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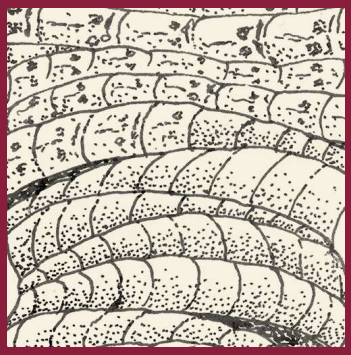


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ABSTRACT BOOK

MICROBIOTA CHARACTERIZATION OF *LEISHMANIA INFANTUM*-ASSOCIATED SKIN ULCERS IN DOGS USING SHOTGUN METAGENOMICS

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Keywords: *canine leishmaniasis; skin microbiome; shotgun metagenomics*

INTRODUCTION. The skin of dogs harbors a wide diversity of bacteria that play an essential role in skin protection. Understanding this microbiota is important for elucidating its composition and behavior. In dogs positive for leishmaniasis and presenting ulcerative skin lesions, this microbial community may also exhibit increased bacterial abundance. Therefore, the present study aimed to characterize the microbiological profile of the skin of these animals using a metagenomic approach.
MATERIALS AND METHODS. Samples were collected from 10 dogs using sterile plastic swabs and stored at −20 °C for subsequent analysis by shotgun metagenomics. The results from the 10 samples revealed a total of 773,554 paired-end (PE) reads, of which 704,239 high-quality reads were obtained after quality control and sequence assembly. A minimum of 48,914 and an average of 70,424 clean reads were generated per sample.
RESULTS AND CONCLUSION. The results showed that most sequences were classified into four predominant phyla: *Firmicutes, Proteobacteria, Bacteroidota,* and *Actinobacteriota*, suggesting that cutaneous lesions in dogs with leishmaniasis harbor significant microbial diversity. Cluster analysis of taxonomic abundance identified 41 taxa, together with their complete taxonomic classification, indicating that the structure of the microbial community varies among individual animals. These findings raise important questions regarding the potential role of the microbiome in lesion development, its biological significance, and its contribution to skin health in dogs. Thus, this study contributes to scientific advancement and may support improvements in clinical practice for the treatment of skin lesions associated with canine leishmaniasis.

NEGLECTED BUT WIDESPREAD: MOLECULAR EVIDENCE OF THE DISTRIBUTION AND GENETIC DIVERSITY OF *KLOSSIELLA EQUI* IN EUROPE

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Keywords: *Klossiella equi; equids; molecular prevalence*

INTRODUCTION. *Klossiella equi* is the only known apicomplexan adeleorinid protist infecting the renal parenchyma of equids. Its monoxenous life cycle begins with ingestion of sporocysts via contaminated feed or water; sporozoites migrate to the renal epithelium, where merogony, gametogony, and sporogony occur, with sporocysts excreted in urine (Vetterling and Thompson, 1972. *J. Parasitol*., 58: 589–594). Infections are usually asymptomatic, but heavy burdens may cause renal tubular rupture and lymphoplasmacytic interstitial nephritis. Despite its worldwide distribution, data from Europe are limited. This study aimed to assess the molecular prevalence of *K. equi* in European equids.
MATERIALS AND METHODS. Between 2023 and 2025, 284 kidney samples were opportunistically collected during postmortem examination from horses (277), mules (6), and one donkey across Italy (46), the Netherlands (100), Spain (30), Romania (96), and the Czech Republic (12). Additionally, 82 urine samples were obtained from a subset of horses from Spain (28), Romania (51), and the Czech Republic (3). Kidney tissue from the corticomedullary junction was sampled. Approximately 0.25 g of tissue was stored at −20 °C until DNA extraction. Urine samples (4–15 mL) were centrifuged at 1,500 rpm for 5 minutes, and up to 0.25 g of pellet was used for DNA isolation. Detection of *K. equi* DNA was performed by conventional PCR targeting the mitochondrial multicopy *rRNA* gene array (*12S/16S*), located between cytochrome c oxidase subunits 1 (*COI*) and 3 (*COIII*), using primers Api_LSUG_F and Haem_RNA_14_R (Léveillé *et al.,* 2019. *J. Parasitol*., 105: 29). A subset of 38 positive samples was further analysed by PCR targeting partial *COI* and cytochrome *b* oxidase (*cytB*). A *COI*-based phylogenetic tree was constructed using unique Adeleorina sequences from GenBank. Cohen's kappa (κ) was estimated to evaluate agreement between kidney and urine samples. Risk factors (country, age, and sex) were analysed using Fisher's Exact Test.
RESULTS AND CONCLUSION. *Klossiella equi* DNA was detected in 33.1% of the equids. Significant differences ($p < 0.05$) were observed among countries, with the highest prevalence in Spain (73.3%), followed by Italy (43.5%), the Netherlands

(33.0%), Romania (17.7%), and the Czech Republic (16.7%). Cohen's kappa was 0.5, suggesting moderate agreement (31.0% in kidneys and 25.6% in urine). No difference was observed according to sex ($p = 1.00$), whereas age was significant ($p < 0.001$), with the highest prevalence in young adults (56.3%) and the lowest in foals (12.5%). A dominant "European haplotype" was identified across all countries; the remaining minor variants were restricted to Italy. The greatest divergence occurred in one donkey (Campania region) and one mule (Latium region). These findings confirm the widespread presence of *K. equi* in European horses. Given its tropism for renal tubular epithelium and association with inflammatory lesions, detection of *K. equi* should be considered in cases of equine nephropathies.

EVALUATION OF RECOMBINANT SALIVARY ANTIGEN OF *PHLEBOTOMUS PERNICIOSUS* AS AN EXPOSURE MARKER IN CATS FROM A *LEISHMANIA INFANTUM* ENDEMIC AREA

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Keywords: *Phlebotomus perniciosus; Leishmania infantum; cats*

INTRODUCTION. *Phlebotomus perniciosus* females are among the main vectors of *Leishmania infantum* in southwestern Europe (Ready, 2013. *Annu Rev Entomol*., 58: 227–250), and exposure to this sand fly has been associated with a higher risk of *L. infantum* infection in dogs and humans (Kostalova *et al.,* 2015. *PLoS Negl Trop Dis*., 6:e0003855; Ortuño *et al.,* 2022. *Transbound Emerg Dis*., 69: 1854–1864). To measure vector exposure, host antibody responses to sand fly salivary proteins serve as suitable markers, and the recombinant SP03B protein as antigen has emerged as a reliable alternative to *P. perniciosus* salivary gland homogenate (SGH) in such assays (Kostalova *et al.,* 2015. *PLoS Negl Trop Dis*., 6:e0003855). Although dogs are the main reservoir for *L. infantum* and play a pivotal role in disease transmission in endemic regions such as Italy, increasing evidence supports the role of cats in the epidemiological cycle (Pennisi *et al.,* 2015. *Parasites Vectors*., 8:302; Foglia Manzillo *et al.,* 2025. *Pathogens*., 14:1194; Cortese *et al.,* 2025. *Animals*., 15:1801). This study evaluated the humoral immune response of cats against *L. infantum* and *P. perniciosus*.
MATERIALS AND METHODS. Blood samples were obtained by convenience sampling from 81 naturally exposed cats in southern Italy. The study area is endemic for *L. infantum*, with documented circulation in cats (Pennisi *et al.,* 2015. *Parasites Vectors*., 8:302; Foglia Manzillo *et al.,* 2025. *Pathogens*., 14:1194; Cortese *et al.,* 2025. *Animals*., 15:1801), supporting the relevance of feline studies. Anti-*Leishmania* IgG was detected by in-house indirect fluorescent antibody test (IFAT, cut-off ≥1:80). IgG against sand fly saliva was assessed by ELISA using SGH and the recombinant protein rSP03B (Kostalova *et al.,* 2015. *PLoS Negl Trop Dis*., 6:e0003855). Sera were diluted 1:200, and results expressed as mean OD ×100 at 450 nm. Cut-offs were defined as mean + 3 SD of negative controls.
RESULTS AND CONCLUSION. A total of 21/81 cats were IFAT-positive (19 at 1:80 and 2 at 1:160), while 60/81 were negative. Median ELISA OD values were 21.9 (2.9–327.4) for SGH and 45.52 (2.9–424.80) for rSP03B ($p < 0.05$). Most IFAT-positive cats were SGH-positive (19/21); however, high SGH seropositivity was also observed in IFAT-negative cats (44/60). Discordant results were observed for rSP03B in both IFAT-negative and IFAT-positive groups. A moderate correlation between SGH and rSP03B was found using Pearson's test ($r = 0.602$, $p < 0.001$), indicating limited agreement. No differences in anti-saliva IgG levels were found between IFAT-negative and IFAT-positive cats for either rSP03B ($p = 0.8739$) or SGH ($p = 0.2203$), suggesting that exposure to *P. perniciosus* does not necessarily correlate with *L. infantum* infection. Although rSP03B is a promising marker of exposure to *P. perniciosus* in dogs, it was not optimal in cats compared with SGH. Further studies are needed to identify