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Research paper

Prevalence of canine heartworm infection in Mississippi animal shelters

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ABSTRACT

Understanding diagnosis of heartworm disease in the context of animal shelters' needs and expectations is crucial for developing guidelines that specifically address the unique shelter population. Accurate and economical heartworm testing strategies are essential. However, current comprehensive guidelines for the management of heartworm disease in dogs are directed toward client-owned animals and do not address needs of animal shelters and other resource-scarce facilities. Additionally, testing recommendations do not take into account regional and local differences in heartworm prevalence across the United States that occur due to abiotic and biotic factors. The objective of this study was to determine the apparent prevalence of *Dirofilaria immitis* microfilaremia and antigenemia in dogs from Mississippi animal shelters. Further, we compare agreement between microfilaria and antigen testing in this population. We performed a cross-sectional study using canine serum and blood bank samples representative of the Mississippi shelter population. Microfilaria testing of whole blood included a blood smear and modified Knott test. Antigen testing of serum was performed using the DiroCHEK® antigen ELISA test. Analyses included descriptive and analytic statistics as well as Cohen's kappa for test agreement. A total of 283 whole blood samples and 363 serum samples, representing 363 dogs from 18 shelters in 17 Mississippi counties, were utilized in this study. Sixty-four (22.6%) whole blood samples demonstrated *D. immitis* microfilariae on the modified Knott test and 125 (34.4%) serum samples had detectable *D. immitis* antigen. Increasing age and low body condition were associated with antigen-positive test results. Only age was associated with microfilaria-positive test results. There was moderate agreement between the antigen ELISA test and the modified Knott microfilaria test and poor agreement between the antigen ELISA and the blood smear. This study provides the first known report of the prevalence of *D. immitis* microfilaremia and antigenemia in Mississippi shelter dogs. We observed that prevalence of both microfilaremia and antigenemia was significantly higher in these sampled dogs compared to previous reports for the owned canine population in Mississippi. Heartworm infection presents unique management challenges for animal shelters. Knowledge of the expected prevalence in the area can be utilized for management decisions related to prevention, testing, and treatment of dogs in shelter populations.

1. Introduction

Heartworm disease can cause significant morbidity and mortality in canids. Accurate and cost-effective detection, treatment, and prevention of this disease is a significant management concern for animal shelters (Colby et al., 2011). While guidelines exist to address each of these issues on an individual canine level (Nelson et al., 2014), recommendations do not address detection, treatment, or prevention of heartworm transmission on a population level in resource-scarce facilities (Polak and Smith-Blackmore, 2014). This issue is compounded for animal shelters operating in the southeastern region of the United States which contends with a higher prevalence of canine heartworm disease than in other parts of the country (Bowman et al., 2009).

In 2009, the southeastern United States had a regional mean heartworm prevalence of 3.9%, which was the highest percentage of heartworm positive cases in the nation; Mississippi had a state-wide heartworm prevalence of 7.4% in owned dogs that reported test results (Bowman et al., 2009). These data are likely a gross underestimation of the true prevalence of heartworm infection in owned dogs in Mississippi as the study relied on convenience sampling and the use of reference laboratories. Dogs included in samples for testing were potentially more likely to have received preventive care and less likely to have been

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infected with heartworm. It is also probable that the prevalence of heartworm infection in Mississippi's stray and shelter dogs is higher than in owned dogs due to lack of preventive care in the former population. A previous study examining the difference in heartworm antigen prevalence between canine populations found that shelter dogs had an almost 10% higher prevalence than owned dogs in the state of Florida (Tzipory et al., 2010). Furthermore, in a study performed on dogs rescued from Mississippi during Hurricane Katrina, Levy and colleagues detected a 34.4% prevalence for heartworm using a combination of antigen and microfilaria testing (Levy et al., 2007).

Not only do heartworm-infected dogs from these shelters enter the local community as pets, they could potentially serve as reservoirs for infection throughout the country if not identified prior to transport. Consequently, it is essential that appropriate prevention, detection, and treatment guidelines be developed to assist these organizations in managing their canine populations. In order to develop such region-specific protocols, the true magnitude of the problem, or prevalence of the disease, must be known. This is especially important when developing detection guidelines, as correct interpretation of diagnostics requires knowledge of pre-test probability of disease (Rohrbach and Patton, 2013). Unfortunately, surveys based on systematic random sampling from southeastern canine animal shelter populations, from which the pre-test probability could be determined, have not been performed.

To fill this gap in knowledge, our objective was to determine the apparent prevalence of *Dirofilaria immitis* microfilaremia and antigenemia in Mississippi animal shelters through the use of a previously developed serum bank representing the state's shelter dog population.

2. Materials and methods

2.1. Serum bank

A blinded cross-sectional study to determine the prevalence of *D. immitis* microfilaremia and antigenemia was performed using blood and serum collected as part of serum banking from Mississippi animal shelters in 2016. Briefly, development of the serum bank began with a phone survey of all Mississippi shelters to determine dog intake in 2015. This survey identified 61 animal shelters within the state. Geographic shelter regions were based on the 9 health districts in the state. Dogs were then sampled from one to three animal shelters in each district to proportionally match dog distribution from the census. Dogs over 6 weeks of age were randomly sampled within each shelter. The total serum bank thus represents 571 dogs from 18 shelters located in 17 counties across the state of Mississippi or 29.5% of the animal shelters identified in the state (Hubbard K., unpublished data). Of the 571 dogs in the serum bank, 365 (63.9%) had recorded ages of 6 months or greater and qualified for heartworm testing. Of the 365 dogs who qualified for testing, 363 had serum sample aliquots available and 283 also had whole blood samples available for testing.

2.2. Animal characteristics

Dogs received a brief physical exam performed by a veterinarian or veterinary students directly overseen by a veterinarian, which included estimation of age by dentition, visual estimation of weight in kilograms (kg), sex, body condition (on a scale of 1–9), and breed (Laflamme, 1997). Animal shelter location was recorded for each dog. Historical information on sampled dogs was recorded when available, and included intake source (owner surrender, stray, etc.) and date of entry to the shelter. When possible, the length of shelter stay at time of sampling was determined by subtracting the date of sampling from the intake date. Age was categorized as 6 months to ≤ 1 year, > 1 year to ≤ 3 years, and > 3 years. Breed included seven categories based on American Kennel Club classifications and were working, herding, hound, sporting, terrier, non-sporting, and toy. Source included the categories owner surrender/returned and stray/animal control. Sex included the categories male and female.

2.3. Diagnostic testing

Samples were transported on ice and refrigerated 24–48 h until processing. Whole blood samples in EDTA were tested for microfilariae. Separate whole blood samples collected without anticoagulant were centrifuged for 10 min at 5000 rpm (Allegra x30 centrifuge, Beckman Coulter, Brea, CA) and serum was collected and stored at −80 °C in 1 mL aliquots. Banked samples were tested in batches using the antigen and microfilaria tests. Each test was performed, and results recorded, by a single individual trained in that assay and blinded to other test results. Thus, the antigen test was run by one individual, the modified Knott by another, and the blood smears interpreted by a third. Blood smears were made by one of the three trained individuals before any assays had been performed. The specific reviewer of the blood smears then stained and read those slides.

2.3.1. Antigen testing

Serum samples (n = 363) were tested for *D. immitis* antigen using a commercially available antigen well-ELISA (DiroCHEK® Heartworm Antigen Test Kit, Zoetis, Parsippany, NJ). Each plate contained a negative and positive control provided by the manufacturer. The well-ELISA was performed as detailed in the package insert. Results were initially interpreted as no antigen detected (0), slight blue color lighter than positive control (1), blue color change equal to positive control (2), blue color change darker than positive control (3).

2.3.2. Microfilaria testing

Microfilaria testing was performed for the 283 dogs with whole blood samples available. We used a modified Knott test on 1 mL of whole blood (Newton and Wright, 1956). Modified Knott results were interpreted as either *D. immitis* microfilaria present (1) or absent (0) based on the morphology of the microfilaria present. This assay was performed by a veterinarian previously trained in performance and interpretation of the assay. Slides were scanned at 100× magnification. Once microfilariae were identified, morphology, including pointed cephalic end, straight tail with pointed end, and subjective width, was assessed at 400× magnification. (Lindsey, 1965). For a subset of 192 whole blood samples, a single drop of blood was also used to create a blood smear for a subset of samples. We stained smears using Diff-Quik (Quik-dip stain, Mercedes Medical, Sarasota, FL) and a single observer blinded to other test results examined the slides for microfilariae. Blood smears were interpreted as microfilaria present (1) or absent (0).

2.4. Statistical analysis

For the purpose of calculating prevalence, ELISA scores of 0 were considered antigen-negative per test instructions and scores of 1, 2 and 3 were combined and considered antigen-positive. Apparent prevalence of canine heartworm antigenemia and microfilaremia in Mississippi was determined as the percent of dogs positive for each test. Confidence intervals accounting for sample clustering by shelter were reported (Thrusfield, 2005). We used geographic information systems software to map the distribution of antigenemia and microfilaremia (QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org).

Statistical analyses for risk factors associated with antigenemia and microfilaria were performed using generalized linear mixed modeling with a binomial distribution and a logit link (PROC GLIMMIX, SAS Institute, Inc., Cary, NC, USA). All models included shelter as a random effect to account for sample clustering. Beginning with a saturated model including all candidate variables and an interaction term for age and length of stay (univariable p-value≤ 0.25) manual backwards elimination was used to sequentially remove non-significant candidate