PREVALENCE OF CANINE TICK-BORNE PROTOZOA ASSOCIATED WITH HEMATOLOGY IN DHAKA CITY

MOST. AKLIMA KHATUN

DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY, DHAKA-1207

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BY

MOST. AKLIMA KHATUN

REGISTRATION NO. 15-06656

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Approved by:

Dr. Mahfuzul Islam
Supervisor
Chairman and Associate Professor
Department of Microbiology and Parasitology
Sher-e-Bangla Agricultural University
Dhaka-1207

Md. Kamrul Hassan
Co-Supervisor
Associate Professor
Department of Microbiology and Parasitology
Sher-e-Bangla Agricultural University
Dhaka-1207

Dr. Mahfuzul Islam
Chairman
Examination Committee
Department of Microbiology and Parasitology,
Sher-e-Bangla Agricultural University, Dhaka-1207

Department of Microbiology and Parasitology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

Memo No: SAU/MIPA/

CERTIFICATE

*This is to certify that the thesis entitled, "**PREVALENCE OF CANINE TICK-BORNE PROTOZOA ASSOCIATED WITH HEMATOLOGY IN DHAKA CITY**" submitted to the Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University, Dhaka-1207, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) IN PARASITOLOGY**, embodies the result of a piece of bona fide research work carried out **Most. Aklima Khatun**, Registration No.**15-06656**, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during this investigation has duly been acknowledged.

Dr. Mahfuzul Islam
Supervisor
Chairman and Associate Professor
Department of Microbiology and Parasitology
Sher-e-Bangla Agricultural University
Dhaka-1207

Date: December, 2022

Dhaka, Bangladesh

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The Author

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ACRONYMS AND ABBREVIATIONS

ABBREVIATIONS		FULL MEANINGS
Dr.	**=**	**Doctor**
M.S.	**=**	**Masters of Science**
mm	**=**	**Millimeter**
mg	**=**	**Milligram**
gm	**=**	**Gram**
µm	**=**	**micrometer**
etc.	**=**	**Et cetera**
viz	**=**	**That is**
e.g.	**=**	**For example**
et al.	**=**	**And others/Associates**
i.e.	**=**	**That is/namely**
No.	**=**	**Number**
$\chi 2$	**=**	**Chi-square**
IgG	**=**	**Immunoglobulin G**
sp.	**=**	**Single species**
spp.	**=**	**Plural species**
X	**=**	**Times (Magnification)**
PCR	**=**	**Polymerase Chain Reaction**
EDTA	**=**	**Ethylenediaminetetraacetic acid**
OIE	**=**	**World Organization for Animal Health**
WHO	**=**	**World Health Organization**
VBP	**=**	**Vector Borne protozoa**
TBD	**=**	**Tick-Borne Disease**
CVBD	**=**	**Canine Vector Borne Diseases**
UK	**=**	**United Kingdom**
USA	**=**	**United States of America**
R/A	**=**	**Residential Area**
SPSS	**=**	**Statistical Package for Social Sciences**

LIST OF SYMBOLS

SYMBOLS		FULL MEANINGS
<	=	**Less than**
>	=	**Greater than**
%	=	**Percentage**
&	=	**And**
+	=	**Plus**
=	=	**Equal to**
^{O}**C**	=	**Degree Celsius**

PREVALENCE OF CANINE TICK-BORNE PROTOZOA ASSOCIATED WITH HEMATOLOGY IN DHAKA CITY

ABSTRACT

Dogs, being companion animals serve a variety of economic, social, and cultural purposes. Among the diseases of dogs, tick-borne protozoa are drawing attention globally for both human and animals. The aim of this study was to observe the prevalence of tick-borne protozoan infections as well as some selective hematological parameters of stray dogs in Dhaka city. A total number of 160 dogs from various places in the study area were selected randomly and examined for both tick and protozoan infection. Only one species of hard tick, *Rhipicephalus sanguineus* was identified where 49 (30.62%) among the study population were found to be infested with this tick. There were differences in tick burdens in two seasons with higher infestation levels in Summer (37.50%) followed by Winter (26.92%). Ticks ranging from 1-16 were removed from dogs where most of the ticks were collected from the neck and chest region ($P < 0.001$). On the other hand, examinations of blood smear confirmed three protozoan species (*Babesia canis, Babesia gibsoni,* and *Hepatozoon* spp.) comprising 23.13% of the overall prevalence. Among the protozoan species, *B. canis* (11.88%) was the most prevalent protozoa. Subsequently, only 10.81% of the infected samples showed multiple infections. In both cases, females were more infected than males. Among the hematological parameters, the RBC counts, Hemoglobin, and PCV of all infected dogs were significantly lower ($P < 0.001$) compared to the healthy group. Moreover, the eosinophil of the infected groups showed higher values (11.00 % and 12.70% for protozoa and ticks, respectively) than the normal range indicating parasitic infections. Therefore, these results suggest the necessity of frequent blood examinations to enhance animals' welfare and disease prevention.

Keywords: Prevalence, blood protozoa, tick, hematological parameters, stray dogs.

Chapter I

Introduction

One of the most popular pets in the world, dogs serve a variety of economic, social, and cultural purposes in society. (Swai et al., 2010). Keeping pet animals enhances people's self-esteem, particularly young people (Paul and Serpell, 1996; Dohoo et al., 1998; Robertson et al., 2000; Knoble et al., 2008). World Health Organization reported that over ninety million dogs are classified as free-roaming (stray dogs), which has significant implications for public health. Nowadays, many stray, lost, or owner-surrendered dogs are kept in shelters to provide a temporary home until they can be reclaimed by the owner (Barrera et al., 2010). New and comfortable shelters for stray dogs are being established worldwide by local communities where they adopt relevant legislation, and implement numerous activities required to stop the spread of stray animals (OIE, 2014). Overcrowding or isolation, unfamiliar environments, limited physical activity, noise, and a limited diet are common problems for shelter animals (Tuber et al., 1999). The confluence of these issues along with the daily admissions of canines from various origins, and the struggle to control vectors, shelters provide favorable conditions for spreading different diseases including protozoal infection (Oliveira-Sequeira et al., 2002). These stray animals are not even tested for parasites, vaccinated, or treated for diseases. Therefore, they serve as the reservoirs for some significant zoonotic parasites. (Dakkak, 2010).

Different microorganisms such as bacteria, viruses, and protozoa can be transmitted by ectoparasites. Among them, tick-borne protozoa are drawing attention globally for both humans and animals. Certain diseases can be spread by different species of tick, and the efficiency of a vector depends on several genetic factors that determine a pathogen's ability to spread diseases (Fuente et al., 2017). *Ixodes ricinus*, commonly known as castor bean tick, acts as a vector of some pathogens, namely, *Anaplasma phagocytophilum Borrelia burgdorferi, Rickettsia monacensis, Babesia divergens,* and tick-borne encephalitis virus, while *Dermacentor reticulatus* works as a vector for *Babesia canis, Anaplasma marginale,* and *Theileria equi*. Meanwhile, *Rhipicephalus sanguineus* is a vector for *Babesia*,

Hepatozoon canis and Ehrlichia canis (Dantas-Torres, 2008; Solano-Gallego, 2011; Rizzoli et al., 2014; Földvári et al., 2016).

The brown dog tick*, R. sanguineus* is distributed globally and is most common in tropical areas. It has relatively few species ranging from yellow to brown in color. Hence, it is difficult to differentiate the species *R. sanguineus* from other species, which have similar morphological characteristics (Estrada-Pena ˜ et al., 2004), but differ in behavior, vector characteristics, and ecology (Walker et al., 2000). *R. sanguineus* has great importance in both medical and veterinary fields as a vector. Moreover, the tick can cause skin lesions, anemia, and tick paralysis in case of heavy infestations in dogs (Otranto et al., 2012). Furthermore, two protozoal diseases of dogs, namely babesiosis and ehrlichiosis are transmitted by this dog tick (Dantas-Torres et al., 2012).

A protozoal disease, named babesiosis is found globally and is caused by numerous species of *Babesia*. (Vial and Gorenflot, 2016). Traditionally, the taxonomy of this genus is identified according to its morphology within red blood cells, while two forms of these protozoa exist, i.e. small forms (e.g., *B. gibsoni*) or large forms (e.g., *B. canis*). Subsequently, different molecular techniques can differentiate the species of *Babesia* that infect dogs (Hamel et al., 2012; Solano-Gallego et al., 2016). Tóthová et al., 2020 also determined the species (large or small forms) by examining the protein profile from the serum of dogs. So far, small *Babesia*, namely *B. gibsoni*, *B. conradae* (Kjemtrup and Conrad, 2006), and recently reported *B. vulpes* (Baneth et al., 2015) have shown clinical signs in dogs. On the other hand, Clinical manifestations occurring by *B. gibsoni* can produce severe conditions which resemble *B. canis* including clinical signs like diarrhea, hemoglobinuria, proteinuria, enlargement of spleen, lymphadenopathy, nephropathy, etc. (Macintire et al., 2002; Birkkenheuer et al., 2004; Lee et al., 2009).

Two recently recognized piroplasm, *Theileria annae* and *Babesia microti* along with other species can cause canine piroplasmosis (Irwin, 2010). Indeed, it creates confusion about these two species *T. annae and B. microti*, which are very similar (Zahler et al., 2000; Camacho et al., 2001; Boozer and Macintire, 2003; Irwin, 2009). There was no evidence for transovarial transmission in ticks or extra-erythrocytic infecting stages which can be a

distinguishing feature of *Theileria* spp. (Zahler et al., 2000). *T. annae* morphologically resembles *Babesia gibsoni*. On the other hand, *B. microti* resembles "true theilerias" such as *Theileria parva* (Zahler et al., 2000; Goethert, 2003; Criado-Fornelio et al., 2003).

Irwin (2010) reports that 12 different piroplasm species have been found in dogs, some of which can only be determined through molecular methods. *B. canis* is endemic and the most prevalent species among the other species. Moreover, *B. gibsoni* and *B. vogeli* are significant among the dog populations in both Old and New World countries (Solano-Gallego and Baneth, 2011; Yisaschar-Mekuzas et al., 2013). *B. gibsoni* occasionally manifests importing sick dogs from endemic places, while *B. vogeli* has been described in Asia (Criado-Fornelio et al., 2003; Solano-Gallego and Baneth, 2011). Transmission of these protozoa could be due to the wide distribution of ticks around the world (Jefferies et al., 2007; Yeagley et al., 2009).

On the other hand, tick-borne diseases among pet owners are increasing day by day (Jones et al., 2018). This is due to the companion animals being a reservoir of tick-transmitted infection. For this reason, the One Health concept was underlined, encouraging medical professionals and veterinarians to unify their energies for preventing tick-borne zoonoses (Shaw et al., 2001; Dantas-Torres et al., 2012). The health of dogs is negatively affected by parasitic diseases, which can result in anemia and, sometimes, thrombocytopenia and leukopenia (Eiras et al., 2013; Kaewmongkol et al., 2017; Piratae et al., 2017; Rautenbach et al., 2017; Thongsahuan et al., 2020). There aren't many comprehensive morphological, molecular, or serological studies of dog blood protozoa in the literature. Unfortunately, in our country, no attempt has been made on the morphology, biology, control strategies, and even the prevalence data of blood protozoa in dogs. Keeping all the points mentioned above, the following objectives were set for the present research project.

- To identify different blood protozoa in dogs as well as their prevalence;
- To observe the prevalence of tick infestation along with their morphology; and
- To analyse the hematological changes of infected blood.

Chapter II

Review of literature

One of the most severe diseases that affect both humans and animals is tick-borne infections which result in high rates of morbidity and mortality (Chomel, 2011). *Babesia* spp., *Anaplasma platys, Hepatozoon canis*, and *Ehrlichia canis* are common tick-borne infections that infect dogs and cause the disorders known as babesiosis, anaplasmosis, hepatozoonosis, and ehrlichiosis, respectively (Baneth et al., 1998; Yabsley et al., 2008; Chomel, 2011). According to Baneth et al. (1998) and Chomel (2011), these pathogens are divided into two primary groups of haemoparasites: protozoans (e.g., *Babesia* spp. and *Hepatozoon* spp.) and Rickettsia (e.g., *Anaplasma* spp. and *Ehrlichia* spp.). According to Lewis et al. (1977) and Nava et al. (2015), these parasites infect domestic dogs via an ixodid tick, *Rhipicephalus sanguineus sensu lato*.

Epidemiological findings of these protozoa have been recorded globally by various authors. Among them, Rani et al. (2011); Pinyoowong et al. (2008) and Ikadai et al. (2004) observed the prevalence in Asian countries. Outside Asia, Shaw et al. (2001); Brown et al. (2006); Dantas-Torres (2008); Hii et al. (2012); Kelly et al. (2013); Williams et al. (2014) conducted research in different countries in Europe, Africa, Australia, Caribbean areas, and South America.

The introduction of these infections in previously unaffected populations is accelerated by a number of common variables, including climate, travel, transportation, and globalization (Harrus and Baneth, 2005). Accurate species diagnosis of these protozoa increases the effective treatments and management of these infections (Bashiruddin et al., 1999). Various methods focusing on sensitivity, accuracy, and speed, including both molecular and serological have been improved. Protozoan infections are routinely assessed and quickly diagnosed by microscopic examination of blood samples; however, this requires skilled staff due to the challenges of species differentiation. (Buddhachat et al., 2012).

In this section of the thesis, we are going to discuss the worldwide prevalence of canine blood protozoa as well as their association with brown dog ticks due to their vector importance.

2.1 Review of blood protozoa

2.1.1 Prevalence of *Babesia*

Babesia is an apicomplexan parasite that can cause severe tick-borne disease, named babesiosis, and has been recorded in some countries throughout the world, including Bangladesh. The protozoa can be transmitted by a tick named, *Rhipicephalus sanguineus*. The disease is characterized by erythrocyte destruction that produces mild to severe systemic clinical symptoms, such as different degrees of anemia, fever, thrombocytopenia, and splenomegaly. Canine babesiosis occurs by two species of B*abesia*, namely B*abesia canis* and *Babesia gibsoni.*

Ikadai et al. (2004) conducted research to identify and evaluate *Babesia gibsoni* infection from whole-blood samples obtained between July 2002 and July 2003. Examined dogs had *B. gibsoni* infections in 3.9% (37 of 945) and 10.9% (15 of 137) of the cases. Despite the relevance of blood protozoan infections for canine morbidity and mortality, very little information on these illnesses has been documented in the Caribbean, to find *Babesia spp*., Kelly et al. (2013) conducted research where the findings confirmed the presence of *Babesia* species in dogs, including *B. vogeli* (12%; 43/372) and *B. gibsoni* (10%; 36/372). However, there was evidence of multiple infections with co-infection.

Pennisi et al., 2012 aimed a study to determine the seroprevalence of *Babesia* spp. in dogs (n=249) from Italy. To concentrate on the specific sanitary risk for tick-borne infections posed by public shelters in southern Italy, they evaluated the seroprevalence in 2 public shelters and 4 privately-owned kennels where various tick-preventive measures were put into place. When compared to other *Babesia* spp., *B. canis* (70%) had the most common infection. Seroprevalence in public shelters was substantially greater than in private kennels. But in both kinds of kennels, *B. canis* seropositivity was comparable.

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Mahmud et al., 2014 investigated the prevalence of protozoa in Sirajganj where they investigated 272 sick pet dogs. The prevalence of protozoa in pet dogs was found 22.42%.

They observed 1.64% Babesia infection among the study sample. Most of the adult dogs were infected while females (55.74%) were more infected than males (44.26%). Terao et al., 2015 performed research in Mymensingh District in Bangladesh to detect 30% of *Babesia gibsoni* where 15 were found infected out of 50 dogs.

Singh et al. (2014) performed research on 214 blood samples from dogs suspected of having canine babesiosis around Ludhiana, Punjab (India). The incidence of canine babesiosis was found to be 7.47% (16/214) in peripheral thin blood smears stained with Giemsa. The study found *Babesia canis* 0.93% (2/214) and 6.54% (14/214) of *Babesia gibsoni* as the predominant species. However, molecular analysis revealed that 33/214 samples tested positive for *B. gibsoni* infection at a rate of 15.42%. Although breed and host sex were not substantially related to the incidence of the disease, the prevalence of *B. gibsoni* was higher in the summer compared to the winter and also in younger dogs. In India, a survey of diseases transmitted to dogs by ticks was carried out by Rani et al. (2011). *Babesia gibsoni* was found at 0.2% by utilizing the blood smear technique.

In two sites in Zambia, William et al. (2014) conducted a survey of multiple hemoparasites in domestic dogs and three kinds of wild carnivores. *Babesia felis*, *Babesia leo*, and a *Babesia spp* (similar to *Babesia lengau*) were found in lions (*Panthera leo*), spotted hyenas (*Crocuta crocuta*), and one lion, respectively. Wild carnivores from Zambia were found to have a high rate and diversity was found in *Babesia spp*. According to Otranto et al., 2019 research, many vector-borne pathogens (VBPs) were shown to be present in various species of carnivores in Iraq. In several American military bases in Iraq, blood samples were taken as part of a feral animal management and zoonotic disease surveillance program. Foxes had the highest frequency of *Babesia* spp. However, a newer *Babesia* species known as *Babesia lengau* was detected among the study samples.

Nur-e-Azam et al., 2016 conducted an epidemiological study of babesiosis in the Chittagong Metropolitan area, in Bangladesh. By using Microscopic Examination, the prevalence was 6.92% and 4.61%. Babesiosis more frequently occurred in adult dogs (10.11%) and male dogs (11.94%) than in younger and female dogs, respectively. In order to find, tick-borne infections in dog blood samples. Moreover, Talukder et al., 2012 conducted a study where they identified 38.23% *Babesia gibsoni* from 26 dogs.

Brown et al. (2016) examined dog blood to find *Babesia canis*, *Babesia vogeli* infection in free-roaming dogs connected in Australia and to estimate the impact of infection by the examination of platelet counts. An indirect method was utilized to quantify the platelet numbers from peripheral blood films taken from 92 of the 215 dogs. 69 (32%) of the 215 dogs tested positive for protozoal disease. Yabsley et al., 2008 identified the tick-borne protozoa in dogs from Grenada, surveying a variety of tick-borne pathogens. According to the findings of this study, several tick-borne pathogens have been found in dogs from Grenada. As a result, tick-borne diseases should be identified by differential diagnosis from dogs displaying thrombocytopenia, leukopenia, fever, or lethargy.

In Malaysia, a study on canine babesiosis was undertaken by Prakash et al., 2018. The presence of *Babesia* protozoa was checked in 240 dogs and 140 *Rhipicephalus sanguineus sensu lato (s.l.)* (Acari: Ixodidae) ticks after being collected from various locations throughout Malaysia. *Babesia vogeli* was found in both dogs and ticks (1.4%), in contrast to *Babesia gibsoni*, which was found in just ticks (1.4%). This study highlighted the first-ever identification of *B. gibsoni* and *B. vogeli* in *R. sanguineus s.l.* ticks from Malaysia in both the adult and nymphal stages, suggesting the possibility that this tick species may be involved in the transmission of canine babesiosis.

2.1.2 Prevalence of *Hepatozoon*

Hepatozoonosis, a vector-borne disease spread by ticks (Ixodidae), can occur in dogs by *Hepatozoon canis*. Numerous writers have identified *Hepatozoon canis* and *Hepatozoon americanum* in dogs. *H. canis* is found throughout the world, whereas *H. americanum* has only been identified in the continent of North America. It is believed that the brown dog tick, *Rhipicephalus sanguineus sensu lato*, is the primary vector of *H. canis*.

In India, a survey of diseases was conducted by Rani et al., 2011 that were transmitted to dogs by ticks. Microscopic examination revealed only *Hepatozoon* in 12 out of 525 blood smears (2.3%; 95% CI: 1.2, 4) in that study, where infections were found with either one or more than two canine tick-borne pathogens. The most frequent TBD pathogen detected infecting dogs in India was *Hepatozoon canis* (30%; 95% CI: 26.0, 34.0).

Despite its significance for dog morbidity and mortality, very little information on blood protozoan infections in the Caribbean has been documented. *Ehrlichia canis*, *Babesia* species, *Anaplasma* species, and *Hepatozoon* species were investigated in the Caribbean in 2013 by Kelly et al. The study confirmed 6% of *Hepatozoon canis* infection among the study samples. Yabsley et al., 2008 conducted a study on dogs from Grenada. The study confirmed 7% *Hepatozoon canis* infection. William et al., 2014 also conducted a survey of several blood protozoa of domestic dogs and three species of wild carnivores from two sites in Zambia. All three wild carnivores (38–61%) and domestic dogs (13%), which have a high prevalence of *Hepatozoon*, were seen. Comparing hyenas and wild dogs to domestic dogs and lions, a noticeably higher prevalence was found.

In several carnivore species from Iraq, Otranto et al., 2019 looked into the prevalence and occurrence of several vector-borne infections (VBPs). In dogs, up to five pathogens have been identified. The most common VBP in jackals was *Hepatozoon canis*. To better understand the prevalence and risk factors for Babesia spp. and *Hepatozoon* spp. infections in wild golden jackals (*Canis aureus*) and red foxes (*Vulpes vulpes*) in Israel, Margalit Levi et al., 2018 conducted a study. In 50/109 (46%) of the jackals and 9/21 (43%) of the foxes, *Hepatozoon canis* was found.

2.2 Review of tick infestation

According to Jungejan and Uilenberg (2004), ticks are blood-sucking arthropods that act as vectors for several protozoa that cause tick-borne diseases (TBDs) in humans and animals. One of the tick species that is found globally is *Rhipicephalus sanguineus*. *Babesia vogeli, Ehrlichia canis, Hepatozoon canis*, and many other organisms of veterinary and medical importance are proficiently transmitted by this tick (Lorusso et al., 2010). *Rhipicephalus sanguineus* is a well-adapted tick to both urban and rural environments (Szabó et al., 2001); it is primarily an endophilous tick, while Sonenshine (1993) found that temperature, humidity, and availability of host affect its distributions. Several countries, including Japan, Brazil, Mexico, France, South Africa, and the United States, have researched *R. sanguineus* in dogs (Koch, 1982; Gilot et al., 1992; Cruz-Vazquez and Garcia-Vazquez, 1999; Jacobs et al., 2001; Shimada et al., 2003; Silveira et al., 2009).

2.2.1 Prevalence of *Rhipicephalus sanguineus*

Shimada et al., 2003 recovered ticks from domestic dogs in Japan where a total of 4122 ticks (1624 larvae, 1200 nymphs, 1016 females, and 282 males) were removed from 1221 dogs during the study periods. They reported 4.8% *Rhipicephalus sanguineus* in those study samples. Although *R. sanguineus* was mainly distributed in a limited area, other ticks were found in wide geographical distributions. Additionally, they discovered that *R. sanguineus* was substantially related to exposure to gardens in urban and suburban regions, where dogs were more likely to live there.

To determine which ticks, infest dogs in the Punjab, Pakistan, Ul-Hasan et al., 2012 undertook research. 60 (11.42%) of the 525 dogs that were tested for tick infestations. Using morphological keys, the stereomicroscope was used to identify the ticks' morphological characteristics and establish their identification. *Rhipicephalus sanguineus* had a 98.33% prevalence rate. A total of 265 adult males, 224 adult females, and 19 nymphal ticks were found in the samples that were gathered from dogs. During the period of the investigation, no larvae were obtained from the infected canines. Throughout the study months, no significant difference was noticed.

Bhadesiya et al., 2014 researched the prevalence of *Rhipicephalus sanguineus* in Gujarat, India. In their study, 104 ticks were collected from 74 dogs, and the overall prevalence of ticks in those areas was recorded as 58.11% where all the tick species were identified as *Rhipicephalus sanguineus*. For dog health monitoring in the region, epidemiological data of morbidity in dogs with tick infestation by *Rhipicephalus sanguineus* in correlation to several epidemiological characteristics such as breed, sex, age, and housing pattern was compiled. In those research regions, epidemiological data on the morbidity of dogs with tick infestations caused by *R. sanguineus* had been gathered and associated with many epidemiological factors, including breed, sex, age, and housing design.

A survey of ticks, as well as haemo-parasites was conducted on 400 stray dogs by Konto et al., 2014 in Maiduguri. On the 384 infected dogs (96.0%), four genera of ticks were found, all of which belonged to the Ixodidae family (hard ticks). The genus *Rhipicephalus* had a rate of 10.8% in those study samples. Dogs of the younger group (6-12 months) were more infested than the adults of the age group of 24-120 months. More females than males

had tick infestation. The most often infected body parts were the perineum and the ear, with 328 (85.4%) and 252 (65.4%), respectively. The greatest mean tick load was recorded in August, with a mean of 462.53.2 (range from 450–475), while the lowest mean tick burden was recorded in February, with a mean of 244.53.8 (ranging from 239–250). All of the dogs that had *Babesia canis* infections had ticks of the species *Rhipicephalus* on them.

Abdullah et al., 2016 documented the results of tick abundance on dogs in the UK They used a participatory approach that allowed relatively cost-effective extensive data collection. A total of 12,096 dogs were examined from where 6555 tick samples were received. After examination, only 13 *Rhipicephalus sanguineus* were identified, although 640 ticks were too damaged for identification. The overall prevalence of tick attachment was 30 %. All of the *R. sanguineus* cases involved dogs that had recently traveled outside of the UK.

Soundararajan (2016) researched dog ticks which are well-recognized vectors of many pathogens affecting dogs and occasionally humans. Ticks were inspected on a total of 352 dogs from Chennai, Tamil Nadu, of various breeds. Only one species, *Rhipicephalus sanguineus* was found among the study population, and the overall tick prevalence was 58.52%. The northeast monsoon (34.46%) had the highest incidence of *R. sanguineus* among the seasons, followed by the southwest monsoon (30.10%), summer (23.79%), and winter (11.65%). R. *sanguineus* tick infestations on adult dogs were higher than those on puppies (67.96% vs. 32.04%). Male dogs (74.76%) had a higher infestation rate than female dogs (25.24).

Saleh et al. (2019) examined a variety of tick species infesting dogs and cats in North America. A total of 10,978 ticks were collected from 1494 dogs from February 2018 to January 2019. where infestation intensities ranged from 1 to 4765 was found in dogs. Four species of ticks were identified and *Rhipicephalus sanguineus* was found in 11.5% of the study population. They reported attachment sites of tick species that differed whereas *R. sanguineus* is much more attached to the head, neck, abdomen, and feet.

de Waal et al. (2020) reported a result of a tick investigation in dogs in Ireland. A total of 120 ticks were collected from 56 dogs where only a single *Rhipicephalus sanguineus* specimen was detected. The most common place where dogs and cats were exposed to ticks

was the garden. More sporting dog breeds (n = 17; 31%) than any other breed had tick infestations. Ticks were a concern for the owners when they discovered ticks on their pets. Pet owners utilized a range of products to control ectoparasites on their animals, though the items weren't as effective as they thought they should be. Moreover, ticks were found in low numbers, according to field samples.

Adetayo et al. (2021) examined tick infestation and density in dogs around Ibadan. Throughout the study, 130 dogs of various breeds, ages, and sexes were inspected. Breeds, techniques of control, age, sex, location, and management constituted the risk factors. The overall rate of tick infection was 56.2% in this study, with *Rhipicephalus sanguineus* being the most often affected dog species and the head area being the most ticks' preferred site of attachment. The most infected dogs were those under 12 months old, while the least infected were those between 24 and 120 months old. Male dogs have fewer ticks than female dogs. However, tick infestation was not significantly influenced by the demographics of dog owners, their understanding of tick infestation, and the age, breed, or sex of the dogs.

Wyk et al. (2022) conducted a study aimed at identifying ticks infesting dogs admitted to the Potchefstroom Animal Welfare Society (PAWS) and detecting tick-borne protozoa they were harboring. A total of 592 ticks were collected from 61 stray dogs where 61% of *Rhipicephalus sanguineus* was detected. Of these ticks, Male and female *R. sanguineus* ticks made up, respectively, 51.5% (186/361) and 48.5% (175/361) of the total population. Blood smears from engorged female ticks were examined under a microscope, and the results showed that 0.5% of *Babesia* spp., 1% of *Anaplasma* spp., and 22% of *Rickettsia* spp.

Grant et al. (2023) collected *R. sanguineus* from hundreds of dogs and cats from different locations across 25 of the 50 states from 2018 to 2021 in the U.S.A. Dogs from 20 states were found to have infestations with temperate lineage, with the majority (83.5%) coming from regions with annual mean daily average temperatures under 20°C. The majority (80.0%) of tropical lineage tick submissions were from regions with an annual mean daily average temperature >20°C, and tropical lineage submissions were less prevalent (19.3%), coming from 15 states. Even while all dogs' travel histories were not known, subsequent

interviews with vets revealed that some tropical lineage infestations in cooler places may have been brought on by recent canine travel.

A thorough report on the epidemiological aspects of tick infestations in dogs in Pakistan was published by Zeb et al. in 2023. 300 dogs were tested during the period and subsequently collected 1150 ixodid ticks. Two ixodid tick genera including six tick species were identified where *Rhipicephalus sanguineus* had a prevalence rate of 41.3% although the overall prevalence found in dogs was 61%. However, the risk factors analysis indicated that many demographic and host management-related characteristics, including host age, breed, exposure to acaricide treatment, and history of prior tick infestation, were linked to a higher risk of tick infection in dogs.

2.3 Haematological studies of dog's blood

Khan et al. (2011) conducted a research on the hematology and serum chemistry values of stray dogs in Bangladesh. They looked at the values concerning bodily condition, age, sex, and reproductive stage. White blood cells, differential leukocyte count, total protein, albumin, glucose, cholesterol, phosphorus, and potassium mean values did not differ significantly between or among sexes, ages, reproductive states, or physical conditions. Neither did hemoglobin, packed cell volume, mean corpuscular hemoglobin concentration, hemoglobin, mean hemoglobin concentration, or hemoglobin. Only statistically significant variations (p 0.02) between sexes were seen for erythrocyte sedimentation rate. Across age categories, there were significant variations in total red blood cell count (p 0.001). Between-body conditions showed a substantial difference in red blood cell count, mean corpuscular volume, and mean corpuscular hemoglobin (p 0.001). Females who were pregnant or not were substantially different from non-pregnant females in terms of red blood cell count, mean corpuscular volume, and mean corpuscular hemoglobin (p 0.001).

Abdel-Rahman et al. (2015) examined 200 dogs emphasizing clinical, hematological, and parasitological parameters. Along with the other parameters, the hematological findings showed that RBC, Platelet, Granulocyte, HCT, and HGB counts all significantly decreased in infected mice compared to healthy animals.

Paiz et al. (2016) did a retrospective study of dog hematological reports in Brazil. They aimed to assess these sick pups' hematological profiles. Two dogs, one with *E. canis* and the other with *Babesia* spp., had co-infected with *H. canis* and other agents, according to an analysis of the hematological data. Only one dog's blood test revealed no alterations in comparison to the reference levels. Anemia was the most prevalent hematological abnormality. Despite the rarity of *H. canis* infection, the majority of affected dogs experienced severe hematological alterations. *Babesia* spp. and *E. canis* infections were discovered in two dogs, therefore the hematological abnormalities in these animals cannot be entirely attributed to *H. canis*.

Bhatta et al. (2018) observed the prevalence of blood parasites in hyperthermic dogs of Kathmandu Valley, Nepal. Hematological tests such as the total leukocyte count (TLC), total erythrocyte count (TEC), packed cell volume (PCV), hemoglobin (Hb), and differential leukocyte count (DLC) were also investigated in that study. Eosinophil count significantly increased whereas TLC, DLC, TEC, PCV, and Hb significantly decreased according to hematology (p 0.05). *Ehrlichia* species and *Babesia* species infections resulted in samples with considerably reduced TLC levels and significantly increased eosinophil counts, respectively. Given that blood parasite incidence is higher in hyperthermic dogs, parasitic infection may be a likely cause of the fever or hyperthermia. Therefore, it is crucial to make a differential diagnosis of hyperthermic cases including hemoprotozoan infections, which is made simpler by identifying changes in blood parameters and the presence of parasites in the blood.

Thongsahuan et al. (2020) examined different hematological characteristics in infected dogs. Anemia, thrombocytopenia, monocytosis, and eosinophilia were among the hematological changes brought on by *Ehrlichia* infections. Anemia, thrombocytopenia, leukocytosis, neutrophilia, and monocytosis were present in the blood samples of *Hepatozoon*-infected dogs. Dogs with *B. canis* infections had higher odds of having anemia, thrombocytopenia, eosinopenia, and lymphopenia.

Boonhoh et al. (2023) investigated the effect of multiple blood parasite infections on the hematological profiles of dogs at a shelter in Southern Thailand. The results showed that all of the infected dogs had significantly lower levels of platelet count, hemoglobin,

hematocrit, and red blood cell count (RBC) compared to the uninfected dogs. Despite the triple-infected dogs having lower RBC, HB, HCT, and PLT values than the double- and single-infected pups, the difference was not statistically significant. They postulated that triple blood parasite infection with *Anaplasma platys, Babesia vogeli*, and *Ehrlichia canis* resulted in more serious sickness than double and single infections. If dogs are naturally infected with one, two, or more blood parasite infections without exhibiting any clinical signs, it may be advantageous for their health and welfare to analyze their hematological profiles.

Chapter III

Materials and Methods

3.1 Ethical approval

Blood samples were aseptically collected by registered veterinarians through proper restraining of dogs to avoid any injuries. All the procedures required for the sample collection were fulfilled, based on the ethical guidelines approved by the Animal Welfare Act, 2019. Moreover, permission for sampling was verbally obtained from the Department of Livestock Services (DLS).

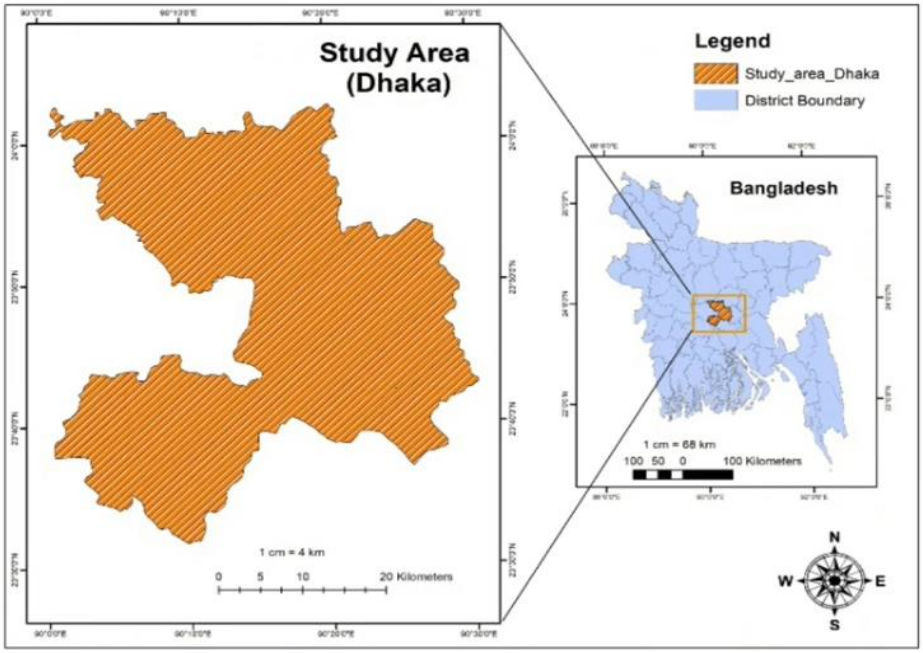


Figure 1: Location of study area

3.2 Study area

This research was carried out in Dhaka, the largest and the capital city of Bangladesh (Figure 1). The city has a total area of 118.29 square miles and is situated at 23°42′N 90°22′E. Tropical vegetation covers the region, which has moist soils that are nearly flat and very near sea level. As a result of the excessive rainfall, Dhaka is vulnerable to floods during the monsoon seasons. The city experiences 2,123 millimeters (83.6 inches) of annual rainfall and an average yearly temperature of 26 °C (79 °F).

3.3 Study period

The cross-sectional study lasted for six months comprising November 2022 to April 2023. Therefore, two seasons, namely Winter (November to February) and Summer (March to April) were covered by this study period.

3.4 Sample size

For this study, 160 street dogs from various places were randomly selected and examined. Based on their availability, dogs of different sexes or age ranges were chosen for the current investigation. Several criteria were taken into account when determining the prevalence study such as dogs' sex, age, and seasonal changes, i.e. summer (March–April) and winter (November–February), respectively. During sampling, 47 individuals were male and the rest 113 were female. Moreover, 24 dogs were of < 1 year, 52 were between 1 and 2 years and the remaining 84 were above 2 years of age. A structured questionnaire was developed including the tentative age, body weight, sex, etc. which was very helpful to collect data from the study population.

3.5 Research laboratories

The investigation was conducted in the Laboratory Parasitology (Figure 2), located at Sher-e-Bangla Agricultural University, Dhaka. The morphological identification of ectoparasites as well as hematological examinations were performed in the laboratory mentioned above after preparation.

Figure 2: Preparation of laboratory for morphological identification

3.6 Restraining of animals

A common versatile tool, ‘Catchpole’ was used to capture and restrain the street dogs. Moreover, a group of trained people from Obhoyaronno - Bangladesh Animal Welfare Foundation helped to restrain the animals. A general anesthesia was performed by using some drugs such as Atropine Sulphate (0.2 mg/kg, SC) and Ketamine (2.0 mg/kg, IV).

3.7 Collection of ticks

The procedure for collecting ticks included several features, among them the inspection of the head region with a particular focus placed on the ears, especially the interior and the region behind the ears. Then, a comprehensive physical examination of the legs, armpits, and space in between the toes was carried out. Subsequently, with the aid of fingers, the fur of the animal was combed from head to tail and vice versa, applying sufficient pressure to find any small lumps. The body was then combed down the length to locate the attachment site of ticks. The entire process took 2-3 minutes to complete. Following this approach, any ticks were extracted using forceps, preserving the tick's mouthparts. Each of the ticks found during the examination was collected to encourage maximum participation for their morphological identification (Figure 3). Collected ticks from each individual were placed into separate vials containing 70% alcohol with proper labeling and stored in a cool place. After arriving at the lab, every sample was given a unique number. Finally, the ticks were identified up to the species level and sex according to the keys and description of (Hillyard, 1996 and Estrada-Peña et al., 2017).

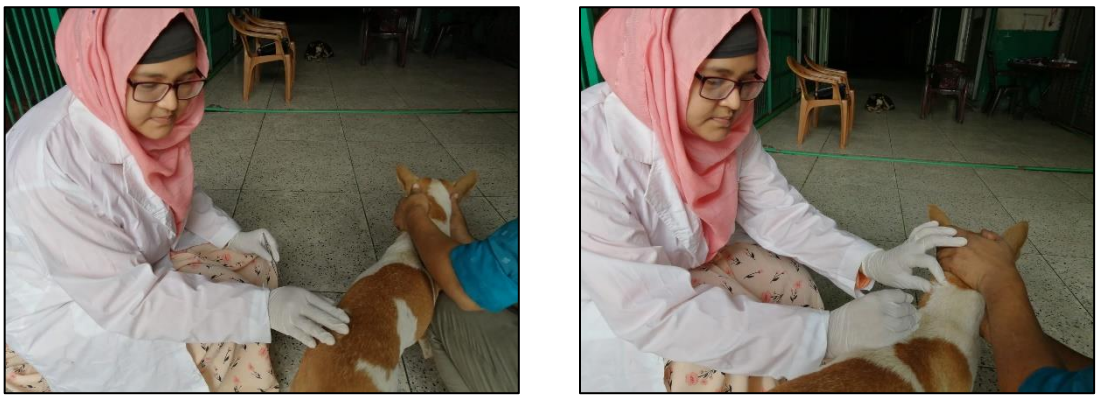


Figure 3: Collection of ectoparasites

3.8 Collection of blood

The superficial and accessible cephalic vein was used as the site for blood sampling. An appropriate aseptic method was followed during the collection process, which included trimming the hair surrounding the sampling site and cleaning it with an antiseptic solution. Using a sterile 21G needle, 1-2 ml of blood was taken from each animal and immediately transferred to an EDTA vial (Figure 4). Then, the vials were transferred to the laboratory maintaining a cool chain, and kept in a refrigerator (4-8 ^{0}C) for further examination. Finger pressure at the sampling site was used to control bleeding after each successful blood collection for around 5-10 seconds and finally, the dog was returned to its pen.

Figure 4: Collection of blood from animals

3.9 Processing of blood for microscopy

3.9.1 Preparation of thin blood smear

At least 2 (Two) thin smears per animal were prepared where the cells were in a monolayer, i.e., not touching one another. For this purpose, blood in the EDTA vial was shaken to mix well, and then a little drop of blood was applied to the pre-cleared, labeled slide near the frosted end. To make a decent smear, a spreader slide was quickly and smoothly drawn forward at about 45° angle (Figure 5). This allowed the blood to spread along the contact line of both slides. A good feather at the slide's edge served as evidence of a successful smear while the correct amount of blood was dispersed in the proper technique. Finally, the smears were allowed to become dry and then fixed in them by dipping them in absolute methanol.

Figure 5: Preparation of thin blood smear

3.9.2 Preparation of Giemsa working solution

A freshly prepared Giemsa working solution was made from a well-prepared commercial stock to detect blood protozoa. For this purpose, 50 ml of 10% Giemsa working solution was prepared each day. Firstly, a 100 ml container was filled with 5 ml of Giemsa stock solution after it had been filtered through Whatman paper. Following that, 45 ml of distilled water was added and mixed well by vigorous shaking. After the preparation of the working solution, the smears were stained within one hour, and the leftover stains were discarded each day.

3.9.3 Staining of blood smear

To conduct proper staining, a Coplin jar was filled with approximately 40 ml of Giemsa working solution, and 2 drops of Triton X-100 were added to the solution. Then, the slides were placed into the working Giemsa solution for 30 minutes. The excess stain was afterward removed from those slides by dipping them three to four times in Giemsa buffer solution. Finally, the stained slides were dried by keeping them on tissue paper (Figure 6).

Figure 6: Staining of blood smear

3.10 Processing of ectoparasites (Tick)

Collected ectoparasites were slide mounted by following several steps, such as clearing, staining, and dehydrating before mounting.

Figure 7: Processing of ticks before staining

Firstly, the ectoparasites were cleared by dissolving in 10% KOH at room temperature overnight which allowed them to pass light through them (Figure 7). After clearing, the specimens were returned to 50% ethanol, followed by distilled water for 30 minutes in each to prepare them for staining. Hematoxylin-Eosin (H & E) dye was used to stain the specimens where the slides were kept in the stain overnight (Figure 8). As the specimens became darker, the excessive stain was removed by keeping them in 3% Acid-Alcohol. Subsequently, the dehydration process was accomplished to prevent the specimen from

spoiling by bacteria. This process was done by passing the specimens through a series of ascending concentrations of ethanol for 30 minutes in each step (Figure 9).

Figure 8: Staining of ticks

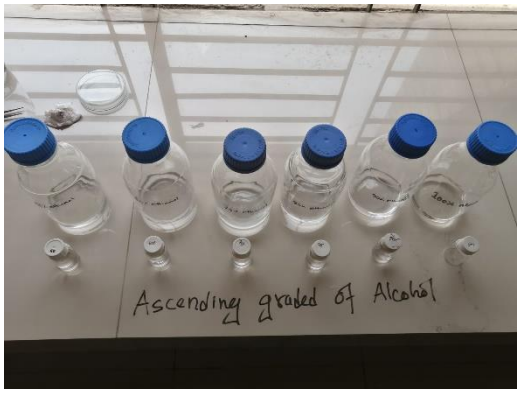


Figure 9: The process of dehydration

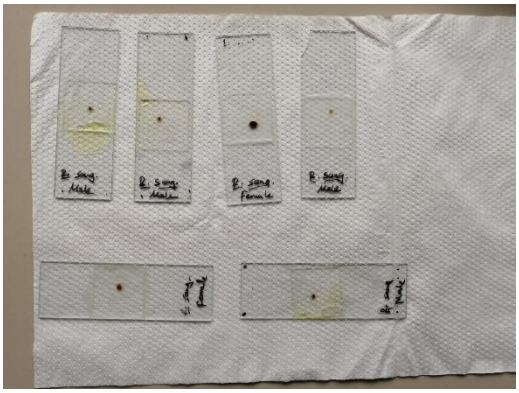


Figure 10: Drying of slides

After the dehydration process, the specimens were cleared by xylene for a few seconds to remove ethanol. Then, the specimens were mounted in a fresh slide with Canada balsam. During the mounting process, needles, fine forceps, and insect pins were used to make good visible slides. After mounting, slides were allowed in a place to become dry for 1-2 days (Figure 10). Finally, the ectoparasites (ticks) were examined under a microscope (4X and/or 10X) for morphological identification according to the keys and descriptions of Soulsby, 1982, Ruprah, 1985, Taylor et al., 2012, Allison and Little, 2013, Saari et al., 2019.

3.11 Hematological parameters of canine blood

Three groups i.e., healthy, infected with ticks, and infected with protozoa were categorized, and 10 blood samples from each group were analyzed for different hematological parameters. These parameters were Red Blood Cells (RBC), Hemoglobin (HGB), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Red Cell Distribution Width (RDW), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cells (WBC), etc.

3.11.1 Total Erythrocyte Count (TEC)

The diluting pipette was filled with blood up to 0.5 mark (Figure 11). The tip of the pipette was then filled with Hayem's solution up to 101 marks. The pipette was shaken in 8(eight) knot fashion for 1 minute to mix up the contents inside. The Hemocytometer slide was filled with the mixed solution and placed under the microscope after discarding 1/3 of the mixture from the pipette. The cells in four corners and one center secondary square (A, B, C, D, and E) were counted (Figure 12). The numbers of cells counted in 5 squares were multiplied by 10,000 and were expressed in million/µl.

Figure 11: Blood loading in diluting pipette

Figure 12: RBC counting under microscope

3.11.2 Hemoglobin (Hb)

The study was premeditated to detect the hemoglobin values by Sahli's method. To conduct this protocol, 0.1N HCl was added to the hematometer tube up to the lowest graduation (20 marks). Blood was filled into a capillary pipette up to 20 µl and immediately transferred to the comparison tube (Figure 13). The tube was left for 5-10 minutes for the lysis of RBC. After this period, a few drops of distilled water were added drop by drop, and stirred the solution with a glass rod. Finally, the color of the tube was matched with the standards in the comparison tube, and noted the reading by holding the haemoglobinometer against good daylight.

Figure 13: Determination of hemoglobin values by Sahli's method

3.11.3 Packed Cell Volume (PCV) or Hematocrit (HCT)

To conduct this protocol, blood was loaded into the Wintrobe tube up to the 10 mark of the right-sided scale. The Wintrobe tube was then placed in the centrifuge machine and centrifuged @ 3000 rpm for 30 minutes. Then, the hematocrit or PCV was recorded using the following formula.

PCV (%) = (Height of packed red cells ÷ Height of the total blood in the tube) × 100

3.11.4 Calculation of MCV, MCH, and MCHC

Mean corpuscular volume (MCV) is an auxiliary indicator, particularly in the differential diagnosis of anemia. MCV was calculated using the following formula.

MCV (fl) = (PCV ÷ Red blood cell) × 100

Another two important red blood cell indicators were MCH and MCHC where both values serve in specifying the type of anemia. MCH and MCHC were recorded using the following formula.

MCH (pg) = (Haemoglobin ÷ Red blood cell) × 10

MCHC (%) = (Haemoglobin ÷ PCV) × 100

3.11.5 Total Leukocyte Count (TLC)

Blood was drawn into the diluting pipette up to 0.5 marks. Subsequently, the tip of the pipette was filled with 0.1 N HCl up to 11 marks. After shaking the pipette in 8(eight) knot fashion for 1 minute, the hemocytometer slide was filled with the solution. The cells in four corner squares (A, B, C, and D) were counted and multiplied by 50. Finally, the TLC was expressed in thousand/µl.

3.11.6 Differential Leukocyte Count (DLC)

At first thin smear of blood was made which was dried in the air. Then, the smear was stained with Giemsa's stain and the slides were dried in air. The stained slide was then placed under a microscope and cell count was started using an oil immersion objective (X100). A total of 200 cells were counted based on their shape and color. The results of DLC were calculated in percentage.

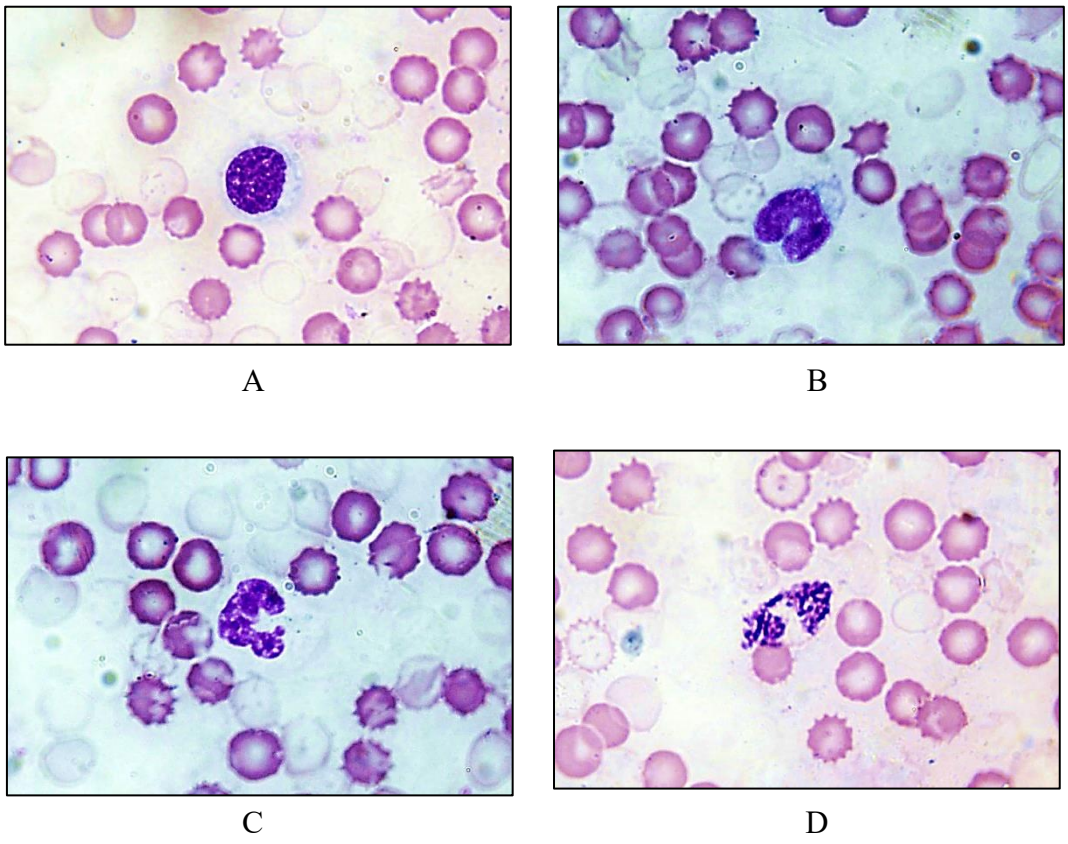


Figure 14: Types of White Blood Cells (100X magnifications); (A) Lymphocyte, (B) Monocyte, (C) Neutrophil, and (D) Eosinophil

3.12 Statistical analysis

The obtained data was imported, stored, and coded accordingly using Microsoft Excel 2016 where all data analyses were performed by using statistical software program (SPSS for Windows, Version 19.0, USA). The results of prevalence were expressed in percentage. Association among various risk factors, namely, sex, age, and season, was carried out by Chi-square (χ2-test). Moreover, the standard error of the mean was also determined for hematological parameters

Chapter IV

Results and Discussion

4.1 Morphological identifications

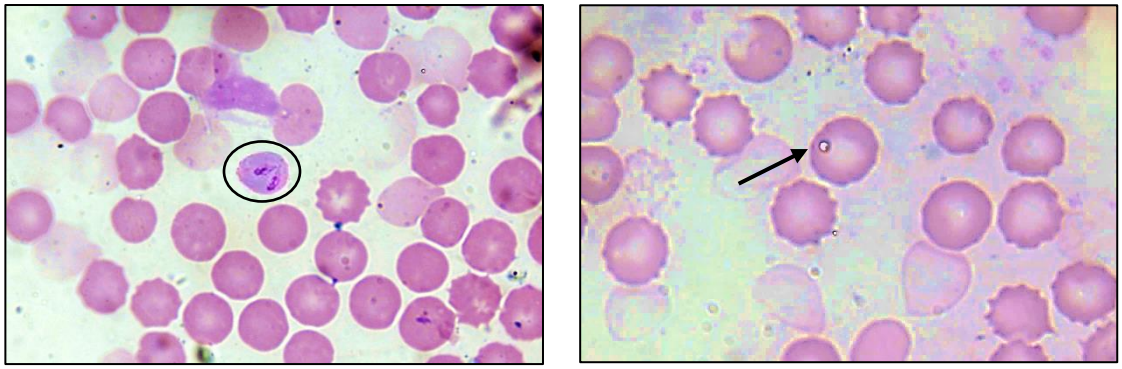
4.1.1 Blood protozoa of dog

160 street dogs were included in this study where samples were collected, smeared, and stained for microscopic identification through a proper scientific way. Three (3) protozoan species, namely, *Babesia canis*, *Babesia gibsoni*, and *Hepatozoon* spp., were identified according to the keys and descriptions of various authors given below.

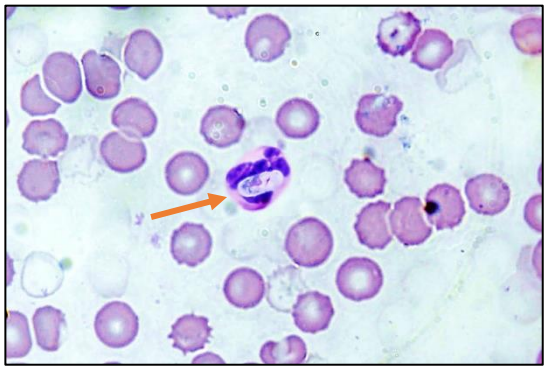
Small babesias (1.0-2.5 μm long), which include *Babesia gibsoni*, and large babesias (2.5-5.0 μm long), which include *Babesia canis*, are separated into two groups based on their morphology.

Babesia spp. are divided into two groups, namely *B. gibsoni* (small babesias) and *B. canis* (large babesias). These species can be identified by their orientation in RBCs where *B. canis* makes an acute angle, while *B. gibsoni* appears single in most cases (Ruprah, 1985). In our study, the shape of *B. canis* was observed as pyriform, where one end was pointed, and rounded the other end (Figure 15A). On the other hand, *B. gibsoni* lacked the usual pyriform shapes and had a signet ring form (Figure 15B).

Moreover, in the stained blood smear under the microscope, *Hepatozoon* spp., was easily identified in the cytoplasm of white blood cells (mostly in neutrophils) where the gamonts were observed elongated, ellipsoidal, and had an eccentrically positioned nucleus (Figure 15C). These silent features confirm *Hepatozoon* spp. according to the descriptions of Allison and Little, (2013); and Saari et al. (2019).



A B

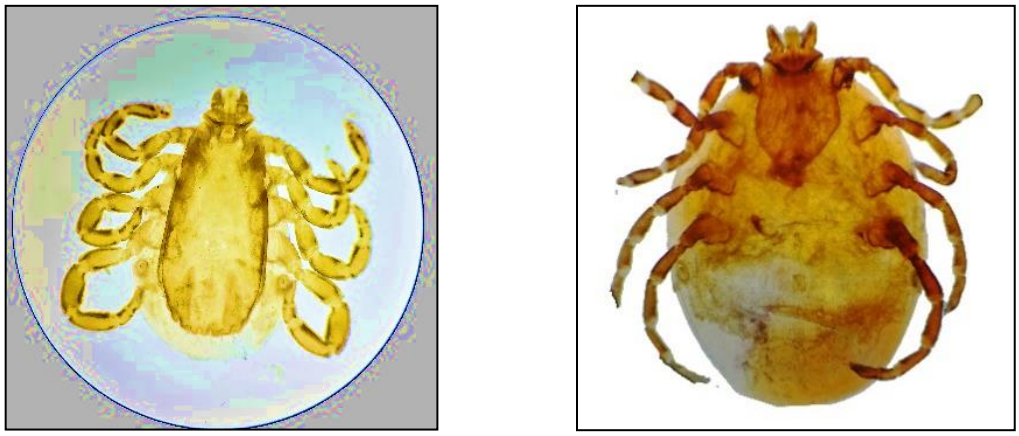


C

Figure 15: Microscopic observation of canine blood protozoa (100X magnifications) A black circle indicates *Babesia canis* (A); A black arrow indicates *Babesia gibsoni* (B); and A White arrow indicates *Hepatozoon* spp. (C).

4.1.2 *Rhipicephalus sanguineus*

Medium-sized, yellowish-brown to reddish-brown ticks having a dark, inornate brown scutum were measured with a scale where unfed males (Figure 16A) and females (Figure 16B) were found on an average of 3.60 mm and 4.23 mm, respectively. The specimens having the best physical integrity were chosen for staining with Haematoxylin and Eosin (H & E) and observed under the light microscope for their morphological identification.



A B

Figure 16: Microscopic examination of *Rhipicephalus sanguineus* (4X magnifications); Male (A) & Female (B)

The capitulum, or anterior portion of the body (Figure 17) was composed of one hexagonal-shaped basis capitulum, which was used to hold several organs, such as one powerful hypostome for sucking blood, two chelicerae for cutting the skin, and two short palps for sensory function. Furthermore, in all the studied specimens in our investigation, setae and sensilla were discovered to be present throughout the body without a distinct pattern of distribution.

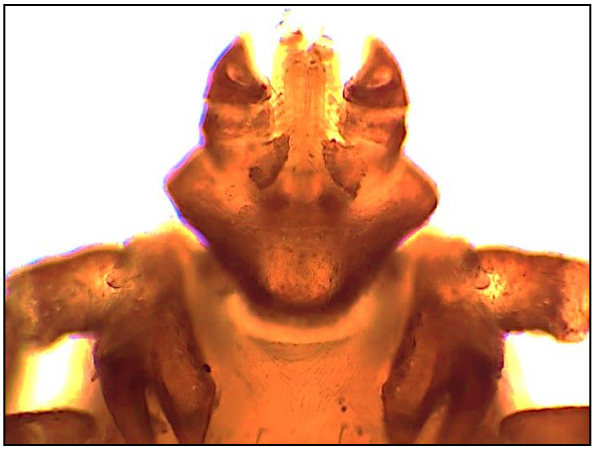


Figure 17: Mouthparts of *Rhipicephalus sanguineus* hexagonal basis capitulam (10X magnifications)

On the foretarsus of the first pair of legs, a unique structure known as Haller's organ had been identified that functioned as a chemosensation. Despite intraspecific heterogeneity among the study samples, festoons were located on the posterior margin of the body and were separated into 11 unique rectangular portions (Figure 18). The size of the caudal process varied across the specimens under study in fed males. The two valves and an anal groove that created the anal orifice were articulated with four setae that are positioned symmetrically on each side.

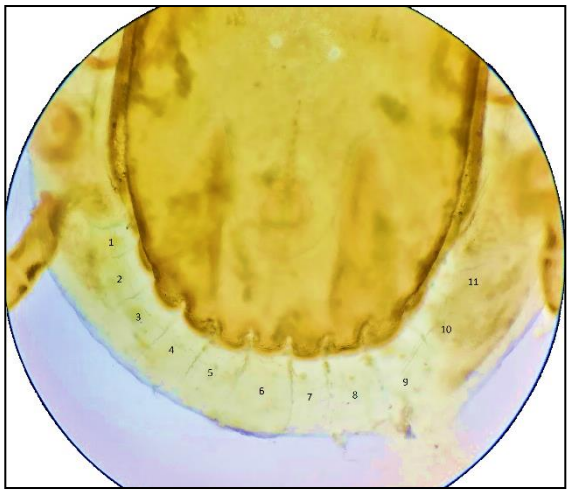


Figure 18: Posterior part of a male *Rhipicephalus sanguineus* (10X magnifications) where the numbers indicate 11 rectangular portions of festoon

Adanal plates and accessory shields were found in male ticks, composing the anogenital region. These structures are highly sclerotized and located on the side of the anus. The adanal plates were long, parallel, and had a sharp posterior margin. The accessory shields varying in form were located beside the adanal plates. The narrow spiracular plates were located behind the last pair of legs. A circular ostial lip was also present in each spiracular plate. The genital plate was located in between 1st and 2nd pair of coxae and exhibited a round structure in all the specimens in our study (Figure 19).

All the characteristics found in our study were supported by various authors, and confirmed this species as *Rhipicephalus sanguineus* (Walker et al., 2005; Guglielmone et al., 2006; Krantz and Walter 2009; Nava et al., 2015; Dantas-Torres et al., 2013).

A B

Figure 19: Male *Rhipicephalus sanguineus* (4X); Dorsal view (A) & Ventral view (B) Both figures indicate palp (pl), hypostome (hy), chelicerae (ch), basis capitulam (bc), coxa (cx), scutum (sc), spiracle (sp), anus (an), adanal plate (ap), festoon (fs), genital apron (ga), caudal plate (cp)

4.2 Prevalence of blood protozoa

4.2.1 Overall prevalence of blood protozoa

The study was carried out throughout six (6) months, specifically from November 2022 to April 2023, covering two predominant seasons in Bangladesh. A total of 160 dogs, consisting of 70.63% females and the rest 29.37% males were bought in a shelter house for spaying and neutering, respectively. Examination of blood smear was performed under a light microscope where 37 out of 160 samples (23.13%) were infected with any of the three species of blood protozoa, namely *Babesia canis*, *Babesia gibsoni*, and *Hepatozoon* spp. (Figure 20).

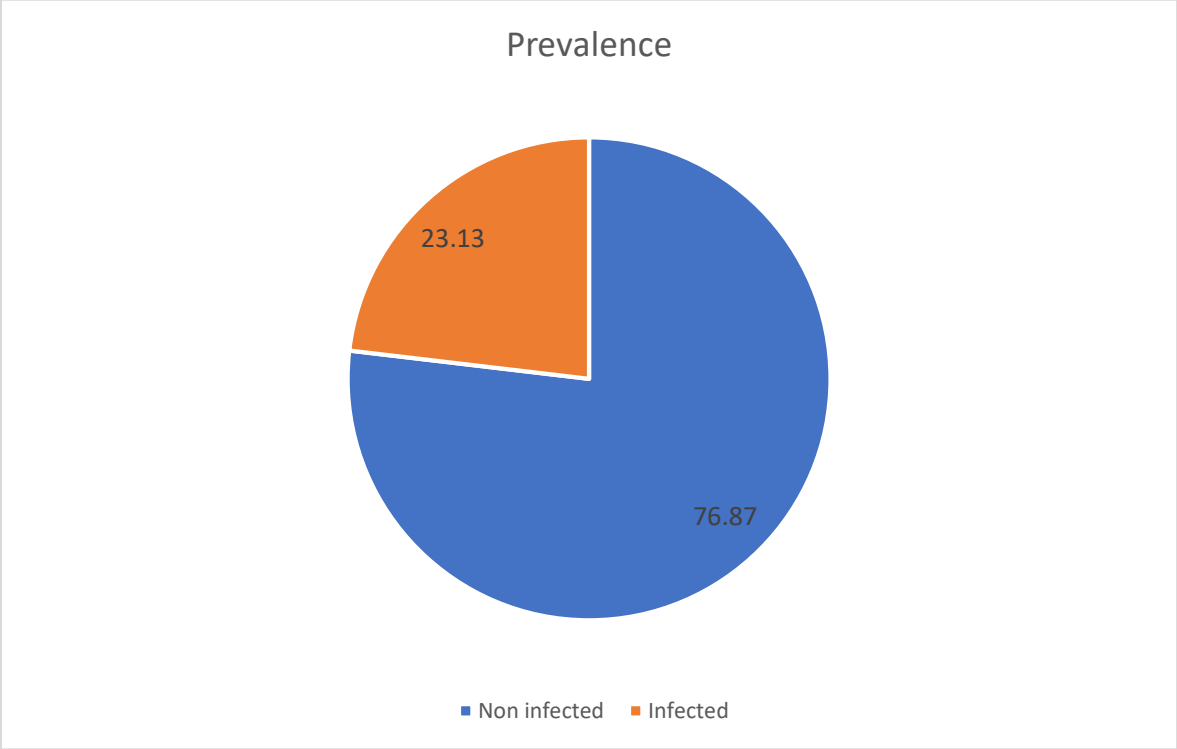


Figure 20: Overall prevalence of blood protozoa in dogs

4.2.2 Species-wise prevalence of blood protozoa

A total of three blood protozoan species in dogs were encountered in this study which is shown in Table 1. Among 160 examined dogs, *B. canis*, *B. gibsoni*, and *Hepatozoon* spp. were detected in 19, 7, and 16 dogs, comprising 11.88%, 4.38%, and 10.00%, respectively.

Although the species-wise prevalence of canine blood protozoa was numerically different, there was no statistical significance among them.

Table 1: Species-wise prevalence of canine blood protozoa

Species	**No. of dogs infected (n=160)**	**Prevalence %**	**P-value**
Babesia canis	19	11.88	0.047
Babesia gibsoni	7	4.38	
Hepatozoon spp.	16	10.00	

4.2.3 Single and Mixed infections of blood Protozoa

Using microscopic examinations, the protozoan infection was calculated at 23.13% where the dogs were infected with one or more canine blood protozoa. Of the 37 positive dogs, 33 (89.19%) dogs were infected with only one species. However, infections with more than one canine blood protozoa were found only in 4 (10.81%) dogs. These co-infections were observed with two (3) and three (1) species of canine blood protozoa, comprising 8.11% and 2.70% of prevalence, respectively. The prevalence of single and mixed infections of protozoa had a statistical significance ($P < 0.001$) which is exhibited in Table 2.

Table 2: Prevalence of single and mixed infections of protozoa

Types of infection	**No. of dogs infected (n=37)**	**Prevalence %**	**P-value**
Single infection	33	89.19	<0.001*
Multiple infections			
Two species	3	8.11	
More than two species	1	2.70	

*= Statistically significant

4.2.4 The occurrence of co-infections of blood protozoa

A total of four blood samples were infected with more than one species of blood protozoa. Most of the co-infection (3) was observed with two species of protozoa where only one sample had infections with three protozoa (*B. canis, B. gibsoni,* and *Hepatozoon* spp.). The occurrence of these co-infections with canine blood protozoa is shown in Table 3.

Table 3: The occurrence of co-infections with canine blood protozoa

Co-infections	**No. of dogs infected**
B. canis + *Hepatozoon* spp.	2
B. gibsoni + *Hepatozoon* spp.	1
B. canis + *B. gibsoni* + *Hepatozoon* spp.	1

4.2.5 Gender-wise prevalence of blood protozoa

The proportion of gender was mentioned before where 70.63% were females and the rest 29.37% were males. In case of gender-wise prevalence, a little difference was observed where females (24.78%) were infected with canine blood protozoa more than males (19.15%). The gender-wise prevalence is given in Table 4.

Table 4: Gender-wise prevalence of canine blood protozoa

Variables	**No. of examined**	**No. of infected**	**Prevalence %**	**P-value**
Male	47	9	19.15	0.442
Female	113	28	24.78	

4.2.6 Age-wise prevalence of blood protozoa

During sampling, all dogs were categorized into three groups, i.e., < 1 year, 1–2 years, and > 2 years. The highest prevalence (29.17%) was seen in the younger groups of age (<1 year), followed by 23.08% in the age group of 1–2 years, and 21.43% in the age group of more than 2 years. These results indicated more canine blood protozoan infections in puppies than in adults. Age-wise prevalence is shown in Table 5.

Table 5: Age-wise prevalence of canine blood protozoa

Variables	No. of examined	No. of infected	Prevalence %	P-value
< 1 year of age	24	7	29.17	0.730
1–2 years of age	52	12	23.08	
> 2 years of age	84	18	21.43	

4.2.7 Prevalence of canine blood protozoa in different locations

As mentioned before, a total of 160 stray dogs from 6 different locations in Dhaka city were included in this study. Microscopic examination of the blood revealed the highest prevalence in Basundhara R/A (27.27%), followed by Mirpur (26.19%), Farmgate (22.22%), Tejgaon (20.83%), Malibagh (19.23%), and Gulshan (12.50%). This area-wise prevalence is given in Table 6.

Table 6: Prevalence of canine blood protozoa in different locations

Areas	No. of examined	No. of infected	Prevalence %	P-value
Farmgate	27	6	22.22	0.923
Mirpur	42	11	26.19	
Malibagh	26	5	19.23	
Basundhara R/A	33	9	27.27	
Tejgoan	24	5	20.83	
Gulshan	8	1	12.50	
Total	**160**	**37**	**23.13**	

4.3 Prevalence of ticks

4.3.1 Overall prevalence of adult ticks in Dhaka city

A total of 160 stray dogs, varying in age and sex, were selected and examined for ticks after performing general anesthesia. Upon visual inspection, 49 of those study populations were found to be infested with brown dog tick, *Rhipicephalus sanguineus*. Area-wise overall prevalence (30.62%) is shown in Table 7. Concerning area-wise infestation (Figure 21), the highest prevalence was encountered in Basundhara R/A (42.42%), followed by Malibagh (30.77%), Farmgate (29.63%), Mirpur (28.57%), Tejgoan (25.00%), and Gulshan (12.50%). The infected dogs produced a total of 278 adult ticks where the average sex ratio was 3.21 indicating more female ticks on the host.

Table 7: Overall prevalence of adult ticks in Dhaka city

Areas	**No. of examined**	**No. of infected**	**Prevalence %**	**Collected female ticks**	**Collected male ticks**	**Sex ratio**
Farmgate	27	8	29.63	38	12	3.16
Mirpur	42	12	28.57	52	19	2.73
Malibagh	26	8	30.77	28	7	4.00
Basundhara R/A	33	14	42.42	62	18	3.44
Tejgoan	24	6	25.00	29	8	3.62
Gulshan	8	1	12.50	3	2	1.50
Total	**160**	**49**	**30.62**	**212**	**66**	**3.21**

4.3.2 Gender-wise prevalence of ticks

In our study, 47 dogs were male and the rest 113 were female. Gender-wise prevalence is shown in Table 8. Among the examined 47 male dogs, 10 (21.28%) were found infected with ticks. On the other hand, 39 dogs out of 113 female dogs comprising 34.51% of the prevalence rate were recorded in females during the study. Considerably, females were infected more the male dogs where there was no statistical difference.

Table 8: Gender-wise prevalence of ticks

Variables	No. of examined	No. of infected	Prevalence %	P-value
Male	47	10	21.28	0.098
Female	113	39	34.51	

4.2.3 Age-wise prevalence of ticks

The prevalence of tick infestation varied depending on the age of the studied samples (Table 9). A total of 160 dogs were grouped into 3 categories, i.e., less than 1 year, 1–2 years, and more than 2 years. Dogs aged less than 1 year showed the highest prevalence (37.50%) followed by 1–2 years (28.85%), and more than 2 years (29.76%) age groups. From these results, it was clear that young dogs were affected by ticks more than adults, although there was no statistical significance among them.

Table 9: Age-wise prevalence of ticks

Variables	No. of examined	No. of infected	Prevalence %	P-value
< 1 year of age	24	9	37.50	0.726
1–2 years of age	52	15	28.85	
> 2 years of age	84	25	29.76	

4.3.4 Seasonal Prevalence of ticks

The present study was initiated in November 2022, when we collected samples from dogs. Since then, the collection of ticks from dogs has continued till April 2023. Therefore, only two major seasons, namely Winter (November–February) and Summer (March–April) were included in our study. There were differences in tick burdens in different seasons with higher infestation levels in Summer (37.50%) followed by Winter (26.92%). Moreover, the monthly prevalence of tick infestation is given in Table 10 where the highest percentage of

tick-infested dogs were examined in March (37.84%) and the lowest percentage was marked in December (23.81%).

Table 10: Monthly prevalence (ectoparasites) of infected dogs

Months	No. of examined	No. of infected	Prevalence %	P-value	Seasons	Prevalence %	P-value
November	32	9	28.13	0.787	Winter	26.92	0.166
December	21	5	23.81				
January	31	8	25.81				
February	20	6	30.00				
March	37	13	35.84		Summer	37.50	
April	19	8	42.11				

4.3.5 Degree of tick infestation

In our study, ticks were removed individually from each dog, with an average of 1 to 16 ticks per dog. To determine the degree of tick infestation, a total 4 categories were identified, firstly, low infestation rate comprising 1-4 numbers ticks, secondly, mild infestation rate comprising 5-8 numbers ticks, thirdly, moderate infestation rate comprising 9-12 numbers of ticks, and finally, high infestation rate comprising 1-4 numbers of ticks. The degree of tick infestation having a statistical significance is included in Table 11.

Table 11: Determination of the degree of tick infestation

Degree of infestation	No. of ticks counted	No. of dogs infected	Prevalence %	P-value
Low (+)	1-4	22	44.89	0.001*
Mild (++)	5-8	16	32.65	
Moderate (+++)	9-12	7	14.28	
High (++++)	13-16	4	8.16	

*= Statistically significant

4.3.6 Regions of infestation by ticks on dog's bodies

Predominately, five (5) regions were identified on the dog's body after reviewing the literature and these areas were the head with ears, neck and chest region, back region, abdomen, and legs. As we mentioned before, 278 adult ticks were collected during this study, while 95 ticks comprising nearly one-third of the total population were found around the neck and chest region, which expressed the highest percentage of other parts of the body with a statistical significance (P <0.001). On the other hand, the Back region comprised the lowest percentage of the availability of ticks. The attachment of ticks on the host's body is exhibited in Table 12.

Table 12: Attachment of ticks on the host's body

Attachment of ticks	No. of ticks counted	Prevalence %	P-value
Head with ears	56	20.14	<0.001*
Neck and chest region	95	34.17	
Back region	19	6.83	
Abdomen	72	25.89	
Legs	36	12.94	

*= Statistically significant

4.4 Haematological parameters of blood

Of the 160 samples collected from dogs, 49 were positive for *Rhipicephalus sanguineus* and 37 were positive for different protozoan infections. The average hematological values obtained from the healthy and infected groups are presented in Table 13. The RBC counts, Hemoglobin, and PCV of all infected dogs were numerically lower compared to the healthy group. The average values RBC (6.23, 4.51, and 4.69), Hemoglobin (15.48, 11.44, and 11.18), and PCV (43.80, 33.50, and 32.10) were recorded from the healthy group, the infected group with protozoa and infected group with ticks, respectively, which indicated the different degree of anemia. The average values of MCV, MCH, and MCHC were found in the normal range. On the other hand, the average WBC count was higher in the infected groups where the average value of WBC for protozoan was 16.90×10^3 cells/µL and the

average value of WBC for tick infestation was 17.30×10^3 cells/µL. When compared to the different leukocyte counts, the eosinophil of the infected groups showed higher values (11.00 % and 12.70% for protozoa and ticks, respectively) than the normal range indicating the parasitic infections.

Table 13: Average values of hematological profiles of dogs infected with protozoa and ticks compared to healthy dogs

Parameters	**Healthy Dog**	**Infected with protozoa**	**Infected with ticks**	**SEM**	**P-value**	**Reference Value**
RBC (10^6 cells/µL)	6.23^a	4.51^b	4.69^b	0.081	<0.001*	5.5-8.5
Hemoglobin (g/dL)	15.48^a	11.44^b	11.18^b	0.265	<0.001*	12-19
PCV (%)	43.80^a	33.50^b	32.10^b	0.901	<0.001*	37-57
MCV (fL)	70.34	74.78	68.56	2.140	0.143	66-77
MCH (Pg)	24.87	25.49	23.89	0.639	0.238	19.5-24.5
MCHC (%)	35.43	34.24	35.06	0.857	0.662	32-36
WBC (10^3 cells/µL)	13.66^b	16.90^a	17.30^a	0.295	<0.001*	6-17
Neutrophil (%)	70.70^a	66.40^b	65.90^b	1.244	0.024	58-85
Lymphocyte (%)	14.50	13.40	11.30	0.928	0.065	8-21
Monocyte (%)	8.80	9.20	10.20	0.460	0.134	2-10
Eosinophil (%)	6.00^b	11.00^a	12.70^a	0.673	<0.001*	0-9

*= Statistically significant

RBC=Red Blood Cell, PCV=Packed Cell Volume, MCV=Mean Corpuscular Volume, MCH= Mean Corpuscular Hemoglobin, MCHC= Mean Corpuscular Hemoglobin Concentration, WBC=White Blood Cell

4.5 Discussion

The present study revealed several blood protozoa in stray dogs with no clinical signs, along with one vector (tick) from the dogs. Nearly one-fourth (23.13%) of the study samples were infected with at least one protozoon *viz B. canis, B. gibsoni,* or *Hepatozoon* spp. The findings of this study were very similar to various reports in southeast Asia where the prevalence of canine blood protozoan infections reached up to 28% (Laummaunwai et al., 2014; Sontigun et al., 2022). However, other authors (Piratae et al., 2015; Juasook, et al., 2021) revealed more prevalence than our study. These reports of higher infection were observed due to their methodology where they applied molecular techniques. Although staining blood smears under a microscope is a quick and low-cost method to diagnose various blood protozoan infections, PCR-based techniques are more sensitive and provide more specific genetic and species information (Sainz et al., 2015; Das et al., 2020).

Among 160 examined dogs, *B. canis*, *B. gibsoni*, and *Hepatozoon* spp. were detected in 19, 7, and 16, dogs, comprising 11.88%, 4.38%, and 10.00%, respectively. Notably, *B. canis* was encountered the highest number found during the study which is similar to the studies in the above-mentioned areas including the Indian Sub-continent (Singh et al., 2014; Jain et al., 2017). Although Piratae et al., 2015 and Thongsahuan et al., 2020 found more infection with *Hepatozoon* spp. than *Babesia*, the variation in species-wise prevalence might be due to the geographic location, distribution of vectors, methods of samplings, etc. In addition to this, both biological (such as ticks) and mechanical (such as biting flies) vectors were commonly seen in the research area. Warm and muggy conditions may promote the development of ectoparasites and the spread of diseases carried by vectors. Moreover, this finding probably reflects the wide distribution of the vector, *R. sanguineus* (Singla et al., 2016; Rucksaken et al., 2019).

Of the 37 positive dogs out of 160 samples, a total of 33 (89.19%) had single infections while the rest 4 (10.81%) had shown mixed infections with two or more canine blood protozoa, comprising 8.11% and 2.70%, respectively. These results illustrate most of the samples were infected with one protozoon species, although the introduction of molecular tests revealed more canine tick-borne protozoan co-infections worldwide (Kordick et al., 1999; Shaw et al., 2001; Mylonakis et al., 2004; Kumar et al., 2007; Yabsley et al., 2008).

In our study, the proportion of gender in the observed population was nearly 2:1 where the females were 70.63% and the rest 29.37% were males. In case of gender-wise prevalence, females (24.78%) were more infected than males (19.15%). Moreover, all dogs were categorized into three groups, where the highest prevalence (29.17%) was seen in the younger groups of age (<1 year), followed by 23.08% in the age group of 1-2 years, and 21.43% in the age group of more than 2 years. It was clear that the prevalence of blood protozoa was found highest in young dogs. These results may be due to various risk variables including immunity, habitat, interaction, etc. (Abdullahi et al., 1990; Samradhni et al., 2005). In case of gender, it had been observed that the prevalence of the protozoa among male and female dogs was inconsistent with those reported by Amuta et al. (2010) and Singh et al. (2011). The physiological stress experienced by females during nursing, oestrus, and pregnancy may be the cause of this greater incidence.

All the dogs selected for the study were examined for ticks after performing general anesthesia. By visual assessment, 49 of those study populations were infested with *Rhipicephalus sanguineus*. A total number of 278 adult ticks were gathered from the infected dogs where the average sex ratio was 3.21 indicating more female ticks on the host. The sex ratio is in complete disagreement with Dantas-Torres and Otranto, 2011 who reported more male ticks. This variation may be due to the more attachment time of females than males.

This study revealed a moderate prevalence of ticks in dogs sampled in Dhaka city and the percentage was 30.62%, which was very similar to Zeb et al., 2013. However, the results of this study showed a lower prevalence than the neighboring countries where the prevalence of ticks in India, Pakistan, and Indonesia have been reported at 45.0%, 53%, and 67.9% respectively (Bhadesiya et al., 2014; Soundararajan, 2016; Grant et al., 2023). On the other hand, Shimada et al., 2003; Ul-Hasan et al., 2012 and Saleh et al., 2019 reported a much lower prevalence than the present study. This fluctuation in prevalence might be brought on by factors such as climate, geographic distribution, sample size, methods of sample collection, etc.

Among the examined 47 male dogs, 10 (21.27%) were found infected with ticks. On the other hand, 39 dogs out of 113 female dogs comprising 34.51% of the prevalence rate were

recorded during the study. Considerably, females were infected more than the male dogs. This may be due to the reason the female dogs have a sitting habit on the ground, nursing their puppies which easily makes them available to be infested with ticks (James-Rugu and Jidayi, 2004). Age-wise prevalence showed young dogs were affected with more ticks than adults which may be related to gradual immunity development and close closeness to the ground (Abdulkareem et al., 2018).

There were differences in tick infestation in different seasons with higher infestation levels in Summer (37.50%) followed by Winter (26.92%). This could be attributed to a number of different climate factors in the research area. Moreover, the monthly prevalence of the highest tick infestation was examined in March (37.83%) and the lowest percentage was marked in December (23.80%). *Rhipicephalus* in stray dogs had shown their activities mainly in Spring. It had been encountered that *R. sanguineus* was found lower in the Winter season (November to January), with a peak activity in March (Bouattour, 2002).

The dog's body was primarily divided into five (5) regions: the head with ears, the neck and chest region, the back region, the abdomen, and the legs. While the back region had the lowest percentage of tick availability, the neck and chest appeared to be the most favored preference sites for ticks on dogs. This supports past studies that head, neck, and legs were the most common tick attachment sites. (Foldvari and Farkas, 2005).

Canine babesiosis and hepatozoonosis are important tick-borne diseases that infect dogs worldwide. The results of this study indicated that both protozoa and ticks were considered risk factors showing significantly lower RBC, HB, and HCT or PCV volumes. However, MCV, MCH, and MCHC values were observed in the reference limits. The results from RBC parameters indicated normocytic normochromic anemia, which is non-regenerative due to bone marrow dysfunction (Fleischman, 2012). These RBC indices, which were computed from blood samples infected with both protozoa and ticks, were below the accepted reference limits and consistent with previously published findings (Salakij et al., 1999; Das and Konar, 2013; Wongsawang and Jeimthaweeboon, 2018; Piratae et al., 2019). In fact, ehrlichiosis was linked to permanent bone marrow damage, according to a prior study (Skotarczak, 2003). Anemia is a common finding in canine blood protozoan infection, which occasionally can be severe (Waner et al., 2001; Baneth et al., 2001; Baneth

et al., 2003; Das and Konar, 2013; Bhadesiya and Raval, 2015; Paiz et al., 2016; Wongsawang and Jeimthaweeboon, 2018).

Moreover, WBC abnormalities were also found in protozoa and tick-infected dogs compared to the healthy ones which was in agreement with Salakij et al. (1999) and Wongsawang and Jeimthaweeboon (2018). However, WBC counts were higher in both cases of infected dogs than in healthy ones, which is indicative of leukocytosis. The high WBC counts hereby observed corresponded to increased eosinophil numbers, which is consistent with the previous findings (Mundim et al., 2008). However, eosinophilia is not exclusive to protozoal infections; it can also be associated with various other conditions, such as allergies, fungal infections, and certain autoimmune diseases. A comprehensive veterinary evaluation, including a thorough history, physical examination, and diagnostic tests, is essential to determine the underlying cause of increased eosinophil counts in dogs. These data support the fact that hematological abnormalities help to identify tick and protozoan infections as well as guide veterinarians in the clinical diagnosis of canine blood parasitic infections (Piratae et al., 2019).

Chapter V

Summary and Conclusion

Ticks are considered one of the important obligate blood-sucking arthropods after mosquitoes. They parasitize many vertebrates and take a blood meal which is distributed all over the world. Besides, they crucially transmit a large number of protozoa, like *Babesia, Theileria, Anaplasma, Ehrlichia, Hepatozoon*, etc. Several ecological parameters, including seasonal variations, tick survival, and tick development, particularly temperature, relative humidity, and vegetation, are linked to the transmission of these ectoparasites. Since dogs are the most common companion pets worldwide, they can transmit pathogens to humans having public health importance.

In Bangladesh, limited studies have been found on the prevalence of ticks in dogs. Therefore, this study was aimed to observe the prevalence of canine tick-borne protozoan infections, as well as their associated hematology. A total number of 160 street dogs were randomly investigated from different locations for this study. Dogs of different sexes and age groups were selected for this study based on their availability. The study was carried out in the laboratory for the morphological identification of ectoparasites as well as hematological examinations after the collection of samples. Examination of blood smear was performed under a light microscope where 37 out of 160 samples (23.13%) were infected with three species of blood protozoa. These species were *Babesia canis*, *Babesia gibsoni*, and *Hepatozoon* spp. The proportion of gender in the observed population was nearly 2:1 where females (24.78%) were more infected than males (19.15%). Moreover, all dogs were categorized into three groups, showing the highest prevalence (29.17%) in the younger groups of age (<1 year), followed by 23.08% in the age group of 1-2 years, and 21.43% in the age group of more than 2 years.

Only one tick species, *Rhipicephalus sanguineus* was found during the study which revealed a moderate prevalence (30.62%) of tick infestation. There were differences in tick infestation according to the seasons with higher infestation levels in Summer (37.50%) followed by Winter (26.92%). The neck and chest appeared to be the highest preferred preference areas for ticks on dogs.

This study demonstrates the variety of canine tick-borne protozoan infections that may be associated with certain hematological changes. Symptoms of these protozoan infections included eosinophilia, leukocytosis, and anemia. Additionally, compared to animals with normal hematological profiles, this study showed that dogs with lower RBC, Hb, and PCV values were more likely to acquire blood parasite infections.

Finally, it can be concluded that the gold standard for diagnosing blood protozoa is the examination of stained blood smears under the microscope. However, PCR provides improved sensitivity and in-depth knowledge of specific species and genetics. These results strongly suggest that necessity of frequent blood examinations is necessary to enhance animal welfare and disease prevention.

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Appendix I

Questionnaire

Department of Microbiology & Parasitology

Sher-e-Bangla Agricultural University

Dhaka-1207

Serial no.:……..

1. Area of Dog's collection:
2. Date of catching:……..
3. Date of anesthesia:……
4. Anesthetic agents: Atropine Xylazine Ketamine
5. Date of examination:…….
6. Collection of blood: Yes No
7. Body weight of dog in Kg:…….. ……..
8. Tentative age of dog in months:……..
9. Sex of dog: Male Female:
10. Information of ticks' collection

Head & Ear	Neck & Chest	Abdomen	Back	Legs	Total

Appendix II

Composition of different chemicals

1. 0.1N HCl

Ingredients	Composition
Distilled Water	96.88 ml
Hydrochloric Acid	1.85 ml
Sodium Hydroxide	0.79 ml
Arsenic Trioxide	0.49 ml

2. Hayem's Solution

Ingredients	Composition
Sodium Chloride	0.5 gm
Sodium Sulphate	2.5 gm
Mercuric Chloride	0.25 gm
Distilled Water	100 ml

3. Giemsa Solution

Ingredients	Composition
Giemsa Powder	3.80 gm
Methanol	250 ml
Glycerin	250 ml

4. 10% Giemsa Working Solution

Ingredients	Composition
Giemsa Solution	10 ml
Distilled Water	90 ml

5. 70% Ethanol

Ingredients	Composition
Ethanol	70 ml
Distilled Water	30 ml

6. 10% KOH

Ingredients	Composition
KOH	10 gm
Distilled Water	100 ml

7. 3% Acid Alcohol

Ingredients	Composition
HCl	3 ml
Absolute ethanol	97 ml
Glycerin	250 ml