A broad insight onto the distribution pattern of Dirofilaria immitis in community dogs in Nepal

Subash Rimal (subashrimal2051@gmail.com)
Tribhuvan University Institute of Agriculture and Animal Science https://orcid.org/0000-0002-9546-1437

Anil Adhikari
Tribhuvan University, Institute of Agriculture and Animal Science

Rabin Acharya
Tribhuvan University, Institute of Agriculture and Animal Science

Dinesh Kumar Singh
Tribhuvan University, Institute of Agriculture and Animal Science

Naresh Prasad Joshi
Livestock Service Department, Government of Nepal

Birendra Shrestha
Tribhuvan University Institute of Agriculture and Animal Science

Krishna Kaphle
Tribhuvan University Institute of Agriculture and Animal Science

Khaled Mohamed El-Dakhly
Beni Suef University Faculty of Veterinary Medicine

Alessio Giannelli
BVBA, Izegem

Research

Keywords: Dirofilaria immits; stray dogs; zoonosis; haematobiochemical; epidemiology; Nepal

Posted Date: February 27th, 2020

DOI: https://doi.org/10.21203/rs.2.24778/v1

Abstract

Background Nematodes of the genus Dirofilaria are widespread vector-borne helminths (VBH) of increasing relevance. Indeed, dirofilariosis is frequently diagnosed in domestic pets, often dogs, associated with a severe clinical condition known as heartworm disease caused by D. immitis . Assessing the distribution pattern of canine dirofilariosis is pivotal to undertake appropriate control measures and define the risk of infection in animals and humans. This study revealed the occurrence of D. immitis in naïve community dogs from Nepal. Methods An epidemiological study was performed in 2019 in Siddharthanagar (Lumbini region, Nepal). A total of 150 blood specimens were examined using direct microscopy, buffy coat centrifugation and modified Knott's method aiming at isolating and identifying Dirofilaria microfilariae. In addition, hematobiochemical parameters, including packed cell volume (PCV), alanine aminotransferase (SGPT), aspartate aminotransferase (SGOT), alkaline phosphate (ALP), creatinine, blood urea nitrogen (BUN), urea and total protein were analyzed, along with a clinical scoring (i.e., body temperature and respiratory rate) of the animals enrolled in this study. Results Out of 150 dogs sampled, 29 (19.3%) had D. immitis microfilariae. The prevalence of the infection varied according to the technique used, ranging from 16.0% to 19.3% based on direct blood smear microscopy and modified Knott method, respectively. The infection rate was significantly (P <0.05) higher in dogs aged more than 3 years. A positive correlation was observed between SGPT and SGOT and the presence of microfilariae (P <0.05). Conclusion The current findings revealed the presence of D. immitis in dogs from Nepal, thus providing an explanation for the diagnosis of this VBH is human cases. Further investigations are warranted to accurately define the prevalence of the infection in other pets, instrumentally to reduce the potential burden on the infection in dogs and, accordingly, to control the spread of this parasite to humans.

Background

Canine heartworm disease is a vector-borne helminth (VBH) infection caused by *Dirofilaria immitis* [1], being particularly notorious for its implications in veterinary and human medicine [2–4]. Infected dogs might suffer from a potentially-fatal clinical condition featured by cardiorespiratory alterations, such as pulmonary hypertension, dyspnoea, ascites, caused by the localization of adult worms within the pulmonary arteries and right heart chamber [5]. Conversely, humans are dead-end hosts for *D. immitis*, as the infection often results in a self-limited pulmonary condition. Nonetheless, the inflammatory response against the parasites induces so-called “coin lesions” potentially misdiagnosed as tumors [6]. Overall, dirofilariosis in dogs and humans is extremely relevant and requires attention by public health authorities and veterinary bodies, ultimately involving a constant need for up to date epidemiological information on the distribution of the parasite [2, 7, 8].

The life cycle of *D. immitis* includes a reservoir and a susceptible host, biologically connected by competent arthropod vector [5]. While feeding on a native dog, infected mosquitoes of approximately 70 species included in the genera *Culex, Aedes, Ochlerotatus, Anopheles, Armigeres or Mansonia* [9] can transmit *D. immitis* infective third-stage larvae (L3) into the definitive host. Larvae migrate into the skin

through the insect bite wound, developing in L4 in 3 days and reaching the pulmonary arteries in 47-67 days [2, 5]. Worms continue their development and growth for 4-5 months, finally reaching the adult stage. Over their lifespan, sexually-mature females release microfilariae into the bloodstream, which are eventually ingested by mosquitoes during their meal. Inside the vector, microfilariae moult into L3 in 10-14 days [5]. Totally, a period of 7 to 9 months is necessary for *D. immitis* to conclude its life cycle, persisting for around 4-5 years in the pulmonary arteries of the infected dog [10]. During this long period, microfilaeremic animals represent a continuous source of microfilariae for female mosquitoes, indirectly regulating the distribution of the parasite and the seasonality of the infection [5, 10].

Dirofilaria immitis has been diagnosed worldwide in dogs from different countries and climatic areas, including temperate, tropical, subtropical countries, such as Southeast Asia. In this continent, however, the literature on dirofilariosis is mainly limited to reports from Korea [11], India [12], Taiwan [13], or China [14], with scant information from Nepal. Although the presence of the parasite has been suspected following an isolated clinical record in a dog with chylothorax [15] and a human dirofilariosis case in an elderly woman [16], no epidemiological data is available from this country, which actually counts a large number of stray dogs, potentially serving as reservoir for the infection [17–18].

In the present study, the occurrence of *D. immits* was investigated in naïve stray dogs and documented for the first time through the use of traditional parasitological and hematobiochemical assays, providing original data of the prevalence of canine dirofilariosis in Nepal.

Methods

Study area

The study was conducted from November 2018 to May 2019 in Siddharthanagar, Nepal (Indo-Nepal border, 28’10”N,84’15”0E), a municipality located 107 m above sea level. The area is featured by a semi-tropical climate with annual temperature of 24.8°C (range 18.7°C - 30.9°C), and rainfall means 143.8 mm (range 8.2-545.6 mm).

Animals and sampling procedures

Blood specimens (approximately 10 ml) containing ethylene diamine tetraacetic acid (EDTA) were randomly collected from stray dogs and sent to the Veterinary Teaching Hospital (VTH, Institute of Agriculture and Animal Science, Tribhuvan University, Siddharthanagar). Animals were categorized into four age classes (i.e.<1, 1–3, 3–5 and >5 years) and subjected to an accurate anatomopathological examination at VTH if found dead during the study period. In particular, dogs were examined for cardiopulmonary parasites, which were identified at species level based on their morphological features [5].

Parasitological examination

Samples were examined by direct microscopy, buffy coat centrifugation and modified Knott's method. In the former, one drop of blood (approximately 20 µl) was placed onto a clean microscope slide with a cover slide and microscopically examined, with samples showing slight undulating movements of larvae considered as positive. In addition, micro-hematocrit tubes were filled with 20 µl blood, centrifuged at 12000 rpm for 5 min and observed for alive microfilariae on the buffy coat. Finally, a third subsample was evaluated according to the modified Knott's method. One ml of EDTA blood was mixed with 9 ml 2% formalin solution and centrifuged at 1500 rpm for 5 min. After discarding the supernatant, 0.1% methylene blue was added to the sediment, which was transferred onto a microscope slide using a plastic pipette. The sample was examined and microfilariae were identified at species level based on their morphometry and morphology of the anterior and posterior extremities [19–22].

Hematobiochemical and biochemical evaluations

The following parameters were analyzed in microfilaria-positive and the -negative dogs: packed cell volume (PCV), alanine aminotransferase (SGPT), aspartate aminotransferase (SGOT), alkaline phosphate (ALP), creatinine, blood urea nitrogen (BUN), urea and total protein. All data were determined by using an advanced microprocessor-based colorimeter and biochemical reagents (Accurex Biomedical Pvt. Ltd., India).

Statistical analysis

Statistical analysis was conducted using Statistical package on social science (SPSS) v.25 and MS Excel were used for data analysis. The analysis was performed using Chi-square test and one sample T-test with comparison of means. The significance level was set at $P < 0.05$.

Results

A total of 150 dogs (81 males and 69 females) were enrolled. Of these animals, 25 (16.7 %) were less than 1 year, 34 (22.7%) aged 1-3 years, 44 (29.3%) aged 3-5 years and 47 (31.3%) over 5 years. Soon after the blood collection, 19 dogs (12.7%) were found dead and showed chylothorax and ascites upon necropsy. Nine (47.4%) of them were infected with *D. immitis* heartworms (range 2-14 adult nematodes) (Fig. 1). Overall, only 41 dogs (27.6%) suffered from elevated respiratory rate (more than 30 acts per minute) whereas the majority (129 animals, 86.2%) had elevated body temperature, ranging from 37.5 to 39.0ºC, but not statistical correlation was observed ($P = 0.611$).

Upon parasitological analysis of blood samples, 29 (19.3%) animals were positive for microfilariae. Larvae measured 305 µm in length and showed a conical front end a thin and straight rear end,

suggestive for *D. immitis* (Fig. 2). The sensitivity of the diagnosis was higher in samples analyzed by the modified Knott method (i.e., 19.3%) than those by direct smear microscopy on blood or buffy coat (i.e., both 16.0%). The prevalence of the infection was higher in female dogs than males (i.e., 21.7% vs 17.3%) but not statically significant (P= 0.49), and in dogs aged 3-5 years (51.7%) than those younger than one year (3.5%) (Fig. 3).

PCV ranged 35%-54% in 79.3% of infected dogs, while it was less than 35% in 13.8% of infected dogs and more than 54% in 6.9% of infected dogs. A significant correlation between SGPT and SGOT was recorded in infected animals (P <0.05) (Table 1).

Discussion

Results of the present study documented the occurrence of *D. immitis* infection in community dogs from Nepal demonstrating that the parasite is circulating among native dogs living in the Indo-Nepal border. With the exception of previous reports from mainland and northeast India [12, 23–25] or Bangladesh [26], no epidemiological data was available from the study area. Interestingly, the prevalence of the infection herein reported is relatively higher than that reported from the eastern Indian boundaries [12, 23–26], and whether this is due to the different climatic conditions, activity of arthropod vectors or availability of a larger population of stray dogs is yet to define. Although it is scientifically proved that mosquitoes are the main intermediate hosts of *Dirofilaria* spp. and are crucial for the larval development of microfilariae into L3 and their transmission [27–30], no information is reported on the species involved into the circulation of these parasites within the Indo-Nepal region. Nevertheless, mosquitoes are active in this area, as it can be inferred from the efforts spent on the control and epidemiological surveillance of other vector-borne diseases, such as Dengue, Zika or other human filarial worms [30–32].

The lack of an overt clinical presentation in *D. immitis* infected dogs is related to the life cycle of this nematode and to the pathological alterations it may cause, which are often detected during the chronic phase of the infection. As confirmed in this study, a significant correlation between dirofilariosis and increased respiratory rate might be observed, although this clinical sign might be associated with other clinical conditions [33–34]. However, when observed during canine heartworm disease, dyspnoea is mainly attributed to the presence of adult nematodes in the arteries interfering with normal cardiopulmonary function, ultimately inducing hemolysis, liver or kidney dysfunction, and respiratory failure [5, 10, 35]. The persistence of *D. immitis* for up to 6 years and long life cycle of the parasite (i.e., 7 to 9 months) also explains the significantly-higher and -lower prevalence of the infection is in dogs older than 5 years and younger than one year, respectively. In the latter, however, a false negative diagnosis cannot be excluded based on the sensitivity of the detection method tested in the present study, only allowing to confirm the occurrence of *D. immitis* when the infection is patent [5, 10]. Comparing the techniques herein tested, the modified Knott's method was more sensitive than the direct detection of larvae either in blood or buffy coat, based on the principle that microfilariae were concentrated into a small volume before being analyzed [36]. The use of indirect diagnostic methods, like serology, might overcome this limitation. Although the direct parasitological methods are straightforward [10, 37], they

are less sensitive and a potentially-low specific compared to antigen or antibodies detection methods, thus reducing the potential for misidentification of microfilariae species [20, 38]. Currently, the identification of *D. immitis* microfilariae has been corroborated by the detection of adult heartworms upon necropsy. Nonetheless, other zoonotic filarioid species might be present in this region, including *Dirofilaria repens* or *Acanthocheilonema* spp. [25].

Finally, the lack of significance between alteration in PCV and positivity for *D. immitis* was not surprising, as previously reported [5, 10]. Oppositely, the increase in SGPT and SGOT might be associated with predominant intracellular activity following the inflammation caused by the parasite, as previously reported [39]. Accordingly, the cellular damage increases alongside the progression of the disease [5, 10]. Although this epidemiological study was limited by the relatively small number of dogs enrolled, the present findings provided new data on the occurrence of *D. immitis* in a remote region of Nepal where no data was available so far, despite the report of human and animal cases [15, 16]. Further investigations are warrant to learn about the distribution of other canine VBD in Nepal.

Conclusion

To the best of our knowledge, this is the first report documenting the occurrence of *D. immitis* in community dogs from the Indo-Nepal region and demonstrating an active circulation of the parasite. The veterinary care in the study area is considered inadequate and this results into free movement of untreated domestic animals and wildlife potentially acting as a source of the infection for large populations of stray dogs potentially serving as a reservoir for the infection [17–18] for humans living within the Nepal/India urban districts. Strict hygienic measures, control programs of stray dogs and adequate control of mosquitoes are urgently demanded in order to reduce the potential threat represented by *D. immitis* and other zoonotic parasites that might be present in this region.

Declarations

Acknowledgement

The authors would like to acknowledge the Veterinary Teaching Hospital, Institute of Agriculture and Animal Science, Tribhuvan University, Nepal Veterinary Council, Faculty members, Dr. Hari Bd. Rana, Mr. Subodh Khanal, Dr. K. R. Subramanaian, Huma Neupane, Tirthraj Ghimire, Sandeep Rimal, Dr. Rajan Bhattarai, Dr. Prabeg Acharya, Dr. Rikesh Shrestha, Dr. Avash Shrestha, Dr. Rabina Ghimire, Dr. Prithvi Thapa, Dr. Sirjana Bamrel, Dr. Astha Thapa, Dr. Monalisha Khanal, Dr. Asmita Shrestha, staff and students who helped in the completion of the work. Authors especially thanks to Heather Thaxter, MA, BA (Hons), PGCE FHEA for final English editing.

Funding resources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical approval

Ethical clearance and publication approval was given by the ethical review committee of the Nepal Veterinary Council.

Conflict of interest

The authors declare that there was no conflict of interest.

Consent of publication

No need the consent of publication

References

1. Tahir D, Davoust B, Parola P. Vectorborne nematode diseases in pets and humans in the Mediterranean Basin: an update. Vet World. 2019, 12: 1630–1643.
2. Otranto D, Deplazes P. Zoonotic nematodes of wild carnivores. Int J Parasitol Parasites Wildl. 2019, 9:370–383.
3. Lee AC, Montgomery SP, Theis JH, Blagburn BL, Eberhard ML. Public health issues concerning the widespread distribution of canine heartworm disease. Trends Parasitol. 2010, 26:168–173.
4. Tasic-Otasevic SA, Bozinovic MST, Gabrielli SV, Genchi C. Canine and human *Dirofilaria* infections in the Balkan Peninsula. Vet Parasitol. 2015, 209:151–156.
5. Bowman DD, Atkins CE. Heartworm biology, treatment, and control. Vet Clin North Am Small Anim Pract. 2009, 39:1127–1158.
6. Li CY, Chang YL, Lee YC. Human pulmonary dirofilariasis coexisting with intercostal neurilemmoma: a case report and literature review. J Formos Med Assoc. 2013, 112:644–647.
7. Bowser NH, Anderson NE. Dogs (*Canis familiaris*) as Sentinels for Human Infectious Disease and Application to Canadian Populations: A Systematic Review. Vet Sci. 2018, 5.
8. Capelli G, Genchi C, Baneth G, Bourdeau P, Brianti E, Cardoso L, Danesi P, Fuehrer HP, Giannelli A, Ionică AM, Maia C, Modrý D, Montarsi F, Krücken J, Papadopoulos E, Petrić D, Pfeffer M, Savić S, Otranto D, Poppert S, Silaghi C. Recent advances on *Dirofilaria repens* in dogs and humans in Europe. Parasites Vectors. 2018, 11:663.
9. Capelli G, Frangipane di Regalbono A, Simonato G, Cassini R, Cazzin S, Cancrini G, Otranto D, Pietrobelli M. Risk of canine and human exposure to *Dirofilaria immitis* infected mosquitoes in endemic areas of Italy. Parasites Vectors. 2013, 6:60.
10. McCall JW, Genchi C, Kramer LH, Guerrero J, Venco L. Heartworm disease in animals and humans. Adv Parasitol. 2008, 66:93–285.
11. Yoon WK, Kim YW, Suh SL, Choi R, Lee SG, Hyun C. Evaluation of cardiopulmonary and inflammatory markers in dogs with heartworm infection during treatment with the 2014 American Heartworm

Society recommended treatment protocol. Parasites Vectors. 2017, 10:535.

12. Sarma K, Nachum-Biala Y, Kumar M, Baneth G. Molecular investigation of vector-borne parasitic infections in dogs in Northeast India. Parasites Vectors. 2019, 12:122.
13. Lu TL, Wong JY, Tan TL, Hung YW. Prevalence and epidemiology of canine and feline heartworm infection in Taiwan. Parasites Vectors. 2017, 10:484.
14. Wang J, Zhu X, Ying Z, Han Q, Liao C, Wang J, Zhao J, Sun J, Lindsay DS. Prevalence of *Dirofilaria immitis* infections in dogs and cats in Hainan Island/province and three other coastal cities of China based on antigen testing and PCR. J Parasitol. 2019, 105:199–202.
15. Singh DK, Adhikari A, Rimal S, Singh K. Chylothorax due to heartworm (dirofilariasis) in a Dog. Blue Cross. 2018, 15:83–86.
16. Pradhan D, Pradhan C, Joshi P. An Encounter with a Worm in Anterior Chamber: A Case Report. Nepal J Ophthalmol. 2018, 10: 98–101.
17. Ng D, Carver S, Gotame M, Karmasharya D, Karmacharya D, Man Pradhan S, NarsinghRana A, Johnson CN. Canine distemper in Nepal's Annapurna Conservation Area - Implications of dog husbandry and human behaviour for wildlife disease. PLoS One. 2019, 14:e0220874.
18. Acharya KP, Acharya N, Wilson RT. Animal Welfare in Nepal. J Appl AnimWelf Sci. 2019, 22:342–356.
19. Evans CC, Bradner JL, Savadelis MD, Nelson CT, Moorhead AR. Acetic acid as an alternative reagent in the modified Knott test. Vet Parasitol. 2019, 276:108975.
20. Magnis J, Lorentz S, Guardone L, Grimm F, Magi M, Naucke TJ, Deplazes P. Morphometric analyses of canine blood microfilariae isolated by the Knott's test enables *Dirofilaria immitis* and *D. repens* species-specific and *Acanthocheilonema* (syn. *Dipetalonema*) genus-specific diagnosis. Parasites Vectors. 2013, 6:48.
21. Traversa D, Di Cesare A, Conboy G. Canine and feline cardiopulmonary parasitic nematodes in Europe: emerging and underestimated. Parasites Vectors 2010, 3:62.
22. Albanese F, Abramo F, Braglia C, Caporali C, Venco L, Vercelli A, Ghibaudo G, Leone F, Carrani F, Giannelli A, Otranto D. Nodular lesions due to infestation by *Dirofilaria repens* in dogs from Italy. Vet Dermatol. 2013, 24:255–256.
23. Patel JR, Devi S, Varshney JP, Jadhav KM. Epizootiological observations on canine microfilaremia in Gujarat state, India. Vet World. 2018, 11:1564–1568.
24. Borthakur SK, Deka DK, Islam S, Sarmah PC. Occult dirofilariosis in dogs of north eastern region in India. J Arthropod Borne Dis. 2015, 10:92–97.
25. MegatAbd Rani PA, Irwin PJ, Gatne M, Coleman GT, McInnes LM, Traub RJ. A survey of canine filarial diseases of veterinary and public health significance in India. Parasites Vectors. 2010, 3:30.
26. Fuehrer HP, Treiber M, Silbermayr K, Baumann TA, Swoboda P, Joachim A, Noedl H. Indigenous *Dirofilaria immitis* in Bangladesh. Parasitol Res. 2013, 112:2393–2395.
27. Kartman L. Factors influencing infection of the mosquito with *Dirofilaria immitis* (Leidy, 1856). Exp Parasitol. 1953, 2:27–78.

28. Taylor AER. The development of *Dirofilaria immitis* in the mosquito *Aedes aegypti*. J Helminthol. 1960, 34: 27–39.
29. Montarsi F, Ciocchetta S, Devine G, Ravagnan S, Mutinelli F, Frangipane di Regalbono, A, Capelli G. Development of *Dirofilaria immitis* within the mosquito *Aedes* (Finlaya) *koreicus*, a new invasive species for Europe. Parasites Vectors, 2015, 8:177.
30. Gupta BP, Haselbeck A, Kim JH, Marks F, Saluja T. The Dengue virus in Nepal: gaps in diagnosis and surveillance. Ann Clin Microbiol Antimicrob. 2018, 17:32.
31. Shakya G, Marasini B, Karki KB, Upadhaya BP, Acharya J, Adhikari S, Manjhi R, Maharjan L, Shrestha L, Ranabhat K, Marahatta SB, Shrestha B, Dhimal M. Outbreak Investigation Following the 2015 Earthquake Disaster in Nepal. J Nepal Health Res Counc. 2018, 16:61–65.
32. Ojha CR, Joshi B, Kc KP, Dumre SP, Yogi KK, Bhatta B, Adhikari T, Crowley K, Marasini BR. Impact of mass drug administration for elimination of lymphatic filariasis in Nepal. PLoS Negl Trop Dis. 2017, 11:e0005788.
33. Stillion JR, Letendre JA. A clinical review of the pathophysiology, diagnosis, and treatment of pyothorax in dogs and cats. J Vet Emerg Crit Care (San Antonio) 2015, 25:113–129.
34. Carretón E, Morchón R, Montoya-Alonso JA. Cardiopulmonary and inflammatory biomarkers in heartworm disease. Parasites Vectors. 2017, 10:534.
35. Dillon AR, Blagburn BL, Tillson M, Brawner W, Welles B, Johnson C, Cattley R, Rynders P, Barney S. Heartworm-associated respiratory disease (HARD) induced by immature adult *Dirofilaria immitis* in cats. Parasites Vectors. 2017, 10:514.
36. Watanabe Y, Yang C-H, Tung K-C, Ooi H-K. Comparison of microfilaria concentration method for Setariadigitata infection in cattle and for *Dirofilaria immitis* infection in dogs. J Vet Med Sci. 2004, 66:543–545.
37. Siwila J, Mwase ET, Nejsum P, Simonsen PE. Filarial infections in domestic dogs in Lusaka, Zambia. Vet Parasitol. 2015, 210: 250–254.
38. Little S, Saleh M, Wohltjen M, Nagamori Y. Prime detection of *Dirofilaria immitis*: understanding the influence of blocked antigen on heartworm test performance. Parasites Vectors 2018, 11:186.
39. Niwetpathomwat A, Kaewthamasorn M, Tiawsirisup S, Techangamsuwan S, Suvarnvibhaja S. A retrospective study of the clinical hematology and the serum biochemistry tests made on canine dirofilariasis cases in an animal hospital population in Bangkok, Thailand. Res Vet Sci. 2007, 82:364–369.

Table 1

Table 1 Biochemical parameters evaluated in sera of examined community dogs in Siddharthnagar sub-metropolitan city, Nepal

Figures

Parameter	Infected dogs	Non-infected dogs	*P* value	T value
Total protein (mg/dl)	7.37 ± 0.36	6.56 ± 0.29	*P* >0.05	1.166
Aspartate Aminotransferase (SGOT) (IU/L)	26.73 ± 3.54	17.98± 2.52	*P* <0.05	2.013
Alkaline phosphatase (ALP) (IU/L)	376.11 ± 39.60	337.35± 53.71	*P* >0.05	0.581
Alanine Aminotransferase (SGPT) (IU/L)	27.25 ± 2.65	19.91± 2.47	*P* <0.05	0.049
Urea (mg/dl)	22.19.12 ± 1.91	25.24± 2.90	*P* >0.05	-0.876
Blood Urea Nitrogen (BUN) (mg/dl)	11.39 ± 01.01	12.21 ± 1.49	*P* >0.05	-0.509
Creatinine (mg/dl)	1.63 ± 0.132	1.46± 0.12	*P* >0.05	0.934

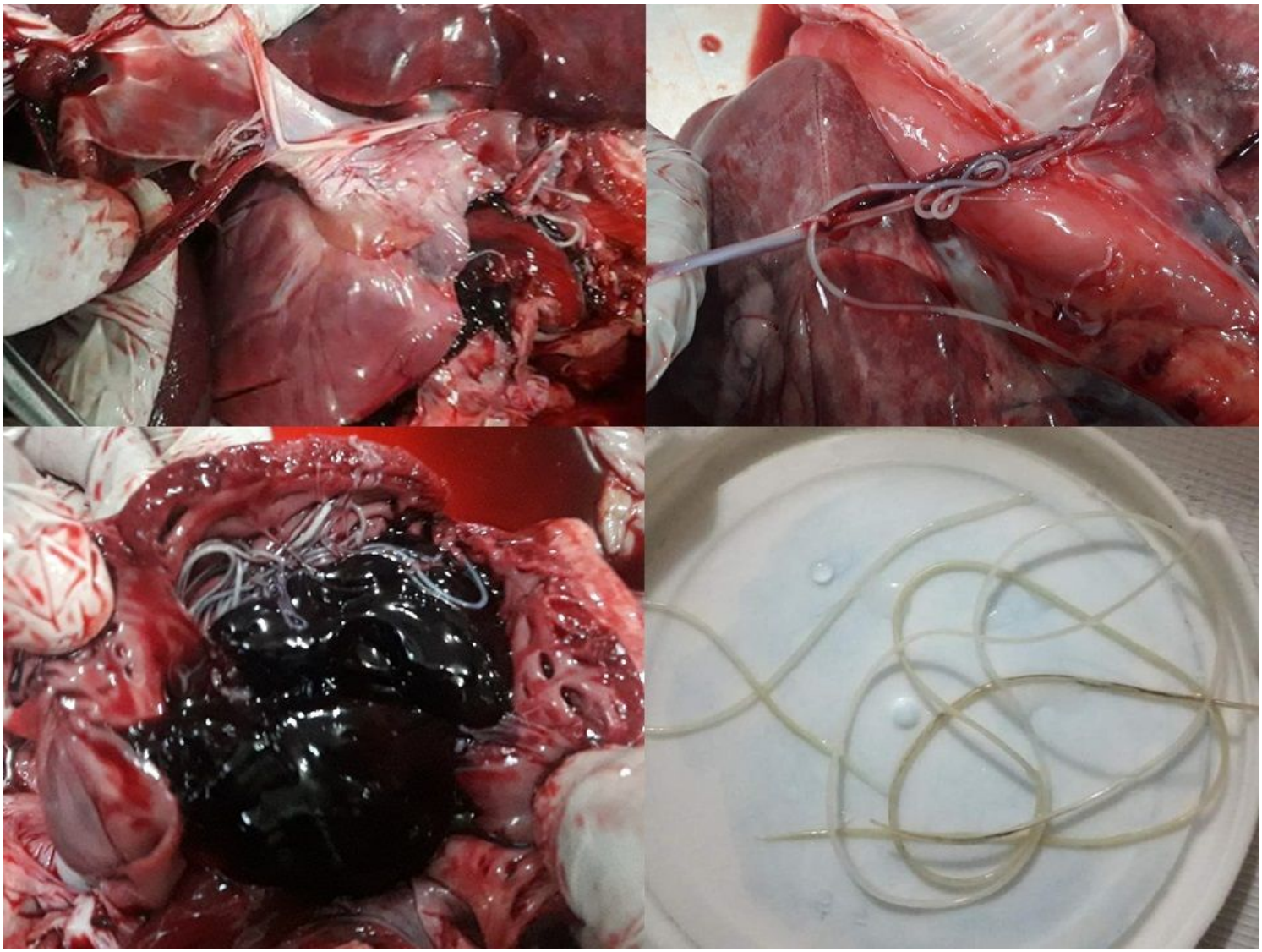

Figure 1

Adult heartworm (Dirofilaria immitis) revealed from Right Ventricle and Pulmonary artery in necropsy finding

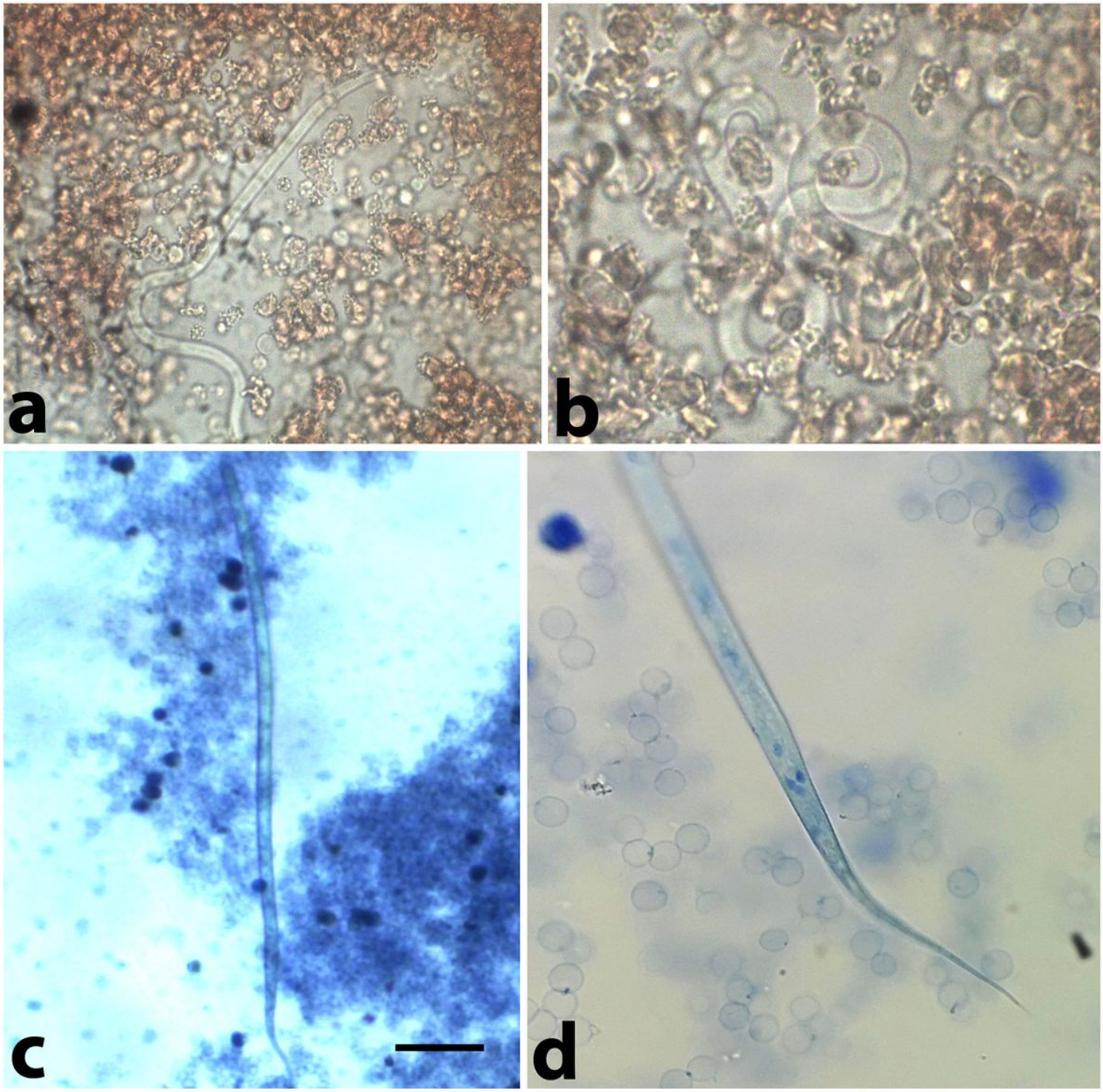

Figure 2

Microfilariae of the recovered Dirofilariaimmitis. a. The whole microfilaria in direct blood smears with x 10. b. Magnified power of the microfilaria with x 20. c. The whole microfilaria of D. immitis. Scale bar= 50 µm. d. The posterior end of D. immitis microfilaria, Note the characteristic thin and straight end

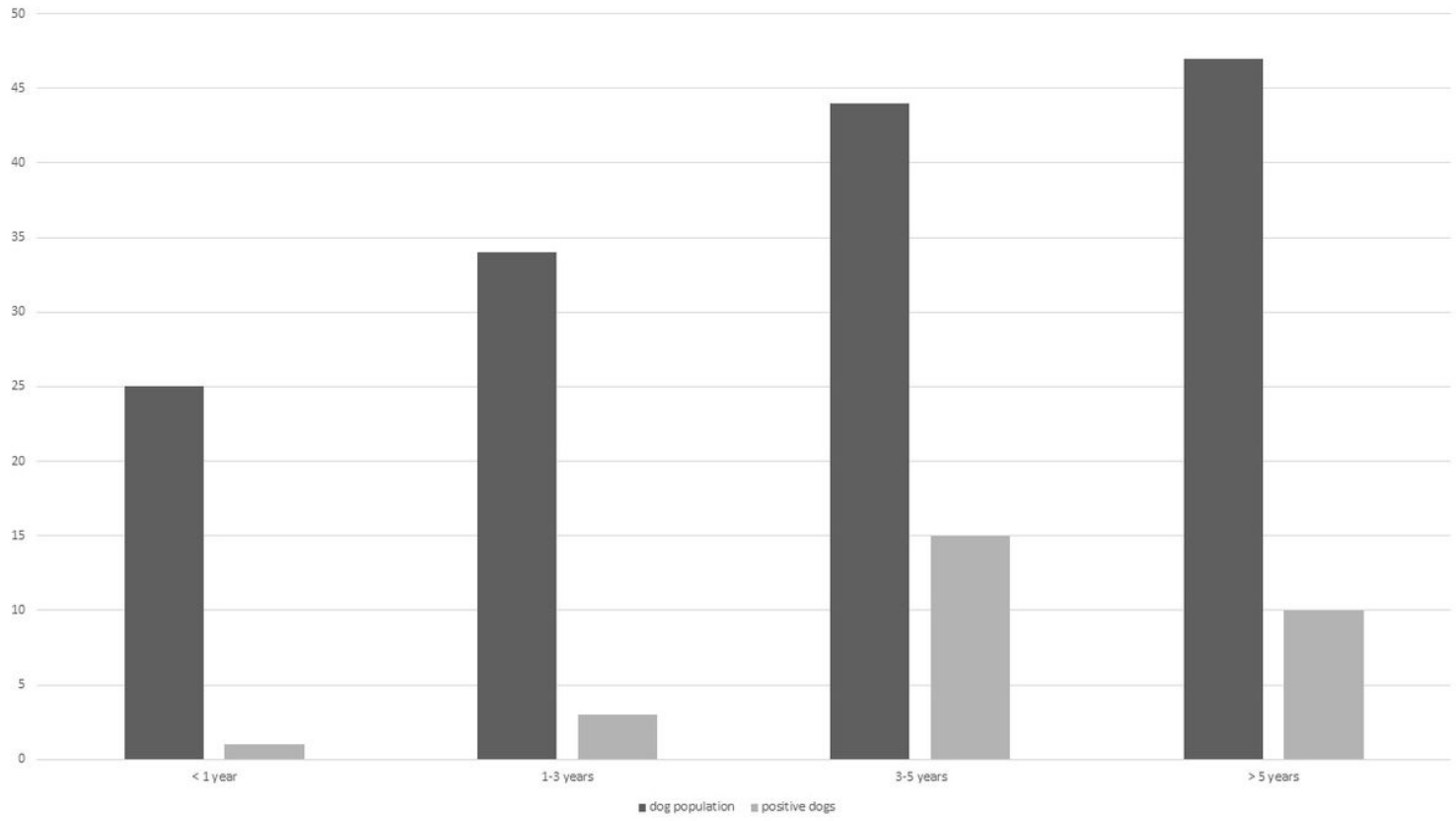

Figure 3

The prevalence of Dirofilaria in community dogs of different aged group of Siddharthanagar Sub-metropolitan city, Nepal using various traditional techniques

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- geographicabstract.pdf