

RESEARCH ARTICLE

TICKS AND PREVALENCE OF TICK-BORNE PATHOGENS IN DOGS OF KATHMANDU VALLEY

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ABSTRACT

The study was conducted from November, 2016 to February, 2017 to assess ticks and tick-borne pathogens in dogs in Kathmandu valley. The total of 411 ticks specimen was collected from different body parts of dog and blood samples were collected from same tick infested 100 dogs (54 owner and 46 street dogs). Parasitological procedures were followed for collection and identification of ticks. All tick samples were kept in tubes with 70% alcohol and 5% glycerine so that they could be identified under a stereomicroscope. Dog blood tests were used to check for hemoparasites using the blood smear method. The findings demonstrated that all of the tick samples that were gathered belonged to the same species, *Rhipicephalus sanguineus*. It is the most prevalent canine ectoparasite in the globe. The result indicated that the favourable predilection sites of *Rh. sanguineus* ticks were more in the region of the back (38%) followed by the inter-digital space (18%), neck (17%), shoulder (14%), head (8%), ears (4%) and less frequent on thigh (1%). The overall prevalence of haemoparasites was 5% with: *Anaplasma spp.* (3%), *Babesia spp.* (1%) and *Anaplasma spp.* and *Ehrlichia canis* (1%). There was significant variation ($p < 0.05$) in the prevalence of haemoparasites and degree of tick infestation. There was no significant difference ($p > 0.05$) of haemoparasites in relation to sex (Male 8.33% and Female 1.92%), age groups (<2years 6.25%, 2-4 years 5.36% and >4 years 3.57%) and ownership (Owner 7.41% and street dogs 2.17%).

KEYWORDS

Ectoparasites, haemoparasites, predilection, stereomicroscope, variation

1. INTRODUCTION

Dog (*Canis lupus familiaris*) being one of the domestic subspecies of the wolf, is a mammal belonging to the Canidae family of the order Carnivora. In addition to being "man's best friend," dogs can be trained to be a variety of useful assistance to humans. Blind individuals can use guide dogs to assist them with daily tasks. Dogs can be trained to do certain police tasks or to serve as "ears" for the deaf. They make the best buddies in the family, of course. Dogs are prone to a wide range of illnesses, conditions, and toxins; some of these impact humans similarly, while others are specific to dogs. Like all animals, dogs can experience heat exhaustion in situations with high humidity and/or excessive temperatures. Dogs are frequently affected by infectious diseases such as canine parvovirus, canine distemper, and rabies (hydrophobia). Dogs can have a wide range of congenital conditions, such as pulmonic stenosis, hip dysplasia, and medial patellar luxation. With the exception of few illnesses that are species-specific, dogs can contract almost any illness that affects humans, including cancer, heart disease, diabetes, hypothyroidism, and dental problems.

Numerous internal and external parasites affect dogs. Dog ectoparasites include lice, ticks, fleas, mites, and so forth. (Colebrook and Wall, 2004). There are reports of high mortality rates from parasites, tick-borne illnesses, and disorders connected to ticks. (Muchenje et al., 2008). Ticks transmit a number of disease agents such as *Anaplasma spp.*, *Babesia spp.*, *Ehrlichia spp.*, *Hepatozoon spp.*, *Borrelia spp.*, etc. all of which cause morbidity and mortality of dogs. Canine internal parasites range in size from small intestinal protozoa, such as *Giardia*, to grotesquely lengthy filarial worms and trypanosomes, which are inter-erythrocytic protozoa.

Numerous people gain a great deal from the intimate bond between dogs and humans in the form of companion animals. However, there's also a chance that this could pose a harm to the public's health because parasite illnesses can naturally spread from dogs to people through intermediary hosts and environmental variables.

Dogs' tick-borne infections are varied and can cause a great deal of morbidity, such as leukopenia, anemia with subsequent hypoxia and organ damage, and thrombocytopenia and hemostasis problems. (Harrus and Waner, 2011; O'Dwyer, 2011; Solano-Gallego and Baneth, 2011). Numerous canine diseases, such as *Ehrlichia canis*, *Babesia canis*, *Hepatozoon cani* (by ingestion of infected ticks), *Babesia platys* or *phagocytophilum*, and *Rickettsia species*, have been linked to ticks as vectors. Though infections with *Borrelia burgdorferi* and *Rickettsia conorii* frequently result in subclinical infections, it is more challenging to assess how these infections relate to a clinical illness in dogs. Dogs also seem to be susceptible to tick-borne viral encephalitis (TBE) and *Coxiella burnetii* (Q-fever), although there are currently not enough comprehensive studies on the clinical effects of these infections.

Attaching firmly to their hosts, ticks facilitate effective blood feeding, the transmission of pathogens, as well as the spread of both ticks and microorganisms to different geographical habitats via migrating animals or traveling pets. As hematophagous arthropods, ticks are well designed to transmit disease agents such as viruses, bacteria, rickettsiae, protozoa, fungi, and nematodes (Kenny et al., 2004). Additionally, it has been documented that they spread pathogens that cause Lyme diseases, ehrlichiosis, babesiosis, tularemia, Rocky Mountain spotted fever, and tick-borne relapsing fever, among other human illnesses. In order to

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execute effective control tactics and programs in the research region, animal health workers and policy makers will benefit from an assessment of tick composition and the identification of more common tick-borne illnesses.

2. MATERIALS AND METHODS

The investigation was carried out in the Kathmandu Valley between November 2016 and February 2017, with all laboratory work done at the Central Veterinary Laboratory's (CVL) entomology lab. The cross-sectional study with purposive sampling was designed for this study. Pet dogs sample were collected from dogs brought to Central Veterinary Hospital (CVH), various animal clinics, from kennel club's and dog breeding farms. Stray dogs sample were collected from various stray dog's organization like Animal Nepal, Kathmandu Animal Treatment (KAT) centre and Sneha's Care and some street dogs brought to CVH. Sample size was estimated according to Daniel formula (Daniel, 1999). Assuming the expected prevalence to be 50%, by applying the given formula, 384 sample size was determined. But in this study total of 100 blood samples was collected from dogs and 411 tick's specimens. Ticks were collected from different body parts of dog using tick remover.

Ticks were stored in tubes containing preservative (70% alcohol and 5% glycerine) according to the obtained body parts and labelled. About 3ml of blood was collected aseptically from the cephalic vein and kept into the EDTA vials. Identification of ticks was done based on morphological features by using Stereomicroscope. Standard keys provided by some researchers was used for identification (Walker et al., 2003). Examination of blood parasite in blood samples was done by using Geimsa Stained Thin Smear Technique Blood was extracted from the EDTA tube using a micropipette, and a little drop of blood was then transferred from the capillary tube onto the microscope slide (WHO, 1991).

The blood sample was then thinly placed onto another slide to create a bullet form with a feathered edge. Blood with a higher viscosity required a larger angle on the slide to disperse the sample, and vice versa. This was

dependent on the blood's viscosity. This makes it possible for the blood smears to be uniform in length and size. After air drying, blood smears were immersed in absolute alcohol for ten minutes in order to fix the slide and organisms. After being air dried once again, they were dyed with a Gimesa stain, which made it possible to see the bacteria and parasites in the smear. The blood smears were viewed with X100 (oil emersion) lenses under a microscope.

Data entry was done, arranged and analysed using MS-Excel. Association of risk factors with prevalence was tested for its significance using chi-square test using OpenEpi: (Open Source Epidemiologic Statistics for Public Health) version 2.3. $P < 0.05$ was taken as significant. Fisher exact value was calculated from Vassarstats.net

3. RESULT

3.1 Tick Identified

Under a stereo microscope, 411 tick samples from 100 dogs were analyzed, and the results revealed the presence of just one type of tick: *Rhipicephalus sanguineus*, also known as the brown dog tick. This tick had an elongated body, was brownish-red in color, and had no decorations. The adult ticks were around 1 centimeter in size when completely engorged, and their anal groove was located behind the anus. *Rh. sanguineus* possesses basic capituli that are hexagonal in shape and have acute lateral angles. The eyes are distinct and slightly convex, and the palp pedicels are short. The back of the body contains festoons, which are spiracles with sparse setae and a comma-like form. When a female tick lays eggs, a large porose region separates it and secretes chemicals. These materials act as a waterproof barrier to preserve the eggs. Adanal plates are narrow and trapezoid in males, though they can also appear large and curved. In fed males, the caudal appendage is wide and protrudes as a small protrusion. Adal plates for accessories are sizable. The shield and additional adanal plates are absent from the female tick. Female vaginal aperture posterior lips are shaped like a broad U, though they can also resemble a broad V.



Figure 1: Dorsal surface of male *Rh. sanguineus* tick



Figure 2: Ventral surface of male *Rh. sanguineus* tick



Figure 3: Dorsal surface of female *Rh. sanguineus* tick



Figure 4: Ventral surface of female *Rh. sanguineus* tick

3.2 Body Parts Wise Distribution of Tick

The ticks were removed from seven different areas/parts of the dog's body: the ears, the head, the neck, the back, the shoulder, the thigh, and the interdigital space. The findings showed that the back region (38%) had the highest number of favorable predilection locations for *Rh. sanguineus* ticks, followed by the interdigital space (18%), neck (17%), shoulder (14%), head (8%), ears (4%) and the thigh (1%). The dog's rear area exhibits an extremely high level of infestation.

3.3 Overall Prevalence of Tick-Borne Blood Pathogens/Haemoparasites

Out of 100 blood samples examined by preparation of blood smear, only 5 samples were found positive for blood parasites and 95 were negative with overall prevalence being 5%. Upon physical examination, infected dogs displayed elevated body temperature, mild anemia, emaciation, dullness, and decreased appetite.

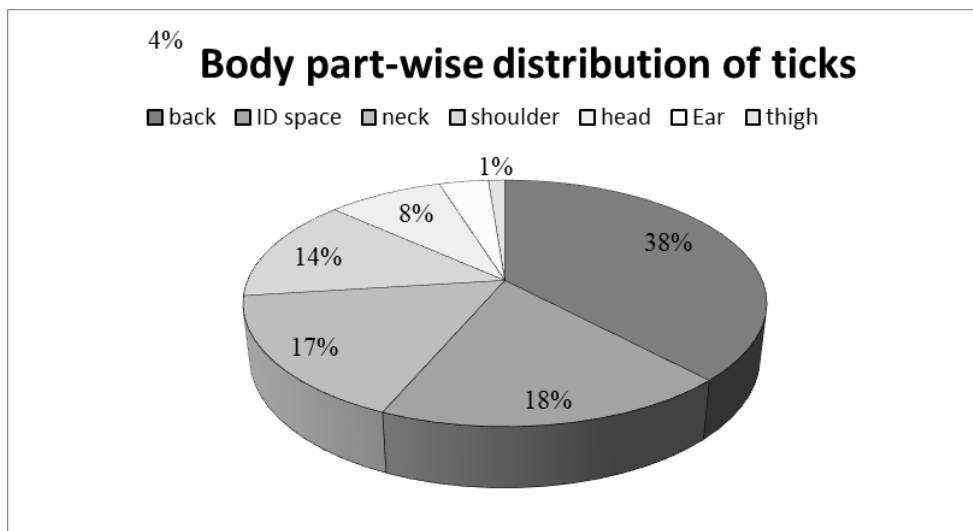


Figure 5: Pie chart showing body part-wise distribution of *Rh. sanguineus* tick

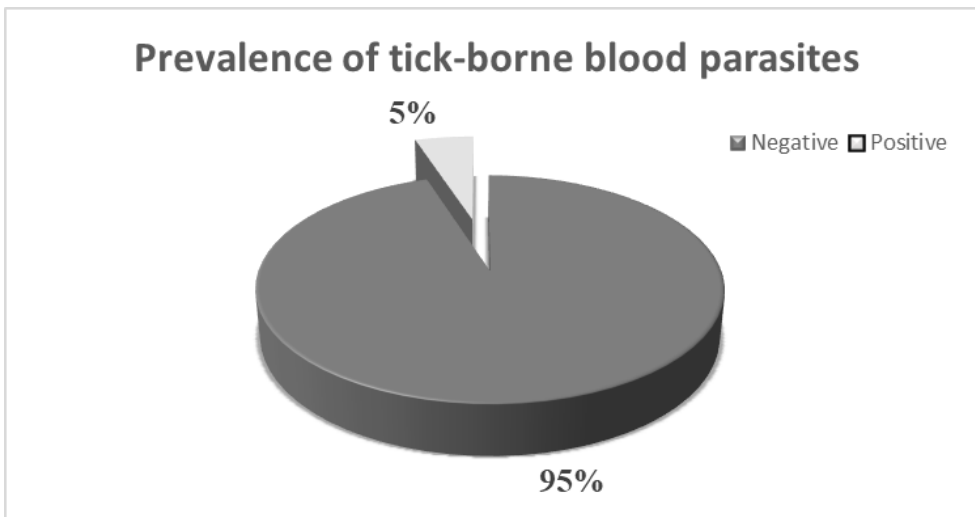


Figure 6: Pie chart showing overall prevalence of tick-borne pathogen

3.4 Species Wise Prevalence of Blood Parasites/Pathogens

Out of 5 positive samples 3(3%) are positive for *Anaplasma spp.*, 1(1%) for *Babesia spp.* and one (1%) had mixed infection of *Anaplasma* and *Ehrlichia canis*.

3.5 Sex Wise Prevalence of Tick Borne Pathogen

Of the 100 blood samples collected, 48 were from dogs that were male and 52 from dogs that were female. There were 4 positive and 44 negative samples out of 48 male samples, and 1 positive and 51 negative samples out of 52 female tests. In men, the prevalence was found to be 8.33%, whereas in women, it was 1.92%. There were no statistically significant

variations in the sex-wise prevalence of blood-pathogens between male and female dogs (Fisher exact= 0.3143).

3.6 Ownership Wise Prevalence of Tick-Borne Blood Parasites

Of 100 blood samples, 54 samples were from owners or pet dogs and 46 samples were from street dogs. Among owner dogs, parasite was detected in 4 samples and among street dog's parasite was detected in 1 sample with the respective prevalence percentage of 7.41 and 2.17. Based on statistical analysis, there was no significant correlation (Fisher exact value = 0.4705, P > 0.05) found between dog ownership and blood parasite infection.

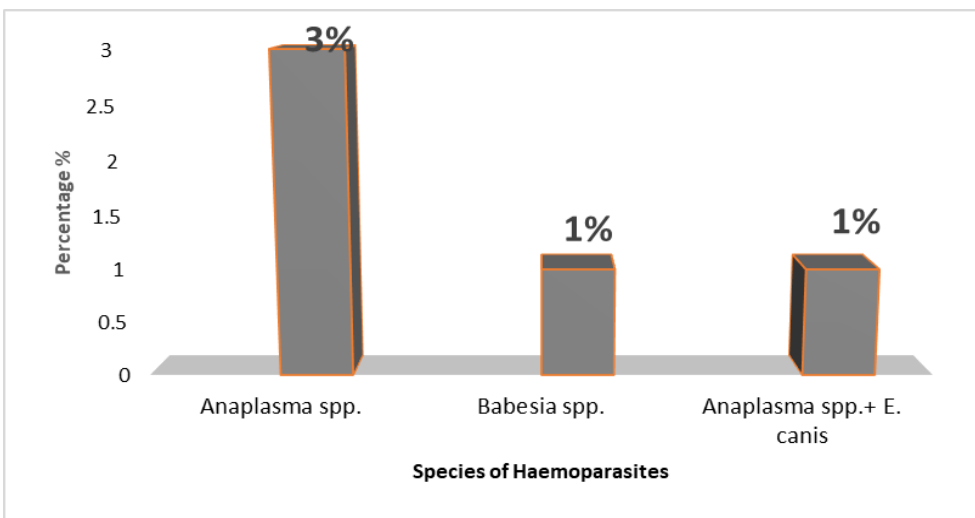


Figure 7: Haemoparasite species wise prevalence of blood pathogen

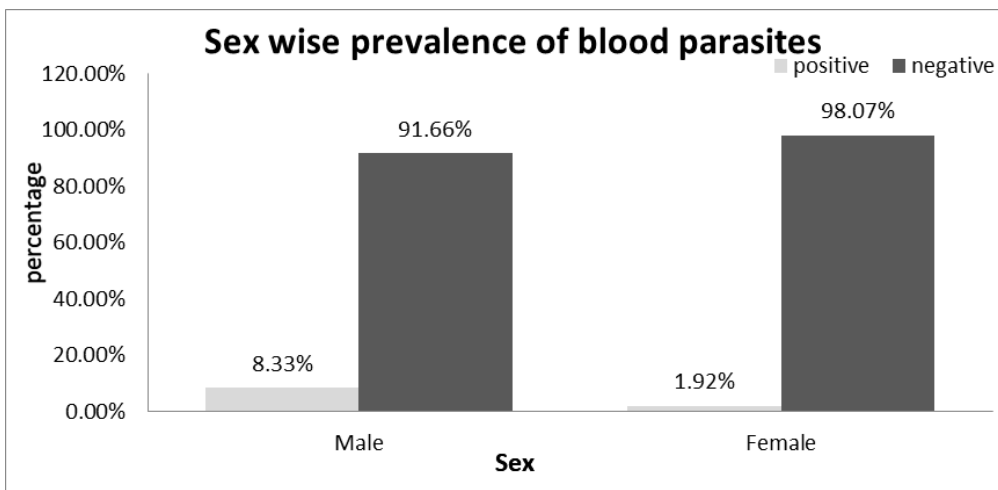


Figure 8: Sex wise prevalence of blood pathogens

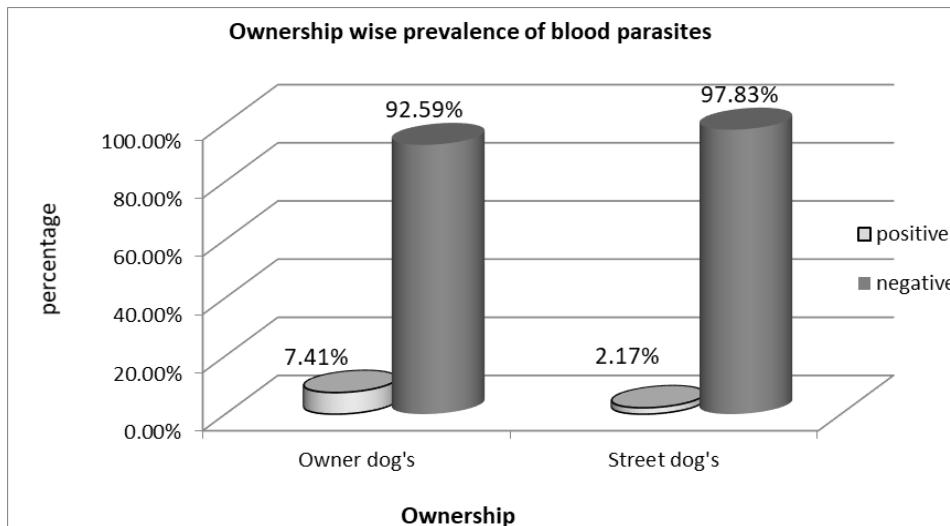


Figure 9: Ownership wise prevalence of blood parasites

3.7 Degree of Tick Infestation Wise Prevalence of Blood Parasites

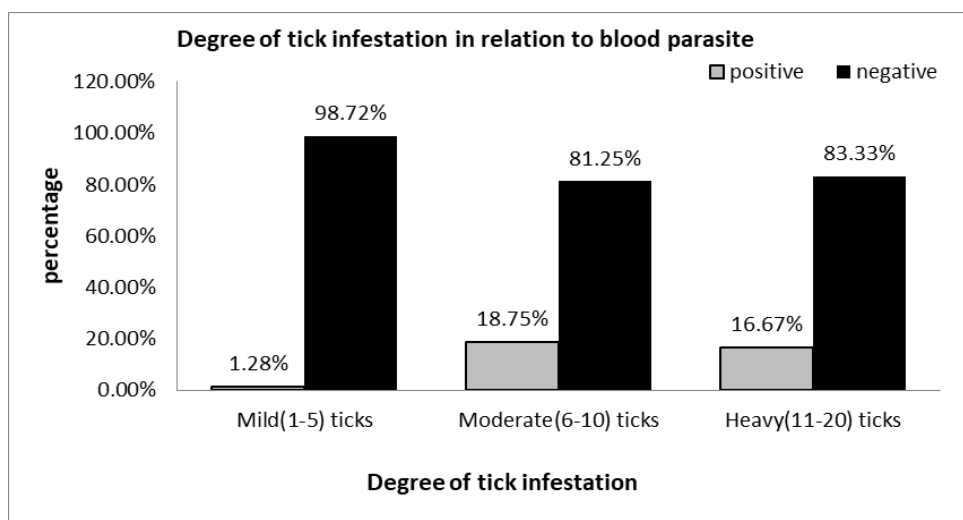


Figure 10: Degree of tick infestation in relation to blood parasite

All the available ticks in the body of dog were collected, counted and the category of degree of tick infestation was recognized. According to a study, there are three levels of tick infestations: light (1-5 ticks), moderate (6-10 ticks), and heavy (11-20 ticks) (Hadi et al., 2016). Out of 100 dogs, 78 dogs were mildly infested, 16 dogs were moderately infested and 6 dogs

were heavily infested. The prevalence of tick borne parasite in mild, moderate and heavily infested dogs was 1.28% (1), 18.75% (3) and 16.67% (1) respectively. Presence of blood parasite showed statistically significantly different with the degree of tick infestation (Fisher Exact value=0.0087)

3.8 Age Wise Prevalence of Tick-Borne Blood Parasites

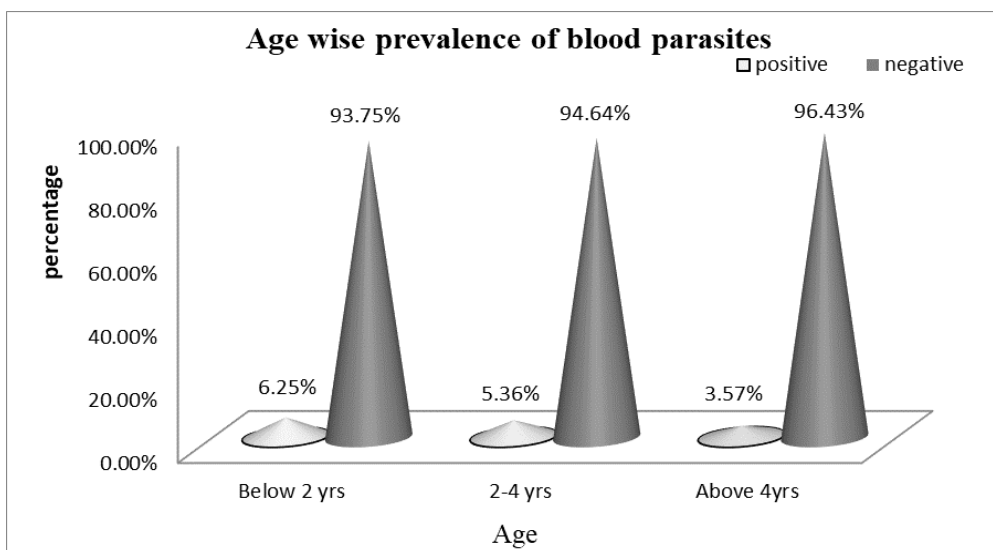


Figure 11: Age wise prevalence of blood parasite

The dogs were categorized into 3 age groups, i.e. i) below 2 years ii) 2-4 years and above 4 years. In below 2 years' age group, 1 was found positive for blood parasite out of 16 samples, in 2-4 years' group 3 were found positive out of 56 samples and in above 4 years' group, 1 was positive of 28 samples, thus prevalence percent being 6.25%, 5.36% and 3.57% respectively. The age wise prevalence showed no statistically significant differences (Fisher Exact= 0.999, $p > 0.05$) among the categorized age groups.

4. DISCUSSION

Only the tick species *Rhipicephalus sanguineus* was recognized in this study. The results of some study found *Rh. sanguineus* species ticks in dogs, are comparable to this one (Hadi et al., 2016; Loftis et al., 2013). The study conducted by Chhetri and Shrestha reported *Boophilus microplus*, *Rh. Sanguineus* and *Haemaphysalis bispinosa* species of ticks in dogs and reported *B. microplus* and *Rh. Sanguineus* tick species (Chhetri and Shrestha, 2011; Shrestha et al., 2005). The current discovery bolsters the theory that species composition changes brought about by climate change may accelerate the appearance of unforeseen events, such as the introduction of new pests and illnesses. Many studies have indicated that the species and prevalence of ticks is geographically variable. The winter season and climate change might be the possible reason that in this study only one species of ticks was identified.

The study conducted by a group researcher revealed that *Rh. Sanguineus* was more commonly discovered in the back region (35.0%), followed by the head, ears, and neck (29.0%), legs and interdigital regions (14.5%), belly (10.9%), and tail 6 (10.9%) (Hadi et al., 2016). They claimed that because it was difficult for the dog to utilize his legs to remove a tick, ticks were mostly located in the back area. According to reports, a number of variables, including host density, time of year, season, and inaccessibility for grooming, influence the tick's attachment site. When spraying individual animals, knowledge of tick predilection spots is useful as it can indicate which body area needs greater attention (Tadesse et al., 2012).

The overall haemoparasite prevalence is comparable to the 5.92% winter prevalence in Odisha, as reported (Adhikari et al., 2013). The result is lower than that of 15%, 14% 17.14% and 12% prevalence of blood parasite respectively in dogs of the Kathmandu valley (Maharjan and Jha, 2014; Manandhar and Rajawar, 2006; Phuyal et al., 2014). The lower prevalence is probably due to, in their study they selected the sample only from febrile, anaemic and symptomatic dogs during summer season whereas in this study the sample were taken from normal dogs having ticks. Similarly, the result was less than that 11.66%, 10.60 %, 11.57% and 8.9% (Gadahi et al., 2008; Srivastava et al., 2014; Senthil et al., 2009; Jegede et al., 2014). This can be the result of the study's different methodology.

Among 100 samples examined, 3 (3%) were positive for *Anaplasma spp*, 1 (1%) were positive for *Babesia spp*, and 1 (1%) had mixed infection of *Anaplasma* and *Ehrlichia canis*. This finding shows the highest prevalence of *Anaplasma spp* followed by *Babesia spp*. The incidence pattern deviates from Manandhar and Rajawar's findings (*Ehrlichia spp*. 11.43% and *Babesia spp*. 5.71%), (*Babesia spp*. 10%, *Ehrlichia spp* 3.4% and *Anaplasma spp* 2%) and (4% were positive *Babesia canis*, *Ehrlichia spp* was detected in 8% of cases) which may be due to difference in geographical regions resulting to differences in tick availability, method of sample collection and sample size (Manandhar and Rajawar's, 2006; Maharjan and Jha, 2014; Phuyal et al., 2014).

There were no statistically significant differences in the sex-wise prevalence of blood-pathogens between dogs that were male or female. Male dogs (8.33%) had a greater infection rate than female dogs (1.92%), which could be attributed to hormonal causes, frequent territory establishment and wandering in search of mates, and the picking of vectors. This is consistent with other experts' past study showing that a dog's gender typically has little bearing on whether the animal is susceptible to parasite infestations (Omudu et al., 2007; Maharjan and Jha, 2014; Manandhar and Rajawar, 2006; Jegede et al., 2014; Nwoha et al., 2013; Gadahi et al., 2008; Srivastava et al., 2014).

According to statistics, there was no meaningful correlation ($P > 0.05$) between dog ownership and blood parasite infection. The odds ratio, however, is 3.6, meaning that a dog owned by an owner has a three times higher risk of infection than a street dog. This outcome is comparable to that of who found no appreciable variations between street and pet dogs (Gadahi et al., 2008). The claim that native dogs are typically passively immune due to mother colostrum transmission of antibodies strengthens the current finding (Soulsby, 1982).

Presence of blood parasite showed statistically significantly different with the degree of tick infestation (Fisher Exact value=0.0087). This outcome differs from that (Hadi et al., 2016). They found no correlation ($p > 0.05$) between the level of tick infestation and *E. canis*, *Babesia*, or *Anaplasma* infections. This finding may be explained by the fact that dogs were not included in the tick sample. The significant association might be supported by the assumption that larger the number of ticks infesting on the body the greater the probability of presence of parasite infected ticks resulting in transmission of pathogen to the host.

The age wise prevalence showed no statistically significant differences (Fisher Exact= 0.999, $p > 0.05$) among the categorized age groups. On the other hand, a greater trend of infection was noted in younger dogs compared to older canines, which is corroborated (Adhikari et al., 2013; Srivastava et al., 2014). Younger dogs' immature immune systems make them more vulnerable to blood parasites, according some study, most of researchers found a higher percentage occurrence in adult dogs (Okubanjo et al., 2013; Senthil et al., 2009; Gadahi et al., 2008). They speculate that it might be brought on by older dogs' decreased immunity or resistance as a result of repeated tick bite exposure.

5. CONCLUSION

The identification of dog tick species and the study of blood parasites in dogs in the Kathmandu Valley were the main objectives of this research. Only one species of tick, *Rh. sanguineus*, was found in this investigation. Five percent of people have blood parasites overall. Three species were found to have high rates of *Anaplasma spp*. (3%) and co-infection with *Ehrlichia spp*. (1%), respectively, with *Babesia spp*. (1%). There was a strong correlation between the level of tick infestation and the occurrence of infection. There were no discernible differences seen in terms of dog ownership, age, or sex. Males had a higher infection rate than females did. Compared to adult age groups, a higher incidence was noted in young canines (less than 2 years old). In pets, the infection rate was higher than street dogs.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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