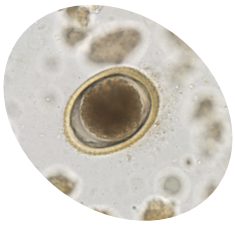
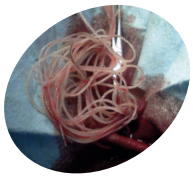
Asia-Pacific Association of Veterinary Parasitology

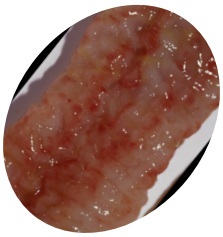
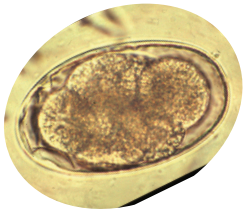
# PROCEEDINGS

## 1st APAVP Congress

### November 6 and 7, 2025

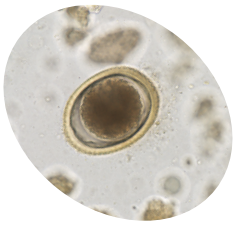
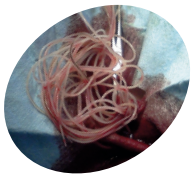
### Le Meridien Hotel, Kuala Lumpur, Malaysia

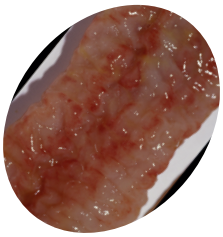
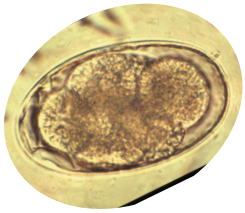




Asia-Pacific Association of Veterinary Parasitology

# ORAL COMMUNICATIONS


**PUBLIC SPACES AS HOTSPOTS OF ZOONOTIC GASTROINTESTINAL PARASITE TRANSMISSION: EVIDENCE FROM SMALL ANIMAL AND SOIL SURVEILLANCE IN MALAYSIA**

Low[1] S.Y., Gun[1] S.Z., Babatunde[2] S.M., Hussain[3] S.S.S., Kamil[4] W.N.I.W.A., Omar[1] A.R., Othman[5] A.H., Azizan[6] T.R.P.T., Moktar[1] M.A., Azman[5] N., Tan[7] Y.M., Aziz[1]* N.A.A.,

*Corresponding author. azlinaaziz@upm.edu.my

[1]*Department of Veterinary Pathology & Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia*

[2]*Farm and Exotic Animal Medicine and Surgery, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia*

[3]*Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Malaysia*

[4]*Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Malaysia*

[5]*Department of Veterinary Laboratory Diagnosis, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia*

[6]*Department of Veterinary Pre Clinical Science, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Malaysia*

[7]*Boehringer Ingelheim (Malaysia) Sdn. Bhd.*

Public spaces such as parks and playgrounds offer social and ecological benefits to communities, but it might also pose public health risks. This epidemiological survey investigated the presence and risk factors of zoonotic parasites in faecal and soil samples collected from 60 public spaces across Kuala Lumpur and Selangor, Malaysia. Eggs and larvae were collected from 71 faecal and 300 soil samples using flotation-sedimentation techniques. Species identification was done using both morphological and conventional PCR. Overall, 88.7% of faecal and 21.3% of soil samples were positive for at least one gastrointestinal parasite. *Cystoisospora* sp. was the predominant protozoa (62.0%) in faecal samples. Hookworm was the most prevalent helminth, detected in 46.5% and 9.3% of faecal and soil samples, respectively. Among hookworm-positive faeces, *Ancylostoma ceylanicum* predominated (84.4% of the identification). Hookworm coinfections with *Cystoisospora* were

commonly detected in faecal samples (37.5%). Viability testing revealed that 37.7% of collected hookworm eggs and 74.6% of collected *Toxocara* eggs were viable. Hookworm burdens were significantly higher in rural areas and beaches. Risk factor analysis revealed that the presence of dustbin was associated with reduced hookworm infections (OR=0.075, 95% CI: 0.007–0.520) in dogs. Higher odds of hookworm were found in cat faeces (OR=4.961, 95% CI: 1.10–25.98) and soil (OR=5.77, 95% CI: 1.54–20.26) from residential parks. Notably, faecal-soil concordance was observed at 43.8% sampling sites, all located in residential parks. It highlights that these areas are potential active transmission hotspots. These findings provide essential information for public health officials to develop targeted interventions to reduce the risk of transmission of zoonotic parasites in public spaces.

**Keywords**: *Ancylostoma*, co-infection, gastrointestinal parasites, public spaces, risk factors

# UPDATES ON ANTHELMINTHIC RESISTANCE AND WORM CONTROL IN SMALL RUMINANTS IN MALAYSIA

**Chandrawathani P. *[1] , Premalatha B.[2] , Zaini C,M. [2], Sam Mohan A.[3]**

*** Corresponding author**
chandra1959@gmail.com

1. **Universiti Malaysia Kelantan**
2. **Veterinary Research Institute, Malaysia**
3. **Windsor Animal Hospital, Penang**

This study carried out from 1990 to 2010, involves the control of gastrointestinal helminths in small ruminants (goats and sheep) in Malaysia, focusing on their prevalence, pathogenic effects, resistance to anthelmintics, and control strategies. Common helminths include *Haemonchus contortus*, *Trichostrongylus* spp., and *Oesophagostomum* spp., which significantly impact productivity through mortality, treatment costs, and reduced growth. Goats are more susceptible to helminth infections than sheep, and young males are particularly vulnerable. Over the years, the economic burden of parasitism has been highlighted, with studies showing high mortality rates in small ruminants due to haemonchosis and pneumonia. Epidemiological studies reveal that infective larvae are present year-round, with no strong correlation between rainfall and worm burden. Resistance to anthelmintics, particularly benzimidazoles, ivermectin, and levamisole, is widespread, exacerbated by frequent and improper drug use. Control strategies include grazing management, where rotational grazing reduces pasture contamination, and the use of medicated feed blocks, which improve nutrition and reduce worm burdens. Medicinal plants, such as neem leaves, show promise as natural anthelmintics. Breeding for resistance has been explored, with some imported breeds showing higher resistance to worms. Biological control using nematophagous fungi, such as *Duddingtonia flagrans,* has demonstrated effectiveness in reducing larval development and pasture contamination. There is a need for integrated parasite management, combining chemical, biological, and management strategies. It calls for educating farmers on sustainable practices, such as rotational grazing, strategic drug use, and improved nutrition. While chemical dewormers remain the primary control method, their overuse has led to resistance, necessitating alternative approaches. The future of helminth control in Malaysia lies in resistance management, exploring medicinal plants, and promoting sustainable practices among farmers.

Keywords: Anthelmintic resistance, small ruminants, control strategies

# EMERGENCE OF NOVEL ZOONOTIC AGENTS OF FILARIASES IN HUMANS AND DOGS IN THE POST-VALIDATION SURVEILLANCE PHASE IN THE ASIA PACIFIC

Colella V[1], Gunaratna I.E.[2], Atapattu U.[1], Young N.D.[1], Manzanell R.[1], Zheng Y.[1], Sumanam S.B.[1], Huggins L.G.[1], Koehler A.V.[1], Liyanage L.[2], Shilpeswarage N.[2], Vallipuranathan M.[2], Khieu V.[3], Gasser R.[1]

*Corresponding author: vito.colella@unimelb.edu.au

[1]Faculty of Science, The University of Melbourne, VIC 3010, Australia

[2]Anti-filariasis Campaign, Ministry of Health, Colombo 5, Sri Lanka

[3]National Centre for Parasitology, Entomology and Malaria Control, Ministry of Health, Phnom Penh, Cambodia

Zoonotic filariases are important but often neglected tropical diseases caused by filarial parasites of the family *Onchocercidae*, transmitted from animals to humans via arthropod vectors. *Brugia* spp. and *Dirofilaria repens* are the most commonly implicated agents. However, limitations in microscopy-based diagnostics have hindered accurate identification of novel or cryptic species, restricting our understanding of the role of zoonotic filariases in the Asia–Pacific region. We employed combined morphological, genetic, and genomic characterisation of a novel zoonotic *Dirofilaria* species from dogs in Sri Lanka. Additionally, we applied a newly developed next-generation sequencing (NGS)-based metabarcoding platform to detect zoonotic filarial parasites in dogs from Cambodia and Bhutan, and in both dogs and humans from Sri Lanka. We present a comprehensive morphological description of adult females, males, and microfilariae, along with mitochondrial and nuclear genome characterisation of a previously unrecognised *Dirofilaria* genotype, now designated *Dirofilaria asiatica*. Metabarcoding revealed that dogs serve as reservoirs of *D. asiatica* in Sri Lanka, Bhutan, and Cambodia. Phylogenetic and sequence type analyses further showed that *Brugia* sequences from dogs were identical to those from human infections in Sri Lanka, and distinct from *B. malayi* strains circulating in Southeast Asia.

Our findings highlight the critical role of advanced molecular tools in detecting cryptic zoonotic filarial species in both dogs and humans. We demonstrate that *D. asiatica* likely accounts for the majority of zoonotic *Dirofilaria* infections across southern and southeastern Asia, including imported cases in travellers from these regions to Australia and Europe. We also provide evidence

that dogs are reservoirs of a novel zoonotic *Brugia* species infecting humans in Sri Lanka. These findings have important implications for filariasis control strategies in the post-validation surveillance phase across the Asia–Pacific.

**Keywords**: Onchocercidae; Filarial Parasites; Neglected Tropical Diseases; Parasite Genetic; Metabarcoding

# High prevalence and cross-species transmission of *Entamoeba gingivalis* and *Trichomonas tenax* in humans, dogs, and cats in Guangxi, China

Binhan Luo,[1] Zhijuan Yin,[1] Shuyu Liang[1], Xiaoqing She[1], Domenico Otranto,[2,3] Fang Fang[1*]

[1]Parasitology Department, College of Animal Science and Technology, Guangxi University, Nanning, China
[2] Department of Veterinary Medicine, University of Bari, Valenzano, Italy
[3] Department of Veterinary Clinical Sciences, City University of Hong Kong

*Corresponding author:
E-mail: fang8730@163.com

Oral protozoa such as *Entamoeba gingivalis* and *Trichomonas tenax* are implicated in periodontal disease, yet their zoonotic potential remains poorly understood. Companion animals may act as reservoirs, facilitating cross-species transmission to humans. This study investigated the prevalence, subtype distribution, and risk factors for oral protozoan infections in humans, dogs, and cats in Guangxi, China.
Oral samples were collected by swabs from 93 humans, 298 dogs, and 325 cats. Species identification and *E. gingivalis* subtyping were performed using nested PCR and sequencing. Phylogenetic analysis was conducted to assess genetic relatedness among isolates. Associations between infection status and demographic or oral health variables were evaluated using chi-square tests and logistic regression.
*Entamoeba gingivalis* was highly prevalent across species (>90%), with mixed ST1/ST2 infections predominating. *Trichomonas tenax* prevalence differed significantly among hosts with a prevalence ranging from 4.30% in human, to 16.62% (cats) and 25.17% (dogs). The phylogenetic analysis clustered *T. tenax* and *E. gingivalis* isolate sequences irrespective of host species. In dogs, *T. tenax* infection was significantly associated with age (OR = 4.77 for seniors) and periodontal disease (OR = 4.10). Mixed *E. gingivalis*/*T. tenax* infections occurred in 4.30% of humans, 23.15% of dogs, and 16% of cats.
The high prevalence and genetic similarity of *E. gingivalis* and *T. tenax* among humans and companion animals suggest cross-species transmission, thus their zoonotic potential. *Trichomonas tenax* infection was significantly associated with age and periodontal disease, whereas *E. gingivalis* appeared to be a commensal in healthy hosts. Findings underscore the need for integrated oral health strategies incorporating veterinary screening to reduce cross-species transmission risks.

**Keywords:** *Entamoeba gingivalis*, *Trichomonas tenax*, zoonotic transmission, periodontal disease, companion animals

**PYRANTEL EVALUATION AGAINST NEMATODIASIS IN DOGS IN SLEMAN, INDONESIA**

Lintang W. Firdausy[1], Joko Prastowo*[2], Dwi Priyowidodo[2], R. Wisnu Nurcahyo[2], Ana Sahara[2], Yudhi Ratna Nugraheni[2], Vika Ichsania Ninditya[2]
*Corresponding author: joko2465@ugm.ac.id

1. Division of Veterinary Medicine, Department of Health and Life Science, Faculty of Health, Medicine, and Life Sciences, Universitas Airlangga, Banyuwangi, East Java, 68425, Indonesia
2. Department of Parasitology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia.

Dogs are among the most popular animals used by humans. Its proximity to humans has the potential for zoonotic transmission. Nematodiasis is a common gastrointestinal infection in dogs. Pyrantel is an anthelmintic that has long been used to treat gastrointestinal nematode infections. However, in recent years, the efficacy of pyrantel pamoate against nematodiasis has decreased in several regions of Australia, America and Brazil. Therefore, this study aimed to determine the efficacy of pyrantel against gastrointestinal nematodiasis in naturally infected dogs. Dog fecal samples were collected from the shelter or directly from owners. Feces were first examined qualitatively and quantitatively to determine the presence and number of nematode eggs. Animals confirmed to have nematodiasis were orally administered an appropriate dose of pyrantel pamoate. Seven days after treatment, another stool examination was performed to determine the progression of infection. The results of the next examination were analyzed for fecal egg count reduction (FECR). Even though three samples had FECRT values < 90% and showed a decrease in sensitivity to pyrantel, pyrantel still had high efficacy in reducing nematode EPG in feces (> 99 %). Based on the research results obtained, it can be concluded that the evaluation of anthelmintics is important as a form of preventing the emergence and spread of resistance in parasites.

**Keywords:** Dogs, anthelmintics, nematodiasis, pyrantel pamoate

**Genetic Diversity of *Anaplasma marginale* in Sri Lanka: MSP4 Gene Analysis**

S.S. Iddamaldeniya[1*], Sharma R.[2]

1.Veterinary Research Institute, Sri Lanka

2.Universiti Putra Malayasia, Malaysia

*Corresponding Author: samanthaidd@gmail.com

(+94)718118361

*Anaplasma marginale*, the causative agent of bovine anaplasmosis, is a tick-borne Rickettsial pathogen that infects the erythrocytes of cattle and poses significant economic challenges to the livestock industry in Sri Lanka. The disease results in reduced productivity, including decreased milk yield, weight loss, and, in severe cases, mortality. Effective epidemiological surveillance and vaccine development require a detailed understanding of the genetic diversity of circulating *A. marginale* strains, as genetic variability can influence pathogenicity, immune evasion, and vaccine efficacy.

In this study, we examined the genetic diversity of *A. marginale* in cattle populations from four geographically distinct districts in Sri Lanka. Blood samples were collected and subjected to PCR amplification targeting the major surface protein 4 (MSP4) gene. Amplified products were cloned into plasmid vectors and sequenced using the Sanger method. Raw sequences were aligned using BioEdit, and polymorphic sites were identified to assess sequence variability. Major haplotypes were selected and colour-coded to facilitate visual comparison, and a hierarchical haplotype network was constructed to evaluate genetic relatedness among clones from the different districts. Sequence analysis revealed multiple polymorphic sites distributed across the MSP4 gene, leading to the identification of several major haplotypes.

Haplotype network analysis demonstrated a combination of unique, region-specific haplotypes and shared haplotypes across districts, suggesting potential cattle movement between regions or vector-mediated gene flow. Estimates of nucleotide diversity ($\pi = 0.0027$) and haplotype diversity ($Hd = 0.81$) indicated moderate genetic variability within the sampled populations. Phylogenetic reconstruction primarily grouped clones according to their geographic origin; however, certain clones from different districts clustered together, reflecting gene flow and ongoing microevolution of the pathogen.

These findings represent the first comprehensive characterization of MSP4-based genetic diversity of *A. marginale* in Sri Lanka. The detection of both conserved and region-specific haplotypes provides critical insight into pathogen distribution and evolutionary dynamics. Such information is essential for designing molecular diagnostics to detect diverse strains and the development effective vaccines. Furthermore, understanding the patterns of gene flow among districts can help guide targeted surveillance and control strategies, including tick management and movement regulations for livestock. Overall, this study establishes a genetic baseline for *A. marginale* in Sri Lanka and underscores the importance of integrating molecular epidemiology into national livestock health programs.

**Exploring Pet Owners' Knowledge and Awareness of Parasitic Infections in Sylhet City Corporation, Bangladesh**

Islam[1] S., Rahman[2] M S., Islam[3] K M., and Islam S.[*3]

*Corresponding authors: saiful.parasitology@sau.ac.bd

1. Department of Anatomy & Histology, Sylhet Agricultural University, Sylhet, Bangladesh
2. University of Arkansas at Little Rock, USA
3. Department of Parasitology, Sylhet Agricultural University, Sylhet, Bangladesh

**Abstract**

Pets can contain parasites along with other infectious diseases. This survey investigates risk factors associated with pet owners' sociodemographic status and categorizes pet animals into different risk groups, as reported by their owners, in Sylhet City Corporation, Bangladesh. Data were collected using a preplanned questionnaire from cat and dog owners at different Pet Clinics. The responses provided details on pets' living conditions and classified them into one of the four levels of risk for ESCCAP infections (A, B, C, and D). The Chi-square test examined associations between risk groups and the owner's sociodemographic factors. This study assessed 197 cat and 32 Owners of dogs need to assess their pets' danger of diseases using ESCCAP guidelines and its relationship with owners' sociodemographic factors. Among dogs, 50% were classified in the highest-risk group (D), requiring monthly deworming, while 54% of cats were in the lowest-risk group (A), reflecting reduced exposure to parasites. For dogs, significant associations were observed between risk groups and owners' education, sex, and veterinary visits and residency ($P<0.05$). Among cat owners, owners residency, responsibility, vet visits, and attitude towards pets significantly associated with different risk groups ($P<0.05$). Deworming compliance was higher among cat owners (55.83%) than dog owners (18.75%), though it remained suboptimal overall. Awareness of zoonotic diseases was low, with only 21.87% of dog owners and 25.38% of cat owners informed. Vaccination rates were higher for cats (56.34%) than dogs (28.12%). Pets in urban areas faced lower risks than those in rural settings ($P<0.001$), underscoring the role of environmental exposure. These findings emphasize the urgent need for comprehensive health education, better veterinary engagement, and targeted interventions to enhance parasite control and reduce zoonotic risks within the One Health framework.

**Keywords:** ESCCAP, Risk assessment, Intestinal parasites, Zoonosis, Parasite control

# A REAL-TIME PCR TO QUANTIFY MICROFILARIAE OF *DIROFILARIA ASIATICA* AND ITS *WOLBACHIA* ENDOSYMBIONT IN THAILAND

Kamkong P.[1], Jitsamai W.[2], Narapakdeesakul D.[3], Taweethavonsawat P.[1,3,*]

*Corresponding author. Piyanan.T@chula.ac.th

1. Parasitology Unit, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

2. Department of Parasitology and Entomology, Faculty of Public Health, Mahidol University, Bangkok, Thailand

3. Biomarkers in Animals Parasitology Research Unit, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand

Canine dirofilariosis is a mosquito-borne disease caused by *Dirofilaria immitis*, *Dirofilaria repens*, and *Dirofilaria asiatica* (formerly known as *Candidatus* Dirofilaria hongkongensis). Among these, *D. asiatica* is frequently regarded as asymptomatic; nonetheless, parasites can cause subcutaneous dirofilariosis. *Wolbachia* is an intracellular bacterium associated with filarial nematode species and is widely used to diagnose and treat dirofilariosis. Therefore, this study aimed to quantify the *Wolbachia* endosymbiont of *D. asiatica* infecting dogs in Thailand. A total of 20 *D. asiatica*-positive blood samples were confirmed using the partial sequencing of *cox1* gene. The quantification of *D. asiatica* and its *Wolbachia* DNA was conducted using a TaqMan real-time PCR technique that targeted the 28S rRNA and *ftsZ* genes, respectively. Cycle threshold (Ct) values were ascertained for each gene, demonstrating that the median Ct values for *D. asiatica* and its *Wolbachia* DNA were 24.82 (range 27.55-37.09) and 32.46 (range 20.69-29.22), respectively. The results of this study indicate that the TaqMan real-time PCR assay is suitable tool for detecting microfilariae of the filarial *D. asiatica* and associated *Wolbachia*. Additional studies are warranted to further investigate *D. immitis* and *D. repens* and their *Wolbachia* endosymbiont, as well as other filarial nematode commonly responsible for lymphatic filariasis, particularly *Brugia malayi* and *Brugia pahangi*.

**Keywords:** Canine dirofilariosis; *Dirofilaria asiatica*; *Wolbachia*; real-time PCR; Thailand

**STUDIES ON DEVELOPMENT OF *BABESIA* PARASITES USING GENE MANIPULATION AND BIOIMAGING ANALYSIS: THE TRAP-ASSOCIATED MOLECULE P200 PLAYS A CRUCIAL ROLE IN INTRAERYTHROCYTIC PROLIFERATION OF THE MEROZOITES**

Kawazu SI[1], Hakimi H[2], Asada M*[1]. *Correspondence. masada@obihiro.ac.jp

1. National Research Center for Protozoan Diseases, Obihiro University of Agricultural and Veterinary Medicine, Inada, Obihiro 080-8555, Japan

2. Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA

Bovine babesiosis is an acute and fatal tick-borne disease affecting cattle industry worldwide, caused by several *Babesia* species. Using time-lapse video microscopy of GFP-expressing *Babesia bovis* merozoites developed in our previous study, we observed the intra-erythrocytic development of the *Babesia* parasites. The time-lapse images revealed the sequential processes of infected erythrocyte rupture, egress, gliding motility, attachment and invasion of merozoites into new erythrocytes. In closely related malaria parasites and Toxoplasma, single transmembrane TRAP-related molecules with TSR or vWF domains adhere to external substrates and transmit the parasite's driving force during this process. However, *B. bovis* possesses multiple TRAP-related molecules and it is unclear which ones are utilised for this process. We previously created single gene knockout parasites for these four molecules, none of which were essential for erythrocytic-stage proliferation. Having now identified p200, which possesses only the TSR domain, as a novel TRAP-related molecule through detailed motif searches, we analysed its function during the erythrocytic stage. The p200 knockdown parasite was created by adding sequences encoding a glmS ribozyme and a Myc tag to the gene. Post-knockdown, parasite infection rates decreased significantly, and numerous merozoites were observed outside erythrocytes in blood smears. This suggests that the knockdown parasites were unable to re-invade erythrocytes. We are currently investigating the involvement of p200 in the erythrocytic-stage using time-lapse imaging analysis.

**Keywords:** *Babesia bovis*; Gliding; Knockdown; Time-lapse imaging analysis; TRAP

# Molecular Characterization and Epidemiology of Pathogenic *Theileria* spp. in Sheep Across Two Dry Zone Districts of Sri Lanka

K.M.H.H.K. Kulasekara[*1], T.S.R. Fernando[1], P.G.I.D. Amarasiri [2], S.S. Iddamaldeniya[2], R.M.R. Priyadharshani[3]
*Corresponding author: hashanikulasekara207@gmail.com

1. Department of Animal Science, Faculty of Animal Science and Export Agriculture, Uva Wellassa University of Sri Lanka

2. Division of Parasitology, Veterinary Research Institute, Peradeniya, Sri Lanka

3. Government Veterinary Surgeon's Office, Ampara, Sri Lanka

Theileriosis is an economically significant tick-transmitted disease in small ruminants caused by parasites of family *Theileriidae.* Sri Lanka is still relying on the microscopic detection of *Theileria spp.*, with a distinct deficiency in molecular identification and characterization of globally recognized pathogenic *Theileria spp.* in small ruminants. This preliminary study was conducted to estimate the molecular prevalence, associated risk factors for *Theileria* infection and perform a phylogenetic analysis of *Theileria spp* infecting sheep in the selected farms across two districts (Anuradhapura and Puttalam) in dry zone, Sri Lanka. Information on the risk factors related to the *Theileria* infection was collected through a structured questionnaire. Blood samples (n = 50) were collected from apparently healthy sheep. Infected animals were identified morphologically through microscopic examination and processed for PCR assays using the species-specific primers for *T.luwenshuni*, *T.uilenbergi* and *T.lestoquardi*. Molecular characterization was done using 18S rRNA gene as a genetic marker for *T.luwenshuni.* Overall infection prevalence was 29/50 (58%) by molecular assays (PCR) whereas only 10/50 (20%) were detected by microscopy which revealed a significant difference between detection methods ($p < 0.05$). Mono-Infection with the *T.luwenshuni* (29/50,58%) was detected, indicating the absence of infection with *T.uilenbergi* and *T.lestoquardi.* While univariable analysis of risk factors revealed that sex and breed were significant risk factors; however, multivariable analysis confirmed that breed (OR = 4.30, 95% CI = 1.14–16.29) as the significant determinant ($p < 0.05$) of *Theileria* infection. A BLAST analysis of 18S rRNA gene sequences revealed a 99% identity with *T luwenshuni*, while the phylogenetic tree indicated that the 18S rRNA gene sequence of *T.luwenshuni* was more strongly clustered with the earlier Sri Lankan reports and Indian isolates, showing a low genetic variation from Chinese cluster. Further investigations across other dry zone districts are mandatory to better define the epidemiological burden and guide effective control strategies.

**Keywords:** Dry-zone sheep; Risk factors; Sri Lanka; Tick-borne haemoparasites; Theileriosis; *T. lestoquardi; T. luwenshuni; T. uilenbergi*

**ASIAN ZOONOTIC SCHISTOSOMIASIS IN THE PHILIPPINES: ONE HEALTH APPROACHES IN ANIMAL AND HUMAN PARASITIC DISEASE CONTRO**L

Kunluang S[1], Ohari Y[2], Angeles JMM[3], Kawazu SI*[1].

*Correspondence. skawazu@obihiro.ac.jp

1. National Research Center for Protozoan Diseases, Obihiro University of Agricultural and Veterinary Medicine, Obihiro, Japan.

2. International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan.

3. Department of Parasitology, College of Public Health, University of the Philippines Manila, Manila, Philippines.

Zoonotic schistosomiasis, caused by the parasite S*chistosoma japonicum*, remains a neglected public health concern in Asian countries, including China, the Philippines, and Indonesia. The presence of animal reservoirs has posed a major challenge in controlling the disease. Previous studies in China have demonstrated that incorporating animal reservoir host control measures led to reduction in human cases. To support the control efforts in the Philippines, we conducted a multi-locus genotyping (MLG) analysis using microsatellite markers (STRs) to investigate the transmission dynamics of *S. japonicum* infections between human and animal hosts in two areas with moderate (M) to high (H) endemicity. Fecal samples were collected from human residents and water buffaloes in the provinces of Leyte (H) and Oriental Mindoro (M). *S. japonicum* eggs were isolated from positive samples, and single-egg genome DNA was extracted for STR/MLG analysis. In Leyte, STRUCTURE analysis and discriminant analysis of principal components (DAPC) showed that the parasite population in water buffaloes clustered with those in humans, suggesting potential zoonotic transmission. By contrast, STRUCTURE and DAPC analyses revealed distinct parasite populations between water buffaloes and humans in Oriental Mindoro, suggesting that infections in this area are less likely to be zoonotic. Overall these findings highlight the need to include animal surveillance in schistosomiasis elimination guidelines. Given the complex life cycle of the parasite, understanding infection dynamics across human and animal hosts is crucial for designing effective, integrated control strategies.

**Keywords:** Asian zoonotic schistosomiasis; One Health approach; STR/MGL analysis

# PET HAMSTERS AS RESERVOIRS OF ZOONOTIC PARASITES: A PILOT MOLECULAR EPIDEMIOLOGICAL STUDY IN MALAYSIA

Low S.Y.[1]; Sadiq M.B. [2]; Omar A.R. [1]; Othman A.H.[3]; Moktar M.A.[4]; Azman N.[4]; Aziz N.A.A. [1]*
*Correspondence: azlinaaziz@upm.edu.my, Tel: +60397693464

1. Department of Veterinary Pathology & Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia
2. Farm and Exotic Animal Medicine and Surgery, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia
3. Department of Veterinary Pre Clinical Science, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Malaysia
4. Department of Veterinary Laboratory Diagnosis, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia

Hamsters are increasingly popular companion animals worldwide, especially in settings where restrictions limit dog and cat ownership. However, information on their gastrointestinal health remain scare as many studies focus on laboratory colonies. Gastrointestinal parasites are common in pet hamsters and might reduced their health. Some of these species are zoonotic and pose risks to owners. This study aimed to investigate the epidemiology of gastrointestinal parasites in pet hamsters from six randomly selected pet shop in Kuala Lumpur, Malaysia. A total of 29 pooled faecal samples were collected and analysed using faecal flotation and PCR. Overall, 72.4% (21/29, 95% CI: 54.3–85.3%) samples were positive for at least one parasite. Multiple infections were frequent, with triple infections being the most common (24.1%). Six parasite genera were identified microscopically, with *Giardia* sp. (55.2%) being the most prevalent, followed by *Enterocytozoon bieneusi* (41.4%), *Hymenolepis* sp. (34.5%), *Aspiculuris* sp. (27.6%), *Capillaria* sp. (20.7%), and *Syphacia* sp. (10.3%). Infection rates were higher in dwarf hamsters (100%) than in Syrian hamsters (65.2%). Sequencing confirmed the presence of zoonotic parasites, including *Hymenolepis nana*, *Giardia duodenalis*, and *E. bieneusi* with genotypes D and Type IV (Group 1). This work provides the first molecular evidence of zoonotic intestinal parasites in pet hamsters in Malaysia. Given the close contact between hamsters and their owners, deworming protocols and owner education are essential to reduce public health risks.

**Keywords**: co-infection, gastrointestinal parasites, hamster, Kuala Lumpur, zoonotic parasites

**TICK-BORNE *RICKETTSIA* AND *BORRELIA* IN *AMBLYOMMA VARANENSE* PARASITIZING ASIAN WATER MONITORS: DIVERSITY, PHYLOGENY, AND ZOONOTIC POTENTIAL**

Myint S.Y.P.P.[1], Aung Z.T.[1], Narapakdeesakul D.[2], Junsiri W.[2], Pongtheerat T.[3], Taweethavonsawat P.[2]*

*Corresponding author. Piyanan.T@chula.ac.th

1. Biomedical Science Undergraduate Program, Department of Medical Sciences, Faculty of Science, Rangsit University, Patumthani, 12000, Thailand

2. Biomarkers in Animals Parasitology Research Unit, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand

3. Unit of Biochemistry, Department of Medical Sciences, Faculty of Science, Rangsit University, Patumthani, 12000, Thailand

Ticks are important vectors of pathogens, including *Rickettsia* and *Borrelia*, posing significant risks to human and animal health. Despite this, little is known about tick-borne pathogens associated with reptiles, particularly Asian water monitors (*Varanus salvator*). This study examined the occurrence and genetic diversity of *Rickettsia* and *Borrelia* in ticks parasitizing Asian water monitors at Khao-Zon Wildlife Breeding Station, Ratchaburi Province, Western Thailand. Thirty adult ticks were collected and identified as *Amblyomma varanense* through morphology and confirmed by partial sequencing of the 16S rRNA gene. Nested PCR targeting the *gltA* and *flaB* genes tested positive for *Rickettsia* and *Borrelia* in 27 (90%) and 22 (73.33%) ticks, respectively. Sequencing of 15 *Rickettsia*-positive and 12 *Borrelia*-positive samples indicated high genetic diversity in *Borrelia* (H = 9) but relative conservation among *Rickettsia* (H = 4). Phylogenetic analysis showed that all *Rickettsia* genotypes clustered within the Spotted Fever Group, forming a distinct lineage from *Rickettsia tamurae*, *Rickettsia monacensis*, and *Candidatus* Rickettsia colombianensi. Most *Borrelia* genotypes grouped with isolates previously detected in Asian water monitors and *A. varanense* ticks from Indonesia and Japan, suggesting a broader distribution. These results reveal high genetic diversity of *Borrelia* and a unique *Rickettsia* lineage in reptile-associated ticks, underscoring their potential role in zoonotic disease epidemiology and the importance of continued surveillance.

**Keywords:** Asian water monitor; tick; *Amblyomma varanense*; *Rickettsia*; *Borrelia*

**MITOCHONDRIAL GENOMES OF *BRUGIA MALAYI* AND *BRUGIA PAHANGI* FROM CANINE AND FELINE HOSTS: AN EPIDEMIOLOGICAL PERSPECTIVE IN THAILAND**

Narapakdeesakul D.[1], Anantaprayoon N.[2,3], Taweethavonsawat P.[1,*]

*Corresponding author. Piyanan.T@chula.ac.th

1. Biomarkers in Animals Parasitology Research Unit, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand

2. Department of Botany, Faculty of Science, Kasetsart University, Bangkok, 10900, Thailand

3. Department of Botany, State Museum of Natural History Stuttgart, Stuttgart, 70191, Germany

*Brugia malayi*, a major causative agent of human lymphatic filariasis (elephantiasis), has traditionally been considered restricted to the Sundaic subregion, encompassing Southern Thailand. Meanwhile, *Brugia pahangi* primarily infects dogs and cats across Asia but can occasionally infect humans. Most previous reports primarily relied on morphological evidence, leaving zoonotic origins insufficiently explored. In this study, 15 microfilaria-positive samples were analyzed by partial *cox1* sequencing, confirming a single infection with either *B. malayi* (7 dogs, 2 cats, 2 lions) or *B. pahangi* (2 dogs, 2 cats). Complete mitochondrial genomes were subsequently assembled through three overlapping fragments, producing genome sizes of 13,643-13,647 bp for *B. malayi* and 13,655-13,656 bp for *B. pahangi*. Both species contained 12 protein-coding, 2 ribosomal RNA, and 22 transfer RNA genes. Intraspecific divergences ranged from 0.012-0.025% in *B. malayi* and 0.021% in *B. pahangi*, whereas interspecific divergence reached 8.59%, indicating that mitochondrial genome markers are reliable tools for distinguishing these filarial species. Importantly, *B. malayi* isolates were detected in central, eastern, and southern regions of Thailand, demonstrating that this parasite has distributed beyond its presumed endemic region and is probably circulated in both canine and feline populations across the country. These findings underscore the necessity for further investigation into the evolutionary history, zoonotic potential, and transmission dynamics of *B. malayi* in Thailand.

**Keywords:** lymphatic filariasis; *Brugia malayi*; *Brugia pahangi*; mitochondrial genome; Thailand

# DETECTION OF IVERMECTIN RESISTANCE AND ITS MITIGATION IN *Rhipicephalus (Boophilus) microplus* OF JAMMU REGION

Nazim K[1*]., Godara R[2]., Katoch R[2]., Rasool S[3].

[*]Corresponding author. kaifa.nazim@gmail.com

[1]Department of Veterinary Parasitology, Khalsa College of Veterinary and Animal Sciences (GADVASU), Amritsar, Punjab, India
[2]Division of Veterinary Parasitology, F.V.Sc & A.H, SKUAST-J, R.S. Pura, Jammu, India
[3]Department of Veterinary and Animal Husbandry Extension Education, Khalsa College of Veterinary and Animal Sciences (GADVASU), Amritsar, Punjab, India

Status of ivermectin resistance was studied in 16 isolates of *Rhipicephalus (Boophilus) microplus* collected from 7 districts of Jammu region using Larval Immersion Test (LIT). The regression graphs of probit mortality of tick larvae plotted against log values of concentrations of ivermectin were utilized for the determination of slope mortality, lethal concentrations ($LC_{50}$ and $LC_{95}$) and resistance factor (RF). Based on the RF values obtained, resistance level was categorized as I, II, III and IV. Out of these 16 isolates examined, 15 were found resistant against ivermectin. Level II resistance was detected in eleven isolates, whereas four isolates (Doda, Sumbli, Kishtwar and Bhaderwah) exhibited level I resistance. Only one isolate (Padder) was found susceptible. Effect of two (MK-571 and Cyclosporin-A) ATP-binding cassette (ABC) transport inhibitors on the efficacy of ivermectin against *R.* (*B.*) *microplus* was studied. Pre-exposure to a single pre-determined sub-lethal concentration of these two synergists has led to reduction in $LC_{50}$ values of ivermectin in different field isolates of *R.* (*B.*) *microplus.* MK-571 showed the highest increase in toxicity against ivermectin in the field isolates of *R.* (*B.*) *microplus.* The highest synergistic factors for MK-571 and Cyclosporin-A, were recorded in RS Pura isolate (2.51 and 1.78, respectively), whereas the lowest value was found for Kishtwar isolate (1.7 and 1.23, respectively).

**Keywords**: Acaricide resistance, Ivermectin, Synergist, *Rhipicephalus (Boophilus) microplus*

# RECOMBINANT K39 ANTIGENS FROM *LEISHMANIA MARTINIQUENSIS* AND *LEISHMANIA ORIENTALIS*: PRODUCTION AND PRELIMINARY EVALUATION OF THEIR DIAGNOSTIC POTENTIAL

Prasitwiset W.[1], Nambooppha B.[3], Tiwanantagorn S.[* 2,4,]

* Corresponding author. saruda.t@cmu.ac.th.

1. PhD Degree Program in Veterinary Science, Faculty of Veterinary Medicine, Chiang Mai University, Muang Chiang Mai, 50100 Thailand

2. Faculty of Veterinary Medicine, Chiang Mai University, Muang Chiang Mai, 50100 Thailand

3. Laboratory of Veterinary Vaccine and Biological Products, Faculty of Veterinary Medicine, Chiang Mai University, Muang, Chiang Mai, Thailand

4. Research Center of Producing and Development of Products and Innovations for Animal Health and Production, Chiang Mai University, Muang Chiang Mai, 50100 Thailand

Leishmaniasis is a neglected tropical disease caused by protozoan parasites of the genus *Leishmania*. In Thailand, *Leishmania martiniquensis* and *Leishmania orientalis* have been increasingly recognized as causative agents. Animal reservoirs, particularly domestic and wild mammals, are suspected to sustain transmission, but their role remains unclear due to limited diagnostic tools. Developing reliable serological methods for animal hosts is therefore critical for surveillance and disease control. Recombinant proteins, such as the kinesin-related antigen rK39, have been widely applied in human diagnostics and may provide potential for reservoir-focused applications.

In this study, the rK39 genes of *L. martiniquensis* and *L. orientalis* were cloned and expressed in Escherichia coli. Recombinant proteins were purified using Ni-NTA affinity chromatography and confirmed by SDS-PAGE and Western blot, showing specific reactivity with anti-His antibodies and positive sera. Their antigenicity was further evaluated by dot blot using serial dilutions of recombinant proteins ranging from 200 ng to 6.25 ng. For *L. martiniquensis* rK39, the lowest concentration consistently yielding a detectable positive signal was 12.5 ng (±6.25 ng), with no cross-reactivity against *L. orientalis* or *L. donovani*. In contrast, *L. orientalis* rK39 produced detectable signals starting at 200 ng and revealed antigenic cross-reactivity with *L. donovani* sera, yet displayed no detectable reactivity with *L. martiniquensis*.

These findings indicate that rK39 from both *L. martiniquensis* and *L. orientalis* display diagnostic potential, though with differing sensitivity and cross-reactivity profiles. Ongoing evaluations using the direct agglutination test (DAT) and enzyme-linked immunosorbent assay (ELISA) aim to further assess their diagnostic performance and applicability in leishmaniasis surveillance in both animal reservoirs and human cases.

**Keywords:** *Leishmania martiniquensis*, *Leishmania orientalis*, rK39 antigen, Serodiagnosis

**INTEGRATING NEMABIOME METABARCODING WITH FECAL EGG COUNT REDUCTION TEST FOR SPECIES-SPECIFIC ANTHELMINTIC EFFICACY AGAIST STRONGYLIDS NEMATODES IN GOATS**

Rompo T.*[1,2], Namboopha B.[2], Singhla T.[2], Hayashi N.[3], Nonaka N.[3], Nakao R.[3], Tiwananthagorn S.[2]

*Corresponding author; Thanakorn.r@dld.go.th

1. Department of livestock development, Ministry of Agriculture and Cooperatives, Bangkok, Thailand.
2. Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand.
3. Laboratory of Parasitology, Graduate School of Infectious Diseases, Faculty of Veterinary Medicine, Hokkaido University, Japan.

Anthelmintic resistance of strongyle nematodes in goats is increasingly reported and challenges routine parasite control. Standard field anthelmintic assessment relies on the fecal egg count reduction test (FECRT). Since the strongyle eggs are morphologically indistinguishable under light microscopy, the identification of resistant species are unachievable. This study integrated FECRT with nemabiome metabarcoding to resolve species-specific responses following anthelmintic treatment. On a goat farm in Chiang Mai, Thailand, Native-Saanen crossbred goats were randomly allocated to the eprinomectin treatment group (0.2 mg/kg subcutaneously, n = 15) and untreated control (n=9). Rectal fecal samples collected on Day 0 and Day 14 were examined by the McMaster method to obtain eggs per gram (EPG). Copro-DNA from the same samples was used for ITS-2 amplicon sequencing on an Illumina MiSeq platform to characterized strongyle taxa and their relative abundances. Individual microscopic EPGs were apportioned by each taxon's read proportion to derive species-adjusted egg counts. Microscopic fecal egg count reduction (FECR), species-adjusted egg count reduction (SECR) and their 90% confidence intervals (CIs) were calculated. Treatment efficacies were interpreted according to the WAAVP 2023 guidelines. FECRT indicated reduced efficacy of eprinomectin, with a 90% CI of -75.24% to 23.60%. SECR indicated reduced efficacy for *Haemonchus contortus* (90% CI: -321.315 to 10.61%) and *Trichostrongylus colubriformis* (90% CI: -43.06 to 70.22%). In contrast, marked susceptibility was observed for *Trichostrongylus axei* (90% CI: 95.68% to 100.00%) and *Oesophagostomum columbianum* (90% CI: 98.66% to 100.00%), indicating species-heterogenous response that was not revealed only by FECRT. Despite quantitative limitations of metabarcoding-derived taxa proportions, assuming of stable taxon-specific ITS-2 copy number and amplification efficiency across time points, FECRT combined with species-adjusted metrics provides practical, species-specific efficacy profiles to guide targeted deworming and support sustainable strongyle nematodes control in goats.

**Keywords;** Anthelmintic resistance, Fecal egg count reduction, Species-specific, Strongyle, Small ruminant

**Trypanosomosis due to *Trypanosoma evansi* in South Asia: Three Decades of Research on Epidemiology, Pathogenesis, Diagnosis and Control Strategies**

**Singla L.D.***

*Corresponding author. ldsinglanavs@gmail.com

Department of Veterinary Parasitology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India

Trypanosomosis caused by *Trypanosoma evansi*, commonly known as surra, remains a significant constraint to livestock productivity across South Asia, particularly affecting equines, bovines, buffaloes, and camels. This presentation summarizes three decades of research on *T. evansi* infection in Punjab, India, examining epidemiological patterns, pathogenesis, diagnostic advances, and control measures. Seroepidemiological surveys using CATT/*T. evansi* revealed variable prevalence rates across different agro-climatic zones of Punjab, with spatial risk analysis identifying lowland areas as high-risk zones due to favorable conditions for mechanical vector proliferation. The infection manifests through diverse clinical presentations ranging from acute to chronic forms, characterized by intermittent fever, progressive anemia, immunosuppression, and multi-organ pathology. Hematological and biochemical investigations demonstrated significant alterations including severe anemia, thrombocytopenia, hypoproteinemia, and oxidative stress markers in naturally and experimentally infected animals. Comparative evaluation of diagnostic techniques revealed that while conventional parasitological methods have limited sensitivity in chronic cases, molecular approaches including PCR, multiplex PCR, and real-time PCR offer superior detection capabilities for both patent and latent infections. Immunological studies employing ELISA and Card Agglutination Test demonstrated high sensitivity for epidemiological surveillance. Experimental trials with levamisole as an immunopotentiator combined with diminazene aceturate showed promising therapeutic outcomes. The concurrent detection of *T. evansi* with tick-borne pathogens (*Anaplasma marginale*, *Babesia bigemina*, *Theileria equi*) highlights the complexity of field infections and diagnostic challenges. Recent investigations into atypical manifestations in canines and potential zoonotic implications emphasize the evolving nature of this disease. The findings highlight the need for integrated control strategies combining improved diagnostic tools, targeted treatment protocols, vector management, and surveillance systems to mitigate the economic impact of trypanosomosis on livestock production in endemic regions.

**Keywords:** *Trypanosoma evansi*; surra; epidemiology; diagnosis; pathogenesis; South Asia; livestock

**HOW OLD IS CANINE HEARTWORM: WHAT DOES POPULATION GENOMICS REVEAL ABOUT THE ORIGIN OF *DIROFILARIA IMMITIS*?**

Power R.I.[1], Abdullah S.[2], Walden H.S.[3], Verocai G.G.[4], Sanders T.L.[4], Luksovsky J.L.[4], Moorhead A.R.[5], Dzimianski M.T.[6], Foster J.M.[7], Michalski M.L.[8], Rojas A.[9,10], Chacón S.C.[11], Deak G.[12], Mihalca A.D.[12], Danesi P.[13], Papadopoulos E.[14], Taweethavonsawat P.[15,16], Bui D.T.[17,18], Do Ngoc A.[19], Sharma R.S.K.[20], Ho S.Y.W.[21], Doyle S.R.[22], Šlapeta J.[1,23]

*Corresponding author: jan.slapeta@sydney.edu.au

1. Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, New South Wales, Australia

2. University of Queensland, School of Veterinary Science, Gatton, Queensland, Australia

3. Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Florida, USA

4. Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, Texas, USA

5. Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

6. Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA

7. New England Biolabs Inc., Ipswich, Massachusetts, USA

8. University of Wisconsin Oshkosh, Oshkosh, Wisconsin, USA

9. Laboratory of Helminthology, Faculty of Microbiology, University of Costa Rica, San José, Costa Rica

10. Centro de Investigación en Enfermedades Tropicales, University of Costa Rica, San José, Costa Rica

11. Department of Veterinary Parasitology, Healthy Pet Veterinary Hospital SC, David, Chiriquí, Panamá

12. Department of Parasitology and Parasitic Diseases, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

13. SCS3 - Parasitology and Mycology Unit, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

14. Laboratory of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

15. Parasitology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

16. Biomarkers in Animals Parasitology Research Unit, Chulalongkorn University, Bangkok, Thailand

17. Department of Parasitology, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi, Vietnam

18. Faculty of Ecology and Biological Resources, Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi, Vietnam

19. Department of Medical Parasitology, Vietnam Military Medical University, Hanoi, Vietnam

20. Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

21. School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, New South Wales, Australia

22. Wellcome Sanger Institute, Hinxton, Cambridgeshire, UK

23. Sydney Infectious Diseases Institute, The University of Sydney, New South Wales, Australia

Heartworms (*Dirofilaria immitis*) are parasitic nematodes causing significant cardiopulmonary disease in canids worldwide. This study presents the largest population genomics analysis of heartworms to date, based on whole-genome sequencing of 127 adult specimens from mammalian carnivores across four continents. Our transcontinental genome-wide analysis reveals a deeper evolutionary origin and dispersal history than previously understood. Rather than being solely driven by recent human-mediated dog movements, ancestral canid hosts played a pivotal role in the global dissemination of heartworms. Admixture analyses suggest an Asian origin for Australian heartworms, consistent with the arrival of dingoes thousands of years ago. Genetic relatedness between European and Central American heartworms reflects modern dispersal linked to colonization and dog migration. These findings highlight the long-standing ecological niche exploited by heartworms and underscore the influence of anthropogenic factors—such as drug pressure, climate change, and global connectivity—on shaping parasite diversity. Understanding the global genomic landscape of D. immitis is essential for developing effective, geographically informed surveillance and control strategies.

**Keywords:** *Dirofilaria immitis*, heartworm, population genomics, evolutionary history, admixture, global dispersal, canids, parasite surveillance, whole-genome sequencing, veterinary parasitology

**COMPREHENSIVE GENETIC CHARACTERIZATION OF HEMOPLASMAS IN DAIRY CATTLE FROM THAILAND USING TWO MARKERS SUGGESTS HIDDEN SPECIES WITHIN THE *MYCOPLASMA WENYONII* LINEAGE**

Thongmeesee K.[1], Tiyamanee W.[2], Narapakdeesakul D.[3], Kamkong P.[3], Wechtaisong W.[1,4], Tiawsirisup S.*[1]

*Corresponding author. sonthaya.t@chula.ac.th.

1. Center of Excellence in Animal Vector-Borne Diseases, Veterinary Parasitology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand

2. Department of Disease Control, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan

3. Veterinary Parasitology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand

4. Academic Service Division, National Laboratory Animal Center, Mahidol University, Nakhon Pathom 73170, Thailand

Cattle, including dairy cattle, are one of the important livestock animals in Thailand. They could be affected by various vector-borne pathogens, including hemotropic *Mycoplasma* spp. (hemoplasmas), leading to production and economic loss. For bovine hemoplasma species, two common species have been described and detected from several countries: *Mycoplasma wenyonii* (*Mw*; *Eperythrozoon wenyonii*) and '*Candidatus* Mycoplasma haemobos' (*C*Mhb; '*Ca*. M. haematobovis'). Although bovine hemoplasmas in Thailand have been documented, comprehensive genetic analyses of those *Mw* sequences as several single species in the same manner as hemoplasmas in cats, pigs, and wildlife animals are limited. This study aimed to study the molecular occurrence and genetic diversity of bovine hemoplasmas in dairy cattle from Thailand using both 16S and 23S rRNA genes. The results showed that 30.8% (161/522, 95% CI: 26.9% to 35.0%) of dairy cattle in Thailand were infected with hemoplasmas based on a PCR assay targeting the 16S rRNA gene. Out of 161 positive samples, 52 samples were successfully sequenced with both 16S and 23S rRNA genes. From genetic analyses, at least four hemoplasma species have been found in Thai dairy cattle, consisting of *Mw*, *C*Mhb, and two putative species hidden within the *Mw* lineage. The name of one hidden species has been proposed with '*Ca*. M. teganodes' sp. nov. Moreover, 16S rDNA analyses also suggested that contaminated plants and water could serve as potential sources of hemoplasma transmission.

**Keywords:** *Candidatus* Mycoplasma haemobos; Dairy cattle; Hemoplasma; *Mycoplasma wenyonii*; Thailand

**MOLECULAR CHARACTERIZATION OF *ORNITHONYSSUS* SP. (MESOSTIGMATA: MACRONYSSIDAE) INFESTING DOG IN SAMUT SAKHON PROVINCE, THAILAND: A CASE REPORT**

Thongmeesee K.[1], Pimolvattana M.[2], Tiawsirisup S.*[1]

*Corresponding author. sonthaya.t@chula.ac.th.

1. Center of Excellence in Animal Vector-Borne Diseases, Veterinary Parasitology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand

2. Mahachaiborirak Animal Hospital, Samut Sakhon 74000, Thailand

Common canine ectoparasites in Thailand include fleas (*Ctenocephalides* spp.), lice (*Heterodoxus spiniger* and *Trichodectes canis*), ticks (*Rhipicephalus sanguineus* tropical lineage; *R*. *linnaei*), and mites (*Demodex* spp., *Otodectes cynotis*, and *Sarcoptes scabei*). However, there was no information regarding dermanyssoid mites (Acari: Parasitiformes: Mesostigmata) infesting dogs in Thailand. In this present study, ectoparasites in the group of dermanyssoid mites from a dog have been reported. A four-year-old, castrated, male Pomeranian was presented with a chief complaint of skin lesions and tiny ectoparasites found on the skin. This dog inherited neurological conditions and received selamectin spot-on regularly, with the ability to access the yard around the owner's house. All ectoparasites have been observed under a microscope by a veterinarian, with mites suspected. Seven individual samples were kept in 70% ethanol and submitted to the Veterinary Parasitology Unit for morphological identification. Microscopically, these ectoparasites could potentially be *Ornithonyssus* sp. mites (approximately 1 mm) with the specific feature of an anus at the anterior half of the anal plate (oval shape). DNA was extracted from a pool of mites. The partial fragment of the *cox1* gene was amplified and sequenced using LCO1490/HCO2198 primers. Nucleotide BLAST results showed that the closest sequences were *Ornithonyssus sylviarum* (MH983585) with 86.15% identity (100% query coverage) and *Ornithonyssus bacoti* with 87.56% identity (68% query coverage). With limited *cox1* sequences in the database, these findings suggested that these mites are *Ornithonyssus* sp., and this is the first report of this mite infesting a dog in Thailand. However, other genetic markers, such as the 28S rRNA gene, should be amplified and compared with GenBank. Finally, a dog was continuously prescribed Revolution® combined with regular grooming, providing effective treatment against infestation of *Ornithonyssus* sp.

**Keywords:** Dog; Ectoparasite; Mite; *Ornithonyssus*; Thailand

**RECOMBINANT K39 ANTIGENS FROM *LEISHMANIA MARTINIQUENSIS* AND *LEISHMANIA ORIENTALIS*: PRODUCTION AND PRELIMINARY EVALUATION OF THEIR DIAGNOSTIC POTENTIAL**

Prasitwiset W.[1], Nambooppha B.[3], Tiwanantagorn S.[* 2,4,]

* Corresponding author. saruda.t@cmu.ac.th.

1. PhD Degree Program in Veterinary Science, Faculty of Veterinary Medicine, Chiang Mai University, Muang Chiang Mai, 50100 Thailand

2. Faculty of Veterinary Medicine, Chiang Mai University, Muang Chiang Mai, 50100 Thailand

3. Laboratory of Veterinary Vaccine and Biological Products, Faculty of Veterinary Medicine, Chiang Mai University, Muang, Chiang Mai, Thailand

4. Research Center of Producing and Development of Products and Innovations for Animal Health and Production, Chiang Mai University, Muang Chiang Mai, 50100 Thailand

Leishmaniasis is a neglected tropical disease caused by protozoan parasites of the genus *Leishmania*. In Thailand, *Leishmania martiniquensis* and *Leishmania orientalis* have been increasingly recognized as causative agents. Animal reservoirs, particularly domestic and wild mammals, are suspected to sustain transmission, but their role remains unclear due to limited diagnostic tools. Developing reliable serological methods for animal hosts is therefore critical for surveillance and disease control. Recombinant proteins, such as the kinesin-related antigen rK39, have been widely applied in human diagnostics and may provide potential for reservoir-focused applications.

In this study, the rK39 genes of *L. martiniquensis* and *L. orientalis* were cloned and expressed in Escherichia coli. Recombinant proteins were purified using Ni-NTA affinity chromatography and confirmed by SDS-PAGE and Western blot, showing specific reactivity with anti-His antibodies and positive sera. Their antigenicity was further evaluated by dot blot using serial dilutions of recombinant proteins ranging from 200 ng to 6.25 ng. For *L. martiniquensis* rK39, the lowest concentration consistently yielding a detectable positive signal was 12.5 ng (±6.25 ng), with no cross-reactivity against *L. orientalis* or *L. donovani*. In contrast, *L. orientalis* rK39 produced detectable signals starting at 200 ng and revealed antigenic cross-reactivity with *L. donovani* sera, yet displayed no detectable reactivity with *L. martiniquensis*.

These findings indicate that rK39 from both *L. martiniquensis* and *L. orientalis* display diagnostic potential, though with differing sensitivity and cross-reactivity profiles. Ongoing evaluations using the direct agglutination test (DAT) and enzyme-linked immunosorbent assay (ELISA) aim to further assess their diagnostic performance and applicability in leishmaniasis surveillance in both animal reservoirs and human cases.

**Keywords:** *Leishmania martiniquensis*, *Leishmania orientalis*, rK39 antigen, Serodiagnosis

**Prevalence of Zoonotic Intestinal Protozoan among Tibetan Pigs in the Qinghai-Tibet Plateau, China**

Wang X.[1], Li H.T.[1], Zhang R.H.*[1]

*Corresponding author. zhang_runhui@swun.edu.cn

1. College of Animal and Veterinary Sciences, Southwest Minzu University, Chengdu, 610041, PR China

Zoonotic intestinal protozoa remain a growing concern worldwide, threatening both livestock production and public health. Among them, *Balantidium* (*B.*) *coli*, *Blastocystis,* and *Enterocytozoon* (*E.*) *bieneusi* are frequently detected in pigs. Tibetan pigs, an indigenous breed on the Qinghai-Tibet Plateau (QTP), are commonly raised under free-range or mixed free-range and captive husbandry, where closely contact with human. In this study, we aimed to investigate the prevalence and genetic diversity of these three protozoa in Tibetan pigs from Ganzi Prefecture, Sichuan Province, China.

A total of 415 fresh fecal samples were collected and DNA was amplified by PCR. Then, the products were sequenced and the genetic diversity of sequences was further analyzed. Consequently, the overall prevalence of three protozoa was 67.2% (279/415). *B. coli* was the most prevalent protozoan (59.0%), that the genotypes of variants A and B were identified. The infection rate of *Blastocystis* was 46.8%, that the subtypes ST5 and ST1 were identified. *E. bieneusi* was occurred in 16.4%, and three genotypes (EbpB, EbpC, Henan-I) were found. Furthermore, the study analyzed the relationship between prevalence and the health status, ages, feeding mode, and different seasons. Infections with *B. coli* and *Blastocystis* were strongly associated with diarrhea ($P < 0.01$). Piglets and free-range pigs showed higher prevalence of *B. coli* and *E. bieneusi* infection, whereas *Blastocystis* was more common in captive pigs ($P < 0.01$). Seasonal peaks were observed in spring for *B. coli* and *Blastocystis*, however in winter for *E. bieneusi.*

These results highlight Tibetan pigs as important reservoirs of these three zoonotic protozoa in the QTP of China. Strengthened control strategies are essential, and the findings support the one health framework by linking animal and human parasite management.

**Keywords**: zoonotic protozoa; Tibetan pigs; surveillance; one Health

**Gastrointestinal Helminths in Dogs and Cats in Malaysia from 2010-2020: A Systematic Review**

Thilakeshwaran, C[1]; Watanabe, M[2]
[1]Department of Veterinary Clinical Studies
Faculty of Veterinary Medicine, University Putra Malaysia, 43400 UPM, Serdang,
Serdang Darul Ehsan
[2]School of Veterinary Medicine,
IMU University, No. 126, Jalan Jalil Perkasa 19
Bukit Jalil, 57000 Kuala Lumpur, Malaysia
*Email: malaikawatanabe@imu.edu.my*

Companion animals play a significant role in the One Health framework, as gastrointestinal (GI) helminths of dogs and cats may pose risks not only to animal health but also to public health. This systematic review aimed to provide an overview of the most commonly reported canine and feline GI helminths in Malaysia between 2010 and 2020, to identify newly reported species during this period, and to evaluate temporal trends.

Ten eligible studies were retrieved from multiple databases based on defined inclusion criteria (publication year, Malaysian study site, language, full text availability, species-specific prevalence data, and separation of canine and feline findings). Key data extracted included study details, diagnostic methods, prevalence values, location, and sample size.

The review revealed limited research on pet populations, with only one study focusing on pet dogs; the remainder involved stray or shelter animals. Consequently, reliable temporal trends could not be established due to data gaps across the decade. Study sites for dogs were primarily Kuala Lumpur, Klang Valley, Selangor, Pahang, and Ipoh, while cats were sampled from Kuala Lumpur, Malacca, Kuantan, Georgetown, Klang Valley, Selangor, and Pahang. Urban–rural differences were notable: feline GI helminth prevalence was higher in rural areas, whereas canine prevalence was greater in urban centres such as Kuala Lumpur and Klang Valley.

The most frequently reported helminths in dogs were *Ancylostoma spp., Toxocara spp*., and *Trichuris spp*., while in cats the predominant species were *Toxocara spp., Ancylostoma spp*., and *Spirometra spp*. In both hosts, *Ancylostoma ceylanicum* was the most common hookworm detected. A novel *Dipylidium* species was also reported in *Ctenocephalides felis* in 2017.

To our knowledge, this is the first systematic review of canine and feline GI helminths in Malaysia, underscoring both their One Health significance and the need for more robust surveillance in companion animals.

**Keywords*:*** dogs; cats; gi helminth; systematic review; Malaysia

**18S rDNA next-generation sequencing uncovers the biodiversity of Gastrointestinal parasites in Tibetan grazing ruminants in China**

Wu S.R.[1], Ying Z.[2],Li H.T.[1], Tang C.[1], Zhang B.[1] and Zhang R.H.[1]*

*Corresponding author. zhang_runhui@swun.edu.cn

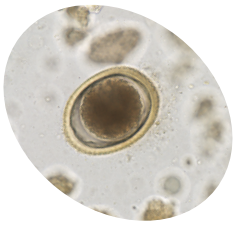
1. College of Animal and Veterinary Sciences, Southwest Minzu University, Chengdu, 610041, PR China
2. Neijiang Vocational and Technical College, Neijiang 641199, China

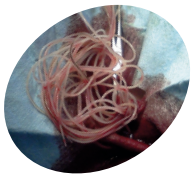
Free-ranging yak, Tibetan sheep, and Tibetan goat on the Qinghai-Tibetan Plateau (QTP) are highly vulnerable to diverse parasitic infections, which may cause underestimated economic losses. This study investigated gastrointestinal parasite biodiversity in local ruminants using 18S SSU rDNA next-generation sequencing. Seventy-nine fecal samples from yak, sheep, and goats in southeast QTP were analyzed. The V3–V4 fragments of 18S rDNA were amplified to profile protozoa and helminth diversity. Associations with host age, health status, and season were also examined.We identified 192 OTUs encompassing 10 phyla and 27 genera. *Entamoeba* (93.67%), *Blastocystis* (75.95%), and *Trichostrongylus* (68.35%) were predominant. Phylogenetic analysis revealed a potential novel *Entamoeba* species and zoonotic taxa including *T. colubriformis* and *Blastocystis* ST10, ST12, and ST14. Notably, *Colpoda* and *Colpodella*—rarely reported zoonotic protozoa showed high prevalence and potential association with diarrhea. Similar parasite assemblages were observed in juveniles and adults, while helminth diversity and prevalence markedly declined in autumn. These findings provide new insights into gastrointestinal parasite diversity in QTP ruminants and contribute to understanding infection risks in grazing livestock.

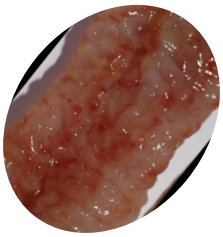
Keywords: Parasite diversity, Yak, Tibetan sheep, Tibetan goat, 18S

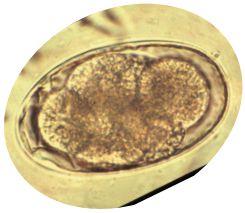
Asia-Pacific Association of Veterinary Parasitology

# POSTERS









**MOLECULAR EVIDENCE OF NOVEL TICK-BORNE HAEMOGREGARINES IN *AMBLYOMMA VARANENSE* PARASITIZING ASIAN WATER MONITORS**

Aung Z.T.[1], Myint S.Y.P.P.[1], Narapakdeesakul D.[2], Junsiri W.[2], Pongtheerat T.[3], Taweethavonsawat P.[2,*]

*Corresponding author. Piyanan.T@chula.ac.th

1. Biomedical Science Undergraduate Program, Department of Medical Sciences, Faculty of Science, Rangsit University, Patumthani, 12000, Thailand

2. Biomarkers in Animals Parasitology Research Unit, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand

3. Unit of Biochemistry, Department of Medical Sciences, Faculty of Science, Rangsit University, Patumthani, 12000, Thailand

Ticks are important ectoparasites of reptiles and are known vectors of haemogregarine protozoa, including *Hepatozoon* and *Hemolivia* species. This study investigated the presence and phylogenetic relationship of haemogregarines in ticks collected from Asian water monitors (*Varanus salvator*) at Khao-Zon Wildlife Breeding Station, Ratchaburi Province, Western Thailand. Thirty adult ticks were obtained and identified as *Amblyomma varanense* based on the morphological characteristics and partial sequencing of the 16S rRNA gene. Screening by nested PCR assay targeting the 18S rRNA gene revealed haemogregarine positive in two ticks (6.66%). Sequence analysis and BLASTN identified two distinct parasite species. The first isolate (TickAm14) exhibited a 99.52% similarity to *Hepatozoon* sp. (HQ292774) previously found in Seychelles wolf snake (*Lycognathophis seychellensis*) in East Africa. The second (isolate TickAm25) showed a 98.92% similarity to *Hemolivia* sp. in *Hyalomma aegyptium* tick collected from Greek tortoise (*Testudo graeca*) in Algeria. Phylogenetic analysis demonstrated TickAm14 clustering with *Hepatozoon* species from African reptiles, whereas TickAm25 was placed in the *Hemolivia* lineage but distinct from closely related taxa, suggesting it may represent a novel species. These findings provide the first molecular evidence of *Hepatozoon* and *Hemolivia* in *A. varanense* ticks parasitizing Asian water monitors in Thailand, emphasizing the diversity of tick-borne haemogregarines in reptiles.

**Keywords:** Asian water monitor; tick; *Amblyomma varanense*; *Hepatozoon*; *Hemolivia*

# Common control methods for worms in small ruminants -

# What you can do to reduce helminthiasis in goats or sheep

Chandrawathani P. *[1] , Premaalatha B.[2] , Zaini C,M. [2], Sam Mohan A.[3]

* Corresponding author

chandra1959@gmail.com

1. Universiti Malaysia Kelantan
2. Veterinary Research Institute, Malaysia
3. Windsor Animal Hospital, Penang

Helminthiasis is one of the most common gastrointestinal infections in small ruminants, which may cause morbidity and mortality which results in losses for the farmer. In Malaysia, the severe anthelmintic resistance issues that have occurred over the past two decades has necessitated the use of alternative methods for worm control. The department of Veterinary services encourages the use of non drug related options for helminth control especially haemonchosis which is prevalent. Due to the hot, wet cli-mate in Malaysia, the strongyle worms and coccidiosis seems to be rampant and more than 80% of farms have these infections. Early detection will allow early remedial treatment and save the animals from death. Biological control using nematophagous fungi is also used in some countries especially for horses and herbal remedies are used commonly in some Asian countries for example neem leaves in India and Malaysia. Other practical strategies to overcome drug resistance will include reversion of resistance by culling the most susceptible animals, natural reversion by diluting resistant genes which will take a long time, or reversion by using drug combinations. Overall, the use of drugs is becoming less popular due to its ineffectiveness and it is hoped farmers will venture into organic farming as helminthiasis is an age old infection which can be managed successfully.

Keywords: Helminthiasis, small ruminants, control options, anthelmintic resistance

**FIELD COMPARISON OF ANTHROPOPHILIC AND ZOOPHILIC MOSQUITO ATTRACTION USING HUMAN AND GOAT BAITS IN KAMPUNG ORANG ASLI LUBOK LEGONG, KEDAH**

Chen C.D.[1]*, Lau K.W.[2,3], Lee I.L.[4], Yazrin M.I.Z.[1,5], Lee K.M.[6], Abdullah N.A.[5]

[1]EntomoBio Research Laboratory, Research Facility Centre, Department of Research Development, High Impact Research Building, Universiti Malaya, 50603 Kuala Lumpur, Malaysia
[2]Mahidol Vivax Research Unit, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchavithi Rd., Rachathevi, Bangkok 10400, Thailand
[3]Center for Toxicology & Health Risk Studies (CORE), Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia
[4]Kulliyyah of Medicine and Health Sciences, Universiti Islam Antarabangsa Sultan Abdul Halim Mu'adzam Shah, 09300 Kuala Ketil, Kedah, Malaysia
[5]Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia
[6]Nanotechnology & Catalysis Research Centre (NANOCAT), Level 3, Block A, Institute for Advanced Studies, Universiti Malaya, 50603 Kuala Lumpur, Malaysia
*Corresponding author: chen_ctbr@um.edu.my

Understanding host-seeking behavior of mosquitoes is crucial for effective vector surveillance and control. This field study was conducted to compare the efficiency and species composition of mosquitoes attracted to human and goat hosts in Kampung Orang Asli Lubok Legong, Kedah. Mosquitoes were collected hourly from 18:00 to 20:00 for three consecutive nights using two trapping methods: Human Landing Catch (HLC) to represent anthropophilic attraction, and Goat-Baited Bed Net (GBBN) collection to represent zoophilic attraction. A total of six mosquito species were collected using each method. From the HLC collection, the dominant species was *Aedes albopictus* (64.29%), followed by *Culex vishnui* (19.05%), *Mansonia dives* (9.52%), *Anopheles barbumbrosus* (2.38%), *Armigeres kesseli* (2.38%), and *Ar. subalbatus* (2.38%). In contrast, the GBBN method captured a different composition, dominated by *Cx. vishnui* (41.44%), followed by *Ar. kesseli* (20.83%), *Ae. albopictus* (16.67%), *Cx. gelidus* (12.50%), *An. barbumbrosus* (4.17%), and *An. kochi* (4.17%). Several species, including *Ae. albopictus*, *Cx. vishnui*, *Ar. kesseli*, and *An. barbumbrosus*, were attracted to both human and goat baits, indicating their opportunistic feeding behavior. Such species may act as potential bridge vectors, capable of transmitting zoonotic pathogens between animals and humans. The findings demonstrate clear differences in host preference, with *Ae. albopictus* showing strong anthropophilic tendencies, while *Cx. vishnui* and *Ar. kesseli* were predominantly zoophilic. This study highlights the importance of integrating both human and animal-baited surveillance methods to better understand mosquito ecology, host preference, and potential zoonotic transmission risks. The results provide valuable insights for developing safer and more representative vector monitoring strategies.

**Keywords:** Mosquito surveillance, Human landing catch, Animal-baited bed net, Host preference, Anthropophilic, Zoophilic

**GENETIC DIVERSITY AND POPULATION CONNECTIVITY OF *Plasmodium knowlesi* INFECTING *Macaca fascicularis* ACROSS PENINSULAR MALAYSIA**

Nor Dilaila M.S.*, Norhadila Z., Zarith S. and Sharma R.S.K.

*Corresponding author. nordilaila06@gmail.com

Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.

*Plasmodium knowlesi* is a zoonotic malaria parasite of primates in Southeast Asia and is currently the most common cause of human malaria in Malaysia. Despite its growing public health importance, information on the parasite's genetic diversity and population structure within its natural reservoir host the Long-tailed macaques (*Macaca fascicularis*) remains limited. The present study was undertaken to determine the genetic diversity, geneflow and population genetics of *P. knowlesi* infecting *M. fascicularis* in Peninsular Malaysia. Macaques were sampled throughout Peninsular Malaysia covering all 12 states. *Plasmodium knowlesi* infections were confirmed by PCR amplification of the 18S SSU rRNA gene. Positive amplicons were subsequently genotyped using ten *P. knowlesi* specific microsatellite loci. Genetic diversity and gene flow analyses were performed using FSTAT v2.9.4, while population structure was examined using Bayesian clustering in STRUCTURE v2.3.4. The relationship between genetic distance and geographical distance was analysed using GENEPOP. Microsatellite analysis revealed moderate to high genetic diversity (5 to 16 alleles per locus; mean expected heterozygosity, He= 0.655), indicating a genetically diverse *P. knowlesi* reservoir in *M. fascicularis* populations. Bottleneck tests showed no evidence of recent population reduction, except in the Southern region. Analysis of molecular variance (AMOVA) attributed 98% of variance to within populations, suggesting high genetic connectivity. STRUCTURE analysis revealed an admixed population divided into three main genetic clusters (K=3). The Mantel test (r=0.079, p=0.438) showed no significant isolation by distance, implying that geographic separation does not restrict parasite gene flow across regions. Overall, these findings demonstrate that *P. knowlesi* populations infecting *M. fascicularis* in Peninsular Malaysia are genetically diverse and well connected, suggesting extensive gene flow likely maintained through macaque movement and the widespread vector distribution, contributing to the ongoing transmission among the macaques.

Keywords: Plasmodium knowlesi, Macaca fascicularis, Genetic diversity, Population connectivity

**Modulation of gut microbiota, epithelial barrier, and immune response by *Blastocystis* sp. in schizophrenic individuals**

Freddy Franklin[1], Chandramathi Samudi Raju[2]*, Arutchelvan Rajamanikam[1*], Sheivanya Gayatrri Kuppusamy[1], Suresh Kumar Govind[1*], Sarah Khairul Othman[1], Kavilasha Venugopal[2], Benedict Francis[3], Luke Sy-Cherng Woon[4] and Jesjeet Singh Gill[3].

1 Department of Parasitology, Universiti Malaya (UM)

2 Department of Medical Microbiology, Universiti Malaya (UM)

3 Department of Psychological Medicine, Faculty of Medicine, Universiti Malaya (UM)

4 Department of Psychiatry, Faculty of Medicine, Universiti Kebangsaan Malaysia (UKM)

*corresponding author

Email addresses

Correspondence: arun04@um.edu.my , chandramathi@um.edu.my & suresh@um.edu.my

## Abstract

Schizophrenia (SZ) is a mental disorder with multifactorial aetiology, including a potential role of gut microbiota. *Blastocystis sp.*, a common gut protozoan, has been reported to alter the composition of gut microbiota. Blastocystis sp. have been identified in SZ patients, yet its pathophysiological relevance remains unclear. Stool samples from SZ (n=78) and non-schizophrenic (NS, n=65) individuals were screened for *Blastocystis sp.*. Gut microbial composition was assessed using 16S rRNA sequencing. Solubilized antigens (SA) from *Blastocystis sp.* were tested on HCT116 and primary colon cells to evaluate epithelial barrier permeability, Claudin-7 expression, and cytokine production.*Blastocystis sp.* infection significantly altered gut microbiota, with increased diversity and elevated Bacteroidota and Prevotella levels in SZ. ECIS analysis showed initial disruption followed by reinforcement of epithelial barrier function upon SA exposure. Annexin V and qPCR assays confirmed increased proliferation and downregulation of Claudin-7, while cytokine profiling indicated elevated IL-2, IL-4, IFN-γ, and TNF-α in SZ-derived SA-treated cells.*Blastocystis sp.* infection may influence gut permeability and immune activation, highlighting its possible role in schizophrenia.

**NOVEL INSIGHTS INTO *WOLBACHIA* ASSOCIATED WITH FILARIAL INFECTIONS IN BURMESE FIGHTING CHICKENS**

Junsiri W[1,2], Narapakdeesakul D[2], Kongtawee R[3], Taweethavonsawat P[2,*]

*Corresponding author. Piyanan.T@chula.ac.th.

1. Department of Animal Science, Faculty of Agricultural Technology and Agro-Industry, Rajamangala University of Technology Suvarnabhumi, Phra Nakhon Si Ayutthaya, 13000, Thailand

2. Biomarkers in Animals Parasitology Research Unit, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand

3. Veterinary Pathobiology Graduate Program, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand

Avian filaria nematodes are often neglected due to their generally nonpathogenic nature. In Thailand, microfilariae have been detected in both wild birds and domestic chickens. Recently, a novel species of the *Onchocercidae* have been reported in Burmese fighting chickens (*Gallus gallus*). However, the detection of *Wolbachia* endosymbiont in *Onchocercidae* sp. has not been reported. Therefore, knowledge about *Wolbachia* and reliable detection methods are important. This study aimed to evaluate a PCR-based approach for detecting *Wolbachia* sp. in *Onchocercidae*-positive chickens using the *wsp* gene. Chickens infected *Onchocercidae* sp. raised in Nakhon Ratchasima Province, eastern Thailand, were investigated for *Wolbachia* endosymbiont. *Wolbachia* bacteria are commonly classified in different supergroups, although they are attributed to one species, namely *Wolbachia pipientis*. Supergroups C, D and J are limited to filariae. Supergroup F encompasses both arthropod and filarial hosts. The PCR successfully amplified the partial *wsp* gene of *Wolbachia* sp. from 5 out of 12 chickens. Phylogenetic analysis of *wsp* sequences derived in this study demonstrated that five sequences belong to two distinct supergroups. Isolates CH06 and CH07 were positioned in supergroup F together with *Wolbachia* endosymbiont of *Cimex lecturarious* (GenBank: DQ842459). Isolates CH05, CH08 and CH09 were positioned in supergroup D closely related to *Wolbachia* endosymbiont of *Brugia* sp. (GenBank:AE017321 and AY527207). However, all of five sequences were constituted a clade phylogenetically distinct from other species, suggesting they may represent a *Wolbacia* endosymbiont of *Onchocercidae.* Therefore, PCR targeting the *wsp* gene enable characterization of genetic features of *Wolbachia*, enhancing our understanding of its role as an endosymbiont in nematodes. Further studies are needed to explore the pathogenicity of filarial nematodes and their *Wolbachia* in chickens, as well as their potential vectors.

**Keywords**: Burmese fighting chickens, *Wolbachia* sp., *wsp* gene, Phylogenetic analysis, Thailand

**DIVERSITY AND NEW HOST RECORDS OF TICKS AND LICE INFESTING EXOTIC PETS IN PENINSULAR MALAYSIA**

Kazim A.R.[1], Vellayan S.†,[2], Low V.L.[3], Houssaini J.[4], Tappe D.[5], Heo C.C.*[1]
*Corresponding author: chin@uitm.edu.my

1. Department of Medical Microbiology and Parasitology, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia
2. Department of Pharmacology and Chemistry, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM) Selangor, 42300 Bandar Puncak Alam, Selangor, Malaysia
3. Higher Institution of Centre of Excellence (HICoE), Tropical Infectious Diseases Research and Education Centre (TIDREC), Universiti Malaya, Kuala Lumpur, Malaysia
4. Cardiovascular Advancement and Research Excellence Institute (CARE), Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia
5. Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

†Deceased on September 20, 2024.

Ectoparasites such as ticks and lice are important vectors of pathogens affecting both animals and humans. Despite the growing number of exotic pets in Malaysia, studies documenting their ectoparasites remain limited. In this study, a series of in-clinic and out-clinic health assessments (from 2018 until 2023) were conducted on various exotic pets to identify ectoparasitic infestations. Each animal was carefully examined by parting the feathers, fur, or rakhis to screen for ticks and lice. Ectoparasites were collected using fine tweezers, preserved in 70% ethanol, and subsequently identified morphologically under a stereomicroscope using established taxonomic keys. A diverse assemblage of ticks and lice was recorded across multiple exotic hosts, including *Amyrsidae phaeostoma* (n = 1), *Lipeurus caponis* (n = 15), *Haemaphysalis doenitzi* (n = 1), and *H. wellingtoni* (n = 30) from Indian peafowls (*Pavo cristatus*); *Rhipicephalus linnaei* from Indian vultures (n = 52) (*Gyps indicus*) and rabbits (n = 62) (*Oryctolagus cuniculus*); *Argas pusillus* (n = 4) from a bat (*Pipistrellus* cf. *stenopsis*); *H. wellingtoni* from swan geese (n = 5) (*Anser cygnoides*), helmeted guineafowls (n = 1) (*Numidia meleagris*), domestic chicken (n = 12) (*Gallus gallus*) and black turkeys (n = 15) (*Meleagris gallopavo*); *Gliricola porcelli* (n = 1) from a guinea pig (*Cavia porcellus*); *Myrsidea splendenticola* (n = 3) from a house crow (*Corvus splendens*); and *Colpocephalum apivorus* (n = 9) from an Oriental honey buzzard (*Pernis ptilorhynchus*). This study provides the first documentation of several tick and louse species infesting exotic animals in Peninsular Malaysia, expanding current host–parasite records. Continued surveillance is recommended to evaluate the potential zoonotic risks posed by these ectoparasites, particularly their role in transmitting vector-borne pathogens to humans and other animals.

Keywords: Veterinary parasitology; tick- and louse-host ecology; exotic pets

# Uncovering gametocyte-specific genes in *Babesia ovata*: toward the development of a transmission-blocking vaccine

Komatsu K.[1#], Nakazaki-Hasegawa K.[1#], Arayaskul N.[1], Ohari Y.[2], Lee J.S.[1], Suganuma K.[1], Asada M.[1] * and Kawazu S.[1]*

*Corresponding author: masada@obihiro.ac.jp, skawazu@obihiro.ac.jp

[1]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan

[2]International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan

# These two authors contributed equally to this work.

Bovine babesiosis causes substantial economic losses to cattle industries worldwide. Current control methods rely on tick control with acaricides and on the use of therapeutic drugs to diminish the parasite from infected animals. However, these approaches have significant drawbacks, including the emergence of resistance and high costs. Therefore, new control strategies are required. In recent years, increasing attention has been given to transmission-blocking vaccines, which induce antibodies in bovine hosts that target sexual-stage *Babesia* in tick. Nevertheless, only a limited number of antigens have been identified for this parasite. In this study, we aimed to identify genes specifically expressed in the gametocytes of *Babesia ovata*, which causes bovine babesiosis in Asian countries. *B. ovata* intra-erythrocytic parasites were cultured *in vitro*, and gametocytes were artificially induced from these parasites. The RNA samples extracted from density gradient–enriched gametocytes and intra-erythrocytic (blood stage) parasites were subjected to RNA sequencing. We identified 116 genes with expression levels more than two-fold higher in gametocytes than in the blood stage. Among these, nine genes predicted to encode transmembrane proteins showed more than five-fold higher expression in gametocytes. Notably, two of these genes were member of the major facilitator superfamily previously implicated in gametocyte development in *Plasmodium* parasites. We plan to examine the gametocyte-specific expression of the currently identified candidate and to conduct neutralization assays using specific antibodies.

**Keywords:** Bovine babesiosis, transmission broking vaccine, gametocyte

**HIGH BURDEN AND SPATIAL CLUSTERING OF CANINE HEMOPARASITIC INFECTIONS IN SOUTHERN THAILAND: A MOLECULAR SURVEY OF FREE-ROAMING DOGS**

Kongtawee R[1] , Junsiri W[2], Narapakdeesakul D[2], Thiptara A[3], Beugnet F[2,5] , Taweethavonsawat P[2,4]*

*Corresponding author: Piyanan.T@chula.ac.th

1. Veterinary Pathobiology Program, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand

2. Parasitology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand

3. Epidemiology and Information Group, Veterinary Research and Development Center (Upper Southern Region), Nakhon Sri Thammarat, 80110, Thailand

4. Biomarkers in Animals Parasitology Research Unit, Chulalongkorn University, Bangkok, 10330, Thailand.

5. Boehringer Ingelheim Animal Health, 29 Av. Tony Garnier, Lyon, France.

Canine vector-borne diseases (CVBDs) are increasingly prevalent in tropical and subtropical regions, particularly among free-roaming dog populations, which serve as key reservoirs for transmission. This study investigated the prevalence and the spatial distribution of CVB pathogens in 318 free-roaming dogs from five sub-districts of Songkhla Province, southern Thailand. Conventional PCR was utilized to detect eight pathogens: *Anaplasma platys*, *Ehrlichia canis*, hemotropic *Mycoplasma* spp., *Babesia vogeli*, *Hepatozoon* spp., *Trypanosoma evansi*, *Dirofilaria immitis*, and *Brugia malayi*. A high overall infection rate of 77.04% was observed, with *Anaplasma platys* (51.89%) and hemotropic *Mycoplasma* spp. (27.04%) being the most prevalent. Co-infections were common, occurring in over 42% of infected dogs. Spatial analysis using kernel density estimation (KDE) revealed pathogen-specific clustering patterns, particularly in the central and southwestern areas of the province. Infection prevalence was significantly associated with host factors such as age and weight. These findings underscore the critical role of free-roaming dogs in the epidemiology of CVBDs and highlight the need for targeted surveillance and vector control strategies under the One Health approach to mitigate zoonotic transmission. This integrative approach provides a comprehensive assessment of pathogen diversity and prevalence while identifying environmental and host-related factors that may influence their geographic distribution.

**Keywords:** Canine vector-borne diseases (CVBDs), Hemoparasites, free-roaming dogs, Molecular diagnosis, Spatial epidemiology

**GENETIC CHARACTERIZATION OF *Ascaris* spp. FROM HUMANS AND ANIMALS IN SELECTED RURAL COMMUNITIES IN CARAGA REGION, MINDANAO, PHILIPPINES USING RIBOSOMAL AND MITOCHONRDIAL MARKERS**

Llanes K.K.[*1], Kozel, K.[2], Betson, M.[2], Paller, V.[1]

[*]Corresponding author: krllanes@up.edu.ph

1. Animal Biology Division, Institute of Biological Sciences, University of the Philippines Los Baños, Pedro R. Sandoval Avenue, Los Baños, Laguna 4031, Philippines

2. Department of Comparative Biomedical Sciences, School of Veterinary Medicine, University of Surrey, Guildford, Surrey, United Kingdom

*Ascaris lumbricoides* and *Ascaris suum* are two of the most widespread intestinal helminths commonly known to affect humans and pigs respectively worldwide. The two species of *Ascaris* are morphologically indistinguishable through microscopy, and are still debated as to whether they represent separate host-specific transmission cycles. The expansion of backyard or free-range pig rearing and organic farming especially in rural areas maximizes the contact between humans, animals, and the contaminated environment that may facilitate the circulation and maintenance of *Ascaris* infection in these communities. Therefore, there is a need for more localized surveys using molecular analysis to reveal insights about the transmission dynamics, host specificity, and zoonotic potential of *Ascaris*. The present study aimed to investigate the molecular identity and diversity of *Ascaris* spp. from humans and animals. Stool samples were collected from humans, dogs, cats, pigs, and water buffaloes from eight municipalities in Caraga Region in a household-based survey. Amplification and Restriction Fragment-Length Polymorphism (RFLP) of the nuclear ribosomal internal transcribed spacer (ITS) showed a restriction pattern suggestive of cross-infection and hybrid *Ascaris* genotypes. Moreover, analysis of cytochrome oxidase I (COI) sequences from *Ascaris*-endemic and non-endemic areas and various host types showed clustering among *Ascaris* samples from this study. No significant genetic differentiation regardless of host species and geographical locations were found based on phylogenetic and network analyses. Altogether, these findings highlight that animals may act as possible contributor to the transmission and maintenance of *Ascaris* infection. Therefore, the zoonotic potential of *Ascaris* spp. should not be overlooked and must be considered in planning for a more efficient control program.

**Keywords:** *Ascaris* spp., zoonoses, PCR-RFLP, Caraga Region, Philippines

**Speed-of-kill comparison of isoxazolines in the combination endectocide products Credelio™ PLUS (lotilaner), NexGard Spectra® (afoxolaner) and Simparica Trio® (sarolaner) against the Australian paralysis tick (*Ixodes holocyclus*) throughout one month.**

**Authors:**

Lyons R.[1]; Ahlstrom L.*[1]; Ellenberger C.[1]; Baker K.[1]; Pittorino M.[1]; Wiseman S.[2]; Schunack B.[3]

*Corresponding author: liisa.ahlstrom@elancoah.com

[1] Elanco Animal Health, Sydney, Australia; [2] Elanco Animal Health, Hook, United Kingdom; [3] Elanco Animal Health GmbH, Monheim, Germany.

**Abstract:**

The Australian paralysis tick (*Ixodes holocyclus*) causes a severe, potentially fatal, toxicosis in dogs. A fast and sustained speed-of-kill throughout the dosing interval is a valuable acaricidal characteristic. Lotilaner has the longest half-life (35 days) of the oral isoxazolines[1], and a single dose kills paralysis ticks for over 11 weeks.[2]

A randomised, blinded, controlled study was conducted to compare the speed-of-kill of the monthly-dosed combination isoxazoline products Credelio™ PLUS (lotilaner, milbemycin oxime), NexGard Spectra® (afoxolaner, milbemycin oxime) and Simparica Trio™ (sarolaner, moxidectin, pyrantel) against *Ixodes holocyclus.* Dogs (n=7/group) were treated (or left as untreated controls) on Day 0 and infested with 10-12 unfed adult female ticks on Days -2, 21, 28 and 35. Tick counts were performed 12, 18 and 24 hours post-treatment and post-reinfestation.

The initial speed-of-kill efficacy (12h post-treatment) was rapid and similar for lotilaner and sarolaner (>80%), and significantly greater than afoxolaner (50%; $P<0.004$). On Day 21, efficacies of lotilaner, sarolaner and afoxolaner were 95.6%, 66.1% and 26.0%, respectively, 12 hours after reinfestation, with lotilaner and afoxolaner exceeding 97% and sarolaner nearly reaching 95% by 24 hours. On Day 28, efficacies of lotilaner, sarolaner and afoxolaner were 86.4%, 21.6% and 3.6%, respectively, 12 hours after reinfestation, with all products exceeding 96% and lotilaner reaching 100% by 24 hours. On Day 35, efficacies of lotilaner, sarolaner and afoxolaner were 76.6%, 23.4% and 6.9%, respectively, 12 hours after reinfestation. Lotilaner exceeded 95% efficacy already by 18 hours, sarolaner by 24 hours, while afoxolaner remained below 90%.

Lotilaner killed reinfesting ticks faster ($P<0.01$ at 12h) than sarolaner and afoxolaner and sustained its rapid speed-of-kill and efficacy beyond the label claim of one month. This offers reassurance to veterinarians and dog owners, addressing concerns about the potential increased risk of tick paralysis towards the end of the dosing interval of acaricides.

**Keywords:** paralysis ticks; *Ixodes holocyclus*; speed of kill; acaricide; isoxazolines; lotilaner; sarolaner; afoxolaner.

**References:** [1] Toutain CE *et al. Parasit Vectors*. 2017 Nov 1;10(1):522. doi: 10.1186/s13071-017-2475-z. [2] Baker K. *et al. Parasit Vectors*. 2018 Aug 29;11(1):487. doi: 10.1186/s13071-018-3061-8.

# FLOW THROUGH TECHNIQUE- A NOVEL IMMUNODIAGNOSTIC ASSAY FOR DIAGNOSIS OF VISCERAL SCHISTOSOMOSIS IN CATTLE

Shivani Mamane*, Jeyathilakan N., Latha B R and Senthilkumar T.M.A.

*Corresponding author: shivanimamane.1339@gmail.com

Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai -600007, Tamil Nadu, India

*Introduction*

Visceral schistosomosis, caused by *Schistosoma spindale*, is a chronic, economically significant trematode infection of cattle with zoonotic potential. Its diagnosis in field conditions is often challenging due to the absence of reliable and rapid diagnostic tools. In this study, a novel pen-side Flow-Through Technique (FTT) was developed and evaluated for the immunodiagnosis of active visceral schistosomosis in cattle from South India.

*Methodology*

The assay utilizes conventionally prepared excretory-secretory (ES) antigens of *S. spindale*, offering high specificity with minimal cross-reactivity. Post-mortem mesenteric examination was employed as the reference standard for evaluating diagnostic performance.

*Results and Conclusion*

Statistical analyses, including the Cochran's Q test and kappa statistics, indicated no significant difference between the FTT and the reference standard, with a strong level of agreement ($\kappa > 0.8$). The FTT demonstrated a diagnostic sensitivity of 92.98%, specificity of 96.74%, and overall accuracy of 95.55%. The assay is simple to perform, rapid, cost-effective, and requires minimal technical expertise, making it ideally suited for field deployment. Its portability and ease of interpretation support its potential as a practical pen-side diagnostic tool for early and reliable detection of *S. spindale* infection in cattle.

***Keywords**:* Visceral schistosomosis, *Schistosoma spindale,* excretory-secretory (ES) antigens, Flow-Through Technique

**PREVALENCE OF *Cryptosporidium* AND *Giardia* IN LIVESTOCK AND POULTRY AND THEIR POTENTIAL USE FOR SOURCE TRACKING OF PATHOGEN CONTAMINATION IN THE SEVEN LAKES OF SAN PABLO, LAGUNA, PHILIPPINES**

Morales A.*[1], Berlin S.[1], Paller V.[1]
*Corresponding author. admorales@up.edu.ph
1. Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños.

The waterborne protozoan parasites *Cryptosporidium* and *Giardia* have emerged as vital tools for source tracking contamination in lake ecosystems as they can withstand harsh environmental conditions, making them ideal indicators of fecal contamination from various sources, such as livestock, wildlife, or human wastes. This study aimed to detect *Cryptosporidium* spp. and *Giardia* spp. from livestock and poultry around the selected Seven Lakes of San Pablo, Laguna, namely Sampaloc, Bunot, and Calibato. A total of 66 fecal samples from livestock (10) and poultry (56) were collected within a 200-meter radius around the vicinity of the lakes and analyzed using immunofluorescence assay (IFA). Of these, 69.70% were found positive for *Cryptosporidium* spp. and 53.03% for *Giardia* spp., with a 34.85% co-infection rate. *Cryptosporidium* spp. prevalence was highest in chickens (72.09%), followed by ducks (63%), cows (60%), then goats and turkeys (both at 50%). While *Giardia* spp. prevalence was highest in goats (100%), followed by ducks (54.55%), chickens (51.16%), carabaos and turkeys (both at 50%), then cows (40%). Nested Polymerase Chain Reaction (PCR) targeting the 18S rRNA gene confirmed the identification of *Cryptosporidium,* although phylogenetic analysis has yet to confirm the species for animal source tracking. Moreover, risk factor analysis indicated that groundwater used for drinking and washing animals was significantly less likely to contribute to protozoan infections compared to surface water sources (OR = 0.25; p = 0.0260). Additionally, the Knowledge, Attitude, and Practice (KAP) analysis did not reveal significant correlations, except for handwashing after handling animal waste, which showed a positive correlation (r = 0.2653; p = 0.0313). This suggests that while handwashing is important, it alone is not sufficient to prevent infections, highlighting the need for a comprehensive approach to parasite control in animals. Meanwhile, Kernel Density Estimation (KDE) was used to model contamination clustering, identifying sharp localized hotspots near poultry waste runoff in Lake Sampaloc, broader zones related to mixed-species farming around Lake Bunot, and a diffuse contamination profile from upland farms affecting Lake Calibato. This study revealed that analyzing *Cryptosporidium* and *Giardia* in water samples can help trace fecal pollution sources, leading to more targeted management strategies to mitigate lake contamination. Monitoring these parasites is vital for public health, as their presence can pose serious risks to aquaculture and recreational waters.

**Keywords:** *Cryptosporidium*; *Giardia*; livestock; poultry; microbial source tracking; immunofluorescence assay; zoonotic parasites; public health; Seven Lakes of San Pablo, Laguna, Philippines

**GENETIC CHARACTERIZATION OF THE MITOCHONDRIAL GENOME OF *Plasmodium knowlesi* INFECTING *Macaca fascicularis* IN PENINSULAR MALAYSIA.**

Norhadila Z.*, Nor Dilaila M.S., Zarith S. and Sharma R.S.K.

*Corresponding author. norhadilazulkifli@gmail.com

Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.

Malaria is considered a major socio-economic disease where approximately half of the world's population are at risk of infection. In recent years, *Plasmodium knowlesi*, once thought to be limited to macaques in Southeast Asia, has been found infecting a large number of humans in the Kapit region of Sarawak, Malaysia, making it the fifth species of *Plasmodium* responsible for human malaria. The present study was undertaken to elucidate the genetic diversity of the mitochondrial genome of *P. knowlesi* infecting non-human primates in Peninsular Malaysia. Long-tailed macaques (*Macaca fascicularis*) were sampled from various locations throughout Peninsular Malaysia. PCR amplification of the 18S SSUrRNA gene was conducted to detect *P. knowlesi*. Representative positive amplicons were subsequently amplified and sequenced to obtain the complete mitochondrial genome of *P. knowlesi*. Genetic diversity analyses were performed using DnaSP and phylogenetic trees were constructed using MEGA12. *Plasmodium knowlesi* exhibited the highest haplotype diversity (Hd=1.000±0.001) but lower nucleotide diversity (pi= 0.0198±0.0006) at the mtDNA control region compared to the three protein-coding genes (*cox1, cox3* and *cytb*). The results from Tajima's D ($T_D$), Fu and Li's F ($F_F$) and D ($F_D$) genetic indices revealed negative values ($T_D$=-1.14876, $F_F$=-5.12328, $F_D$=-4.03023), suggesting events of population size expansion. The phylogenetic tree illustrated a distinct separation between populations from Malaysia Borneo and Peninsular Malaysia. The data obtained on the genetic characterization of the mitochondrial genome of *P. knowlesi* constitutes the first attempt to provide a detailed representation of the infection in wild macaques in Peninsular Malaysia. The close phylogenetic clustering, the relatively high haplotype diversity (Hd) and low nucleotide diversity (pi), coupled with the negative values obtained for Tajima's D, Fu and Li's D and F test may indicate population expansion and selective fitness of beneficial genotypes. These attributes may contribute to the persistent *Plasmodium* infection observed among both macaques and humans in the region.

**Keywords**: *Plasmodium knowlesi*, *Macaca fascicularis,* genetic diversity, mitochondrial genome

# INNOVATIVE ANTHELMINTIC STRATEGIES: EVALUATING THE EFFICACY OF ENCAPSULATED *TRACHYSPERMUM AMMI* AGAINST *HAEMONCHUS CONTORTUS*

**Authors:**

Lalawmpuii K[1*]; Singla LD[1]; Sharma S[2]; Devi LG[1]; Choudhury D[2]

[1]College of Veterinary & Animal Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab

[2]Thapar Institute of Engineering and Technology, Patiala, Punjab

*Corresponding author: omomi44585@gmail.com

In this comprehensive study, we investigated the anthelmintic efficacy of the ethanolic extract of *Trachyspermum ammi* (Ajwain). Utilizing gas chromatography-mass spectrometry (GC-MS), we screened the phytochemical constituents of *T. ammi*. Subsequently, we assessed its anthelmintic activity through adult and larval mortality tests, comparing it to conventional synthetic anthelmintics. To enhance anthelmintic efficacy, we encapsulated the Ajwain extract in solid lipid nanoparticles (SLN) using beeswax as lipid source and Poloxamer-407 as surfactant, employing a water-oil-water (w/o/w) double emulsion technique. The extract-loaded solid lipid nanoparticles (ext-SLN) showed sustained release of extract till 48 h. The anthelmintic effects of extract-loaded SLNs were rigorously examined against *Haemonchus contortus*, yielding insightful data on their efficacy. The SEM images of treated adult worms revealed notable morphological alterations and the enzymatic parameters suggested that ext-SLN induced a concentration-dependent decline in antioxidant enzyme activities. Further, the oxidative stress induced by ext-SLN treatment was observed using DCFDA dye that showed excessive fluorescence in the gastrointestinal tract and later diffused throughout the body. This multifaceted approach underscores the promising potential of herbal extracts in combating nematode infections, paving the way for innovative and effective anthelmintic strategies in veterinary medicine.

**Key words**: Ajwain; *Haemonchus contortus*; Solid Lipid Nanoparticles; *Trachyspermum ammi.*

**VECTOR-BORNE PARASITES OF DOGS AND CATS IN WEST ASIA**

Sazmand, A.[*1]

*Corresponding author: alireza.sazmand@basu.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamedan 6517658978, Iran

Vector-borne diseases (VBDs) are of growing concern, and their increasing incidence has been attributed to several factors, such as climate change and animal movements. Their distribution depends on a complex combination of biotic and abiotic factors, making their control extremely difficult. In tropical and subtropical regions, including Western Asian countries, where the climate is more suitable for various arthropod vectors, and animals have limited access to healthcare services, including diagnosis and treatment of these diseases, the impact of VBDs is heavier. The situation becomes even more complex when the VBDs are zoonotic. In this lecture, the current situation of selected vector-borne infections of dogs and cats in Western Asian countries, *i.e*., leishmaniosis, dirofilariosis, hepatozoonosis, babesiosis, anaplasmosis, ehrlichiosis, rickettsiosis, and the less-known tick-borne filarial nematodosis “cercopithifilariosis” will be presented.

**Keywords:** *Dirofilaria*; *Hepatozoon*; *Babesia*; *Anaplasma*; *Ehrlichia*; *Rickettsia*; *Cercopithifilaria*; canine; feline; vector-borne; hemoparasite; One Health

# AMBIENT TEMPERATURE STORAGE IN DESS SUPPORTS MOLECULAR STUDIES OF BENZIMIDAZOLE RESISTANCE FROM CANINE HOOKWORM EGGS

Chen Y.-J.†[1], Li V.†[1], Suwandy M.†[1], Mitrea I.B.[2,3], Hayward D.[4], Jaensch S.[4], Francis E.K.[1], Šlapeta J.[1,5]

*Corresponding author: jan.slapeta@sydney.edu.au

†Contributed equally

1. Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, Sydney, NSW, Australia

2. Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

3. Department of Pharmacology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

4. Vetnostics NSW – North Ryde Laboratory, Macquarie Park, NSW, Australia

5. Sydney Institute for Infectious Diseases, The University of Sydney, NSW, Australia

Surveillance of benzimidazole resistance in canine hookworms is essential for veterinary and public health, yet current molecular diagnostics depend on refrigerated faecal samples, limiting their use in field and remote settings. This study demonstrates that dimethyl sulfoxide, EDTA, and saturated NaCl (DESS) is a reliable ambient-temperature preservation medium for canine faeces, eliminating the need for cold chain logistics. Faecal samples stored in DESS for up to 106 days yielded DNA of comparable quality to refrigerated controls, enabling successful PCR amplification and next-generation sequencing of ITS-2 rDNA and tubb-1 amplicons. Hookworm species (*Ancylostoma caninum, Uncinaria stenocephala*) and resistance-associated SNPs, including F167Y, were consistently detected across treatments. These findings validate DESS as a robust, field-friendly solution for molecular parasitology, supporting expanded surveillance of anthelmintic resistance in regions where refrigeration is unavailable or impractical.

**Keywords:** DESS, ambient temperature, *Ancylostoma caninum*, *Uncinaria stenocephala*, benzimidazole resistance, β-tubulin, SNP, nemabiome, anthelmintic resistance, field diagnostics

# PRELIMINARY STUDY OF BORRELIA SPP. IN SOFT TICK (*RETICULINASUS* SP.) FROM THAILAND

Tuangpermsub S.[1,2], Arnuphapprasert A.[3], Riana E. [4], Ngamprasertwong T.[5], Kaewthamasorn M.[1]*

*Corresponding author. Morakot.k@chula.ac.th

1. Center of Excellence in Veterinary Parasitology, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.

2. Veterinary Pathobiology Graduate Program, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.

3. Faculty of Veterinary Science Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat, Thailand.

4. Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU), Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao PDR.

5. Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.

*Borrelia* is a genus of spirochete bacteria capable of causing diseases in humans and various animal species. Phylogenetically, *Borrelia* is divided into three groups: the *Borrelia burgdorferi* sensu stricto group, the relapsing fever *Borrelia* group, and the Echidna–Reptile associated *Borrelia* group. These pathogens represent an important public health concern globally. As vector-borne organisms, *Borrelia* species are primarily transmitted by ticks, which serve as major vectors facilitating disease transmission in many regions. Although *Borrelia* has been extensively studied in both hard and soft ticks worldwide, no previous research has investigated its occurrence in bat-associated ticks in Thailand. This study aimed to detect *Borrelia* in soft ticks collected from Thai bats using molecular techniques. Tick samples were collected between February 2018 and February 2023 from bats in ten provinces of Thailand. Species identification was performed using morphological characteristics with standard taxonomic keys and confirmed by molecular analysis through conventional PCR targeting the mitochondrial 16S rRNA gene. Detection of *Borrelia* was carried out using nested PCR targeting the *flaB* gene fragment. A total of 1,031 bats, representing seven families, 11 genera, and 28 species, were examined. Thirty-four bats were infested with 95 soft tick larvae, all identified as *Reticulinasus* sp. Of 48 pooled tick samples, four tested positive for *Borrelia* and were confirmed by Sanger sequencing. The nucleotide sequences of all positive samples showed the highest similarity to *Candidatus* Borrelia fainii from Zambia (GenBank accession no. AP027070), which was previously reported in a human case involving a *Reticulinasus* tick bite. This study provides the first evidence of *Borrelia* detection in bat-associated soft ticks in Thailand, highlighting their potential zoonotic significance and emphasizing the need for further investigations into their epidemiology and public health implications.

**Keywords:** Bat ticks; *Borrelia*; *Reticulinasus* sp.; Tick-borne pathogens; Thailand.