

neurological function. In 2024, rats from Florida, United States, were collected and evaluated for the presence of RLW. Hearts and lungs were removed and evaluated grossly and histologically for the presence of adult and larval worms. Grossly, 2 to 54 entire or fragmented adult worms were recovered from the right ventricle and pulmonary artery of 8 naturally infected rats. Microscopically, the lungs of each rat had numerous larvated ova and first stage larvae (L1) embedded throughout the pulmonary capillaries and arterioles, with associated marked granulomatous to lymphoplasmacytic interstitial pneumonia and perivasculitis. Large pulmonary arteries exhibited a spectrum of changes including moderate to severe villous myointimal proliferation, medial hypertrophy and fibrosis, arteritis, and occasional thrombosis in response to luminal nematode adults. The arterial findings mirror those in dirofilariasis caused by the canine heartworm, *Dirofilaria immitis*, in which this distinct intimal proliferation and atherosclerotic changes are the hallmark of disease and representative of significant pulmonary hypertension. The similarities between the two parasites may provide a better understanding of angiostrongyliasis in the rat host and how regardless of parasite species, parasite presence can result in similar organ and tissue damage.

19. Probe-based real-time qualitative PCR (qPCR) targeting *Wolbachia* in combination with other standard diagnostic tests for detection of *Dirofilaria immitis*

T.L. Sanders, M.A. Kelly, A. Tapia, M. Schech, E. Orr, P. Weerathne, H. Hakimi, and G. G. Verocai | Department of Veterinary Pathobiology, School of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

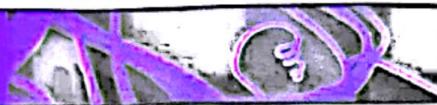
Dirofilaria immitis, the causative agent of canine heartworm disease, is among the most clinically relevant parasite of dogs. Current diagnostic recommendations for heartworm detection in dogs are an antigen detection test paired with a microfilaria detection test (MFDT). However, there are limitations to both types of tests that can yield inconclusive results, warranting a need for additional confirmatory tests. *Wolbachia*, an obligate gram-negative intracellular bacterium, is an endosymbiont of filarial nematodes including

heartworm. *Wolbachia* is transmitted transovarially, with evidence of detection in microfilariae, making it a potential target for additional confirmatory tests. The objectives were to: I) develop and optimize a real-time quantitative PCR (qPCR) to detect *D. immitis*-specific *Wolbachia* and II) assess its performance by screening blood of shelter dogs. Genomic DNA was extracted from 452 samples and subjected to qPCR targeting a partial region of the *ftsZ* gene of *Wolbachia*, a highly conserved region that allows for high sensitivity and specificity. Initially, we determined the detection limit of the qPCR using a 10-fold serial dilution of two sample types, DNA of *D. immitis* adult female and *D. immitis*-positive dog blood. Using these results, we generated a standard curve and limit-of-detection (LOD) for both assays. The LOD was determined as 1.5 pg of DNA for an adult female compared to the LOD for microfilariae in dog blood of 750 pg. To validate the test, we compared the performance of *Wolbachia*-qPCR to other MFDTs, including modified Knott's (MK) and a *D. immitis*-qPCR. All sera were also tested for *D. immitis* antigen using the DiroCHEK® (Zoetis) antigen test using pre- and post-immune complex dissociation (ICD) via heat treatment. Overall, 74 (16.3%) samples tested positive *Wolbachia*-qPCR, 78 (17.2%) in MK and 92 (20.3%) in *D. immitis*-qPCR. Heartworm antigen was detected in both pre-ICD with 22.3% (101/452) and post-ICD in 27.2% (123/452) of dogs. Kappa statistics show near perfect agreement between the newly developed *Wolbachia*-qPCR and both MFDTs, modified Knotts (0.90) and *D. immitis*-qPCR (0.83). Our data support that *Wolbachia* detection using this probe-based qPCR can be introduced as an additional out of clinic option for patients with inconclusive heartworm test results.

20. Evaluation of matrix metalloproteinase-9 (MMP-9) and interleukin-6 (IL-6) as biomarkers for pulmonary parenchyma damage in canine heartworm

B.R. Morales,¹ N. Costa-Rodríguez,¹ D.J. Vera-Rodríguez,¹ S.N. García-Rodríguez,¹ J.A. Montoya-Alonso,¹ R. Morchón García,² and E. Carretón¹

¹Internal Medicine, Faculty of Veterinary Medicine, Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas De Gran Canaria, Spain



²Zoonotic diseases and One Health Group, Laboratory of Parasitology, Faculty of Pharmacy, University of Salamanca, Spain

Matrix metalloproteinases (MMPs), particularly MMP-9, are found in low amounts in healthy adult lungs but are significantly more abundant in various lung diseases in both humans and dogs. Interleukin-6 (IL-6), like other inflammatory cytokines, is elevated in lung diseases in response to infections and tissue damage. However, dysregulated IL-6 synthesis plays a pathological role in chronic inflammation and autoimmunity, actively contributing to the pathogenesis of various lung diseases in humans. To date, no specific studies have been conducted on dogs with heartworm to assess pulmonary parenchyma damage. In this study, MMP-9 and IL-6 biomarkers were evaluated in 40 dogs with heartworm that underwent cardiopulmonary evaluation prior to adulticide treatment (echocardiography, radiography, microfilariae detection, and parasite load determination). The dogs were divided into three groups: Group A (n=14), asymptomatic dogs; Group B (n=15), symptomatic dogs without significant radiographic alterations in the pulmonary parenchyma; and Group C (n=11), symptomatic dogs with pulmonary radiographic changes. Serum samples were collected and analyzed using specific kits: CANINE MMP-9 and CANINE IL-6 ELISA Kits (Assay Genie, Dublin, Ireland). MMP-9 and IL-6 were pathologically increased in 79.64% and 88.56% of dogs, respectively. No statistically significant differences were found between groups, although Cohen's d values indicated a moderate effect for IL-6, suggesting a clinically relevant trend. Additionally, no significant differences were found based on parasite load or the presence/absence of microfilariae. Canine heartworm is characterized by chronic and acute inflammatory lesions in both the pulmonary arteries and parenchyma; however, few studies have evaluated the damage at this level. These results showed alterations of MMP-9 and IL-6 in a high percentage of infected dogs, even those asymptomatic and with no appreciable radiographic alterations. Furthermore, the immune response in heartworm infection in dogs is predominantly immunosuppressive and Th2-type, with Th2 lymphocytes secreting IL-6, among other cytokines. Further research is needed, although the results suggest that MMP-9 and IL-6 could serve as

useful indicators to objectively assess and monitor the clinical status of pulmonary parenchyma in canine heartworm. To this aim, more studies with larger sample sizes and the inclusion of additional biomarkers are currently in development.

21. Mosquito diversity and molecular analysis of *Dirofilaria immitis* prevalence within the Cumberland Gap region ☺

C. McKain, L. Allinson, D. Escandell, K. Smartt, E. Williams, S. Williams, and C. Faulkner | Lincoln Memorial University College of Veterinary Medicine, Harrogate, Tennessee, USA

Heartworm disease, caused by *Dirofilaria immitis*, is a significant threat to canids, felids, and other veterinary important species, with mosquitoes serving as essential vectors. Understanding local mosquito fauna is crucial for effective prevention. This study investigated mosquito populations and their association with *D. immitis* prevalence in the Cumberland Gap Region, aiming to identify potential vectors and assess temporal transmission shifts. Mosquitoes were collected using CDC modified light questing and gravid traps throughout 2024, for 64 trapping nights. Specimens were morphologically identified, and DNA was extracted for PCR to detect *D. immitis* DNA. A total of 7,335 mosquitoes, representing 15 species, were collected. Gravid traps were dominated by *Culex* species with *C. pipiens* most abundant in June (83%) and July (53%), and *C. erraticus* predominated in August (57%). Other species captured to a lesser extent in the gravid traps include: *Aedes japonicus*, *Ae tri/hen*, and *Anopheles punctipennis*. Questing traps were dominated by *C. pipiens*, *Ae vexans*, *Ae albopictus*. The occurrence of *D. immitis* DNA identified in positive mosquito pools from 2020 (*C. pipiens* and *Ae albopictus*) confirms their role as vectors involved with local parasite transmission in the canine population. This ongoing project will continue to analyze temporal mosquito species shifts, and further gel electrophoresis will definitively confirm *D. immitis*. Continued multi-seasonal mosquito collection will provide a comprehensive understanding of vector populations, informing regional heartworm prevention efforts.