

**RESULTS AND CONCLUSIONS:** 2,92 x 109 particles with a median diameter of 107 nm were detected using NTA and iNTA. The iNTA also revealed an inner part of *Anisakis* EVs that could contain 5%-20% of non-water material, suggesting their effective biological cargo, consistently with estimates previously obtained only for EVs of *Leishmania* parasites (Kashkanova et al., 2022. Nat Methods 19, 586-93). HIO treated with *Anisakis* EVs showed a significantly reduced concentration in specific mediators of inflammation and cell cycle regulation as IL-33R, CD40, CEACAM-1, IL-1b, GM-CSF, IL-15 and IL-23. These factors are often associated with anti-helminthic strategies, confirming parasite immunomodulatory effect in the accidental human host, potentially allowing *Anisakis* to establish and persist in its multicellular niche. Furthermore, the use of an innovative EVs characterization technique will be useful also for the study of other helminths EVs, since the knowledge in this field is very limited.

# *IN VIVO* ASSESSMENT OF EFFICACY OF THIOSEMICARBAZONES IN MICE EXPERIMENTALLY INFECTED WITH *TOXOPLASMA GONDII* OOCYSTS

Semeraro M.*[1], Boubaker G.[2], Scaccaglia M.[3], Imhoff D.[2], De Sousa M.C.[2], Haenggeli K.[2], Vigneswaran A.[2], Genchi M.[1], Kramer L.H.[1], Pelosi G.[3], Bisceglie F.[3], Armando F.[1], Vismarra A.[3], Hemphill A.[2]

*[1]University of Parma, Department of Veterinary Science, Parma, Italy; [2]University of Bern, Institute of Parasitology, Bern, Switzerland; [3]University of Parma, Department of Chemistry, Life Sciences and Environmental Sustainability, Parma, Italy*

**Keywords:** *Toxoplasma gondii*, Thiosemicarbazones, *In vivo*.

**INTRODUCTION:** Thiosemicarbazones and their metal complexes have been reported to have a wide range of *in vitro* biological activities, as against cancer cells, bacteria, and protozoan parasites (Beraldo et al., 2004. Mini-Rev Med Chem, 4:31-9; Pelosi, 2010. The Open Cristallogr Journal, 3:16-28). In a previous study, *in vitro* three gold (III) thiosemicarbazone complexes (C1-C3) and one ligand (C4) were screened for their activity on *Toxoplasma gondii* and toxicity on host cells. Two compounds: one gold (III) complex (C3) and its salicyl-thiosemicarbazone ligand (C4) selectively inhibited the proliferation of *T. gondii* RH with half-maximal inhibitory concentrations (IC50) in the range of ~ 50 nM, while the viability of host cells was not affected at concentrations up to 25 μM (Semeraro et al., 2024. ACS Infect Dis, under revision). Moreover, neither compound impaired activation and proliferation of *in vitro* stimulation B- and T cells. Thus, this study aimed to determine the *in vivo* efficacy of C3 and C4 in the murine model of cerebral toxoplasmosis.

**MATERIALS AND METHODS:** Eight-week-old female CD-1 mice were experimentally infected by per-oral application of 120 TgShSp1 (type II) oocysts. Oral C3 and C4 (10mgkg-1/day) treatments were initiated 3 days post-infection and continued for five doses (one per day). After four weeks mice were euthanized and parasite burden in different organs was determined by quantitative real-time PCR (qPCR).

**RESULTS AND CONCLUSIONS:** Results from qPCR analysis revealed no significant difference in parasite load in the brains and eyes of treated mice compared to untreated controls. However, C4-treated mice exhibited significantly lower parasite burden in the heart compared to the control (untreated mice) and C3-treated groups. In conclusion, studied compounds are not effective against infection *in vivo*, since the number of cysts in the brain and eyes was not significantly reduced. This is probably due to the inability of C3 and C4 to cross the blood-brain barrier, similar to most of the drugs used for the treatment of toxoplasmosis. A pharmacokinetic study will clarify how much of the compounds are metabolized and if there could be an effect on *T. gondii* changing the route of administration.

# IMMUNOMODULATORY ACTIVITY OF *HELICOBACTER PYLORI* NEUTROPHIL ACTIVATING PROTEIN (HP-NAP) ON- INTRAMACROPHAGE AMASTIGOTES OF *LEISHMANIA INFANTUM* IN A CANINE MODEL

Mazza G.*[1], Proverbio D.[2], Parapini S.[3], Basilico N.[1], Taramelli D.[4], D'Elios M.M.[5], De Bernard M.[6], Bruschi F.[7]

*[1]University of Milan, Department of Biomedical, Surgical and Dental Sciences, Milan, Italy; [2]University of Milan, Department of Veterinary Medicine and Animal Science, Veterinary Transfusion Research Laboratory, Milan, Italy; [3]University of Milan, Department of Biomedical Sciences for Health, Milan, Italy; [4]University of Milan, Department of Pharmacological and Biomolecular Sciences, Milan, Italy; [5]University of Siena, Department of Molecular and Developmental Medicine, Siena, Italy; [6]University of Padova, Department of Biology, Padova, Italy; [7]University of Pisa, Department of Transaltional Research, N.T.M.S, Pisa, Italy*

**Keywords:** *Leishmania* spp., HP-NAP, Cytokines.

**INTRODUCTION:** Leishmaniasis is a neglected tropical disease caused by an obligate intracellular, protozoan parasite of the genus *Leishmania*. Its manifestations vary from cutaneous to potentially fatal visceral forms depending on the infecting species and the host's immune response. The resolution of *Leishmania* infections depends primarily on a type I immune response characterized by the production of interleukin-12 (IL-12) which activates T cells to secrete IFN-γ, which in turn stimulates the microbicidal mechanisms of macrophages. *Helicobacter pylori* Neutrophil Activating Protein (HP-NAP) is a bacterial protein shown to possess both pro-inflammatory and immunomodulatory properties. HP-NAP can stimulate neutrophils to produce oxygen radicals and inflammatory cytokines. Its role in macrophage activation against *Leishmania* is not known. The aim of the present study was to investigate the *in vitro* effects of HP-NAP on *L. infantum*-infected macrophages.

**MATERIALS AND METHODS:** Peripheral blood mononuclear cells (PBMC) were obtained from heparinized whole blood samples collected from healthy dogs. PBMC were isolated through density gradient centrifugation on Ficoll-Hypaque. Monocytes were purified by adherence to Lab-Tek chamber slides, then treated with 0.1 μM of PMA for 72 h to achieve differentiation into macrophages. Canine macrophages were infected with stationary phase promastigotes of *L. infantum* (promastigote: cell ratio 10:1) for 24 h. Infected macrophages were then treated with HP-NAP (20-1.25 μg/mL) for 72 h. At the end of the incubation, the slides were fixed and stained with Giemsa. The percent of infected macrophages and the mean number of amastigotes per cells were calculated. The production of IL-12 and IL-10 by *L. infantum* -infected macrophages treated with HP-NAP was evaluated in the supernatants by ELISA.

**RESULTS AND CONCLUSIONS:** Canine macrophages heavily phagocytize *L. infantum* promastigotes. After 24 h of infection, the percentage of infected macrophages and the mean number of amastigotes per cells were 82±16 and 7.4±2, respectively. Treatment with 20 μg/mL HP-NAP reduced the percentage of infected macrophages to 68±14 and the mean number of amastigotes per cell to 4.8±0.9. Consequently, the infection index, calculated as the percentage of infected macrophages multiplied by the number of amastigotes per infected macrophage, decreased from 608 in controls to 332 in HP-NAP-treated samples. The decrease in macrophage infection was dependent on the concentration of HP-NAP. IL-12, a cytokine that drives anti-leishmanial T helper 1-type immune response, was induced by HP-NAP in infected macrophages, differently from IL-10 which remained undetectable under all experimental conditions. These data suggest that HP-NAP can inhibit parasite growth and induce the secretion of IL-12, a critical cytokine for macrophage resistance to intracellular pathogens.

# PREVALENCE OF FEMALE GENITAL SCHISTOSOMIASIS AND ACCEPTABILITY AND PERFORMANCE OF OPERATOR-COLLECTED AND SELF-COLLECTED VAGINAL SWABS FOLLOWED BY PCR AMONG WOMEN LIVING IN NORTH-WESTERN TANZANIA

Scarso S.*[1], Ursini T.[1], Mugassa S.[2], Othman J.B.[3], Mazzi C.[1], Leonardi M.[1], Prato M.[1], Pomari E.[1], Mazigo H.D.[2], Tamarozzi F.[1]

*[1]Department of Infectious, Tropical Diseases and Microbiology, IRCCS Sacro Cuore Don Calabria Hospital, Negrar di Valpolicella, Italy; [2]School of Public Health, Catholic University of Health and Allied Sciences, Mwanza, Tanzania, United Republic of Tanzania; [3]Department of Medical Parasitology, Catholic University of Health and Allied Sciences, Mwanza, Tanzania, United Republic of Tanzania*

**Keywords:** Female Genital Schistosomiasis, Molecular diagnosis, *Schistosoma haematobium*.

**INTRODUCTION:** Female genital schistosomiasis (FGS) is a neglected disease caused by infection with *Schistosoma haematobium*. The World Health Organization has prioritized the improvement of diagnostics for FGS and previous studies provided promising results on the use of PCR-based detection of *Schistosoma* DNA on genital specimens. We assessed the prevalence of FGS among women living in an endemic district in Tanzania, applying and preliminary comparing self-collected and operator-collected cervical-vaginal swabs followed by PCR, and explored the acceptability of these sampling procedures.

**MATERIALS AND METHODS:** A cross-sectional study was conducted involving 211 women from two villages in the Maswa District, located in North-western Tanzania. We collected urine samples, along with both self-collected and operator-collected cervical-vaginal swabs. A detailed questionnaire was also administered to assess the participants' comfort levels with the various diagnostic procedures. All collected DNA was initially isolated from the genital swabs and then transported at room temperature to Italy for further PCR analysis.

**RESULTS AND CONCLUSIONS:** Prevalence of urinary schistosomiasis, as assessed by eggs in urine, was 8.5% (95%CI: 5.1-13.1). DNA was pre-isolated from genital swabs and transported at room temperature to Italy for molecular analysis. Prevalence of active schistosomiasis, urinary schistosomiasis, and FGS were 10.0% (95%CI: 6.3-14.8), 8.5% (95%CI: 5.1-13.1), and 4.7% (95%CI: 2.3-8.5), respectively. When real-time PCR was performed after a pre-amplification step, the prevalence of active schistosomiasis increased to 10.4% (95%CI: 6.7-15.4), and FGS to 5.2% (95%CI: 2.6-9.1). Of note, more cases were detected by self-collected than operator-collected swabs. The vast majority of participants (95.3%) declared that they were comfortable/very comfortable about genital self-sampling, which was indicated as the preferred sampling method by 40.3% of participants. The results of this study show that genital self-sampling followed by pre-amplified PCR on room temperature-stored DNA is a useful method from both technical and acceptability point of views. This encourages further studies to optimize samples processing, and identify the best operational flow to allow integration of FGS screening into women health programmes, such as HPV screening.

# EFFECTS OF MALARIA PIGMENT HEMOZOIN ON MACROPHAGE PLASTICITY IN AN *IN VITRO* MODEL OF PRIMARY HUMAN MONOCYTE-DERIVED MACROPHAGES

Perego F.*[1], Ghezzi S.[2], Vicenzi E.[2], Poli G.[3], Basilico N.[1], D'Alessandro S.[4]

*[1]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy; [2]Viral Pathogens and Biosafety Unit, Division of Immunology, Transplantation, and Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy; [3]Vita-Salute San Raffaele University School of Medicine, Milan, Italy; [4]Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy*

**Keywords:** *Plasmodium falciparum*, Hemozoin, Macrophage plasticity.

**INTRODUCTION:** Malaria is a vector-borne parasitic disease caused by a protozoan parasite belonging to the genus *Plasmodium*. Among the six species causing disease in humans, *P. falciparum* is the most lethal. During erythrocytes' invasion, to avoid the toxic effects of free heme derived from haemoglobin catabolism, parasites synthetize hemozoin (HZ). After the rupture of infected red blood cells, HZ is released in the bloodstream and ingested by phagocytic cells. Among the latter, macrophages represent one of the major cell types involved in innate immune response. Macrophages can be polarized to a multitude of intermediate phenotypes between two extreme subsets: M1 (pro-inflammatory) and M2 (anti-inflammatory, tissue-remodelling). Modulation of macrophages plasticity is a key point to determine the outcome of the disease. Despite evidence of HZ to be a potent modulator of the immune response, if and how it can interfere with macrophages plasticity is still little known. Thus, the aim of this project is to unravel the effects of HZ from *P. falciparum* in an *in vitro* model of primary human monocytes-derived macrophages polarization.

**MATERIALS AND METHODS:** Primary human monocyte-derived macrophages (MDM) were differentiated from peripheral blood monocytes of healthy donors; they were polarized towards M1 pro-inflammatory phenotype (IFNɣ+TNFα), M2 anti-inflammatory phenotype (IL-4) or left unpolarized (M0) in presence or not of hemozoin from *P. falciparum*. After 24h incubation, images were taken with an optical microscope to confirm HZ phagocytosis in all the conditions tested; mRNA levels of TNFA, IL1B, PPARG and ARG1, genes typically modulated in M1 or M2 macrophages, were evaluated by Real-Time PCR; IL-1β, IL-10, IL-6, CXCL8 concentrations in supernatants were quantified by ELISA.

**RESULTS AND CONCLUSIONS:** Stimulation of MDM with IFNɣ+TNFα (M1) significantly increased TNFA and decreased PPARG mRNA levels. In contrast, cell stimulation with IL-4 (M2) significantly induced PPARG, confirming macrophage polarization towards M1 and M2 phenotypes, respectively. HZ induced significantly higher levels of TNFA mRNA in M0 and IL1B mRNA both in M0 and M1 phenotypes. A slight, non-significant increase was also observed in TNFA and IL1B gene expression in both M1 and M2 phenotypes. These results suggest a pro-inflammatory effect of malaria pigment independent from the initial inflammatory state of macrophages. Conversely, both PPARG as well as ARG1 mRNA levels were not modulated by HZ in all the conditions tested. The levels of the pro-inflammatory CXCL8 chemokine in the supernatants were reduced in both M1 and M2 MDM vs. M0. In contrast, CXCL8 secretion was increased by HZ independently from macrophage polarization. The concentrations of IL-1β, IL-10, IL-6 were not detected by ELISA. In summary, these results not only confirm that HZ is a pro-inflammatory stimulus in M0 macrophages, but also suggest a role in modifying macrophages plasticity towards a pro-inflammatory state.

# *IN VITRO* EFFICACY OF RED LAPACHO (*TABEBUIA AVELLANEDAE*) AGAINST *GIARDIA DUODENALIS*

Rigamonti G.*[1], Lalle M.[2], Chiaradia E.[1], Klotz C.[3], Brustenga L.[1], Tognoloni A.[1], De Santo R.[4], Veronesi F.[1]

*[1]University of Perugia, Department of Veterinary Medicine, Perugia, Italy; [2]Department of Infectious Diseases, Unit of Foodborne and Neglected Parasitic Diseases, Istituto Superiore di Sanità, Rome, Italy; [3]Department of Infectious Diseases, Unit 16 Mycotic and Parasitic Agents and Mycobacteria, Robert Koch-Institute, Berlin, Germany; [4]Department of Environment and Health, Unit of Human Exposure to Environmental Contaminants, Istituto Superiore di Sanità, Rome, Italy*

**Keywords:** *Giardia duodenalis*, *Tabebuia avellanedae*, *In vitro* activity.

**INTRODUCTION:** *Giardia duodenalis* is a widespread protozoan affecting mammals, including humans and dogs. Affected dogs exhibit a range of symptoms from subclinical to severe abdominal pain and diarrhea (Ballweber et al., 2010. Trends Parasitol, 26(4):180-89). Giardiosis may become chronic, thus requiring repeated treatment with synthetic drugs like fenbendazole (FBZ) and metronidazole (MTZ) (Lalle and Hanevik, 2018. Infect Drug Resist, 11:1921-33). In the last years, drug resistance is rising, especially in human medicine (Argüello-García et al., 2020. Adv Parasitol, 107:201-82). Although no cases of drug resistance are reported in the veterinary field, a recent study showed a lack of efficacy of FBZ in treatment of canine giardiosis (Kaufmann et al., 2022. Parasite, 29:49). Consequently, therapeutic alternatives are required. Medicinal plants as been traditionally used as anti-parasitic compounds, but systematic evaluation under controlled experimental condition is often lacking. Here we have examined the efficacy of *Tabebuia avellanedae* dry extract (TD) and hydroalcoholic extract (TH), as well as one of its active compounds, beta-lapachone (beta-lap), as potential treatment against *G. duodenalis* infection in dogs.

**MATERIALS AND METHODS:** *In vitro* anti-giardial activity of compounds (IC50 values after 48 h) was evaluated by ATP-content assay using reference isolates of *G. duodenalis* Assemblage A and B (Chen et al., 2011. Antimicrob Agents Chemother, 55(2):667-75). MTZ was used as reference drug. *In vitro* cytotoxicity effects were evaluated using human Caco-2 and canine MDCK cell line (CC50 values at 6, 12, 24 and 48 h) and selectivity index (SI = IC50/CC50) evaluated at 48h. Therefore, we evaluated the *in vitro* cytotoxic effects of three concentrations of all the compounds on intestinal Organoid Derived Monolayers (ODMs) after 48h evaluating both alteration of transepithelial electrical resistance (TEER) and organoids viability.

**RESULTS AND CONCLUSIONS:** We observed good anti-*G. duodenalis* activity of all the compounds. Both the Caco-2 and MDCK cell viability assay produced similar results for TD, with only the highest concentration (2 mg/ml) showing toxicity at 12, 24 and 48 hours, while no cytotoxicity was recorded for TH. As expected due to his anticancer activity (Gomes et al., 2021. Phytochem, 186:122713), beta-lap was toxic against both Caco-2 and MDCK cells. A significant SI value was recorded for TH; however, the SI values for TD and beta-lap warranted further assessment using alternative biosystems to verify the safety of the compounds. A remarkable low toxicity was observed for TD and beta-lap on ODMs, while no toxicity was detected for TH. Our *in vitro* results pointed out on a potential therapeutic applicability of *T. avellanedae*. This is the first time that organoids were used for testing antigiardial compounds. Future studies will focus on evaluating *T. avellanedae* using an *in vitro* model of *Giardia*-host co-culture, as well as an animal model of giardiasis.

# *LEISHMANIA* SPP. DETECTION AMONG EQUIDS AND DIPTERAN INSECTS IN CANINE LEISHMANIASIS ENDEMIC AREAS

Carbonara M.*[1], Mendoza-Roldan J.A.[1], Bezerra-Santos M.A.[1], De Abreu Teles P.P.[1], Lia R.P.[1], Locantore F.[1], Iatta R.[2], Volf P.[3], Otranto D.[1]

*[1]Department of Veterinary Medicine, University of Bari, Valenzano, Bari, Italy; [2]Interdisciplinary Department of Medicine, University of Bari, Bari, Italy; [3]Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic*

**Keywords:** Equine leishmaniasis, *Leishmania infantum*, *Leishmania martiniquensis*.

**INTRODUCTION:** Equids may be infected by *Leishmania infantum* when living in canine leishmaniasis (CanL) endemic areas or by *Leishmania martiniquensis*, as it has been reported in horses from Switzerland and Germany (Mhadhbi and Sassi, 2019. Equine Vet J, 52:28-33). This study aimed to evaluate the circulation of both *Leishmania* species among equids in Southern Italy, as well as to identify dipteran vectors from the same habitats.

**MATERIALS AND METHODS:** From March to October 2023, blood and sera were collected from equids (n=98; n=42 horses and n=56 donkeys) living in ten sites from Apulia, four sites from Basilicata, one from Sicily and one from Veneto regions. In addition, when animals presented dermatological signs, biopsies and cytological slides were taken from skin lesions. Sand flies and biting midges were collected from two sites where *Leishmania*-seropositive equids were found. Blood samples (n=98) and skin lesions (n=56) were tested for *Leishmania* spp. by three conventional (cPCR, targeting ITS1, 18S rRNA and HSP70 genes) and two real time PCRs (qPCR); sera were tested by immunofluorescence antibody tests (IFAT, 1:80 cut-off dilution) for *L. infantum* and *L. martiniquensis*. Insects were morphologically identified and female specimens (n=268 sand flies, n=7 biting midges) analyzed for *Leishmania* DNA, using the same tools mentioned above; engorged sand flies (n=16) were screened for blood-meal detection by cPCR.

**RESULTS AND CONCLUSIONS:** While on cytological smear examination, no *Leishmania* spp. amastigotes were observed, one donkey, presenting nodules on the muzzle, scored positive for *L. infantum* by qPCR and one horse with a hyperkeratotic area on the tail was positive for *Leishmania* sp. by cPCR. In addition, 19.4% animals were seropositive for *L. infantum*, with five of them also for *L. martiniquensis*. Both equids positive for *Leishmania* spp. DNA scored seronegative. Of the 356 sand flies collected, 4.5% females (i.e., n=8 *Sergentomyia minuta*; n=3 *Phlebotomus perniciosus*, n=1 *Phlebotomus perfiliewi*) were positive for *Leishmania* spp. DNA (i.e., n=7 for *L. tarentolae* and n=5 for *L. infantum*), as well as one biting midge (i.e., *Culicoides imicola*) for *L. infantum*. Moreover, human and equine DNA were detected in *S. minuta* and *Ph. perniciosus* specimens, respectively. Data suggest that horses and donkeys living in endemic areas for CanL are exposed to *Leishmania* spp., being bitten by infected insect vectors. In such areas, equids presenting skin lesions should be searched for *Leishmania* spp. by molecular tools, due to the cross reactions (e.g., *L. infantum* vs *L. martiniquensis*) that may occur at IFAT. Overall, we advocate for a more in-depth evaluation of *Leishmania* species among equids, given that the role of these hosts in the circulation of both *Leishmania* spp. needs further investigations.

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# MOSQUITO VECTORS OF *DIROFILARIA* SPP. IN SARDINIA (ITALY): PRELIMINARY DATA OF A LONGITUDINAL STUDY

Cavallo L.*[1], Nonnis F.[1], Zeinoun P.[1], Carta C.[1], Arshad F.[1], Tosciri G.[2], Pasini C.[3], Napoli E.[4], Brianti E.[4], Venco L.[5], Perugini E.[6], Cavallero S.[6], Bellini I.[6], Tamponi C.[1], Scala A.[1], Gabrielli S.[6], Pombi M.[6], Varcasia A.[1]

*[1]University of Sassari, Department of Veterinary Medicine, Sassari, Italy; [2] ATS Sardegna, ASSL Lanusei, Department of Animal Health, Lanusei, Italy; [3]Istituto Zooprofilattico Sperimentale della Sardegna"G.Pegreffi", Oristano, Italy; [4]University of Messina, Department of Veterinary Sciences, Messina, Italy; [5]Veterinary Hospital Città di Pavia, Pavia, Italy; [6]Sapienza University of Rome, Department of Public Health and Infectious Diseases, Rome, Italy*

**Keywords:** Mosquitoes, *Dirofilaria* spp., Sardinia.

**INTRODUCTION:** Up to 70 mosquito species included in the genera *Aedes*, *Ochlerotatus*, *Anopheles* and *Culex* are implicated in the transmission of *Dirofilaria immitis* and *Dirofilaria repens*, with *Culex pipiens* and *Aedes albopictus* representing the main vectors (Genchi et al., 2009. Vet Parasitol, 163:286-92). Canine dirofilariosis is endemic in Sardinia Island, but despite the high prevalence observed, information about the transmitting mosquito species are still scarce. Therefore, this study aims were to assess the mosquito species composition in Sardinia, and the occurrence of *Dirofilaria* spp. in mosquito vectors, to define their local epidemiological role in transmission.

**MATERIALS AND METHODS:** An entomological survey was carried out from September 2022 to November 2023 in five peri-urban sites of Sardinia distributed across the whole region: Sassari, Tortolì, Oristano, Vallermosa, and Sant'Antioco. In each site, monthly samplings, consisted of 24-hour collections targeting host-seeking mosquito females, using two CDC-light traps and two BG-Sentinel traps were performed. Female specimens were identified at the Laboratory of Parasitology of the University of Sassari, Italy, according to morphological keys (Severini et al., 2022. Rapporti ISTISAN 22/3). All mosquitoes were sorted according to species, sex, gonotrophic stage, and then pooled for further molecular analyses aimed at investigating the presence of *Dirofilaria* spp. DNA.

**RESULTS AND CONCLUSIONS:** A total of 1,218 mosquitoes (77.8% females) were captured, belonging to eleven species (unidentified 22.3%): *Aedes detritus* (26.8%), *Aedes caspius* (21.5%), *Cx. pipiens* (14.6%), *Ae. albopictus* (10.9%), *Culiseta* spp. (1.3%), *Aedes mariae* (0.9%), *Aedes rusticus* (0.6%), *Aedes geniculatus* (0.3%), *Culex theileri* (0.2%), *Anopheles plumbeus* (0.1%), and *Culex modestus* (0.1%). Most of mosquitoes were represented by unfed females (74.7%), followed by blood-fed females (2.3%) and males (21.9%). Preliminary data reported *Dirofilaria* spp. DNA in *Cx. pipiens* (i.e., 15.2%, 10.3%, 2.1% from Tortolì, Vallermosa and Sant'Antioco, respectively) and in *Ae. albopictus* (i.e., Sant'Antioco,10.6%). Further analyses are in progress to test all the collected samples and to identify the *Dirofilaria* parasites at the species level. In this study *Ae. detritus*, *Ae. caspius*, *Cx. pipiens*, and *Ae. albopictus* were the most abundant species, although they were found at a lower prevalence than reported in previous findings from Sardinia (Foxi et al., 2018. Vet Ital, 54:243-49), where *Cx. pipiens* and *Ae. albopictus* represented 80.1% and 15.6% of the total catches, respectively. These species, recognized as competent vectors of *D. immitis* and *D. repens*, pose a potential threat to human and animal health, stressing the importance of implementing entomological surveillance within a One Health perspective.

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# TESTING OF NOVEL SENSOR FOR AUTOMATIC COUNT AND IDENTIFICATION OF *AEDES* AND *CULEX* ADULTS ACROSS ITALY

Micocci M.*[1], Bernardini I.[2], Soresinetti L.[3], Varone M.[4], Di Lillo P.[4], Severini F.[2], Montarsi F.[5], Epis S.[3], Salvemini M.[4], Manica M.[6], della Torre A.[1]

*[1]Sapienza University of Rome, Department of Public Health and Infectious Diseases, Rome, Italy; [2]Istituto Superiore di Sanità, Department of Infectious Diseases, Rome, Italy; [3]University of Milan, Department of Biosciences and Pediatric Clinical Research Center "Romeo Ed Enrica Invernizzi", Milan, Italy; [4]University of Naples Federico II, Department of Biology, Naples, Italy; [5]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy; [6]Fondazione Bruno Kessler, Center for Health Emergencies, Trento, Italy*

**Keywords:** Mosquitoes, Monitoring, Machine learning.

**INTRODUCTION:** Mosquito-borne diseases are becoming a major public health concern also in Italy, where West Nile virus is endemic and risk of exotic arbovirus transmission is rising, as revealed by the two Dengue outbreaks occurred in 2023. Although effective vector monitoring is increasingly requested to acquire data for evidence-based risk predictions and effective mosquito control, conventional monitoring methods are time- and labor-intensive and difficult to be implemented widely and across the entire season. This evidence is leading to attempts to develop new approaches requiring lower human efforts, such as tool for automatic mosquito count and identification. To this goal, a specific Sensor combined with supervised machine learning algorithm was developed (González-Pérez et al., 2022. P&V, 15:190). We here summarize the results obtained by testing this Sensor in Italy in summer 2023 in order to assess the accuracy of the automatic count and identification of *Aedes* and *Culex* females and males and whether the presence of the Sensor affects the trap catching capacity.

**MATERIALS AND METHODS:** The same experimental design was implemented in Bergamo, Padua, Rome and Procida island (Naples). In each area, 3 types of traps - one BG-Mosquitaire (BG-M), one BG-M equipped with the Sensor, and 4 Sticky Traps (considered as a single trap due to their lower catching capacity and used for future determination of BG-M collection rate) - were rotated in 3 different sites each 48h. Each sampling scheme was replicated 3 times/area. Collected mosquitoes were counted and identified both automatically by the Sensor and by visual inspections.

**RESULTS AND CONCLUSIONS:** A total of 3,829 mosquitoes were captured. No significant difference was observed between the overall mosquito captures with the BG-M with vs. without the Sensor (Chisq NS). Overall, the Sensor detected 4% fewer mosquitoes than the operator (Chisq NS). The correlation between the two identification methods is very high for *Aedes* (Pearson cor 0.985, pval <0.0001) and lower for *Culex* (Pearson cor 0.601, pval <0.0001). Results of the linear model estimate that for every 100 individuals of each species identified by the Sensor, the traps had on average actually captured 104 *Aedes* and 66 *Culex*, respectively (Chisq NS). A systematic slight overestimation of *Aedes* males vs. females is observed.
In conclusion, the Sensor proved to be highly accurate in counting and identifying *Aedes* adults collected by BG-M, accounting for its high potential for continuous monitoring with minimal human effort. Moreover, the Sensor opens the possibility to study mosquito circadian rhythms across seasons by recording the exact time of individual mosquito collection. This goal will be pursued across Italy in 2024-25 in the framework of research activities carried out within Research Node 2 of INF-ACT project funded by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases.

# PREVALENCE AND DISTRIBUTION OF FILARIIDAE SPECIES IN DOGS FROM SARDINIA, ITALY

Nonnis F.*[1], Cavallo L.[1], Atzeni D.[1], Grussu I.[1], Zeinoun P.[1], Sini M.F.[1], Carta C.[1], Arshad F.[1], Tosciri G.[2], Napoli E.[3], Brianti E.[3], Venco L.[4], Cavallero S.[5], Bellini I.[5], Gabrielli S.[5], Pombi M.[5], Tamponi C.[1], Scala A.[1], Varcasia A.[1]

*[1]Department of Veterinary Medicine, University of Sassari, Sassari, Italy; [2]ATS Sardegna ASSL Lanusei, Animal Health Department, Lanusei, Italy; [3]Department of Veterinary Sciences, University of Messina, Messina, Italy; [4]Veterinary Hospital Città di Pavia, Pavia, Italy; [5]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Roma, Italy*

**Keywords:** Dirofilariosis, Dog, Sardinia.

**INTRODUCTION:** Canine vector-borne diseases represent a growing concern in veterinary and human medicine, and among these, scientific interest is focused on the spread, diagnosis, and control of filarial worms. Due to their pathogenic potential, *Dirofilaria immitis* and *Dirofilaria repens* represent the most well-studied species, while knowledge about other filarial nematodes such as *Acanthocheilonema reconditum* is considerably less characterized. To obtain a more comprehensive picture of the current distribution of *D. immitis*, *D. repens* and *A. reconditum* in Sardinia, Italy, a survey on canine filariasis was carried out.

**MATERIALS AND METHODS:** Owned and sheltered dogs (n = 655) older than 1 year and with no history of chemoprophylactic treatment against filarioses were included in the study. Data on sex, age, lifestyle, use of ectoparasiticides, and presence of clinical signs were acquired for each animal. Blood samples were collected from enrolled dogs and stored in tubes with anticoagulant (K3EDTA) and serum collection tubes with clot activator. The samples were processed by modified Knott's test (Genchi et al., 2021. Vet Parasitol, 298:109555), for the detection and identification of microfilariae using morphometric criteria (Magnis et al., 2013. Parasit Vectors, 6:48), and by antigenic test (SNAP4DX, IDEXX Laboratories) for the detection of *D. immitis* specific antigens.

**RESULTS AND CONCLUSIONS:** Microfilariae were found in 11.8% (77/655) of the examined animals. *Dirofilaria repens* was the most frequent species detected with a prevalence of 6.2% (41/655), followed by *D. immitis* (38/655; 5.8%), and *A. reconditum* (15/655; 2.3%). The prevalence was significantly higher in males (14.7%, 49/334) than female dogs (8.6%, 27/315; $\chi 2 = 5.8329$; $P = 0.0157$). No correlation was detected between age and microfilaremia ($\chi 2 = 0.325578$; $P=0.5683$); also, the prevalence was higher in dogs living outdoor than indoor (12.2%, 75/616 vs 7.1%, 2/28), even if no statistical associations were observed ($\chi 2 =0.2550$; $P =0.6136$). Thirty-eight (10.8%) out of 353 tested dogs scored seropositive for *D. immitis* antigen. The percentage of microfilaremia herein detected is lower compared to a previous study conducted in Sardinia (Pipia et al., 2014. Parasitol Res, 113:1505-09) where microfilariae of *A. reconditum* were retrieved in 12.6% (86/684), *D. immitis* in 8.9% (61/684), and *D. repens* in 8.9% (61/684) of the examined dogs. Based on the above data, dirofilariosis in dogs is still present throughout the Island. Given the zoonotic potential of these parasites, and the higher suitability of Sardinia to mosquito vectors, preventative and control measures are strongly advocated to minimise the risk of infection to humans and animals.

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# MOSQUITO SURVEILLANCE AT POINTS OF ENTRY: A LONGITUDINAL MONITORING IN PORT AND AIRPORTS OF VENETO REGION

Manzi S.*[1], Vettore S.[2], Bonetto D.[2], Martini S.[2], Gradoni F.[1], Poletto E.[1], Danca L.[1], Toniolo F.[1], Gobbo F.[1], Russo F.[3], Severino V.[4], Vaia F.[4], Ziprani C.[4], Montarsi F.[1]

*[1]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy; [2]Entostudio S.r.l., Ponte San Nicolò (PD), Italy; [3]Prevention, food safety and veterinary, Veneto Region, Venice, Italy; [4]Ministry of Health, General Direction of Prevention, USMAF-SASN Veneto-F.V.G.-T.A.A. U.T., Venice, Italy*

**Keywords:** Invasive mosquitoes, *Aedes koreicus*, Points of entry.

**INTRODUCTION:** Over time, international travel and trade have significantly modified the geographic distribution of mosquito-borne diseases (Franklinos et al., 2019. Lancet Infect Dis, 19:e302-12). The current global health emergency of dengue disease is increasing the threat level to people traveling, vehicles entering and imported goods from endemic countries (WHO, 2023). In Italy, special control activities are recommended at ports and airports, which are common entry points for both pathogens and invasive mosquito species (Notes ex Directorate General for Preventive Health, Office 3 Prot. No. 4753 of 14/02/2024, Prot. No. 8083 of 14/03/2024 and Prot. No. 4753 of 14/02/2024). The surveillance of invasive mosquitoes is already in place in some parts of Italy, and a specific monitoring project has been implemented in Veneto region since 2018. This project aims to prevent the spread of new mosquito vectors through early detection and focused control intervention in ports and airports of the region.

**MATERIALS AND METHODS:** A pilot survey (2018) and an entomological monitoring (2019-2023) was carried out at the port of Venice (Marghera) and the airports of Venice and Treviso. In each of the selected sites, 7 ovitraps and 3 BG-Sentinel traps (BG-S) baited with BG-Lure worked continuously for one week from June to October. Eggs collected in ovitraps were counted and hatched in laboratory. Hatched larvae and adults from BG-S were morphologically identified. In case of doubtful identification, a molecular confirmation was carried out using PCR and sequencing (Cameron et al., 2010. J M Med Entomol, 7:527-35).

**RESULTS AND CONCLUSIONS:** Overall, a total of 24,355 adults and 58,414 eggs belonging to 13 species were collected. The 99.6% of adult mosquitoes were successfully identified and the most represented species were *Ae. albopictus* (n. = 12,311; 50.7%) and *Cx. pipiens* (n. = 11,483; 47.3%). Two adults of *Ae. koreicus* were collected the 29th July and the 8th October 2019 at Treviso airport and one the 15th July 2020 at Venice airport. Hatched eggs belonged all to *Ae. albopictus* except one egg of *Ae. koreicus* found in Venice airport in 2018 (12th July 2018). Although this species was already reported in Veneto region (Belluno 2011, Treviso and Vicenza 2012, Verona 2014, Padova 2017), this was the first finding of *Ae. koreicus* in Venice province (Gradoni et al., 2021. Data in Brief, 36:107047). Since its discovery at the Venice airport, *Ae. koreicus* was detected in the municipality of Mirano (18 km far from Venice airport) within the framework of another monitoring plan. The species could have been introduced via airport and then spread to another area. Even if a direct link cannot be established, these findings suggest strengthening the control of invasive mosquitoes, focusing on potential entry routes.

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# ECO-CLIMATIC FACTORS SHAPING THE DISTRIBUTION OF *ANOPHELES COLUZZII* MALARIA VECTOR ACROSS WEST AND CENTRAL AFRICA

De Marco C.M.*[1], Caputo B.[1], Virgillito C.[1], Frosi L.[1], Pichler V.[1], Filipponi F.[2], Manica M.[3], della Torre A.[2]

*[1]Sapienza University of Rome, Department of Public Health and Infectious Diseases, Rome, Italy; [2]National Research Council, Institute for Environmental Geology and Geoengineering (CNR-IGAG), Montelibretti (RM), Italy; [3]Fondazione Bruno Kessler, Center for Health Emergencies, Trento, Italy*

**Keywords:** Malaria vectors, *An. gambiae* complex, Satellite observation data.

**INTRODUCTION:** *Anopheles coluzzii* (CO) is a primary vector of human malaria in sub-Saharan Africa west of the Rift Valley. Contrary to the sympatric species *An. gambiae* (GA) which dependent from rain-dependent breeding sites, CO has adapted to permanent breeding sites created by activities such as irrigation, deforestation, and urbanization. The objective of this work was to identify major factors determining CO distribution and to predict its future distribution in different Shared Socioeconomic Pathways (SPP) scenarios.

**MATERIALS AND METHODS:** An electronic literature search was independently conducted by three investigators on PubMed, WoS, and Scopus databases following PRISMA Statement. The search strategy combined "(*Anopheles gambiae* OR *Anopheles coluzzii*)" with "frequencies", "distribution", "identification" and "molecular form" keywords, and focused on 2001-2022 articles. Screening of abstracts was conducted using the RayyanQ program (Ouzzani et al., 2016. Syst Rev, 5:210.) along with two main eligibility criteria: the occurrence or frequency of CO/GA and reporting studies conducted only in West and Central Africa. Data collected for each study included species abundance/site, GPS coordinates, country, sampling period, individual stage, collection method, molecular identification method, and insecticide study details. For each sampling site, Malaria related maps (MAP), essential climate variables (C3S), high-resolution land cover and vegetation index (PROBA-V) were collected and harmonized to a 5x5 km spatial grid at the sampling coordinates. In addition, to evaluate the species distribution in the future, we extracted climate predictors for years 2040, 2060 and 2080 from the IPSL-CM6A-LR global climate model.

**RESULTS AND CONCLUSIONS:** Results of the systematic review yielded 948 papers; 32 were excluded for not meeting abstract eligibility criteria and 661 were excluded upon full-text examination. The ultimate datasets include a total of 211,580 individuals from 2,118 sampling sites. GLM results suggest that CO occurrence is mostly influenced by temperature, precipitation and by distance from the coast, confirming coastal "bimodality" distribution and the significance of coastal habitats in shaping its spatial distribution (Fossog et al., 2015. Evol Appl, 8:326-45). Future projections suggest that the probability of occurrence of CO may escalate in future decades, therefore affecting the risk of malaria transmission. This study offers insights into the distribution patterns of the malaria vector *An. coluzzii* and its ecological and climatic determinants. Continual species distribution modelling using both present and projected ecological variables is crucial, given the evolving global landscape. Nevertheless, for enhanced predictive accuracy of Species Distribution Models (SDMs), we advocate for the integration of further ecological parameters, including species dispersal strategies, human impacts on local environments and the implementation of mosquito control measures into future projections.

# SAND FLIES LONGITUDINAL STUDY IN FOUR REGIONS OF THE ITALIAN TERRITORY (2019-2021): SPECIES DISTRIBUTION, SEASONAL DYNAMICS AND PATHOGEN DETECTION

Mangiapelo C.*[1], Bernardini I.[1], Bianchi R.[1], Fiorentino E.[1], Foxi C.[2], Mosca A.[3], Calzolari M.[4], De Liberato C.[5], Di Muccio T.[1], Satta G.[2], Gradoni L.[1], Bongiorno G.[1], Angelini P.[6]

*[1]Istituto Superiore di Sanità, Department of Infectious Diseases, Unit of Vector-borne Diseases, Rome, Italy; [2]IstitutoZooprofilattico Sperimentale della Sardegna, Sassari, Italy; [3]Istituto per le piante da legno e l'ambiente IPLA S.p.A, Turin, Piedmont, Italy; [4]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy; [5]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Rome, Italy; [6]Servizio Prevenzione collettiva e Sanità pubblica, Bologna, Emilia-Romagna, Italy*

**Keywords:** Sand flies, Seasonal dynamics, Sand-fly borne diseases.

**INTRODUCTION:** Phlebotomine sand flies are tiny blood-feeding insects whose geographic range and seasonal activity are expanding, following the global rise in temperatures due to climate and environmental changes. In Italy they are vectors of zoonotic visceral and cutaneous leishmaniasis caused by *Leishmania infantum* and also associated with Phleboviruses: Fermo, Sicilia, Naples and Toscana viruses. Aim of this study is to report sand fly species distribution and seasonality in four italian regions, providing an epidemiological frame linked to sand flies natural infection by *Leishmania* spp. and Toscana Virus.

**MATERIALS AND METHODS:** A longitudinal study as part of West Nile virus surveillance was carried out from 2019 to 2021, giving an overall picture of upper central part of the Italian peninsula: Piedmont, Emilia-Romagna, Latium and Sardinia. Samplings were performed twice a month, using CDC and BG-sentinel traps, recording environmental data. Subsamples were morphologically identified and molecularly tested by RFLP and RT-PCR for pathogens detection.

**RESULTS AND CONCLUSIONS:** A total of 96,747 specimens were collected, of which almost 9,000 morphologically identified, belonging to four species: *Phlebotomus perfiliewi* was the prevalent one (81.1%), followed by *Ph. perniciosus* (14.9%), *Sergentomyia minuta* (3.7%) and *Ph. mascittii* (0.3%). Results confirm the presence of all above-mentioned species in Piedmont, with *Ph. perniciosus* as prevalent species (63.4%). Emilia Romagna features a 99.6% prevalence of *Ph. perfiliewi* and the remaining 0.4% of *Ph. perniciosus* and *Ph. mascittii*. Lazio showed the same trend as Emilia Romagna, with 99.7% of *Ph. perfiliewi* and 0.3% of *Ph. perniciosus*. Sardinia had a 61.8% prevalence of *Ph. perniciosus*, followed by *S. minuta* (20.3%) and *Ph. perfiliewi* (17.9%). Thereafter, seasonal dynamics was examined, showing following trends: in Emilia Romagna *Ph. perfiliewi* species had a bimodal peak of density, in July and September, while in Latium it was characterized by a single peak in July. In Piedmont *Ph. perniciosus*, *Ph. perfiliewi* and *Ph. mascittii* showed a monomodal distribution in July and August, in 2020 and 2021 respectively. In Sardinia *Ph. perniciosus* and *S. minuta* showed a bimodal peak, while *Ph. perfiliewi* followed a trimodal distribution. Molecular analyses to detect *Leishmania* spp., performed on a total of 4,062 specimens, showed a positivity rate in Piedmont (11.2%) and Sardinia (11.1%). Toscana Virus analyses, performed on a total of 3,321 specimens, revealed a positivity in Latium (25%) and for the first time in Piedmont (1.7%) and Sardinia (0.5%). These preliminary results confirm ubiquitous presence of *Ph. pernicious*, even if *Ph. perfiliewi* proves to be the most abundant species. This study confirm the link between seasonality and latitude (Bulent et al., 2016. PLoS Negl Trop Dis, 10-2). Lastly, it reveals a fair circulation of examined pathogens.

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# EVALUATION OF *PHLEBOTOMUS PERFILIEWI* AS COMPETENT VECTOR SPECIES FOR *LEISHMANIA TROPICA* TRANSMISSION

Bernardini I.*[1], Mangiapelo C.[1], Bianchi R.[1], Fiorentino E.[1], Di Muccio T.[1], Orsini S.[1], Scalone A.[1], Pombi M.[2], Di Luca M.[1], Bongiorno G.[1]

*[1]National Institute of Health, Department of Infectious Diseases, Vector-borne Diseases Unit, Rome, Italy; [2]University of Rome "Sapienza", Department of Public Health and Infectious Diseases, Parasitology Unit, Rome, Italy*

**Keywords:** Phlebotomine-borne diseases, Sand fly, Vector competence.

**INTRODUCTION:** In the current epidemiological scenario, where vector-borne diseases are increasing, the wide geographic distribution in southern Europe of *Leishmania tropica* and its proven vector, *Phlebotomus sergenti*, may give rise to new endemic foci through the introduction of infected humans to areas where this vector is present. On the other hand, the introduction of this *Leishmania* species in non-endemic areas of Europe, where sandfly vectors of the local parasites (e.g. *L. infantum*) are well established, may potentially introduce new *Leishmania-Phlebotomus* vector systems thus increasing the chance of transmission and pathogenicity for the vertebrate hosts (Maia, 2024. J. Comp Pathol, 209:6-12). In this context, the aim of this study is to evaluate the potential vector competence of an Italian population of *P. perfiliewi* for non-endemic *L. tropica*.

**MATERIALS AND METHODS:** Experimental infections were performed in 2022-23 using wild females of *P. perfiliewi* from Tuscany and two *P. perniciosus* colonies as positive control maintained in standard conditions (26±2°C; 80±10% RH; 50% sucrose solution; 14:10h light:dark). Infection replicates of sandfly females were carried out in laboratory BSL2 with 106 promastigotes/ml−1 of *L. tropica* through artificial feeding. From day 0 up to 11 post bloodmeal (PBM) females were microscopically dissected to evaluate infection success and parasite development. To confirm dissection results, molecular analyses were performed by nested-PCR and genotyped by PCR-RFLP.

**RESULTS AND CONCLUSIONS:** A total of 5,923 engorged females were obtained in 8 experiments starting from nearly 100% infected specimens on day 0 PBM, altogether viable parasites were recorded in 23.6% (n= 120/513) of specimens dissected over a 11-day period, with a peak at day 8 PBM (21.7%, n= 26). Within 4-5 days PBM morphological progression of parasite was observed with a light load of nectamonad stage between midgut and hindgut in over 100 females, while around 6 and 8 days PBM moderate load of leptomonad stage was found in the cardia and torax (n= 60 females) until 9 days PBM when heavy load of metacyclic promastogotes in the stomodeal valve occurs in 88 females. Finally, molecular screening confirmed 23% of positive out of 145 dissected females, while in 29% only *L. tropica* DNA was detected. Based on this, the high prevalence of infectious stages suggests that *P. perfiliewi* could be considered as a permissive species supporting development of *L. tropica* and then a potential vector for the establishment of non-endemic transmission foci.

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# TEMPORAL AND SPATIAL PROFILING OF *AEDES ALBOPICTUS* IMMUNE RESPONSES TO CHIKUNGUNYA VIRUS (CHIKV) INFECTION

Dipaola M.G.*[1], Fortuna C.[2], Bevivino G.[1], Severini F.[2], Di Luca M.[2], Salvemini M.[3], Arcà B.[1], Lombardo F.[1]

*[1]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy; [2]Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; [3]Department of Biology, University of Naples Federico II, Naples, Italy*

**Keywords:** *Aedes albopictus*, Transcriptomic, Immune response.

**INTRODUCTION:** The global spread of the *Aedes albopictus*, an aggressive daytime-biting vector of several human pathogens, actually represents an important health problem. It has been described as one of the 100 worst invasive species of the world, showing a rapid spread from its native home range (South-East Asia) to several other countries. *Aedes albopictus* is able to transmit several arboviruses of public health significance, including the chikungunya virus (CHIKV) (Paupy et al., 2009. Microbes Infect, 11:1177-85). After the ingestion of a viremic blood meal, CHIKV starts its journey inside the mosquito, involving the overcome of immune barriers, such as midgut and haemocoel, in order to reach and infect salivary glands, where it replicates for the last time before to be transmitted to new hosts.

**MATERIALS AND METHODS:** In this work, we used RNA-seq to investigate mosquito immune genes modulated into midgut and carcasses collected from CHIKV-infected and uninfected *Ae. albopictus* at 1 and 5 days post-infection. Reads obtained were assembled using the FOSHAN (FPA) genome assembly, obtaining a transcriptome of 55,149 contigs, filtered for FPKM value > 1. Afterwards, differential gene expression (DE) analysis and Pfam and GO enrichment analysis were performed, and a group of DE genes was selected to RTqPCR expression validation in different tissues collected from *Ae. albopictus* adult females.

**RESULTS AND CONCLUSIONS:** DE analysis and Pfam and GO enrichment analysis highlighted that midgut immune responses involve mainly the activation of RNAi, ubiquitination, immune deficiency (IMD) and autophagic pathways, while haemocytes-mediated immune responses entail immune receptor activity, antimicrobial peptides production (AMP) and melanization. In addition, analysis by RTqPCR of dissected female tissues confirmed the enriched expression of a member of leucine rich-repeat family, the AMP Holotricin, and a prophenoloxidase (PO) activating factor in the haemolymph. This research provides a temporal and spatial profiling of *Aedes albopictus* immune response against CHIKV, leading the foundation for future control strategies to limit CHIKV spread.

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# VECTOR-BORNE PATHOGENS SURVEY IN SHELTERED DOGS IN ROMANIA: THE ROLE OF DOG REHOMING IN CHANGING DISEASE DISTRIBUTION

Sturiale E.*[1], Napoli E.[1], Palazzolo V.[1], Di Bella S.[4], Blanda V.[4], Guercio A.[4], D'Amico G.[2], Gabrielli S.[3], Brianti E.[1]

*[1]Dipartimento di Scienze Veterinarie, Università degli Studi di Messina, Messina, Italy; [2]Departement of Parasitology and Parasitic Disease, University of Agricultural and Veterinary Medicine, ClujNapoca, Romania; [3]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy; [4] Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Italia, Palermo, Italy*

**Keywords:** Dogs rehoming, Vector Borne Diseases, Epidemiology.

**INTRODUCTION:** Most of the Vector Borne Pathogens (VBPs) of zoonotic concern are considered emerging. The large movements of infected/diseased dogs among areas at different degree of endemicity plays a crucial role in the spread of VBPs. These movements are influenced by several factors including economic, cultural, natural disasters and war conflicts. In 2022, the impact of the war between Russia and Ukraine resulted in the exodus of a third of Ukrainian population and the uncontrolled rehoming of stray or abandoned dogs in neighbouring countries. Aim of the study was to investigate the prevalence of *Babesia* spp., *Rickettsia* spp., *Anaplasma* spp., *L. infantum*, and *Dirofilaria* spp. in a population of sheltered dogs in Romania, including dogs recently relocated from Ukraine.

**MATERIALS AND METHODS:** A cross-sectional study was conducted in a population of 156 dogs housed in four shelters in Romania located in the North (S1; 59 dogs), in the centre (S2; 29 dogs), and in the South (S3, 30 dogs; S4, 38 dogs) of the country. In the S1 all the enrolled dogs were relocated from Ukraine. Genomic DNA was extracted from the blood samples and analyzed through end-point PCRs for *Dirofilaria* spp., *Anaplasma* spp., *Rickettsia* spp. and *Babesia* spp., and through Real Time (RT) PCR for *L. infantum*. Moreover, circulating microfilariae were detected by the modified Knott's test.

**RESULTS AND CONCLUSIONS:** Out of the 156 dogs, 42 (26.9%) scored RT-PCR positive for *L. infantum*, and 4 (2.6%) dogs were PCR positive for *Rickettsia* spp. *Leishmania* positive dogs were recorded in all the study sites with the highest prevalence (i.e., 40%; 12/30) in S3, followed by S1 (28.8% 17/59), S2 (20.7% 6/29) and S4 (18.4% 7/38). Upon Knott test circulating *D. immitis* microfilariae were detected in 11 (7.1%) dogs hosted in S1. The PCR analyses confirmed all the Knott's test positive samples and added three *D. repens* and *D. immitis* co-infected samples, and a sample positive for *D. repens* thus resulting in an overall prevalence of 9% (14/156) for *D. immitis* and 2.6% (4/156) for *D. repens*. Some co-infections due to *Rickettsia* spp., *D. immitis* and *D. repens* were detected in association with *L. infantum*. Interesting, *Dirofilaria* positive dogs were housed in S1, and all of them were rehomed dogs from Ukraine a few months before the survey. Also, some of these dogs were co-infected with *Leishmania*. The prevalence for *L. infantum* and *Dirofilaria* spp. observed in the present study is similar to those reported for endemic areas and stands for a large circulation of these VBPs. All the *Dirofilaria* positive dogs and many *L. infantum* infected animals were relocated dogs from Ukraine. The results herein reported demonstrate how the relocation of dogs can be a driver for VBPs spread.

# *IN VITRO* EFFICACY AGAINST POULTRY *EIMERIA* SPP. AND CYTOTOXICITY OF NATURAL ESSENTIAL OILS CONSTITUENTS

Zoroaster A.*[1], Iori S.[2], Frangipane Di Regalbono A.[1], Giantin M.[2], Raffaelli M.[3], Perrucci S.[3], Dacasto M.[2], Marani R.[4], Veronesi F.[5], Diaferia M.[5]

*[1]University of Padua, Department of Animal Medicine, Production and Health, Padua, Italy; [2]University of Padua, Department of Comparative Biomedicine and Food Science, Padua, Italy; [3]University of Pisa, Department of Veterinary Sciences, Pisa, Italy; [4]La Veterinaria s.r.l, Perugia, Italy; [5]University of Perugia, Department of Veterinary Medicine, Perugia, Italy*

**Keywords:** Poultry, *Eimeria* spp., Essential oil constituents.

**INTRODUCTION:** Coccidia (*Eimeria* spp.) are intestinal protozoa widespread in intensive poultry farming, impairing feed consumption and growth of infected animals (Dalloul & Lillehoj, 2006. Expert Rev Vaccines, 5:143-63). Essential oils and their main constituents (EOCs) may represent an alternative to anticoccidial drugs, overcoming the risk of drug residues in chicken products and the problem of antimicrobial resistance. However, the mode of action and the potential toxicity of many extracts and natural compounds remain inadequately elucidated (Yang et al., 2021. Evol Bioinform, 17:1176934320938391). The aim of the present study was to investigate the effects of EOCs on the sporulation and morphology of chicken *Eimeria* spp. oocysts and their cytotoxicity in a porcine enterocyte established cell line (IPEC-J2).

**MATERIALS AND METHODS:** A number of 105 oocysts/ml were exposed to thymol (TH), cinnamic aldehyde (CA), eugenol (EU), carvacrol (CR), and a blend named energy poultry (EP), containing CA, CR, and EU. The compounds were emulsified with 5% ethanol and 5% Tween 80 in saline solution at five concentrations (0.1, 1, 2, 5, 10%). An incubation at room temperature for 48 hours was performed; as control group oocysts in water were used. Following exposure, the oocysts were placed in 2.5% K2Cr2O2 solution for 72 hours to allow sporulation. The efficacy of tested compounds was determined by comparing the percentage of non-sporulated, sporulated, and degenerated oocysts to those of the control group. A t-test for statistical analyses was used ($p<0.05$). TH and EP, exhibiting the highest efficacy on oocysts, were further tested for their cytotoxic potential on differentiated (21-days post-seeding) IPEC-J2 cells. The Alamar blue cytotoxicity assay was used. Cells were exposed to increasing concentrations of TH (range 2.5-600 µg/mL) and EP (range 0.05-50 µg/mL) for 24, 48 and 72 hours to determine the half-maximal inhibitory concentration (IC50). Cells incubated with the vehicle alone (0.1% final concentration) were used as control.

**RESULTS AND CONCLUSIONS:** At 5 and 10%, TH (96%), EP (91%) and CR (90%) caused a significant ($p<0.05$) oocyst degeneration compared to the untreated controls. The activity of EP was attributed to a synergistic action between CA and CR. At 0.1- 2% their effectiveness was negligible. Concerning cytotoxicity, the IC50 calculated for TH was ~50 µg/mL at each time point (24, 48 and 72 hours). Conversely, EP caused 30% cell mortality at the highest tested concentration (50 µg/mL). The *in vitro* assessment of cell monolayer integrity is ongoing. As a whole, the results here obtained are encouraging and suggest the use of EOCs as a valid green approach for the control of avian coccidiosis. In vivo studies are also scheduled to obtain a complete overview of the host-parasite interaction as well as of EOCs therapeutic efficacy.

# ELECTRONIC DEVICES FOR SUSTAINABLE STRATEGIES TO CONTROL CYSTIC ECHINOCOCCOSIS IN GRAZING AREAS IN SOUTHERN ITALY

Nocerino M.*[1], Pepe P.[1], Bosco A.[1,2], Ciccone E.[1,2], Lattero N.[1,2], Maurelli M.P.[1,2], D'Orilia F.[2], Cringoli G.[1,2], Rinaldi L.[1,2]

*[1]University of Naples Federico II, Department of Veterinary Medicine and Animal Production, CREMOPAR, Naples, Italy; [2]Regional Reference Centre for Animal Health (C.Re.San.), Campania region, Italy*

**Keywords:** Echinococcosis, Geospatial data, Canids.

**INTRODUCTION:** Cystic echinococcosis (CE), caused by the larval stage of *Echinococcus granulosus*, has a worldwide distribution and is considered one of the most severe parasitic zoonosis of grazing sheep in the Mediterranean region. The lifecycle of *E. granulosus* involves canids as definitive hosts and usually sheep and other herbivore species as intermediate hosts. CE has a worldwide distribution but exhibits the highest prevalence in communities where pastoral activities predominate, as the Mediterranean areas (Deplazes et al., 2017. Adv Parasitol, 95:315-493). Free-roaming dogs (owned and unowned) are the major source of echinococcosis and the most challenging category in dog population management for the control of CE (Kachani et al., 2014. Acta Trop, 139:99-108). The present study, conducted in an area of southern Italy (Salerno province) highly endemic for CE, investigated the combined use of Geographical Information Systems (GIS) and electronic devices (e.g., GPS collars, drones, camera traps) to identify the spatio-temporal patterns of free-roaming dogs and to design new anthelmintic treatment strategies for wild canids gravitating near the CE positive sheep farms.

**MATERIALS AND METHODS:** In five sheep farms positive to CE, one adult sheep and two shepherd dogs were tracked for one month using 15 GPS wearable devices. The spatial and temporal point location data were compared to determine the movement patterns of the animals. For each farm, a specific deworming strategy based on the delivery anthelmintic baits (laced with praziquantel) using a tailored unmanned aerial vehicles (UAVs), was developed. Camera traps were used in the field trial to remotely test three types of PZQ-based baits by evaluating the bait acceptance by target animals (i.e. stray dogs), the integrity over time and the mechanical resistance after the release.

**RESULTS AND CONCLUSIONS:** The mean daily walking distance travelled not significantly differ between sheep and dogs in the farms monitored. The farthest distances from the farms (1,500mt) were travelled between 10.00 and 17.00. The PZQ-laced baits with a double layer of highly palatable chews showed the greatest resistance in the environment while preserving the attractiveness and palatability up to 10 days, also withstood heights of 25 m. This study confirms the importance of geospatial technology in supporting parasite control strategies. Furthermore, the application of anthelmintic baits using UAVs allows the capillary and automatic distribution of anthelmintics, minimizing waste of time and resources.

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# SURVEY ON THE INTESTINAL HELMINTHIC COMMUNITY IN A FOX POPULATION OF NORTHEASTERN ITALY

Ferraro E.*[1], Zarantonello A.[1], Obber F.[2], Celva R.[2], Da Rold G.[2], Cenni L.[3,4,5], Hauffe H.C.[5], Massolo A.[3,6,7], Simonato G.[1], Cassini R.[1], Marchiori E.[1]

*[1]University of Padova, Department of Animal Medicine, Production and Health, Padova, Italy; [2]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, PD, Italy; [3]Ethology Unit, Department of Biology, University of Pisa, Pisa, Italy; [4]Applied Ecology Research Unit, Research and Innovation Centre, Fondazione Edmund Mach, Trento, Italy; [5]Conservation Genomics Research Unit, Centre for Research and Innovation, Fondazione Edmund Mach, San Michele All'Adige, Italy; [6]Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada; [7]UMR CNRS 6249 Chrono-Environnement, Université Franche-Comté, Besançon, France*

**Keywords:** Red foxes, Helminths, Zoonosis.

**INTRODUCTION:** Red foxes (*Vulpes vulpes*), common across Europe, inhabit diverse habitats and frequently coexist with human settlements, playing a significant role in transmitting wildlife-origin parasites and infectious diseases, both of veterinary and zoonotic importance (Veronesi et al., 2023. Pathogens, 12:178). In this context, knowing the parasite fauna and studying its ecological determinants are crucial for risk-based surveillance. This study aimed to i) retrieve data on the intestinal helminth fauna of the red fox from a mountainous region of Northeastern Italy, the Autonomous Province of Bolzano, and ii) to conduct an ecological analysis based on its parasite profile.

**MATERIALS AND METHODS:** Between 2019-2020, 150 red foxes from Bolzano Province were collected during a population control campaign. From each carcass, the small intestine was collected and the Scraping, Filtration and Counting technique (SFCT) was performed (Marchiori et al., 2023. Front Vet Sci, 9:1085996). Parasites were isolated, counted and identified to the lowest taxonomic level possible. Prevalence, mean intensity, mean and relative abundance were computed for each parasite species. Samples were categorized based on the geographic area (west, centre, or east of the province), season (warm, cold), host sex and age (young, adult). Pearson's Chi-square test and non-parametric tests (Kruskal-Wallis Independent Samples Test and Mann Whitney U-test) were used to assess significant differences in prevalence and relative abundance among categories. Statistical analyses were performed using IBM SPSS Statistic 28.0 software.

**RESULTS AND CONCLUSIONS:** Out of 150 red foxes, 114 (76%) were positive for at least one parasite species (95%CI: 69-82%). We identified three nematodes species, i.e., *Toxocara canis* (44%; 95%CI: 36-52%), *Uncinaria stenocephala* (25%; 95%CI: 19-33%), and *Pterigodermatites affinis* (9%; 95%CI: 5-14%), and four cestodes, i.e., *Taenia crassiceps* (22%; 95%CI: 16-29%), *Echinococcus multilocularis* (16%; 95%CI: 11-23%), *Taenia polyacantha* (21%; 95%CI: 15-28%), and *Mesocestoides* spp. (21%; 95%CI: 1629%). All taxa showed an aggregated distribution with mean intensity values up to about 5-10 parasites/host, apart from *E. multilocularis* (2,757.7 parasites/host). Geographical distribution was homogeneous for nematodes, whereas *T. polyacantha* and *Mesocestoides* spp. showed higher prevalence and abundance in the West and East areas, and *E. multilocularis* in the central and eastern ones. Few species had a seasonal pattern with *T. polyacantha* peaking in the cold season, while *U. stenocephala* and *P. affinis* in the warm season. Finally, *U. stenocephala* was significantly more abundant in young foxes.
This study provides insights into the composition of the intestinal helminthic fauna of red foxes in Bolzano Province, highlighting the prevalence and distribution of various helminthic species, of both veterinary and public health interest, contributing to understand their ecological dynamics and parasite profiles of this red fox population.

# GENETIC VARIABILITY IN THE ANISAKID *CONTRACAECUM OSCULATUM* B OVER A TEMPORAL SCALE: A TOOL FOR MONITORING THE FOOD WEBS IN SUB-ARCTIC SEA?

Belli B.*[1], Palomba M.[2], Cipriani P.[3], Bao Dominguez M.[3], Giulietti L.[3], Levsen A.[3], Nascetti G.[2], Mattiucci S.[1]

*[1]Department of Public Health and Infectious Diseases, Sapienza-University of Rome, Rome, Italy; [2]Department of Ecological and Biological Sciences, Tuscia University, Viterbo, Italy; [3]Institute of Marine Research (IMR), Bergen, Norway*

**Keywords:** Anisakids, Genetic variability, Sub-Arctic Sea.

**INTRODUCTION:** The life cycle of anisakid nematodes depends on abiotic factors and on the stability of food-webs (Mattiucci and Nascetti, 2008. Adv Parasitol, 66:47-148). Anthropogenic changes in the Arctic Sea altering the demography of the hosts populations of anisakids, can even affect their endoparasite populations. A reduction in hosts (mainly definitive) population size over time can lead to the decreased anisakid population size and, as a consequence, to possible genetic erosion events in their gene pools (Mattiucci and Nascetti, 2008. Adv Parasitol, 66:47-148; Palomba et al., 2023. Parasitology, 150:1139-57). The anisakid nematode *Contracaecum osculatum* B is one of the three sibling species of the *C. osculatum (s.l.)* complex distributed in Arctic Sea (Nascetti et al., 1993. Int J Parasitol, 23:105-20). At the adult stage is a parasite of the seals *Phoca groenlandica*, *Phoca vitulina* and *Halichoerus grypus*, while its larvae (L3) are common in the fish *Gadus morhua* and *Mallotus villosus* (Levsen et al., 2016. Parasitol Res, 115:4281-91; Levsen et al., 2023. Parasitology, 149:1-49).
This study aimed to investigate the genetic variability of *C. osculatum* B from the same areas, over a period of approximately 35-years.

**MATERIALS AND METHODS:** Genetic variation of historical specimens (N= 250) of *C. osculatum* B collected from various seals in the Barents Sea and sub-Arctic areas in 1985-1986, was compared with contemporary ones (N= 226) sampled in cod and capelin from the same areas in 2021-22. The parasites were first sequenced at the diagnostic mtDNA cox2 (Mattiucci et al., 2008. Parasite, 15:408-19), followed by their genotyping at 7 DNA microsatellite loci (SSRs) developed for the first time in this study. Population genetics was performed by ARLEQUIN v3.5, DnaSP v6, TCS 1.21 and BEAST v2.5.

**RESULTS AND CONCLUSIONS:** High polymorphism was observed at both nuclear and mitochondrial level in the two samples of *C. osculatum* B. However, in the current population, reduction in the number of alleles was observed at each of the SSRs loci; additionally, rare mtDNA haplotypes appear to be lost. In contrast, contemporary samples of *C. osculatum* B showed new rare alleles at SSRs, and unique mtDNA haplotypes, not observed in the historical ones. The demographic Bayesian Skyline Plot performed on the genetic data, showed an initial parasite population decrease followed by a period of demographic stability, and, more recently, a population increase. The results suggest demographic fluctuations of the parasite populations in the studied area over the study period. This finding likely reflects fluctuations experienced by its hosts recorded in the same period and area (Gjøsæter et al., 2009. Mar Biol Res, 5:40-53). Estimating the genetic variability and population size of anisakids in the sub-Arctic waters may represent a tool to monitor the stability of marine trophic webs over spatial and time scales, as demonstrated in the Antarctic ecosystem (Mattiucci et al., 2015. Int J Parasitol PW, 4:356-67).

Granted by MUR-PRA.

# PREVALENCE AND MOLECULAR CHARACTERIZATION OF *BALANTIOIDES COLI* IN FATTENING PIGS RAISED IN NORTHERN ITALY

Allievi C.*[1], Ponce-Gordo F.[2], Villa L.[1], Valleri M.[1], Zanon A.[1], Zanzani S.A.[1], Mortarino M.[1], Manfredi M.T.[1]

*[1]Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Lodi, Italy; [2]Department of Microbiology and Parasitology, Universidad Complutense de Madrid, Madrid, Spain*

**Keywords:** *Balantioides coli*, Pigs, Zoonosis.

**INTRODUCTION:** *Balantioides coli* is the only ciliated protozoan of both human and veterinary interest and colonizes the large intestine of a wide range of hosts as humans, primates, pigs, rodents and birds (García-Rodríguez et al., 2022. Food Waterborne Parasitol, 26:e00143). The lack of good sanitary and biosecurity measures and the close contact between humans and pigs, which are the main source of human infection, favor its emergence and spread. So far, rRNA genotypes A and B, and sequence variants A0, A1, B0 and B1 have been reported in pigs, while only genotype A and sequence variants A0 and A2 have been detected in humans; however, the presence of genotype B in humans cannot be ruled out with current data (Ponce-Gordo et al., 2011. Protist, 162:774-94). Given the scarcity of available data on *B. coli* circulation in pigs in Italy, a study was planned to record its prevalence and to trace its genetic variants.

**MATERIALS AND METHODS:** A total of 440 faecal samples were collected from pigs weighing 170 kg and aging 9 months old raised in 22 farms located in northern Italy. All samples were analyzed by two different copromicroscopic methods: the sedimentation and the FLOTAC® dual technique, using FS2 (sodium chloride, NaCl) and FS7 (zinc sulphate, ZnSO4) flotation solutions. The Cohen's kappa coefficient was performed to evaluate the agreement between the copromicroscopic methods. Then, a conventional PCR targeting the complete ITS1-5.8S rRNA-ITS2 region and the last 117 bp (3' end) of the SSU-rRNA sequence was performed on one positive sample from each farm and PCR amplicons showing the expected size were purified and sequenced. The sequences were compared with those available in the GenBank database using the BLASTn algorithm and when more than one sequence was detected in the chromatograms, the PCR products were cloned.

**RESULTS AND CONCLUSIONS:** A total of 422 samples out of 440 were positive by sedimentation technique (95.9%), while 377 samples out of 440 were positive by FLOTAC® technique with zinc sulphate solution (85.7%) and 39 by FLOTAC® technique with sodium chloride solution (8.9%). The Cohen's kappa coefficient highlighted a moderate concordance between sedimentation and zinc-based FLOTAC® technique (0.47) while it was slight between sedimentation and salt-based FLOTAC® technique (0.15), confirming that this latter method is not suitable for *B. coli* detection. Of the 22 sequenced samples, 19 had genotype B, 2 had genotype A, and 1, which showed mixed sequences including both genotype A and B, after cloning, revealed the specific sequence variants A0 and B1.
This study demonstrated a high prevalence of *B. coli* in pigs in Italy and the genotype B was predominant. Considering that epidemiological studies conducted on *B. coli* are fragmentary, further insights are necessary, particularly in humans, to trace both its distribution and genetic polymorphism and define its public health significance.

# HELMINTH INFECTIONS IN ALPACAS (*VICUGNA PACOS*) IN ITALY: A NATION-WIDE SURVEY

Castaldo E.*[1], Buono F.[1], Scarcelli S.[1], Roncoroni C.[2], Ciaramelli A.[3], Capasso M.[1], Veneziano V.[1]

*[1]Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples, Italy; [2]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy; [3]Veterinary Practitioner, Viterbo, Italy*

**Keywords:** Camelids, Endoparasites, Faecal egg count.

**INTRODUCTION:** Domestic South American Camelids (SACs) include alpacas (*Vicugna pacos*) and llamas (*Lama glama*), native to Andean countries representing the economy engine for fibre, hides, and meat production. In the last decades their popularity increased in Europe, and they are bred mainly as pets (Sala et al., 2024. Vet Res Commun). Among health concerns, gastrointestinal helminths result in severe clinical diseases that compromise welfare and productivity. Helminths SAC specific and helminth fauna shared with small ruminants have been described mainly in South America (Cañal & Beltrame, 2022. Int J Parasitol Parasites Wildl, 19:222-42) while in Europe few data are available. This survey aims to assess the prevalence, distribution and risk factors associated with main helminth infections in alpacas raised in Italy.

**MATERIALS AND METHODS:** The survey was performed from January 2023 to March 2024. Individual faecal samples were collected from 1621 alpacas bred in 60 sites and individual Faecal Egg Counts (FECs) were performed using Mini-FLOTAC technique with floatation medium of saturated ZnSO4 (specific gravity 1.350). Baermann test and sedimentation technique were used for the diagnosis of lungworms and trematode, respectively. A questionnaire survey was conducted to assess management and worm control practices used by Italian alpaca owners. Statistical analysis included helminths with prevalence rate upper 4.0% and Chi square test was used to evaluate significant associations ($p \leq 0.05$).

**RESULTS AND CONCLUSIONS:** A prevalence of 62.4% (1011/1621) in single or mixed helminth infections was reported. Gastrointestinal strongyles (GIS) were the most common parasite (53.0%), followed by *Nematodirus* spp. (14.9%), *Trichuris* spp. (9.4%), *Capillaria* spp. (4.7%), *Nematodirus battus* (3.0%), *Moniezia* spp. (2.0%), *Dicrocoelium dendriticum* (1.1%), *Strongyloides* spp. (0.6%) and *Dictyocaulus* spp. (0.1%). *Fasciola hepatica* eggs were not detected. Animals <2 years were significantly associated with helminth infections; BCS <3 was significantly associated with *Nematodirus* spp., *Trichuris* spp. and *Capillaria* spp. infections while permanent grazing was significant for GIS, *Nematodirus* spp. and *Trichuris* spp. infections. Anthelmintic treatments were performed in 53/60 farms (88.3%) mainly on FEC results (73.6%) while 15.1% and 11.3% dewormed once or twice a year, respectively. The commonly used drug was Fenbendazole (41.4%). Alpacas had access to pasture in 53 (88.3%) farms and grazing was permanent in 39 (73.6%). Dung was removed from pasture in 38 (71.7%) and in 18 (47.4%) on daily basis. Alpacas can harbour different helminth species and GIS are the commonest. Although no selective treatment threshold is available in SAC, 47.0% and 38.5% had a FEC=0 and ≤50 EPG of GIS, respectively. These results reflect good management practices and suggest the development of a Parasitological Assistance Plan in SACs (PAPSAC) for monitoring alpaca infection status and for indication of treatment necessity limiting the anthelmintic resistance.

# MOLECULAR SURVEY ON TICK-BORNE PROTOZOA IN DOG AND CAT POPULATIONS FROM SOUTHERN ETHIOPIA

Grillini M.*[1], Tadesse H.[2], Li Q.[1], Franzo G.[1], Dotto G.[1], Tessarin C.[1], Cassini R.[1], Frangipane Di Regalbono A.[1], Kumsa B.[3], Simonato G.[1]

*[1]Department of Animal Medicine, Production and Health, University of Padova, Legnaro, Italy; [2]Arba Minch Agricultural Research Center, Southern Agricultural Research Institute, Arba Minch, Ethiopia; [3]Department of Veterinary Parasitology and Pathology, Addis Ababa University, Bishoftu, Ethiopia*

**Keywords:** Tick-borne protozoa, Dog, Cat.

**INTRODUCTION:** Tick-borne pathogens (TBPs) pose a significant health risk to dogs and cats, particularly in tropical low-income countries where ticks are widely distributed due to climatic conditions and limited control measures (Heylen et al., 2021. Parasit Vectors, 14:576). If not appropriately treated, dogs and cats may be exposed to TBDs and become competent reservoir of the zoonotic ones (Otranto et al., 2017. Trends Parasitol, 33:813-25; Kostopoulou et al., 2020. Parasit Vectors, 13:282). Although the value of pets as companion animals is rising in many areas of sub-Saharan Africa, the epidemiology of TBPs in these animals has not been adequately studied yet (Heylen et al., 2021. Parasit Vectors, 14(1):576; Madder et al., 2022. Parasit Vectors, 15(1):321). This study aimed to investigate the prevalence of TBPs (i.e., *Hepatozoon*, *Cytauxzoon* and *Babesia* species) from clinically healthy owned dogs and cats living in areas of Gamo zone, Southern Ethiopia.

**MATERIALS AND METHODS:** Animal signalment, location, lifestyle, and agroecology were collected. Blood samples were analysed using real-time and end-point PCR targeting the SSU-rRNA gene (Tabar et al., 2008. Vet Parasitol, 151:332-36). The nucleotide sequences were edited by ChromasPro v.2.1.8 (Technelysium Pty Ltd., Brisbane, Australia), compared to those present in GenBank®, and spent for phylogenetic analysis using IQ-Tree (Trifinopoulos et al., 2016. Nucleic Acids Res, 44:W232-5). Prevalence values and their 95% confidence intervals (95%CI) were calculated by EpiTools using the Wilson method (Brown et al., 2001. Statist Sci, 16(2):101-33). The Pearson Chi-square test or the Fisher's exact test, when appropriate, was used to compare variables proportions for all pathogens ($p<0.05$).

**RESULTS AND CONCLUSIONS:** Blood samples were collected from 273 dogs and 109 cats. *Hepatozoon canis* (147/273; 53.8%; CI:48-60%) and *Babesia canis rossi* (9/273;3.3%; CI:2-6%) were detected in dogs, while *Hepatozoon felis* (33/109;30.3%; CI:22-40%), *Hepatozoon luiperdije* (6/109;5.5%; CI:3-12%), *H. canis* (1/109;0.9%; CI:0-5%) and *Babesia leo* (1/109;0.9%; CI:0-5%) were identified in cats. No cat was positive for *Cytauxzoon* spp. Dogs from rural areas were found to be at higher risk for *H. canis* ($p<0.001$), whereas dogs from highland were significantly less infected ($p=0.022$). On the contrary, *B. canis rossi* was found at higher prevalence in the highland ($p=0.001$) and in young dogs ($p=0.003$). Cats from highland were significantly more infected ($p=0.002$). Phylogenetic analysis was performed for *Hepatozoon* species. Most of the strains could be classified in the *H. felis*-complex due to the genetic variability within the species herein reported. No geographical clustering appeared, suggesting a quite broad circulation and strain interchange between the different districts of the Gamo Zone. Our findings contributed to a better knowledge of the epidemiology of TBPs in pets in Ethiopia. Some aspects of the transmission dynamics remain still poorly understood and call for further studies.

# INDICE DEGLI AUTORI

## A



## B

## C

## D

## E



## F



## G

## H



## I



## J



## K



## L

## M

## N



## O



## P

## Q



## R

## S

## T



## U



## V



## W



## Y

## Z

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