BVA Undergraduate Travel Report 2015

Investigating the prevalence of Canine Heartworm (*Dirofilaria immitis)* and 3 other Vector Borne Diseases on Viti Levu, Fiji

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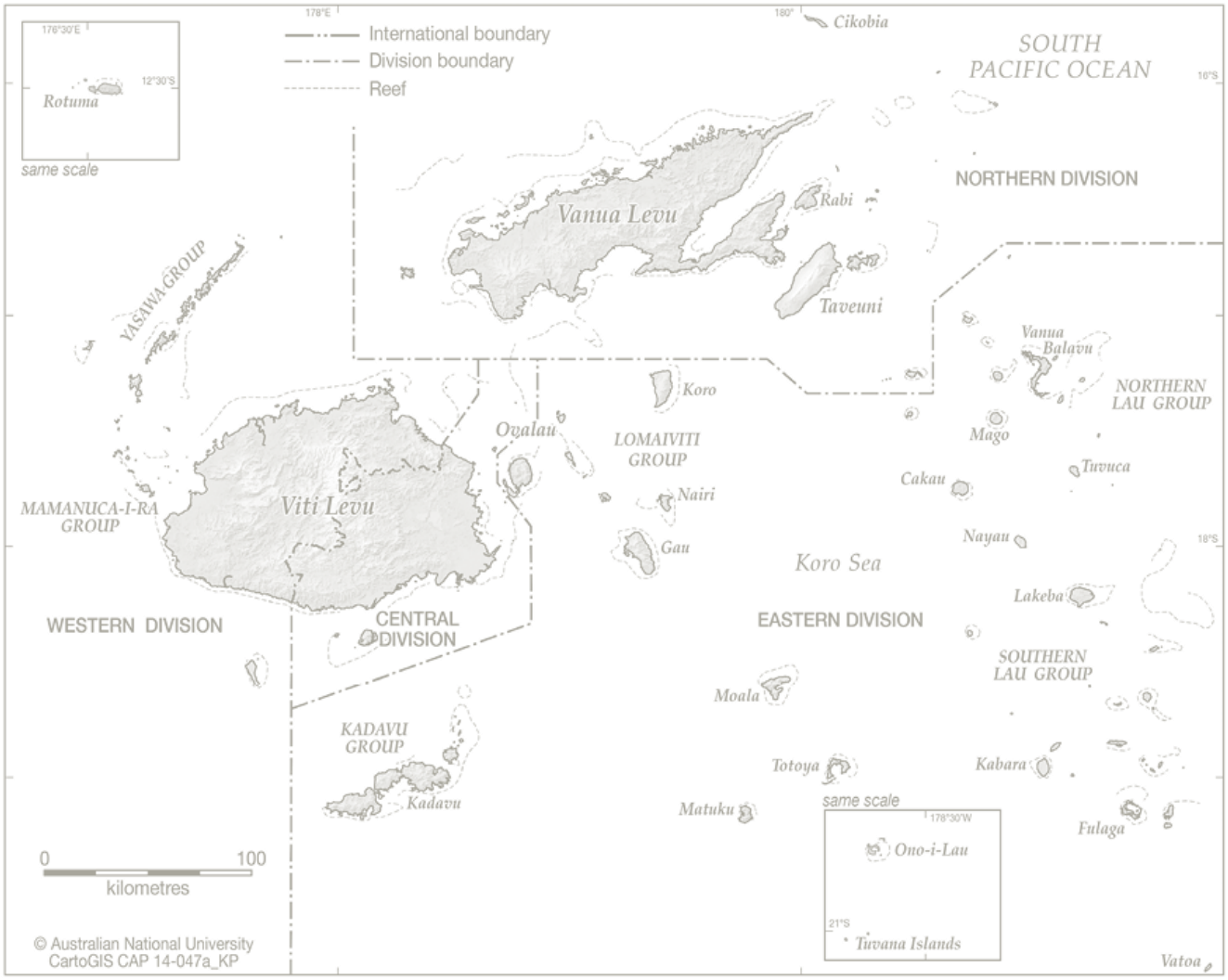
Animals Fiji, Namaka, Viti Levu, Fiji



Introduction

Fiji forms an archipelago of 333 islands located in the South Pacific, ENE of the East Coast of Australia. It is renowned for diving, beautiful beaches and a rich cultural heritage. The West Charity Trust Society, now operating and hereafter referred to as Animals Fiji, was founded in 2011 to support the Nadi branch of the Society for the Prevention of Cruelty to Animals Fiji (the SPCA) operating out of Fiji's capital, Suva. In July 2012 the SPCA could no longer support their Nadi branch and Animals Fiji began to operate self sufficiently, providing the only veterinary services in the western division of Viti Levu, Fiji's largest island. They have subsequently expanded to open a clinic in Savusavu providing the northern divison with its first access to veterinary services. Legislation governing animal welfare in Fiji is virtually non-existent and non-enforceable, with the majority of legislation nearly 60 years old. Coupled with the limitations associated with operating as a charity and virtually no support from the Fijian government, Animals Fiji faces a constant challenge in supporting both the domestic and occasionally food producing animals of Fiji. The main goal of Animals Fiji is breeding control within the free roaming and owned population of domestic animals of Fiji, largely through spey/neuter outreach programs. The clinic faces a wide range of problems not limited to hookworm, transmissible veneral tumour (TVT), acute paraquat intoxication and a number of other more common ailments. The lack of community knowledge and financial stability for most families facilitates progression of these cases to severe states, but Animals Fiji has a "no turn away" policy. Since its inception, Animals Fiji has noted a prominent number of animals infected with and suffering from clinical symptoms associated with *Dirofilaria immitis* (canine heartworm) and were keen to know more about the overall prevalence of the disease and the effectiveness of their treatment protocol within the shelter.

Dirofilaria immitis, colloquially referred to as the canine heartworm, is an intravascular nematode parasite of the superfamily Filarioidea, that commonly inhabits the right ventricle and pulmonary arteries of the canine heart (1) causing both cardiac and pulmonary insufficiency. The Onchocercidae family, of which the genus Dirofilaria is a member, contains 75 or so genera that are characterised by microfilaria (Fig 1A,1B) found in the skin or blood, all of which use biting vectors (2) that ingest this transmissible stage. The lifecycle of canine heartworm is complex and long compared to other nematodes, and a number of mosquito species have been implicated in its transmission including *Anopheles, Aedes* and *Culex* species (2). Infection of vectors occurs during a bloodmeal on an infected microfilaraemic host animal, before development through 3 larval stages within the malphigian tubules of the mosquito vector (3). The infective L3 is inoculated in the final host, larvae migrate through the subcutaneous tissue and into the vasculature, and development to adulthood occurs within approximately 6-8 months (1, 3). A summary of the lifecycle is given below (Fig. 2).

Adult male heartworms residing in the pulmonary vasculature vary in size from 12-20 cm long, while females are generally longer reaching up to 30 cm in length (1, 2), and it is these adult worms that are responsible for the pathophysiology associated with heartworm infection. Immature adults cause eosinophilia and damage within the vasculature that culminates in endothelial damage, coupled with sloughing and fibrosis of the lungs leading to respiratory distress (1). Mature adult worms have been shown to release vasoactive substances responsible for vasoconstriction, pulmonary hypertension and subsequent cardiac overload and failure (4); observed pathologically as compensatory concentric ventricular hypertrophy. The most serious manifestation of heartworm disease is caval syndrome. This occurs with distribution of the worm burden to the right ventricular inflow, resulting in regurgitation at the tricuspid valve, in conjunction with severe haemolytic anaemia as a result (5). The amount of damage observed is host specific and clinical signs are not common in heartworm infected animals. If clinical signs are observed, they are commonly those associated with pulmonary insufficiency (exercise intolereance, dyspnoea, lethargy, syncope) and right sided heart failure (ascites, hepatosplenomegaly and jugular distension). It has also been reported that a number of the clinical signs associated with heartworm are due to the symbiotic *Wolbachia* bacteria (6-8), that is responsible for interstitial and perivascular inflammation, and consideration of this should be given during decisions on treatment protocols administered (8).

The epidemiology of *Dirofilaria immitis* is also highly complex with a wide range of variables influencing its spread throughout a population. Although Grieve et al. (3) summarised a number of the important factors in Table 1. they also concluded that their list was not exhaustive and that further work is still to be done. Briefly, the risk factors were split into vector (including environmental factors, seasonality and flight range), host (including infection status, outdoor activity and pathophysiological response) and parasite (occult, fertility and periodicity) groups and infection has been reported across all the US states, South and Central America, Asia, Europe, Africa and Australia (9), but never previously in Fiji. Although infection rarely reaches patency in humans, *Dirofilaria immitis* is one of a number of zoonotic filariae responsible for causing human dirofilariasis. (10) It typically causes asymptomatic granulomatous coin lesions in the pulmonary tissue, in regions where heartworm is endemic in the canine and wild animal population (10-12), and should be a differential for human cardiopulmonary disease.

Dilofilaria immitis is routinely treated in canines under the guidelines prescribed by the American Heartworm Society (13) using the adulticidal therapy, melarsomine. There are two protocols routinely employed by shelters as outlined by Polak and Blackmore; (14): a three-dose protocol where the animal receives a 2.5 mg/kg dose of melarsomine, followed by two injections 1 month after, or a standard two dose protocol consisting of two injections 24 h apart. The three-dose protocol is the treatment of choice due to its increased safety and efficacy, but shelters such as Animals Fiji often struggle to manage the requirements for return and for client compliance. As Polak and Blackmore correctly infer (14), financial constraints on many individuals in the developing world necessitate slow-kill macrolide endectocide treatment, and this is regularly practiced at the clinic. McCall and Venco et al. report that dogs treated with this method alone develop progressive pulmonary pathology and that macrolides are an inefficient and suboptimal adulticidal approach. (15, 16). Due to the financial constraints associated with treatment, particularly in developing countries, prevention is recommended as a first line approach in managing heartworm infection. Chemoprophylaxis is routinely carried out with macrocylic lactones that kill the L3 and L4 stages of the parasite at up to 30-60 days of age, and monthly oral administration of ivermectin at 6 µg/kg or moxidectin at 3 µg/kg provide effective prevention against heartworm infection (10, 13) and a more financially extensive method for managing this disease.

Briefly this study also aimed to provide some information on the prevalence of tick-borne disease diagnosed serologically. The methods described below provide information on antibody to *Anaplasma phagocytophilum, Ehrlicia canis and Borrelia burgdorferi.* Tick borne disease is common worldwide, spread by both *Ixodes ricinus* (*A.phagocytophilum, B. burgdorferi*), *Rhipicephalus sanguineus* (*Ehrlicia canis*) and many other tick species (17, 18). Although *A. phagocytophilum* and *B. burgdorferi* are commonly considered zoonotic pathogens responsible for human granulocytic anaplasmosis and lyme borreliosis respectively, *E. Canis* has not yet been classified as such (17, 18). These pathogens cause non-specific, variable clinical signs in practice not limited to fever, malaise, vomiting, diarrhoea, anorexia and lethargy (18, 19) and are notoriously difficult to diagnose. Accurate diagnosis is commonly by polymerase chain reaction (PCR) (18, 19) but resource extensive environments are not conducive to this technique. Haematological examination can add further certainty to ambiguous clinical signs, but the detail required for accurate diagnosis is outside the scope of this report (18).

Prevention is key in the management of these diseases, centred on acaricides and permethrin based repellents preventing transmission by vector hosts, and prophylactic vaccination is available for *B burgdorferi* but not yet for *E canis* and *A phagocytophilum* (18, 19). If patent infection does result from vector exposure, treatment protocols centre on antibiotics of the tetracycline class for all three infections, and this may be of relevance to Animals Fiji, given the symbiosis of *Dirofilaria immitis* with the *Wolbachia* bacteria that is key in the pathogenesis of heartworm infection (8, 18, 19).

The primary aim of this study was to give an estimate of the prevalence of canine heartworm, *Dirofilaria immitis* on Viti Levu, Fiji and a brief analysis on the efficacy of the treatment regime employed by Animals Fiji. We also aimed to provide further insight into the prevalence of *Anaplasma phagocytophilum, Ehrlicia canis and Borrelia burgdorferi* prevalence.

Methods

The sample population for this study was made up of canine animals presented to and in residence at the Animals Fiji Nadi Clinic, and animals tested during outreach work to Nawaka and Narewa villages with Animals Fiji across July and August 2015. For each animal tested, a brief history was obtained using a predetermined verbal script and testing was carried out for the direct benefit of the animal. Approval for inclusion of results in this study was sought from the owners of animals and the clinic involved. The results of each test directed the treatment and management of each animal, as advised by Animals Fiji, and exclusion of results were only made where the owner did not consent to inclusion in the study.

For each animal, a sample of blood was taken from the cephalic or jugular vein, using standard aseptic technique and at routine diagnostic volumes. This was transferred to a 2 ml EDTA tube. Haematological examination for *Dirofilaria immitis* microfilaria was carried out using 3 methods: direct examination of wet blood, and thick and thin blood smear examination. Thick and thin blood films were air dried then fixed in methanol at (>80%) and stained with 10% Giemsa (field-stain) solution. Although the author recognises that concentration methods increase the diagnostic sensitivity of haematological examination for microfilaria, facilities in the ‘shelter’ environment were limited and not conducive to this method of examination. All slides were made permanent with DPX slide mount and morphological examination was carried out on a random selection of microfilaria positive slides to distinguish between *Dirofilaria immitis* , *Dirofilaria repens* and *Dipetalonema reconditum/ dracunculoides* (20). Haematological examination was carried out blind to the results of other diagnostic tests.

For each sample *Dirofilaria immits* antigen testing was carried out using the IDEXX (21) 4Dx Snap diagnostic test (Fig 3), as per the manufacturer's instructions for testing. A sample was taken to be positive when the test well developed a stronger colour than the negative control. An animal was taken to be *Dirofilaria immitis* positive when test results indicated the animal was antigen positive (indicating current or occult infection) **or** positive by haematological examination for microfilaria, by at least one of the above methods.

As the IDEXX 4Dx test (21) also provides information on antibodies for *Ehrlicia canis, Anaplasma phagoctophilum* and *Borrelia burgddorferi*, ticks were collected from animals where present and practical. Ticks were collected for morphological examination and species identification and for subsequent polymerase chain reaction (PCR) on those ticks shown to be vectors for infectious disease.

Statistical analysis was carried out using SPSS statistical software (22) and the GraphPad QuickCalcs website (23). An independent samples t-test was used to analyse the relationship between age and infection status (SPSS) and a fishers exact test for prevalence (two tailed) used for the relationship between both gender and neuter status.

Ethical approval for this project was sought from the University of Liverpool Veterinary Ethics Research Committee (Reference VREC341) and was granted on 6/7/15. Subsequent amendments to the study design were approved on 15/7/15.

Results

In total, 60 canine haematological samples were taken across the testing period. 10 of these animals had a previous history of heartworm prevention (16.7%). Of the 60 animals tested, 20 (33.3%) were antigen-positive (Table 2). When testing for microfilaria at haematological examination, 18 animals were positive for microfilariae (mf) at wet preparation or thick/thin blood smear, and 2 animals were regarded as occult infections (antigen +, mf -). There were no animals that were microfilariae positive but heartworm antigen negative, and further morphological examination is ongoing.

The mean age of animals tested was 4.56 ± 6.88 yr. Statistical tests showed that the relationship between age and infection status was not statistically significant. (p=0.525) (Table 3)

The relationships between prevalence according to gender and neuter status were not significant (p = 0.2612; p=0.0523) (Table 4; Table 5). However, the test for neuter status was strongly suggestive of correlation

Although some geographical separation was present within the tested group, the sample size was not large enough to collate any statistically significant relationships.

Of the animals tested, 8/60 (13.3%) were antibody positive for *Anaplasma phagocytophilum*. None of the animals tested were antibody positive for *Borrelia burgdorferi* or *Ehrlichia canis.*

100 % of the tick species collected were identified as *Rhipicephalus sanguineus.*

Discussion

The climate, vector population and large number of feral canines are all likely factors contributing to the results of this study, indicating that heartworm (*Dirofilaria immitis)* is endemic in the canine population in this area with a prevalence of 33.3%, and a cause of concern for both the government of Fiji and veterinary care providers. In particular, mosquitoes of the genus *Aedes* are already implicated throughout the Pacific in the spread of zika virus, chikungunya, dengue fever (24, 25) and other vector-borne diseases, and implicit breeding sites such as discarded tyres and water drums are likely to represent a perfect environment for development of those species that are also vectors for *Dirofilaria immitis*. Although a number of vector control measures have been put in place, including a larval source reduction campaign, implemented by the Ministry of Health in Fiji in 2003 (25), *Aedes* remains a vector for both human and animal infections, and environmental management should remain a target for the government in Fiji and its veterinary care providers in managing *Dirofilaria immitis* and more serious human infections.

However, it is clear from the results of this study that the treatment and prevention strategies administered by Animals Fiji have been efficacious in managing the prevalence of heartworm infection. As the sole veterinary care provider in the western division of Fiji, Animals Fiji is the only provider able to perform desexing operations on the island. This study showed a strongly suggestive relationship between those animals that have been neutered and those that were not infected. (p=0.0523). As clients who are likely to present their animals for de-sexing are likely to be more assiduous with the care and welfare of their animals, we have inferred that increased presentation to Animals Fiji does have a negative effect on the likelihood of subsequent patent infection with *Dirofilaria immitis.* Only one of the

animals found to test positive for *Dirofilaria immitis* had a previous history of heartworm prevention, but it is not clear how long this protocol was followed and how strictly it was adhered to and therefore is unlikely to reflect that efficacy of the treatment protocols administered at the shelter. It is most likely that this reduction in prevalence has occurred as a combination of the increase in awareness and education about heartworm that owners receive with increased presentation to Animals Fiji after de-sexing/adoption, and stricter adherence to prevention protocols. With this in mind the most progress in reducing the prevalence is likely to be made with emphasis on further educating the community, with particular focus on those clients with animals under 2.5 yrs of age. These animals have a high probability of harbouring a low number of adult worms and are commonly found to be amicrofilaraemic, leading to misdiagnosis and false-negative test results (26). Age is a contentious issue in heartworm infection, with numerous studies reporting varying findings on the relationship between infection status and age, and this study showed that the relationship was not significant. However, it is sensible to assume that older animals are more likely to become infected, as a result of increased vector exposure, and reduced imunocompetence (27). A further study with increased sample size would clarify this hypothesis.

There are a number of considerations with regard to the testing methods used in this study that should be analysed alongside the prevalence. Infection with *Dirofilaria immitis* is inherently complex, with states of occult infection, as a result of prepatent infection with young adults, low parasite burdens or single gender infections (27) commonplace, and detection of those animals with such infections is extremely difficult. In particular, the IDEXX 4Dx Snap test (28) makes specific reference to its inability to detect those animals harbouring male-only infections and this is likely to result in an underestimate of the true prevalence. Additionally, although we are aware that a proportion of the animals have previously had heartworm prevention (16.7%), we cannot say how this affects the prevalence, given the inconsistency in the level of prevention administered. It is also important to recognise that the sensitivity (true positive rate) of the direct smear test for microfilariae was reported to be low compared to concentration techniques such as the Knott test, in cases where the worm burden of animals was particularly low (26). It is likely that numerous individuals in this study fall into this category, and that the low sensitivity and therefore low negative predictive value result in a number of false negative results. Unfortunately, facilities at the shelter did not warrant any further development of the protocols, which would have included concentration techniques and further identification of mf by histochemical differentiation (29, 30) and PCR. Finally, an increased sample size would increase the statistical power of the study and provide further clarification on the prevalence, but indications on the limitations of the study suggest an overall underestimation of the prevalence.

There are a numbers of challenges faced by shelters like Animals Fiji in treating *Dirofilaria immitis*, as discussed by Dunn et al. (31), and the cost associated with heartworm treatment and prevention is reported to be the biggest limitation on providing basic care to infected shelter animals. Large numbers of shelters admit a great number more patients than they can care for and effectively manage, and end up euthanizing them before a successful outcome is achieved. In this situation in particular, the cost associated with heartworm treatment does not usually fit with the financial resource structuring of many charitable organisations. As already mentioned, macrolide endectocide treatment (slow kill) is not recommended due to increased pathological lesions (15, 16) in the pulmonary tissue, but may fit better financially into the limited resource environment of many shelters. Both practices are carried out at Animals Fiji, with a prior emphasis on prevention rather than treatment, and a clear explanation is provided of the differences in efficacy, price and the risks associated. However, the ultimate decision is client-led and limited education throughout Fiji presents treating veterinarians with an inherent problem, given the language barrier and lack of full understanding of treatment protocols. This leads to low client return rates and a poor protocol completion rate.

The IDEXX 4Dx SNAP test (21) provided a convenient, accurate and easy method of diagnosis for *Dirofilaria immitis*, accessible to lay staff in the shelter, and this will aid in both continuation of the study and diagnosis. However, the cost precludes its regular use at the shelter. As previously mentioned, facilities within the shelter environment are limited, but investment into concentration techniques with the concurrent increase in diagnostic accuracy (26) would be a valuable and efficient investment, and I would suggest this would be the most efficacious next step in successful diagnosis and management of *Dirofilaria immitis.*

Finally, it is clear that although not yet characterised definitively, tick-borne *Anaplasma phagocytophilum* is also common in Fiji (13.3%). Although more commonly reported as a host of *Ehrlicia canis* (18) it is interesting to note that 100% of the tick species were identified as *Rhipicephalus sanguineus*. Amplification is ongoing to determine whether these ticks are indeed carrying *Anaplasma phagocytophilum* from seropositive animals, but the severity of zoonotic clinical disease in humans warrants careful consideration from the Ministry of Health in Fiji of these results and further investigation to aid vector control. The non-specific nature of the presentation of anaplasmosis in canines also means that is likely to be underdiagnosed in the shelter environment at Animals Fiji and should feature on differential lists for non specific disease in many animals.

This study provides the first information on heartworm and anaplasmosis prevalence in Fiji in the canine population. Studies from Europe (11, 27, 32, 33), the USA (34) and the AHS (13), including data from the Companion Animal Parasite Control (CAPC) prevalence maps (35) cite heartworm prevalences much lower than that found in this study, as cause for implementation of successful treatment and prevention protocols in their respective areas. Animals Fiji has been critical in taking steps to approach the problem in Fiji, and it is clear that their treatment protocols are having a positive impact on the prevalence across the country. Further work should centre on education and increasing knowledge and understanding of the disease throughout the community, with development and investment into techniques that allow efficacious and accurate diagnosis of the disease. Amicrofilaraemia and asymptomatic animals are common, and routine prophylaxis for both heartworm and anaplasmosis should be included in all presentations to the clinic and during outreach and education work, for the benefit of both animal welfare and public health.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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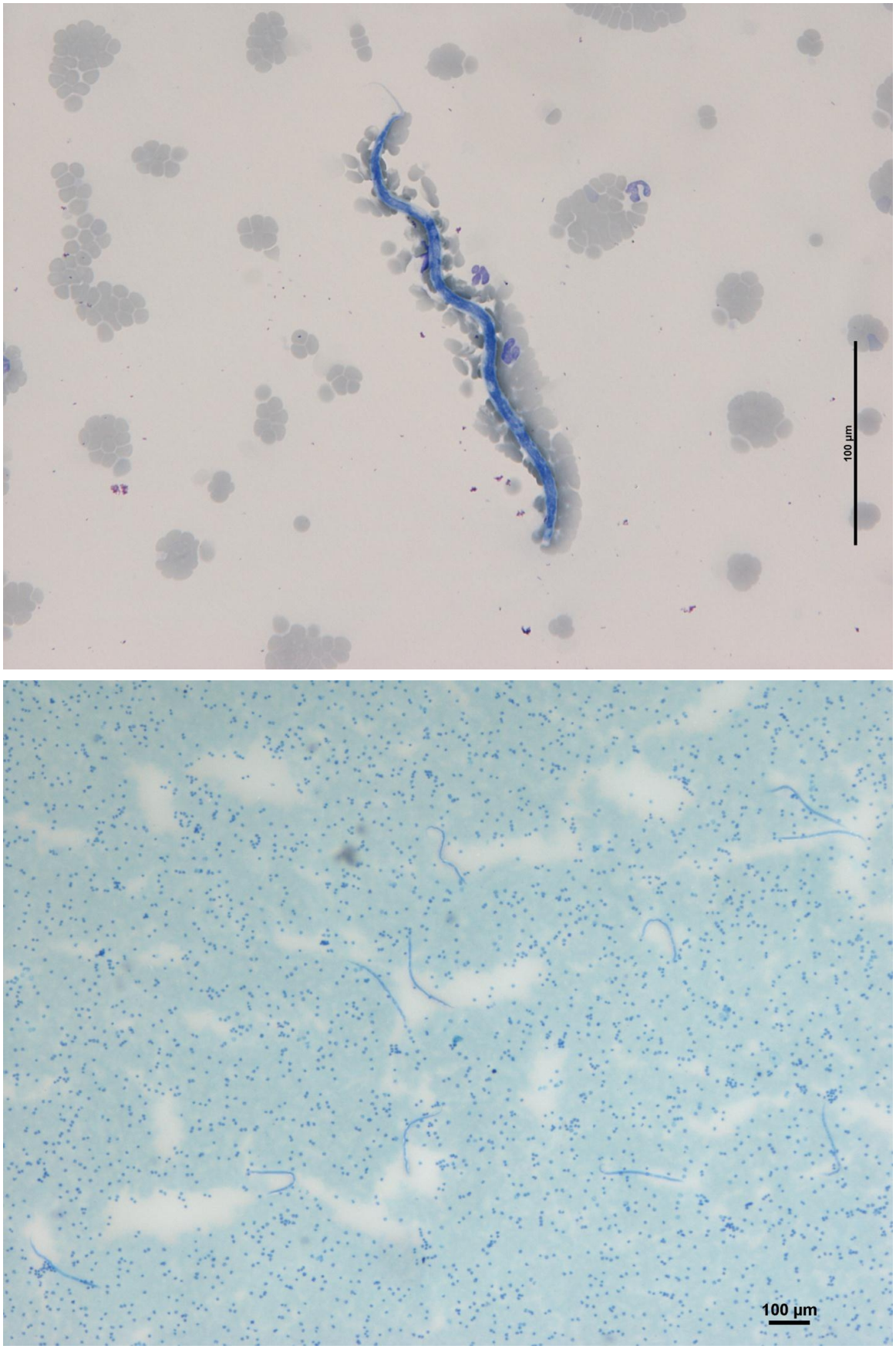
Appendix



Figures 1A & 1B: Microfilaria from a thin and thick blood smear respectively examined at 20X and 5X optics on a Nikon DS - 5Mc – U1 Digital Photomicrographic Camera System, Nikon Instruments Europe 2016

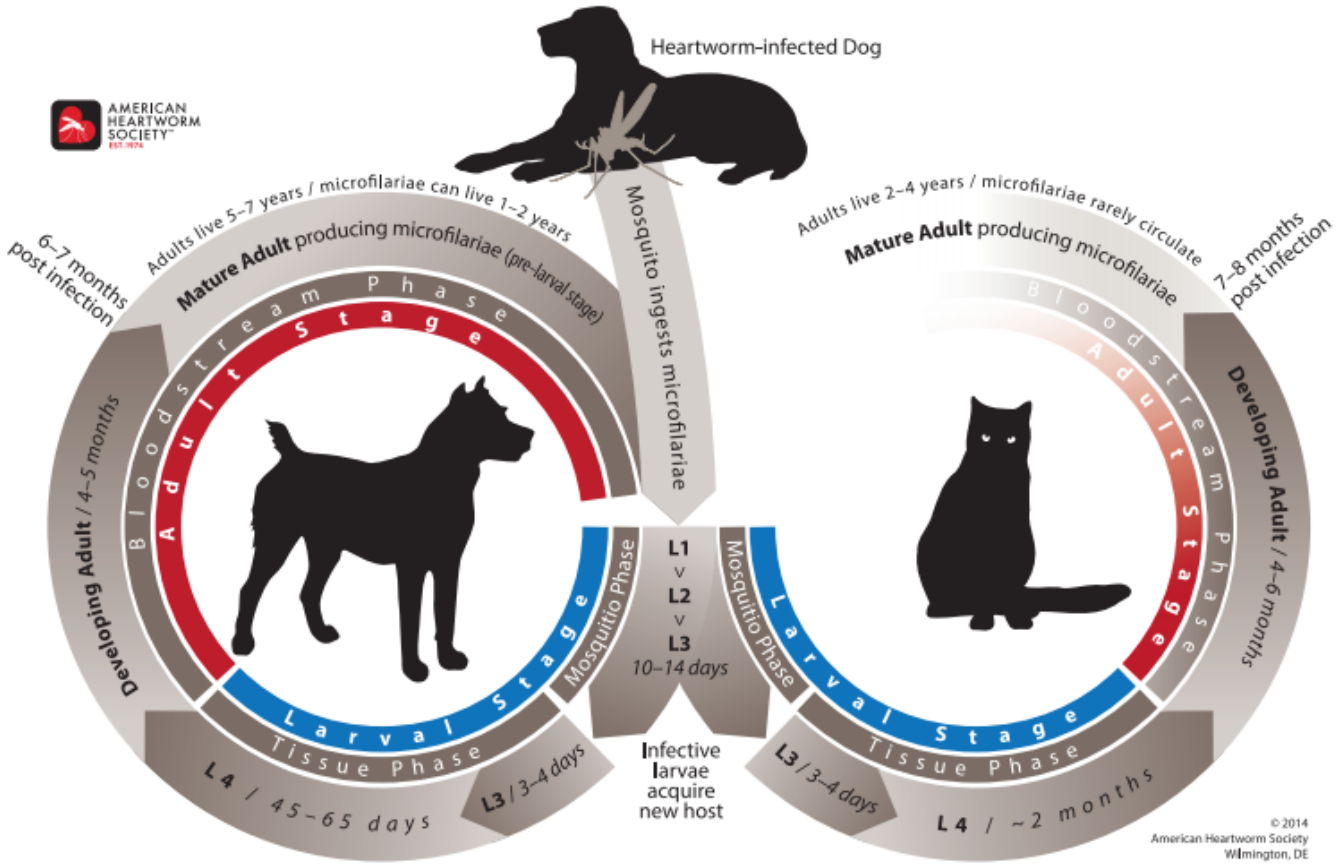


Figure 2: Lifecycle of *Dirofilaria immitis* (reproduced from Current Guidelines for the Prevention, Diagnosis and Management of Heartworm Infection (*Dirofilaria immitis)* Infection in Dogs (Revised July 2014) (13)

Factors affecting transmission of D. immitis *between dogs*

Mosquito	Dog	Parasite
Susceptibility	Uninfected	Occult (ectopic, uni-sexual) infection
Ability to transmit by bite	Exposure to vector	Fertility
Preference for canine hosts	Preventive medication	Number of worms—severity of infection (vector or host)
Longevity	Protective immunity?	Infectivity (vector or host)
Frequency of feeding	Proximity to reservoir of infection	Periodicity
Seasonality	Outdoor activity	Strain differences?
Flight range	Infected	
Domesticity	Occult (immune-mediated) infection	
Population size	Microfilarial periodicity	
Age structure	Exposure to vector	
Environmental factors	Pathophysiologic response and extent of disease	
Temperature	Successful curative chemotherapy	
Relative humidity		
Rainfall		
Light intensity		
Proximity to reservoir of infection		

Table 1: Table outlining epidemiology of vector, host and parasite relationships in *D.immitis* infection. Reproduced from Grieve et al. (3)

Figure 3: IDEXX 4Dx Snap Test showing an antigen positive result for *Dirofilaria immitis* infection (21)

	Number of Samples Tested Positive	Total Number of Samples	Estimated Prevalence	IDEXX 4Dx Test Sensitivity	IDEXX 4Dx Test Specificity
***Dirofilaria immitis* antigen positive**	20	60	(20/60) 33.3%	99.0% (94.3-99.9%)	99.3% (97.4-99.9%)
***Anaplasma phagocytophilum* antibody positive**	8	60	(8/60) 13.3%	90.3% (85.8-93.7%)	94.3% (90.7-96.7%)
***Ehrlicia canis* antibody positive**	0	60	(0/60) 0%	97.1% (94.0-98.8%)	95.3% (92.7-97.2%)
***Borrelia burgdorferi* antibody positive**	0	60	(0/60) 0%	94.1% (88.3-97.6%)	96.2% (92.9-98.3%)

Table 2: Table showing antigen and antibody test results of IDEXX 4Dx testing and test accuracy for haematological sampling (28)

	Mean (yy.)	Standard Deviation	Range
Infected Animals	4.83	2.67	4.83 ± 5.34
Non Infected Animals	4.42	3.83	4.42 ± 7.65
Entire Sample	4.59	3.44	4.56 ± 6.88

Table 3: Table outlining mean age of animals separated by infection status and as an entire sample

	Male	Female	Total
Number of Infected Animals	10	10	20
Number of Non Infected Animals	27	13	40
Total	37	23	60
Odds Ratio	2.08		

Table 4: Table outlining infection status by gender and odds ratio for infection

	Neutered	Entire	Total
Number of Infected Animals	5	15	20
Number of Non Infected Animals	21	18	39
Total	26	33	59
Odds Ratio	3.50		

Table 5: Table outlining infection status by neuter status and odds ratio for infection.