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# Prevalence of heartworm infection in the feral cat population of Grand Cayman

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## Abstract

**Objectives** The aim of this study was to determine the presence and prevalence of heartworm infection in the feral cat population of Grand Cayman.

**Methods** During the study period, feral cats were routinely trapped and euthanized for population control by the municipal animal shelter. Cats older than 6 months of age were obtained for post-mortem examination shortly after euthanasia. The heart, lungs, pulmonary vasculature, thoracic and abdominal cavities were examined for the presence, location and number of mature heartworms. Sections of caudal lung were evaluated histologically and serologic tests were performed to screen for additional evidence of heartworm exposure.

**Results** Mature heartworms were identified in the pulmonary vasculature of 4/36 cats (11.1%). An additional nine cats showed histopathologic changes in the lungs consistent with heartworm exposure, and one cat had a positive antibody test.

**Conclusions and relevance** The results indicate a minimum heartworm prevalence of 11.1% within this population of feral cats, consistent with published necropsy reports from other endemic localities. Considering the histopathologic changes observed in this group, the true prevalence is likely higher and underscores the importance of heartworm prevention for the companion cat population of the island.

**Keywords:** *Dirofilaria immitis*; heartworm infection; heartworm prevalence

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## Introduction

*Dirofilaria immitis* is the causative agent of heartworm disease and is known to have a wide global distribution.<sup>1–4</sup> While the domestic dog and other canids are considered the definitive host, domestic cats can also become infected with *D. immitis*.<sup>1,4,5</sup> The biology of heartworm infection in the cat has been described in the literature.<sup>1–5</sup>

Cats, like dogs, become infected when a third-stage larvae (L3) is transferred from a mosquito to the cat via the mosquito bite wound. Soon after infection, the larvae molt to the fourth stage (L4) within the subcutaneous tissues and begin migrating through the body. The L4 then undergo a final molt within the host to become immature adults. In the dog, this final molt occurs approximately 2 months after infection and a similar time frame for this molt is thought to also occur in the domestic cat.

These immature adults then enter a peripheral vein and make their way to the caudal pulmonary arteries.

Importantly, the arrival of these immature adults in the pulmonary circulation of the cat is accompanied by a marked acute inflammatory response resulting in the prominent clinical syndrome of heartworm-associated

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respiratory disease. Many infections are eliminated during these early phases by the feline host.

Some worms will go on to become mature adults and may show a period of transient patency 7–8 months post-infection. Cats infected with mature heartworms typically display a low worm burden, often with only 1–4 adult worms present. Aberrant migration of fourth-stage larvae is observed more frequently in the cat than the dog. As such, ectopic heartworms can be found in various anatomic sites, including the thoracic cavity, abdominal cavity and the central nervous system.

The prevalence of heartworm infection in the Cayman Islands has not previously been described in the literature, in either canine or feline populations. In this study, the authors took advantage of an ongoing population control program to determine the presence and prevalence of heartworm infection in the feral cat population on the main island of Grand Cayman, in the Cayman Islands.

## Materials and methods

This study was granted ethical approval by the Institutional Animal Care and Use Committee at St Matthew's University, School of Veterinary Medicine prior to commencement. During the study period of April 2013 to April 2014, feral cats were trapped and humanely euthanized according to existing government protocols and then released to the authors for the purposes of this study. For each cat, basic information was collected on sex, reproductive status, weight and estimated age (based on dentition). Cats estimated to be younger than 6 months of age (by observation of incomplete eruption of permanent dentition) were excluded from the study, as they were considered unlikely to have adult *D immitis* present.

Serum samples were obtained from each animal and stored at  $-20^{\circ}\text{C}$  for later analysis. Each cat then had a systematic gross necropsy performed to identify mature heartworms. The thoracic and abdominal cavities were first surveyed for the presence of worms, to screen for aberrant worm migration. The heart and lungs were then removed en bloc and the cardiac chambers and major blood vessels were opened and examined. The pulmonary arteries were opened and traced to their terminations in each lung lobe. Finally, abdominal organs were opened or sectioned to screen for aberrant migration. Adult heartworms were identified based on the finding of a filarial nematode that was pale white,  $<2$  mm wide and 5–20 cm in length. Mature worms were collected and counted. The location of each worm and any gross pathology observed during necropsy were recorded.

Sections from either the right or left caudal lung were collected from each cat and placed in 10% buffered formalin for histologic evaluation. A veterinary pathologist evaluated the sections, characterized pathologic changes and subjectively graded lesions with respect to severity. Banked serum samples were submitted to Texas A&M

Veterinary Medical Diagnostic Laboratory for serum antigen (DiroCHEK) and antibody (Heska Solo Step FH) testing. Both the pathologist and the diagnostic laboratory were blinded to the gross necropsy findings.

## Results

Thirty-six cats were sampled, including 21 males (17 sexually intact and four castrated) and 15 females (13 sexually intact and two spayed). Estimated ages ranged from 8 months to 24 months (median 17.6 months, interquartile range 8–16 months). Four of the 36 cats (11.1%) had adult *D immitis* present on gross necropsy. Three of the four cats had two adult worms present and the fourth had one adult worm. Two worms were located in the main pulmonary artery, two were located at the termination of the left caudal lobar artery, two were located at the termination of the right caudal lobar artery and one was present at the termination of the left cranial lobar artery.

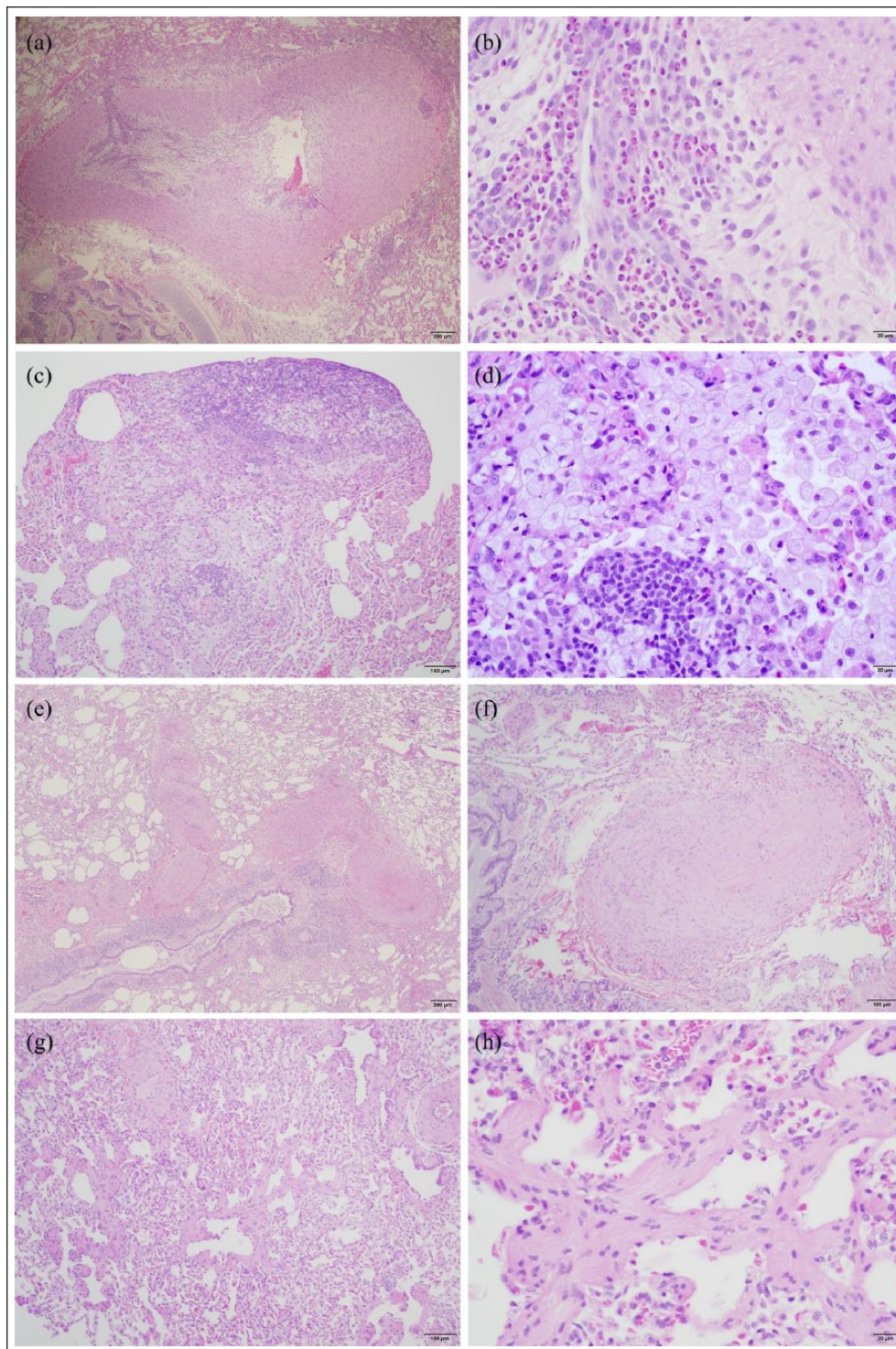
All four cats with adult heartworms had concurrent gross lung pathology. Lesions observed included multifocal white discoloration of the caudal lung lobe ( $n = 1/4$ ) and intimal surface of the right caudal lobar artery ( $n = 1/4$ ), circumferential brown discoloration around the left caudal lobar artery ( $n = 1/4$ ), diffuse red mottling of the lung surfaces ( $n = 1/4$ ) and similar red discoloration localized to the caudal lung lobes ( $n = 1/4$ ). Seven cats without adult worms had similar gross lung lesions observed during post-mortem examination.

A variety of changes were observed on histologic examination of the lungs (Figure 1). In the four cats with grossly identifiable heartworms, these changes included eosinophilic arteritis/endoarteritis/periarteritis, pulmonary arterial medial hypertrophy/hyperplasia, terminal airway and adjacent alveolar interstitial smooth muscle hypertrophy/hyperplasia/fibrosis and subpleural granulomas. These changes were graded as moderate to marked in severity. Similar lesions were identified in nine cats that did not have grossly identifiable heartworms. An additional five animals showed mild, non-specific histopathologic changes. No histopathologic changes were observed in the remaining 18 cats.

On serologic testing, one cat had a positive antigen test and one cat was antibody-positive. Results for each cat are summarized in Table 1.

## Discussion

In analyzing and interpreting the results, cats could be classified into four distinct categories: (1) those with necropsy-confirmed heartworm infection ( $n = 4/36$ ); (2) those with evidence consistent with and/or suggestive of heartworm exposure ( $n = 10/36$ ); (3) those with mild, non-specific histopathologic changes ( $n = 5/36$ ); and (4) those with no evidence of heartworm exposure ( $n = 17/36$ ). These results and categories are reported in Table 1 and further discussed below.



**Figure 1** Representative photomicrographs of pulmonary lesions in cats from this study. (a,b) Images from a cat with mature heartworms at necropsy. (c–h) Images from cats without mature heartworms detected at necropsy. (a) A large pulmonary artery branch showing smooth muscle hypertrophy and hyperplasia of the tunica media and prominent endoarteritis (bar = 200  $\mu$ m). (b) High magnification of (a) showing thickening of the tunica intima with myofibrosis, eosinophilic infiltration and endothelial hypertrophy/hyperplasia (bar = 20  $\mu$ m). (c) A subpleural granuloma (bar = 100  $\mu$ m). (d) Higher magnification of (c) showing foamy macrophages and aggregate of lymphocytes within the alveolar parenchyma (bar = 20  $\mu$ m). (e) Several pulmonary artery branches showing marked smooth muscle hypertrophy and hyperplasia of the tunica media (bar = 200  $\mu$ m). (f) A medium-sized branch of the pulmonary artery showing marked medial hypertrophy and hyperplasia with severe luminal encroachment (bar = 100  $\mu$ m). (g) Thickening of the walls of terminal airways and the adjacent alveolar interstitium with smooth muscle and/or fibrous connective tissue (bar = 100  $\mu$ m). (h) High magnification of (g) (bar = 20  $\mu$ m). Hematoxylin and eosin staining

**Table 1** Classification of study animals based on evidence of heartworm exposure

Cats with necropsy-confirmed heartworm infection (n = 4)				
Cat	Worms on necropsy	Histopathology*	Serum antigen	Serum antibody
1	2	A	–	–
2	2	B	+	–
3	1	B	–	–
4	2	B	–	–
Cats with evidence suggestive of heartworm exposure (n = 10)				
Cat	Worms on necropsy	Histopathology	Serum antigen	Serum antibody
1	–	B	–	–
2	–	B	–	–
3	–	B	–	–
4	–	B	–	–
5	–	D	–	+
6	–	B	–	–
7	–	A	–	–
8	–	B	–	–
9	–	B	–	–
10	–	B	–	–
Cats with non-specific histopathologic changes (n = 5)				
Cat	Worms on necropsy	Histopathology	Serum antigen	Serum antibody
1	–	C	–	–
2	–	C	–	–
3	–	C	–	–
4	–	C	–	–
5	–	C	–	–
Cats with no evidence of heartworm exposure (n = 17)				

\*Histopathology grades were assigned based on the severity and extent of lesions that are consistent with and/or suggestive of heartworm infection. The grading scale is as follows: A = moderate to marked eosinophilic arteritis/endoarteritis, with at least moderate pulmonary arterial medial hypertrophy/hyperplasia (PAMH), and at least moderate terminal airway and adjacent alveolar interstitial smooth muscle hypertrophy/hyperplasia/fibrosis (TA&IMH); B = at least moderate PAMH or TA&IMH observed extensively in sections, a subpleural granuloma and/or at least moderate perivascular eosinophilic infiltrates; C = only occasional areas with moderate PAMH or TA&IMH noted; D = mild to no histologic lesions observed

In cats, prevalence studies based on the presence of mature heartworms on post-mortem examination reflect the minimum detectable burden of infection within that population.<sup>6,7</sup> A search of the literature for other global reports on the necropsy prevalence of *D immitis* in feral cats show prevalences ranging from 0.5–14.0% (Table 2).<sup>2,6–19</sup> The results of this study (11.1% necropsy prevalence) are consistent with prevalences reported from other heartworm-endemic localities.

Each necropsy-positive cat in this study had 1–2 mature heartworms present, which is in accordance with previous reports of heartworm burden in the domestic cat.<sup>1,5,13</sup> Five of the seven mature worms recovered at necropsy were found at the termination of a lobar artery, highlighting the importance of tracing the branches of the pulmonary arterial tree to this level during the necropsy procedure to accurately identify disease status.

While gross necropsy will identify the majority of cats with adult worms, the true prevalence of heartworm infection is likely higher because most infections do not reach maturity and necropsy can fail to identify *D immitis* infections in the immature stages of development.<sup>4–7</sup> For these reasons, serologic and histologic assessments were conducted to look for additional evidence of *D immitis* exposure.

The histopathologic changes observed in this group of cats are consistent with and/or suggestive of heartworm infection. Eosinophilic arteritis/endoarteritis and pulmonary granuloma formation are highly suggestive of *D immitis* infection.<sup>20,21</sup> Other microscopic lesions, such as pulmonary arterial hypertrophy and hyperplasia, while not specific for heartworm infection, tend to be seen with increased frequency and severity in affected animals.<sup>21–23</sup> These histopathologic changes were observed in cats

**Table 2** Reported prevalences of *Dirofilaria immitis* infection in feral cat populations, as determined by gross necropsy surveys, in the preceding 50 years (1968–2018)

First author	Study location	Study year(s)	Sample size	Necropsy prevalence (%)
Carleton <sup>2</sup>	Northwest Georgia	2001–2002	184	2.1
Levy <sup>6</sup>	North Florida	1998 and 2001	630	4.9
Snyder <sup>8</sup>	Gainesville, Florida	1998	330	5.8
Nelson <sup>9</sup>	Southeast Texas	1997–1998	259	9.7
Hermesmeier <sup>7</sup>	Southeastern Michigan	1997	239	2.5
Labarthe <sup>10</sup>	Rio de Janeiro, Brazil	1993–1994	125	0.8
Roncalli <sup>11*</sup>	Japan	1959–1995	3775	0.5–9.5
Kendall <sup>12</sup>	Sydney, Australia	ND	200	1.0
Guerrero <sup>13</sup>	Beaufort, South Carolina	1990	47	4.0
Guerrero <sup>13</sup>	Baton Rouge, Louisiana	1990	50	10.0
Guerrero <sup>13</sup>	Savannah, Georgia	1990	72	7.0
Patton <sup>14</sup>	Eastern Tennessee	1985–1990	122	2.5
Guerrero <sup>13</sup>	Savannah, Georgia	1989	92	1.0
Ryan <sup>15</sup>	Brisbane, Australia	1989	100	1.0
Courtney <sup>16</sup>	Central Florida	1984–ND	712	3.1
Elkins <sup>17</sup>	Western Kentucky	ND	100	14.0
Willard <sup>18</sup>	Alabama	1984–1985	108	2.8
Ryan <sup>15</sup>	Selangor, Malaysia	1977–1978	100	2.0
Talbot <sup>19</sup>	Papua and New Guinea	ND	117	6.8

\*Retrospective report of 20 necropsy studies

ND = not defined

with and without grossly identifiable heartworms. This is consistent with other studies describing the pathophysiology of feline heartworm infection and suggests that some cats in this study with moderate to marked histopathologic changes, but without grossly identifiable worms at necropsy, were affected with heartworm-associated respiratory disease.<sup>21–23</sup> By extension, this supports the premise that the true prevalence of heartworm infection is greater than can be reported based on gross necropsy findings alone.

Alternatively, some cats in this study could have had other pulmonary parasitic infections, such as *Aelurostrongylus abstrusus*, *Toxocara cati*, *Paragonimus kellicotti*, *Capillaria aerophila* or other non-infectious processes, that were not detected in this survey and can cause similar pathologic changes to the lung.<sup>3,17,24</sup>

Serologic testing for heartworm infection in the cat is known to be challenging owing to both the biology of *D immitis* in the cat and available diagnostic tests.<sup>1,5,8,15</sup> The Solo Step antibody test screens for the presence of antibody mounted in response to all *D immitis* life stages that can be seen in the cat.<sup>8</sup> While the sensitivity of this test has previously been reported to be low (31.6%), the specificity is high (99.0%), so positive results can be viewed with some confidence.<sup>8</sup> In our study, there was only one cat with a positive antibody test. It was interesting to note that this cat showed no other evidence of heartworm infection on the assessments we made. This cat could have tested positive because it was in the early stages of

infection, cleared a previous infection and was demonstrating persistent antibody, or had an ectopic infection that was not detected by necropsy.<sup>8</sup>

The remaining 35 cats tested antibody-negative, including those with mature heartworms. It has been reported that 14–20% of cats with necropsy-confirmed heartworm infections test antibody-negative.<sup>3,4</sup> Another study reported that 68% of necropsy-positive cats had at least one negative antibody test.<sup>6,8</sup> In cats with experimentally induced infections antibody status can vary over time post-infection.<sup>22</sup> All of these factors make interpretation of a negative antibody test very difficult. The large number of negative antibody tests in this study could be due to a variety of factors, including the low sensitivity of the test, the variable timing at which blood samples were obtained post-infection, our small sample size and testing of cats that were never actually exposed to *D immitis*.

Antigen testing in the cat is directed at a protein associated with mature female worms.<sup>1,3,4,8</sup> As such, this test is unlikely to detect animals with immature worms, low worm burdens and/or single-sex male infections.<sup>1,3,4,8,13</sup> Nonetheless, a positive antigen result is considered to be very specific for infection.<sup>8,15</sup> One of the four cats with adult heartworms in this study had a positive antigen test. The three antigen-negative cats with adult heartworms may have tested negative for the reasons stated above. Worms were not sexed as part of this study.

Heat treatment of serum samples prior to heartworm antigen testing has been proposed as a technique to

improve the performance of antigen tests, presumably owing to the disruption of antigen–antibody complexes in the sample.<sup>25</sup> Samples submitted to the diagnostic laboratory used in this study are not routinely heat treated. Had they been, more cats may have tested antigen-positive.

On the island of Grand Cayman, there are several trap–neuter–return programs in place. In some of these programs, cats may be exposed to injectable ivermectin while under veterinary care. It is interesting to note that none of the cats that were spayed or castrated had adult worms present, though our sample size was too small to comment further on any impact that exposure may have had on disease outcome.

Some limitations of this study include the small sample size and that the central nervous system was not examined for ectoparasite migration.

## Conclusions

These findings demonstrate a minimum heartworm prevalence of 11.1% within this population of feral cats that is consistent with published necropsy reports from other localities with endemic *D immitis*. Given the biology of this parasite in the cat, and the histologic and serologic findings presented herein, it is likely that the true prevalence of infection is higher than can be detected by necropsy alone. Accordingly, veterinary healthcare providers in Grand Cayman should emphasize the importance of heartworm prevention to cat owners in the Cayman Islands.

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