The first report on the occurrence of Dirofilaria species in stray dogs in Siddharthnagar, Lumbini, Nepal

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Research

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Abstract

Backround: Dirofilaria species are important zoonotic filarioid nematodes transmitted by mosquitoes causing a heartworm disease in canines worldwide. Map of the distribution pattern of dirofilariois in Nepalese context is lacking.

Methodology: A study was done to access the occurrence of dirofilarioids among stray dogs population in Siddharthanagar, sub-metropolitan city of Lumbini, Nepal. A total of 150 blood specimens were examined using direct microscopy, buffy coat centrifugation and modified Knott's method. Haematobiochemical 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 evaluated. Furhter, the clinical performance, comprising the body temperature and respiratory rate, were also assessed.

Results: The dirofilariod is recognized as Dirofilaria immitis . Overall prevalence of dirofilariosis was reported to be 19.33% (29/150). Meanwhile, microfilariae were noticed to be 16.0, 16.0 and 19.33 % using the direct blood smear microscopy, buffy coat and modified Knott technique, respectively. The infection rate was significantly ($P < 0.05$) higher in aged dogs with high respiratory rates. The response of sex was non-significant. Among haematobiochemical parameters, only SGPT and SGOT were significantly ($P < 0.05$) elevated. The body temperature was not altered.

Conclusion: Coinciding with the existence of stray dog communities and the recent zoonotic appearance of such filarioids, further investigations including molecular approaches, are urgently needed to accurately differentiate both dirofilarioids and other filarioid nematodes in both pets and humans.

Introduction

Dirofilariosis or canine heartworm disease, caused by the filarioid nematodes Dirofilaria immitis and D. repens, is a mosquito-borne disease maninly affecting domesric dogs, Canis familiaris, and other wild canids carrying potential for both veterinary and zoonotic aspect (Lee et al. 2010; Tasic-Otasevic et al. 2015). Inspite of advanced and appropriate diagnostic tools, effective preventiev measures and increased awareness among veterianrians, infection rates of the disease are still remarkably observed (McCall et al. 2008; Genchi et al. 2014; Tasic-Otasevic et al. 2015). Findings explain that in canines, the disease emerges in severe form as a result of D. immitis infection, while in humans, both species induce mild dermatological affections to severe cardiopulmonary lesions (Pampiglione and Rivasi 2000; Genchi et al. 2011a; Simón et al. 2012; Albanese et al. 2013; Ionică et al. 2015; Tasic-Otasevic et al. 2015).

Few decades ago, as a result of the availability of mosquito vectors and the anthropogenic climatic conditions, particualrly the high temperature, the global distribution pattern and diversity of Dirofilaria spp. towrads the eastern and northern countries significantly increased (Genchi et al. 2009, 2011b; Pantchev et al. 2011). So far, approximately 70 mosquito species are potentially increminated in the transmission of Dirofilaria spp. in both natural and experimental conditions (Cancrini and Kramer 2001;

Morchón et al. 2012). The potential transmission often occurs in tropical climates as well as the warmest periods of the temprate distrcits, via which the mosquito activitiy is maximized (Vezzani and Carbajo 2006; Vezzani et al. 2011; Bocková et al. 2015). D. immitis occurs in tropical, subtropical and temperate districts (Martin and Collins 1985), while D. repens is found in the old world, particularly via the Mediterranean areas, South Asia and sub-Saharan Africa (Lock 1988; Pampiglione et al. 1995; Cringoli et al. 2001).

The distribution pattern, biology and veterinary medical significance of dirofilarioids are greatly lacking, probably due to the misdiagnosis of the clinical picture observed during the infection or due to the lack of diagnostic expertise (Siwila et al. 2015). The mosquito vectors in Kathmandu valley and Rupandehi are Aedes albopictus, Culex quinquefasciatus, Aedes aeypti (Darsie et al., 1991). The development of D. immitis to the third larval stage in mosquitoes occurs at a threshold temperature of 14 °C (Genchi et al. 2010).

Among previous literature on dirofilariosis in Asia, the prevalence of heartworms in stray dogs was 40.0% in South Korea (Song et al. 2003), 11.38% (by the use of traditional diagnostic tools) in the Assam and Mizoram area of the Northeastern state of India (Borthakur et al. 2015) and 13.18% in Henan province of central China (Wang et al. 2016). In Nepal, no large-scaled works studied dirofilariosis in dogs, however, sporadic cases were reported. Among those, Singh et al. (2018) revealed the occurrence of Dirofilaria-induced chylothorax in a dog admitted to the Veterinary Teaching Hospital, Bhairahawa. Meanwhile, Pradhan et al. (2018) reported a case of human dirofilariosis in the anterior chamber of the eye in elderly female. Therefore, the current study is considered the first that, preliminarily, explore the occurrence of dirofilariosis in stray dogs, by the use of traditional parasitological and hematobiochemical assays, in Nepal.

Materials And Methods

Study area and sampling

Blood specimens were collected from 150 randomly selected dogs in the Siddharthnagar sub-metropolitan city (Indo-Nepal border), Nepal (coordinates: 28° 10' 0" N, 84° 15' 0" E).The selected dogs constituted of 81 males and 69 females. Dogs were categorized into 4 groups on the basis of their age; dogs aged less than one year (25), dogs aged 1-3 years (34), dogs aged 3-5 years (44) and dogs aged more than 5 years (47). Specimens were sent to the Veterinary Teaching Hospital, institute of Agriculture and Animal Science, Tribhuvan University, Siddharthanagar, sub-metropolitan-1, Lumbini, Nepal.

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Direct microscopy

One drop of venous blood was placed on a clean microscope slide with a cover slip. Examination under a low microscopy with magnification power 10× was done. Undulating movements of larvae were

observed.

Preparation of buffy coat

In micro-hematocrit tubes, blood specimens were centrifuged at 12000 rpm for 5 minutes and the movements of live microfilariae were visible in the buffy coat.

Modified Knott's method

A sample of the whole blood was drawn into a syringe containing EDTA. One milliliter of the blood was mixed with 9 mL of a 2% formalin solution, and the mixture was centrifuged at 1500 rpm for 5 min. After discarding the supernatant, one drop of 0.1% methylene blue was added to the sediment and mixed well. The stained sediment was transferred into a microscope slide using a Pasteur pipette to collect the entire sample and it was examined using the 10× microscope objective. Microfilariae were recognized based on morphology and morphometry and counted, per milliliter of blood. Microfilariae were observed in an extended position with blue-stained nuclei. The tail of *D. immitis* is straight, while that of *D. repens* is slightly curved (Traversa et al. 2010; Magnis et al. 2013).

Hematobiochemical evaluations

Ten milliliters of blood sample was collected from each dog. Four mililiters of blood were poured into tubes containing ethylene diamine tetraacetic acid (EDTA), and the remaining blood was kept into clot activator vials to obtain serum.

For evaluation of hematological parameters, packed cell volume (PCV) was calculated for both microfilaria-positive and the negative specimens. Biochemically, alanine aminotransferase (SGPT), aspartate aminotransferase (SGOT), alkaline phosphate (ALP), creatinine, blood urea nitrogen (BUN), urea and total protein of each specimen were considered. All were determined by using advanced microprocessor-based colorimeter and biochemical reagents of the autozyme reagent of 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. Analysis was performed using Chi-square test and one sample T-test with comparison of means.

Results

Dirofilarioid microfilariae were observed in blood of examined dogs using various traditional tools. Morphologically, the recovered microfilaria measured approximately 350 µm long, the anterior end was more or less pointed, the body was straight and the posterior end was characteristically thin and straight.

It was suggested that the species recovered was *Dirofilaria immitis* based on that the anterior end was more or less pointed and the posterior end was thin and straight (Fig. 1). The overall prevalence of *Dirofilaria* infection was 19.33%. Infection rates using direct smear microscopy, buffy coat observation and modified Knott technique were 16.0, 16.0 and 19.33%, respectively (Fig. 2).

It was found that 72.40 % of infected dogs had a normal respiration, and 27.60% suffered from elevated respiratory rate (Fig. 3). Meanwhile, the majority (86.20%) of infected dogs had elevated temperature ranged from 37.5-39ºC (Fig. 4). Regarding sex, the current study revealed that, among male dogs, 17.30% had dirofilariosis, while 21.70% of females had microfilariae (Fig. 5). Concerning the age, the present investigation explored that dogs aged 3-5 years had the highest infection rate (51.70%), while those aged less than one year were the lowest (3.50%) (Fig. 6).

Regarding hematobiochemical parameters, it has been found that the value of PCV was almost normal (35-54%) in 79.30% of infected dogs, while it was less than 35% in 13.80% of infected dogs and more than 54% in 6.90% of infected dogs (Fig. 7). It is worthy to mention that, among biochemical assessments, it has been detected that SGPT and SGOT had only significant values (at $P<0.05$) (Table 1).

Discussion

The veterinary care in the Nepal-Indian border is considerably inadequate so, husbandry and domestic animals and stray dogs as well as the wildlife might be a potential source of parasitic infections. Large populations of stray dogs are present in the urban districts of Nepal/India. The present study reported the first occurrence of dirofilariosis at a prevalence of 19.33% in Nepal. In the closely related areas, in the eastern boundary, Borthakur et al. (2015) have reported that the prevalence rate of 11.38% in Assam and Mizoram. Similarly, Ranjbar-Bahadori et al. (2010) reported that the prevalence of D. immitis was 12.29% in Garmsar area, the central part of Iran.

The mosquito is the main vector for Dirofilaria spp. The larval development to the infective third stage in mosquito vectors, like Anopheles, Culex and Aedes, depends on several factors. The ability of an invertebrate host to survive, invasion of microfilariae and the consequent development is essential for evaluating the vector competence (Kartman 1953; Taylor 1960; Montarsi et al. 2015). The overall hygiene, the improper disposal of garbage, and the inadequate animal welfare, all contribute the dispersal of mosquito vectors, the intermediate host, as well as stray dogs, the definitive host. In Nepal, huge populations of stray dogs are present, and there are no adequate preventive measures.

Currently, the occurrence of dirofilarioids in the blood of stray dogs was evident by the use of blood examinations tests. It is known that traditional methods for detecting filarial parasites is by observing and identifying their larvae (microfilariae) in the blood/skin specimens, usually after application of various staining techniques (McCall et al., 2008; Siwila et al. 2015). Unfortunately, morphological characters including the length and width of each filarioid species greatly vary among commonly released literature as well as they are closely associated to the fixation techniques used. Such variation

might lead to misidentification of the recovered microfilariae, particularly when several Dirofilaria spp. or Acanthocheilonema spp. are detected in the surveyed district (Magnis et al. 2013; Little et al. 2018). Both direct smear and the modified Knott's methods are used for identification of microfilariae, particularly for the heartworm, Dirofilaria immitis, but the later is more reliable as it is more sensitive test depending on the concentration of microfilariae, while the direct smear is a quick test but non-concentrative. Therefore, the priority of choice is for the modified Knott's method (Watanabe et al. 2004)

The duration of the life cycle of Dirofilaria species in stray dogs is approximately 3–7 months, with the diagnosis was often late and the primary symptoms pass unnoticed, therefore, veterinary health problems evoke when clear clinical symptoms appear. In the present work, a significant relationship between dirofilariosis and the respiratory rate [$\chi 2$ (1, N = 150, 7.73, P = o.o21)] of infected dogs was given. This might be attributed to that microfilariae pass within the peripheral circulation and gain access to lungs producing the heartworm associated respiratory disease (HARD). The adult Dirofilaria spp. can survive up to 6 years inside the infected dog, and this might explain the significant higher infection rate [$\chi 2$ (1, N = 150, 12.44, P = 0.006)] in old-aged dogs and the lower prevalence in dogs aged less than one year. It is worthy to mention that the sex of dogs had no significant effect [$\chi 2$ (1, N = 150, o.474, P = 0.491)] relative to dirofilariosis. Clinically, the alteration in the body temperature was non-significant [$\chi 2$ (1, N = 150, 0.985, P = 0.611)].

Hematologically, values of the PCV was non-significant [$\chi 2$ (1, N = 150, 2.571, P = 0.277)]. Biochemically, it has been found that SGPT and SGOT were significantly elevated with unclear clinical symptoms indicating that the infected dogs were subclinically infected. Such enzymatic alteration might be suggested to be due to the predominant intracellular activity which occurs as a result of the damaged tissue cells. Similar findings were obtained by Niwetpathomwat et al. (2007) in Thailand. With the progress of the disease, more cellular damage takes place and the typical clinical picture of the disease evokes. To the best of our knowledge, this is the first report denoting the occurrence of dirofilariosis in stray dogs in Siddharthanagar sub-metropolitan (Indo-Nepal Border), Lumbini, Nepal. Due to being a mosquitoes-transmitted disease, strict hygienic measures regarding eradication of stray dogs, with the proper disposal, as well as adequate control of mosquitoes are urgently demanded in terms of zoonosis and hygiene.

Declarations

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Ethical approval

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

Conflict of interest

Authors declare that there was no conflict of interest.

Consent of publication

No need the consent of publication

References

1. Albanese F, Abramo F, Braglia C, Caporali C, Venco L, Vercelli A, Ghibaudo G, Leone F, Carrani F, Giannelli A, Otranto D (2013) Nodular lesions due to infestation by *Dirofilaria repens* in dogs from Italy. Vet Dermatol 24:255–256.
2. Bocková E; Iglódyová A, Kočišová A (2015) Potential mosquito (Diptera:Culicidae) vector of *Dirofilaria repens* and *Dirofilaria immitis* in urban areas of Eastern Slovakia. Parasitol Res 114: 4487–4492
3. Borthakur SK, Deka DK, Islam S, Sarma DK, Sarmah PC (2015) Prevalence and molecular epidemiological data on *Dirofilaria immitis* in dogs from Northeastern States of India. The Scientific World Journal, 2015, 265385. doi:10.1155/2015/265385
4. Cancrini G, Kramer L (2001) Insect vectors of *Dirofilaria* spp. In: Heartworm Infection in Humans and Animals, eds Simón F, Genchi C, editors. (Salamanca: Ediciones Universidad de Salamanca;), 63–82
5. Cringoli G, Rinaldi L, Veneziano V, Capelli G (2001) A prevalence survey and risk analysis of filariosis in dogs from the Mt. Vesuvius area of southern Italy. Veterinary Parasitology 102: 243–252
6. Darsie RF, JR, Pradhan SP, Vaidya RG (1991) Notes on the mosquitoes of Nepal. I. New country records and revised *Aedes* keys (Diptera, Culicidae). Mosquito Systematics 23: 39-45
7. Genchi C, Bowman D, Drake J (2014) Canine heartworm disease (*Dirofilaria immitis*) in Western Europe: survey of veterinary awareness and perceptions. Parasites & Vectors 7, 206 http://www.parasitesandvectors.com/content/7/1/206
8. Genchi C, Kramer LH, Rivasi (2011a) Dirofilarial infections in Europe. Vector Borne Zoonotic Dis. 11, 1307–1317
9. Genchi C, Mortarino M, Rinaldi L, Cringoli G, Traldi G, Genchi M (2011b) Changing climate and changing vector-borne disease distribution: the example of Dirofilaria in Europe. Vet Parasitol 176: 295– 299.

10. Genchi M, Pengo G, Genchi C (2010) Efficacy of moxidectin microsphere sustained release formulation for the prevention of subcutaneous filarial (*Dirofilaria repens*) infection in dogs. Vet. Parasitol. 170, 167–169
11. Genchi C, Rinaldi L, Mortarino M, Genchi M, Cringoli G (2009) Climate and Dirofilaria infection in Europe. Vet Parasitol 163: 286–292.
12. Ionică AM; Matei IA; Mircean V; Dumitrache MO; D'Amico G; Győrke A; Pantchev N; Annoscia G; Albrechtová K; Otranto D; Modrý D; Mihalca AD (2015) Current surveys on the prevalence and distribution of *Dirofilaria* spp. and *Acanthocheilonema reconditum* infections in dogs in Romania. Parasitol Res 114: 975–982
13. Kartman L (1953) Factors influencing infection of the mosquito with *Dirofilaria immitis* (Leidy, 1856). Exp Parasitol. 2:27–78. doi: 10.1016/0014-4894(53)90005-8
14. Lee AC, Montgomery SP, Theis JH, Blagburn BL, Eberhard ML (2010) Public health issues concerning the widespread distribution of canine heartworm disease. Trends Parasitol 26 (4):168–173
15. Little S, Saleh M, Wohltjen M, Nagamori Y (2018). Prime detection of *Dirofilaria immitis*: understanding the influence of blocked antigen on heartworm test performance. Parasites & vectors, 11(1): 186. doi:10.1186/s13071-018-2736-5
16. Lock JB (1988) *Dirofilaria* sp.: taxonomy and distribution. In: Boreham PFL, Atwell RB (Eds.), Dirofilariosis. CRC Press, Boca Raton, FL, pp. 1–28.
17. Magnis J, Lorentz S, Guardone L, Grimm F, Magi M, Naucke TJ, Deplazes P (2013) 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, *6*, 48. doi:10.1186/1756-3305-6-48
18. Martin TE, Collins GH (1985) Prevalence of *Dirofilaria immitis* and *Dipetalonema reconditum* in greyhounds. Aust. Vet. J. 62 (5): 159–163.
19. McCall JW, Genchi C, Kramer LH, Guerrero J, Venco L (2008) Heartworm disease in animals and humans. Adv. Parasitol. 66, 193–285
20. Montarsi F, Ciocchetta S, Devine G, Ravagnan S, Mutinelli F, Frangipane di Regalbono, A, Capelli G (2015). Development of *Dirofilaria immitis* within the mosquito *Aedes* (*Finlaya*) *koreicus*, a new invasive species for Europe. Parasites & vectors, 8, 177. doi:10.1186/s13071-015-0800-y
21. Morchón R, Carretón E, González-Miguel J, Mellado-Hernández I (2012) Heartworm Disease (*Dirofilaria immitis*) and their vectors in Europe – New distribution trends. Front Physiol 3: 196. doi: 10.3389/fphys.2012.00196
22. Niwetpathomwat A, Kaewthamasorn M, Tiawsirisup S, Techangamsuwan S, Suvarnvibhaja S. (2007) 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. 82(3): 364-369.
23. Pampiglione S, Canestri Trotti G, Rivasi F (1995) Human dirofilariosis due two *Dirofilaria* (*Nochtiella*) *repens*: a review of world literature. Parassitologia 37: 149–193.

24. Pampiglione S, Rivasi F (2000) Human dirofilariosis due to *Dirofilaria* (*Nochtiella*) *repens*: an update of world literature from 1995 to 2000. Parassitologia 42, 235–254
25. Pantchev N, Etzold M, Daugschies A, Dyachenko (2011) Diagnosis of imported canine filarial infections in Germany 2008-2010. Parasitol Res 109: S61–S76.
26. Pradhan D, Pradhan C, Joshi P (2018) An Encounter with a Worm in Anterior Chamber: A Case Report. Nepal J Ophthalmol 10 (19): 98-101
27. Ranjbar-Bahadori S, Mohri M, Helan JA, Jamshidi K, Kashefinejad M (2010) Clinico-pathologic evaluation of the canine heartworm infestation. Research Journal of Parasitology 5 (2): 90-98.
28. Simón F, Siles-Lucas M, Morchón R, González-Miguel J, Mellado I, Carretón E, Montoya-Alonso JA (2012) Human and animal dirofilariosis: the emergence of a zoonotic mosaic. Clin. Microbiol. Rev. 25, 507–544
29. Singh DK, Adhikari A, Rimal S, Singh K (2018) Chylothorax due to heartworm (dirofilariasis) in a Dog. The Blue Cross 15: 83-86
30. Siwila J, Mwase ET, Nejsum P, Simonsen PE (2015) Filarial infections in domestic dogs in Lusaka, Zambia. Veterinary Parasitology 210 (3–4): 250-254
31. Song KH, Lee SE, Hayasaki M, Shiramizu K, Kim DH, Cho KW (2003) Seroprevalence of canine dirofilariosis in South Korea. Vet Parasitol. 114(3): 231-236.
32. Tasic-Otasevic SA, Bozinovic MST, Gabrielli SV, Genchi C (2015) Canine and human *Dirofilaria* infections in the Balkan Peninsula. Veterinary Parasitology 209: 151–156
33. Taylor AER (1960) The development of *Dirofilaria immitis* in the mosquito *Aedes aegypti*. J Helminthol. 34: 27–39. doi: 10.1017/S0022149X00020307
34. Traversa D, Di Cesare A, Conboy G (2010). Canine and feline cardiopulmonary parasitic nematodes in Europe: emerging and underestimated. Parasites & vectors, 3, 62. doi:10.1186/1756-3305-3-62
35. Vezzani D, Carbajo AE (2006) Spatial and temporal transmission risk of *Dirofilaria immitis* in Argentina. Int J Parasitol 36: 463–1472
36. Vezzani D, Mesplet M, Eiras DF, Fontanarrosa MF, Schnittger L (2011) PCR detection of *Dirofilaria immitis* in *Aedes aegypti* and *Culex pipiens* from urban temperate Argentina. Parasitol Res 108: 985–989
37. Wang S, Zhang N, Zhang Z, Wang D, Yao Z, Zhang H, Ma J, Zheng B, Ren H, Liu S (2016) Prevalence of *Dirofilaria immitis* infection in dogs in Henan province, central China. Parasite 23:43.
38. Watanabe Y, Yang C-H, Tung K-C, Ooi H-K (2004) Comparison of microfilaria concentration method for *Setaria digitata* infection in cattle and for *Dirofilaria immitis* infection in dogs. J Vet Med Sci 66 (5): 543-545

Table

Table 1

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

Figures

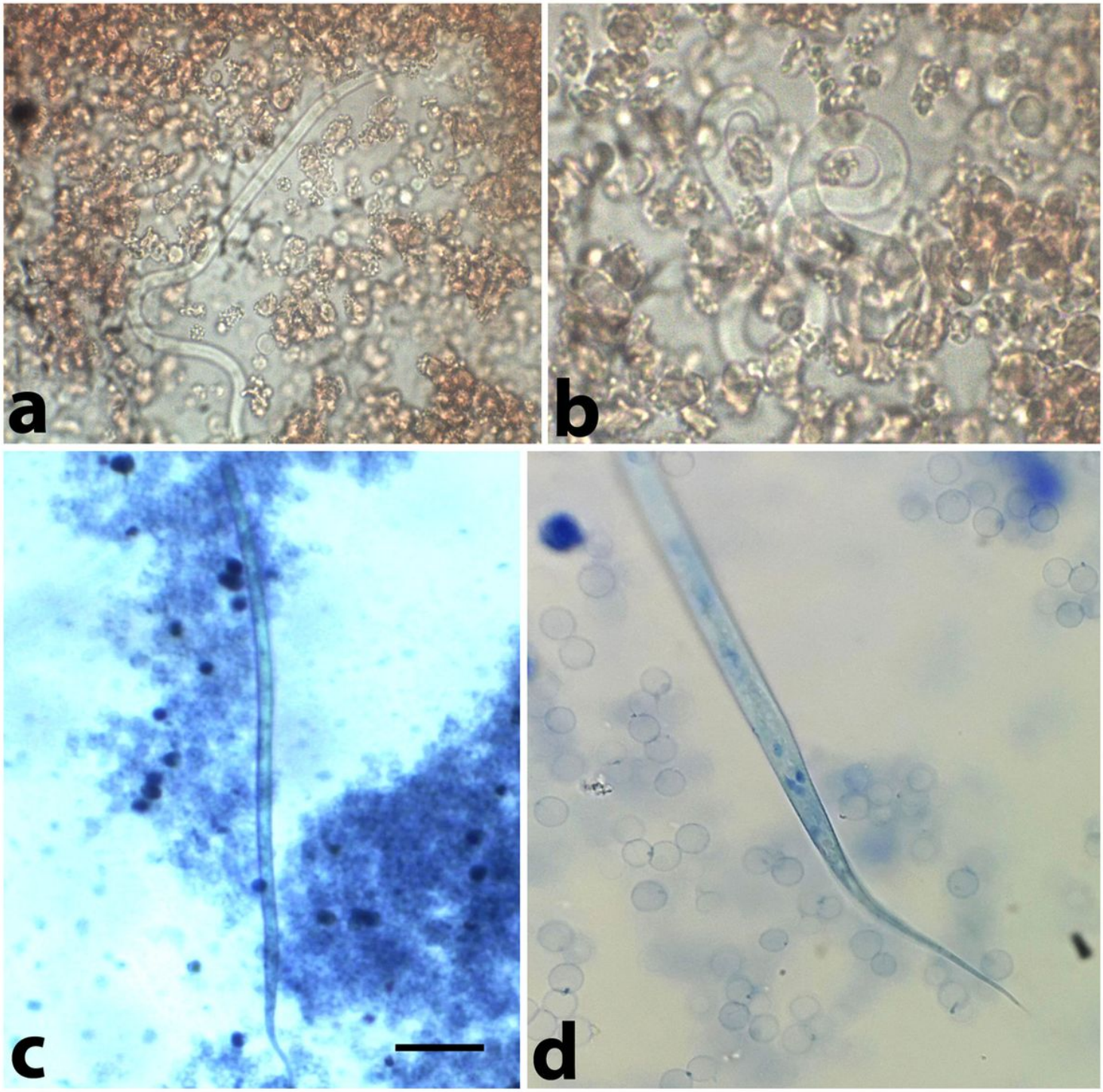

Figure 1

Microfilariae of the recovered Dirofilaria spp. a. The whole microfilaria in direct blood smear 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.

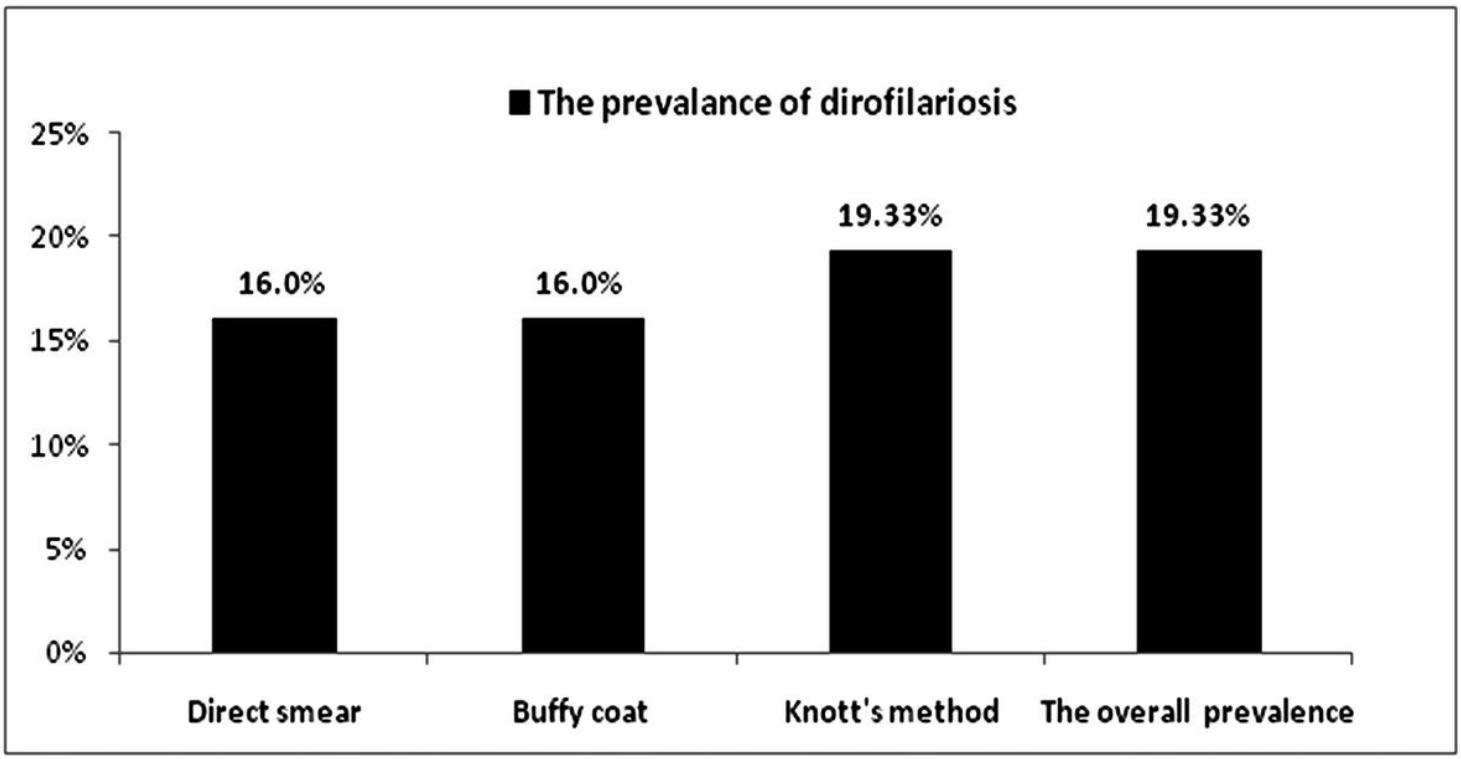

Figure 2

The prevalence of Dirofilaria spp. infection in stray dogs of Siddharth Nagar Sub-metropolitan city, Nepal using various traditional techniques.

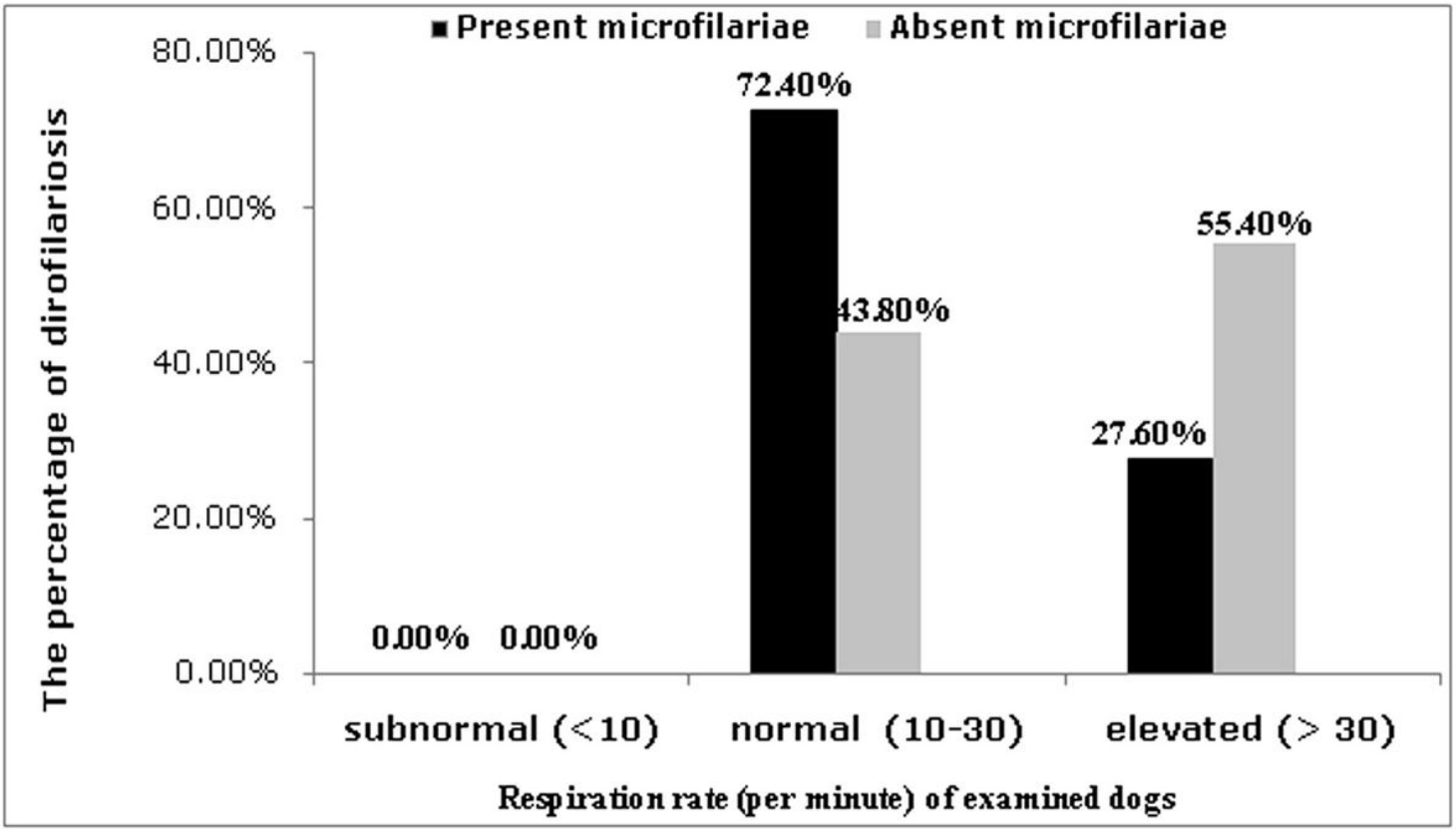

Figure 3

The relationship between dirofilariosis and the respiratory rate of stray dogs.

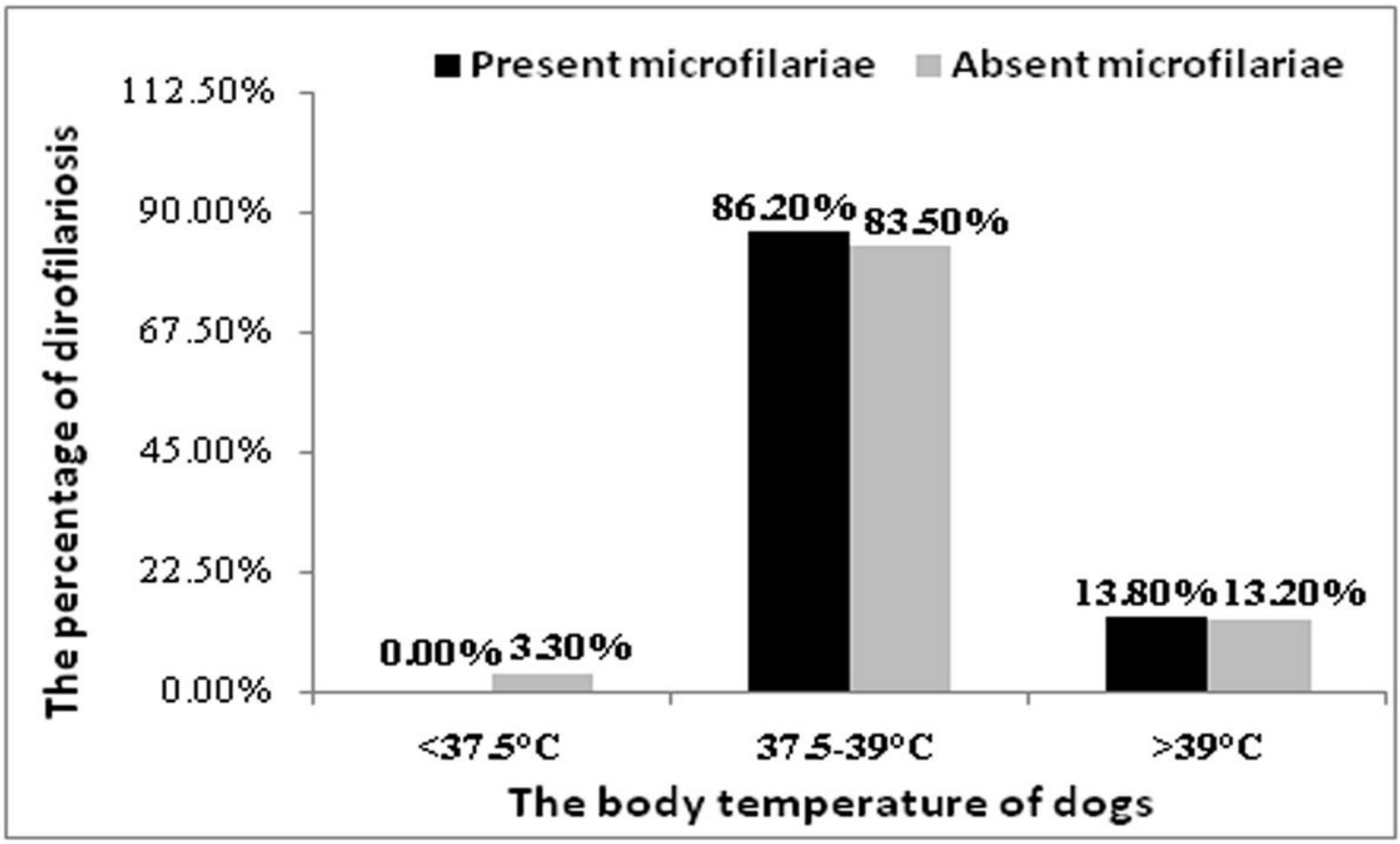

Figure 4

The relationship between dirofilariosis and the body temperature of stray dogs.

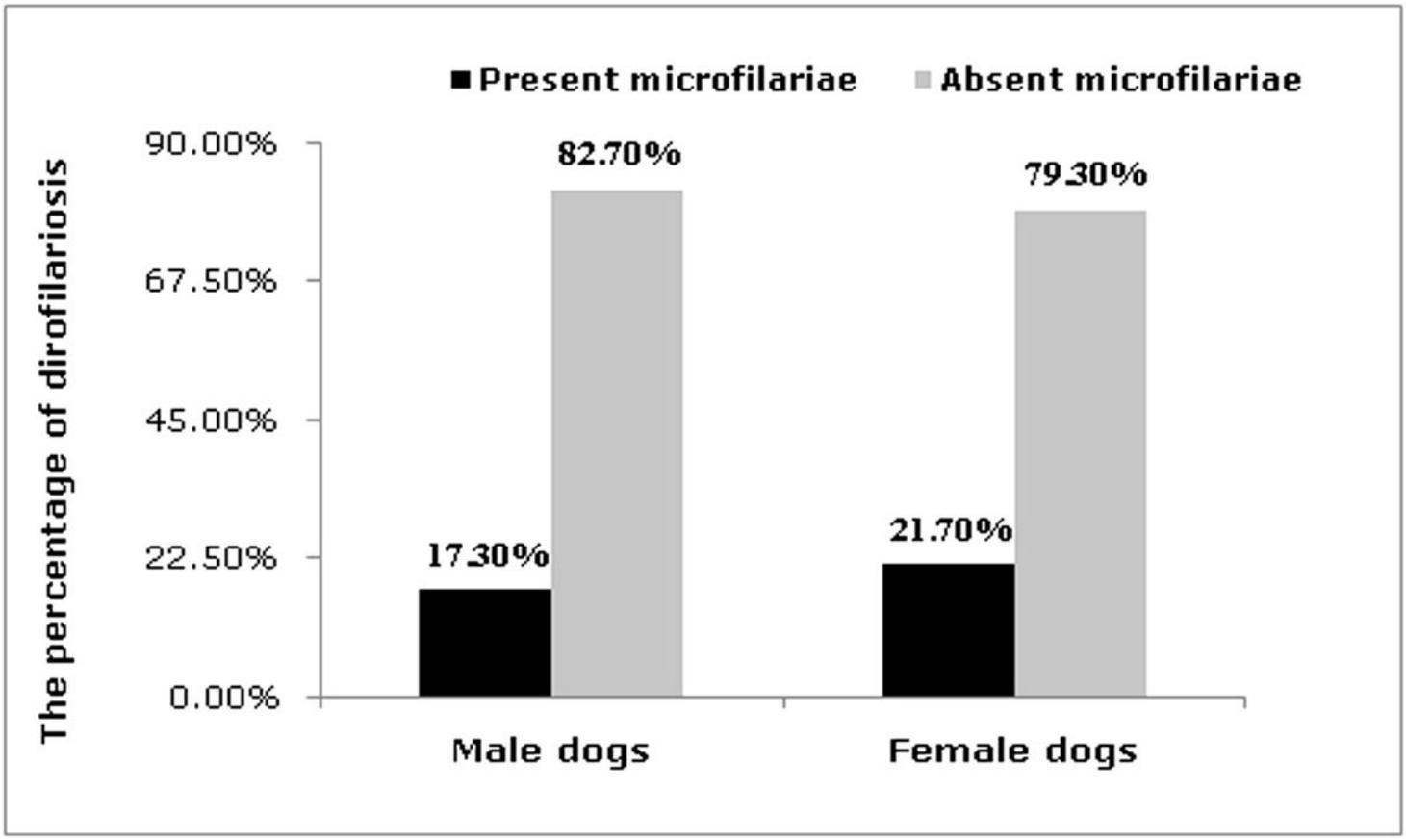

Figure 5

Dirofilariosis in stray dogs relative to the sex.

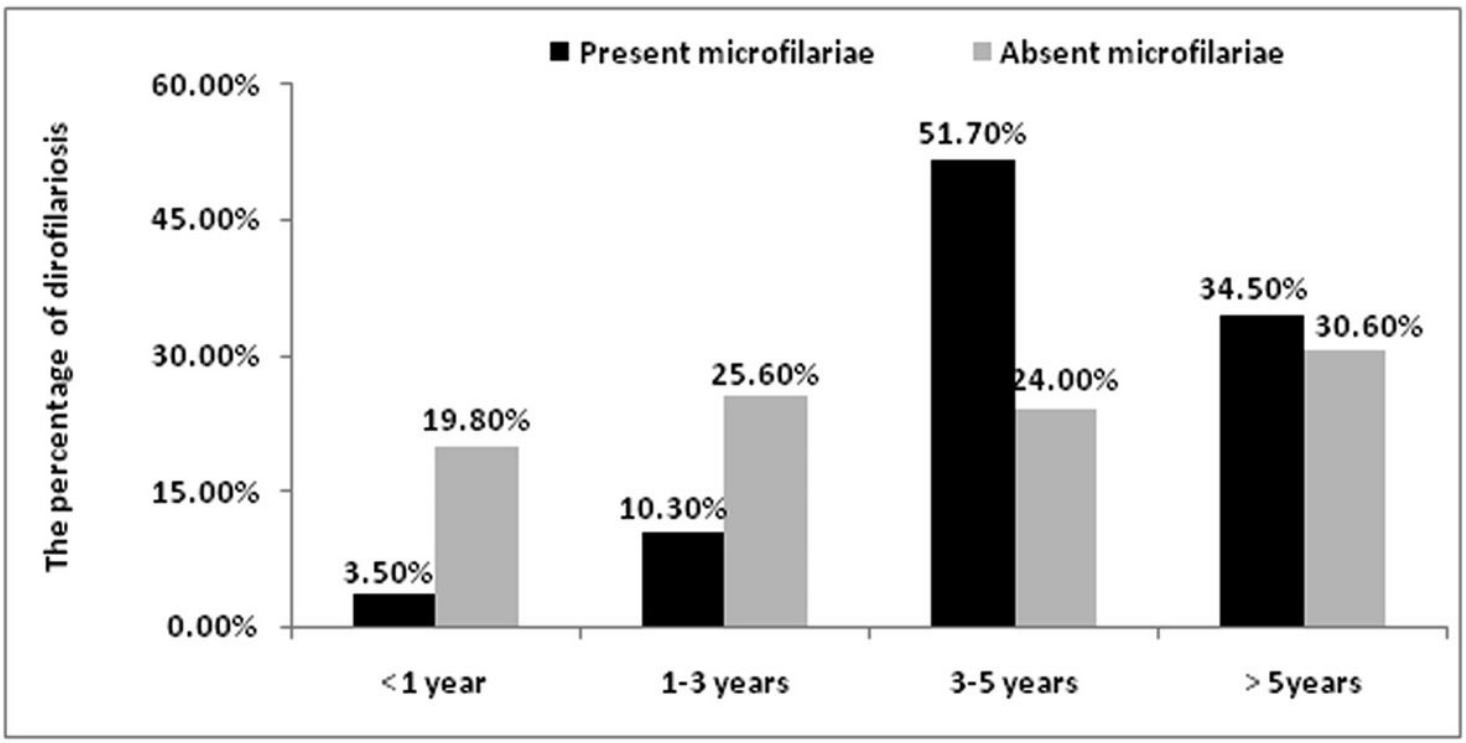

Figure 6

The relationship between dirofilariosis and the age of stray dogs.

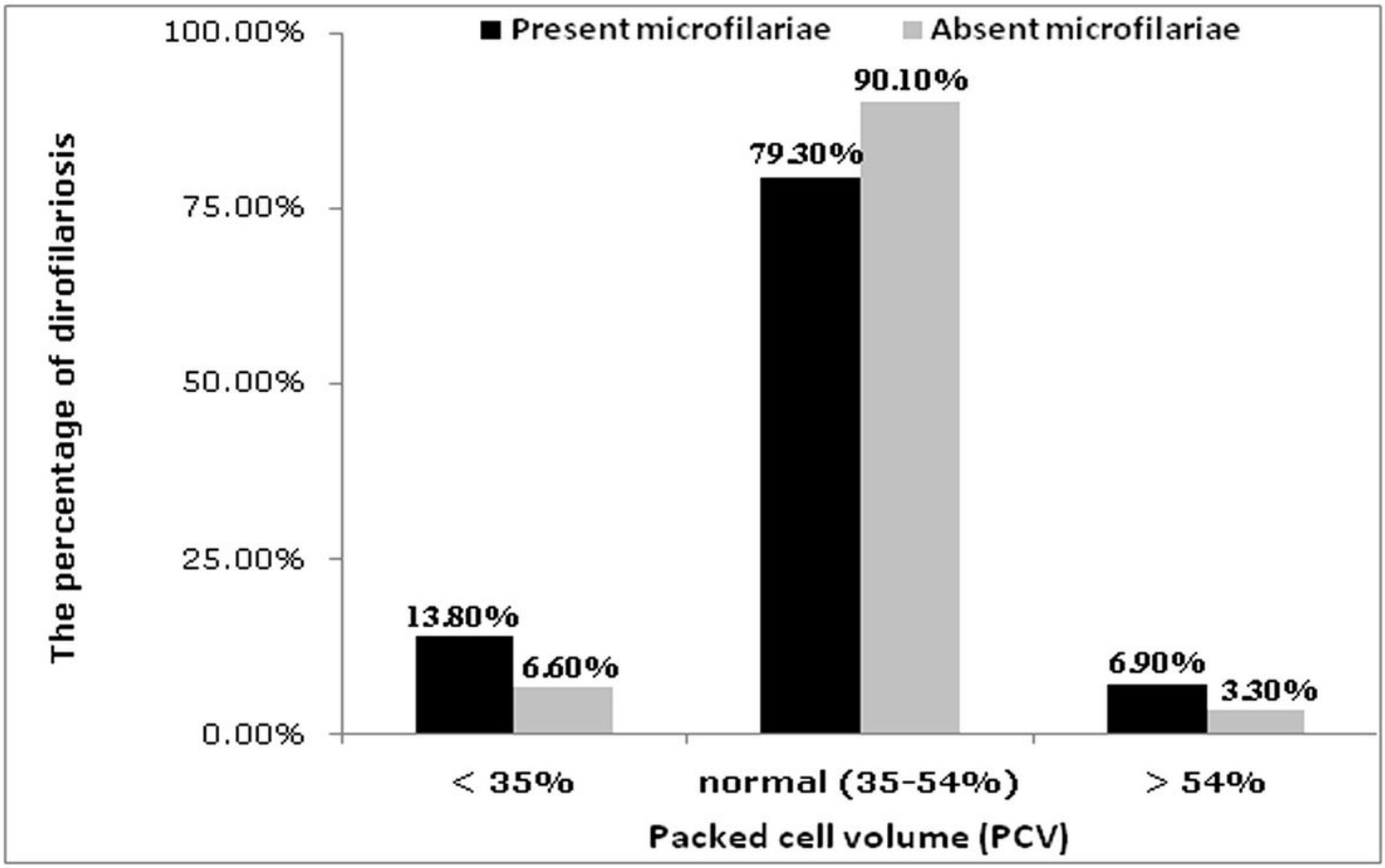

Figure 7

The relationship between dirofilariosis and packed cell volume (PCV) of stray dogs.