PREVALENCE OF BLOOD PARASITES OF DOGS (PET AND STRAY) IN BHAKTAPUR MUNICIPALITY

Semsal Tamang

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In the partial fulfillment of the requirements for the award of the degree of Master of Science in Zoology with special paper Parasitology

Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

2023

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself and has not been submitted elsewhere for the award of any degree. All source of information have been specifically acknowledged by reference to the author(s) or institutions(s).

Date:-..14-05-2023....

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Semsal Tamang

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल।
Kirtipur, Kathmandu, Nepal.

☎ ०१-४३३१८९६
01-4331896
Email: info@cdz.tu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

Central Department of Zoology
T.U., Kirtipur

RECOMMENDATION

This is to recommend that the thesis entitled **"PREVALENCE OF BLOOD PARASITES OF DOGS (PET AND STRAY) IN BHAKTAPUR MUNICIPALITY"** has been carried out by Semsal Tamang for the partial fulfillment of Master's Degree of Science in Zoology with special paper Parasitology. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

Date:-..14.-05.-.2023

.....

Janak Raj Subedi

Lecturer

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल।
Kirtipur, Kathmandu, Nepal.

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Email: info@cdz.tu.edu.np
URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

LETTER OF APPROVAL

On the recommendation of supervisor "Janak Raj Subedi" this thesis submitted by Semsal Tamang entitled **"PREVALENCE OF BLOOD PARASITES OF PET AND STRAY DOGS IN BHAKTAPUR MUNICIPALITY"** is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

Date:- 14-05-2023

.....

Professor Dr. Kumar Sapkota

Head of Department

Central Department of Zoology

Tribhuvan University

Kirtipur, Kathmandu, Nepal

त्रिभुवन विश्वविद्यालय
TRIBHUVAN UNIVERSITY
प्राणी शास्त्र केन्द्रीय विभाग
CENTRAL DEPARTMENT OF ZOOLOGY
कीर्तिपुर, काठमाडौं, नेपाल ।
Kirtipur, Kathmandu, Nepal.

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URL: www.cdztu.edu.np

पत्र संख्या :-
च.नं. Ref.No.:-

Central Department of Zoology
T.U., Kirtipur

CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Semsal Tamang entitled **"PREVALENCE OF BLOOD PARASITES OF DOGS (PET AND STRAY) IN BHAKTAPUR MUNICIPALITY"** has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

EVALUATION COMMITTEE

.....	.....
Supervisor	Head of Department
Janak Raj Subedi	Prof. Dr. Kumar Sapkota
Lecturer	Professor
Central Department of Zoology	Central Department of Zoology
Kirtipur, Kathmandu, Nepal	Kirtipur, Kathmandu, Nepal
.....	.....
External Examiner	Internal Examiner
Dr. Tulsi Ram Gompo	Assoc. Prof. Dr. Kishor Pandey
Senior Veterinary Officer	Central Department of Zoology
Central Veterinary Laboratory	Tribhuvan University
Kathmandu, Nepal	Kirtipur, Kathmandu, Nepal

Date of Examination:-..04/07 2023

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
CDC	Centers for Disease Control and Preventions
CME	Canine Monocytic Echrlichiosis
CVBD	Canine Vector-borne Disease
EDTA	Ethylene Diamine Tetraacetic Acid
PCR	Polymerase Chain Reactions
RBC	Red Blood Cell
SPSS	Statistical Package for Social Science
TU	Tibhuvan University
WHO	World Health Organization

ABSTRACT

Dogs are the companion's animals that are kept primarily for social benefits and utilitarian purposes. They can be affected by various kinds of blood parasites that are zoonotic to human and are worldwide in distribution. A cross sectional study was conducted from September 2022 to February 2023 to investigate the prevalence of blood parasites of dogs (pet and stray) in Bhaktapur municipality. Altogether, 150 blood samples, 50 from pet and 100 from stray dogs were collected with the help of a trained veterinary assistant from saphenous or cephalic vein of dogs. Subsequently, the blood samples were examined through microscopic examination for the detection of blood parasites. The overall prevalence of blood parasites was recorded as 27.33% in dogs. On individual analysis, twenty percent of pet samples and thirty one percent of stray dog's samples were found to be infested with various types of blood parasites. Altogether, four species of parasites with higher prevalence of *Anaplasma* spp. (18.67%), followed by *Babesia* sp. (6.67%), *Ehrlichia* sp. (2.67%) and *Hepatozoon* sp. (1.33%) were observed in the samples. The younger dogs recorded higher infestation (31.25%) followed by older (>12 months) (27.2%) and puppy (22.22%). Female dogs showed higher prevalence (27.65%) as compared to male (27.18%). Higher infestations of parasites were recorded from local breed (21.33%), followed by Japanese breed (1.33%). In the study, the burden of parasites was slightly higher in dogs infested by ticks (52.30%) than tick free dogs. From tick infestation dogs, *Anaplasma* spp., *Babesia* sp. and *Hepatozoon* sp. of blood parasites were recorded. Single infection of blood parasites was found higher (25.33%) than double infection (2%). There were no significant association between factor such as age, sex, breed and tick infestation with difference in the detection of blood parasites ($P>0.05$). However, there was a significant relationship found in species of blood parasites and concurrency of blood parasites ($P< 0.001$). Some of these blood parasites such as *Babesia* and *Ehrlichia* are zoonotic to human and cause significant production loss in the animals. We suggest effective treatment, control and prevention program to break transmission chain of blood parasites among the companion animals.

CHAPTER I

INTRODUCTION

1.1 Background

Companion animals such as cat, dogs and birds were domesticated for social benefit and utilitarian purposes that is for companionship, hunting, military or police activity; guarding, supporting for blind or deaf person (Day *et al*., 2012). On the other hand, they were domesticated to enhance physiological and psychological well being of many human (Beck and Meyers, 1996) and for their emotional advantages (Dotson and Hyatt, 2008). Among companion animals, dog was the first domesticated animal which carries out many roles and also called as "Man's Best Friend" in the western world. Egyptian police began to use dog from early last century due to their great ability to detect the smell which cannot be detected by human, (Traub *et al.,* 2005; Gracenea *et al.,* 2009; Ahmed *et al.,* 2014).

Dogs and other canines are affected by various types of endo and ecto-parasites such as many protozoa, bacteria and helminths which are transmitted by diverse range of arthropod vectors including ticks, fleas, lice, triatomines, mosquitoes, tabanids and phlebotomines sand flies (Dantas- Torres, 2008). The disease caused by these arthropods vectors called as canine vector-borne disease (CVBP) that cause a poor performance in dogs and cause economic losses to the owner (Soulsby, 1982; Pam *et al.*, 2018). Dogs serve as reservoirs, carrier and transmission of many pathogens including parasites (Robertson *et al.*, 2000). Not only this, from the public point of view, they were also the source of zoonotic pathogens (Day, 2016) including hemoparasites (Dantas- Torres, 2008). Mostly stray dogs, plays main role in transfer of infection to human and animals than domesticated dogs (Harandi *et al.*, 2011). The factors that affect the prevalence of the parasites include age, gender of the dogs, anthelmintic treatments, immune status, owner's social status (Matallah *et al.*, 2018), living conditions, animal density, nutritional status (Premaalatha *et al.*, 2018) and breed (Scaramozzino *et al.*, 2008). Mainly young children, pregnant women, immune suppressed and elderly people are at risk of severe disease (Irwin, 2002).

Zoonotic disease has become a major problem for human health, since human live in close with companion animals. According to World health organization (WHO), there

exist 200 known type of disease that is naturally transmissible from vertebrate animals to human. Dogs are exposed to ecto-parasites that transmit haemo-parasites which are zoonotic important common dermatological disease of dogs and cats (Moriello, 2003). In tropical and subtropical regions across the world, tick borne hameoprotozoan diseases such as Babesiosis, Hepatozoonosis and Trypanosomiasis are major affecting canine species because of high prevalence of tick vector (Adhikari *et al.*, 2016). The diseases have seasonal characters primarily because the population density of Arachnids like ticks varies throughout the year which tends to be more active during warmer months (Bowman, 2009; Pavlovic *et al.*, 2012). The factors such as climate change, habitat management, increase in tick number and socio-economic condition plays main role in increase in these diseases (Godfrey and Randolph, 2011). To minimize or managed this diseases, there need to be understand interaction among these factors.

Canine babesiosis is a worldwide significant tick borne disease (Solano-Gallego *et al.*, 2016) and an emerging infectious disease (Solano-Gallego and Baneth, 2011) where member of genus *Babesia* readily parasites red blood cells causing progressive anemia and/or a sever inflammatory reaction associated (Koster *et al.*, 2015). Babesiosis is intraerythrocytic which are commonly called as piroplasms due to pear-shape forms (Telford *et al.*, 1993, Smith *et al.*, 2013). The larger species, *Babesia canis* (approximately 3-5µm), transmitted by tick *Dermacentor reticularis* is consider endemic in Europe (Solano-Gallego and Baneth, 2011) whereas smaller *B. gibsoni* (approximately 1-3 µm) transmitted by *Haemaphysalis bispinosa* and *H. longicornis* is consider endemic in Asian. Similarly, *B. rossi*, transmitted by *H. elliptica* in South Africa, *B. vogeli*, transmitted by *Rhipicephalus sanguineus* in tropical and sub-tropical region (Irwan, 2009). This leads to chronic or subclinical to acute and fatal, depending on present of virulence of *Babesia* species and host susceptibility (Schoeman and Leisewitz, 2006). *Babesia* was transmitted across the placenta (Fukumoto *et al.*, 2005), through a blood transfusion from infected dogs (Stegeman *et al.*, 2007) and in case of *Babesia gibsoni*, via dog bites and other aggressive activities (Jefferies *et al.*, 2007).

Hepatozoon canis is apicomplexan protozoan parsites which is transmitted through the ingestion of tick *R. sanguineus* not by the ticks bite. It is because the infective stage doesn't reach the salivary glands of the vectors (Baneth *et al.*, 2001). The disease caused by Hepatozoon is called hepatozoonosis. *Hepatozoon canis* and *H. americanum* are the agent of canine hepatozoonoses that ranges from subclinical infection to a sever life-

threatening illness characterized by high levels of parasitemia, fever, anaemia and lethargy (Baneth *et al.*, 2000; Baneth *et al.*, 2003). Prevalence of Hepatozooncanis (31.43%) in Nepal has been reported by Daiz- Reganon *et al.*, (2020). Co-infection of *H. canis* with other infectious agents such as *Ehrlichia*, *Leishmania* and parvovirus is a common condition (Baneth and Weigler, 1997; Sahu *et al.*, 2016).

Anaplasma and *Ehrlichia* species belongs to the family of Anaplasmataceae. *Anaplasma* is an intracellular rickettsial organism that causes canine Anaplasmosis. There are two species of *Anaplasma* that has been identified as pathogenic in dogs. It infects granulocytes, predominantly neutrophils but also eosinophils, where it exist and reproduce in membrane-bound vesicles, forming micro colonies called morulae. *A. platys* frequently infect platelets and cause an infectious canine cyclic thrombocytopenia and *A. phagocytophilum* which parasitizes neutrophils cause granulocytic anaplasmosis (Mylonakis *et al.*, 2004). *A. phagocytophilum* is spread through ticks of genus *Ixodes* such as *Ixodes scapularis* in Northeast and Midwestern United states, *Ixodes pacificus* in West coast (CDC, 2019), *Ixodes ricinus* in Europe (Sainz *et al.*, 2015). Most dogs naturally infected with *A. phagocytophilum* probably remain healthy (Folley, *et al.*, 2001). There is no case reports of fatalities in dogs recorded till today.

Ehrlichia species, which affects dogs, humans and other domestic and wild animal species have a worldwide distribution with a high infection rates and disease primarily in tropical and subtropical areas. The disease cause by it is called Ehrlichiosis (Lanza-Perea *et al.*, 2009). *E. canis* is a gram negative intracellular rickettsia, which is the causative agent of Canine Monocytic Ehrlichiosis (CME) causing various signs from asymptomatic to sever illness associated with pancytopenia, hemorrhage and bone marrow hypoplasia (Harrus and Waner, 2011). In global context, prevalence of CVBD was shown higher in North Carolina (82%) by Balakrishnan *et al.*,(2014). In national context, research conducted by Daiz- Reganon *et al.*, (2020) showed higher infections (81.43%).

Most of dogs in Nepal are free to roam and suffer from malnutrition, disease and lack basic health care (Massei *et al.*, 2016). In Bhaktapur as well, we found a large number pet and stray dogs. Bhaktapur municipality is one of municipality among four different municipalities of Bhaktapur district. Due to migration of people from different regions of Nepal, rapid urbanization is found. Along with rapid urbanization, dog's numbers are also increasing day by day as the dogs live in close association with human. The main aim of

this study is to know the prevalence of blood parasites in both stray and pet dogs of Bhaktapur municipality and access the awareness of pet owner about blood parasites of dogs and to recommended preventive measures of canine blood parasites.

1.2 Objectives

a. General objective

i. To study the prevalence of blood parasites of dogs (pet and stray) in Bhaktapur Municipality.

b. Specific objective

i. To estimate the distribution of blood parasites with dog types, gender and their age category.

1.3 Significance of the study

Bhaktapur municipality is a rapidly urbanizing municipality of Bhaktapur districts. Urbanization has created several issues for stray animals. A huge number of stray dogs are found roaming around the open streets. Also, the increasing trend of pet rearing is promoting more pet dogs as well. The people are much more concerned about their pet's health as well as some of stray dogs of their community and take them to the clinic or hospitals for their treatment. However, dogs carried to clinic/hospitals were treated symptomatically without using any diagnostic methods. In context of Nepal, diagnosis of parasites through blood examination is not common practice. Recently published journal reveal that there is a high prevalence of blood parasites in hyperthermic dogs (Bhatta *et al.*, 2017). But most of the dogs which don't show any symptoms can also act as reservoirs and carriers of blood parasites.

There is no organized research on canine blood parasites in this municipality. In this study, investigation effort has been focused on prevalence of blood parasites of dogs (pet and stray) in Bhaktapur municipality and to recommended preventive measures of canine blood parasites. This study will also form a base for the future investigators those who were investigating on this topic.

1.4 Limitation of the study

- Some of the blood samples of stray dogs were collected during castration of stray dogs and some with the help of community member so it's easy to collect more blood samples of stray dogs than pet dogs.
- In the present study, microscopic examination was done to identify parasites but it is better to used molecular or serological methods to diagnosed blood parasites.

CHAPTER II

LITERATURE REVIEW

2.1 Background

Those organisms that live in the blood of their animal host (range from single-celled protozoa to more complex bacteria and rickettsia) are called blood parasites. Parasitic blood infection targets red blood and white blood stream leading to anemia and death of animals. Canines are more susceptible to many blood parasitic diseases such as babesiosis, leishmaniasis, anaplasmosis etc. which are transmitted by diverse range of arthropod vectors including ticks, fleas, lice, triatomines, mosquitoes, tabanids and phlebotomines sand flies (Dantas- Torres, 2008) that cause poor performance in animals and the economic loss of the owner's mortality and morbidity of animals (Soulsby, 1982; Pam *et al.*, 2018,). Dogs serve as reservoirs, carrier and transmission of many pathogens including parasites (Robertson *et al.*, 2000). Not only this, from the public point of view, they were also the source of zoonotic pathogens (Callisto, 2013) globally. Many researches have been done in worldwide considering of significant effect of blood parasites in canine including dog. Different areas, countries or continent have different research and analysis. To some extent, some of research which is similar to this research has been reviewed.

Oduye and Dipeolu (1996) reported 49% of blood parasites with higher infection of *Babesia canis* in dogs of Ibadan. Hii *et al.*, (2012) from Australia reported 29.13% of canine vector born disease. In the same manner, Pam *et al.*, (2013) found the prevalence of haemo (43%) and gastrointestinal parasites (39%) in dogs in Vom, Jos south local government, Plateau state and species of *Babesia canis*, *Oncicola canis*, *Dipllidium caninum* and *Diphylobotum latum*. From Europe, Hamel *et al.*, (2015); Andersson *et al.*, (2017); Hofmann *et al.*, (2019); Jurkovic *et al.*, (2019) reported 6.1%- 52% of infection of blood parasites. Similarly study conducted by Balakrishnan *et al.*, (2014) on serological and molecular prevalence of selected canine vector born pathaogens in blood donor candidates, clinically healthy volunteers and stray dogs in North Carolina recorded 82% of dogs have been exposed to or were infected with one or more CVBD pathogens.

Gadahi *et al.*, (2008) conducted a survey on prevalence of blood parasites in stray and pet dogs in Hyderabad area: comparative sensitivity of different diagnostic techniques for the

detection of microfilaria and recorded *Dirofilaria immitis*, *Dipetalonema reconditum* and *Babesia canis*. The parasitic species observed in the study conducted by Kumar *et al*., (2009) in canines in Chennai city identified various haemoprotozoa and rickettsia. The haemoprotozoans such as *Babesia gibsoni* (84.9%), followed by *Ehrlichia canis* (6 %), *Hepatazoan canis* (4.8%), Babesia *canis* (3.9%) and *Trypanasoma evansi* (0.4%) were found as a major haematoprotozan among positive cases. Survey done by Rani *et al.*, (2011) in India reported 2.3% infection of blood parasites through microscopy examination while 49.7% infection through PCR methods. Research done by Bhattacharjee and Saman (2013) in hospital dogs of India recorded 57.31% prevalence of haemoparasites of which pet (58.03%), working (54.54%) and stray (63.64%) infections were recorded.

Another study done in dogs in and around Bhubaneswar, Odisha by Sahu *et al*., (2014) found positive for *Babesia gibsoni*, *Babesia canis*, *Hepatozoon canis* and *Trypanosoma evansi*. In same manner, study had done in Cyprus by Attipa *et al.*, 2016, in Turkey by Guven *et al.*, (2017). Similarly, a survey of parasites in stray dogs from Pudupasar and chow kit area of Kuala lumpur done by Premaalatha *et al.*, (2018) recorded *Babesia canis*, *Babesia gibsoni*, *Ehrlichia canis*, *Hepatozoon canis* and microfilaria of *Dirofilaria immittis*. The study has done by Cavalho *et al*., (2020) and found 14% prevalence of canine visceral leishmaniasis among 385 dogs. In Combodia, Inpankaew *et al.*, (2016); Nhuong *et al*,. (2020) had done in CVB blood parasites. Out of 474 blood sample collected, 52.95% sample was found to be positive, with 50.60% for *E. canis*, 39.84% for *H. canis* and 9.56% for *B. canis* in the research done by Thongsahuan *et al.*, (2020) in Southern Thailand. Likewise, Iatta *et al.*, (2021) conducted research on vector-borne pathogens in dogs of different regions of Iran and Pakistan which showed 46.9% of haemoparasite infections. Most frequently detected parasite was *Hepatozoon canis* (41.4%), followed by *Anaplasma platys* (6.4%), *Ehrlichia canis* (3.4%), *Rickettsia* spp. (2.2%), *Babesia vogeli* (1.0%) and *L. Infantum* (0.3%). Study conducted by Rajakaruna *et al.*,(2021) found 25.3% of hemoparasites among Sir Lanka Air forced working dogs (22.5%), free roaming (26.3%) and privately-owned (26.2%). 57.4% of infected dogs were asymptomatic, which was significantly higher in free-roaming dogs (96.3%). Similarly, Sathish *et al.*, (2021) recorded *Anaplasma platys* as higher infection followed by *B. gibsoni*, *Ehrlichia canis*, *Babesia canis* and co-infection of *B. gibsoni* and *A. platys* and *B. canis*, *B. gibsoni* and *A. platys* in India by a multiplex PCR.

Jefferies *et al.*, (2007) studies on blood, bull terriers and Babesiosis: further evidence for direct transmission of *Babesia gibsoni* in dogs of Southern Australia and found 9.27% of collected sample positive for *B. gibsoni*, in Central America by Wei *et al.*, (2014). Similarly, survey done on small *Babesia* species in canine of Kerala, South India by Jain *et al.*, (2017) reported 47.3% prevalence of *B. gibsoni* in molecular evidence, while 26.67% in light microscopy. Dogs with chronic *B. gibsoni* infection were found to have anaemia and thrombocytopenia. In same manner, similar research from some of Asian country were done in Bangladesh, China, Iraq by Terao *et al.*, (2015); Guo *et al.*, (2020); Badawi and Yousif (2020). Similarly from Nigeria, Sasaki *et al.*, (2007); Obeta *et al.*, (2020) reported 2.3% and 10.8% *B. canis* infection and factor associated with the infection were age, use of dogs, tick infestation and seasons.

Jittapalapong *et al.*, (2006) conducted research on detection of *Hepatozoon canis* in stray dogs and cats in Bangkok, Thailand and recorded 2.6% prevalence of infection of dogs by microscopy and by PCR 11.4% and found that *H. canis* is indigenous to Thailand. Vertical transmission is also a possible route for *H. canis* infection (Schafer *et al.*, 2022). Similarly, *Hepatozoon canis* was reported by Spolidorio *et al.*, (2009) in the state of Espirito Santo, southeastern Brazil, by El-Dakhly *et al.*, (2013) in Japanese island and peninsulas, by Azmi *et al.*, (2016) from Palestine.

Anaplasma is an intracellular rickettsial organism that causes canine Anaplasmosis. It is another important haemoparasites. In North America, Anaplasma has been reported in Grenada, Haiti, Cuba (Yabsley *et al.*, 2008; Starkey *et al.*, 2016; Silva *et al.*, 2016). *A. platys* were first reported in Cuba in dogs and ticks in 2016. Similarly in Europe, infected dogs were found from Sweden, Germany, Greece (Egenvall *et al.*, 1997; Kohn *et al.*, 2011; Angelou *et al.*, 2019). Likewise, infection was found from Brazil, Western Morocco, Costa Rica, (Melo *et al.*, 2016; Khatat *et al.*, 2017, Springer *et al.*, 2019). Including Nepal (Bhatta *et al.*, 2018), different parts of Asia such as India, Thailand, Malaysia, Iraq (Bhattacharjee and Sarmah 2013; Liu *et al.*, 2016; Konto *et al.*, 2017; Ahmed and Khalaf, 2020) has reported infected dogs with *Anaplasma*.

Very few studies were conducted in blood parasites of dogs in Nepal. Most of the study conducted was based on the microscopy methods. Only few studies were based on molecular detection methods. *Trypanosoma* was reported from Makwanpur district by Maharjan and Mishra (2006). Subedi and Shrestha (2012) reported *Babesia canis*,

Babesia gibsoni and *Ehrlichia spp.* Similarly, Phuyal *et al*., (2017) conducted a research on prevalence of blood parasites in dogs of Kathmandu valley and reported 12% infection with *Babesia canis* and *Ehrlichia spp*. In the same manner, the research done by Bhatta *et al*., (2018) in hyperthermic dogs of Kathmandu valley reported *Babesia sp.*, *Ehrlichia sp.*, *Anaplasma sp.* and *Trypanosoma sp*. The study conducted by Daiz- Reganon *et al*., (2020) in stray dogs in Nepal reported a *Hepatozoon canis* (31.43%), *Anaplasma platys* (31.43%), *Ehrlichia canis* (27.14%), *Leshmania donovani* (18.57%), *Theileria spp.* (12.86%), *Babesia vogeli* (12.86%) and *Babesia gibsoni* (2.86%).

Dogs that are associated with different haemoparasites can pose a financial burden in worldwide including Nepal. Dog that were infected with haemoparasites have different sign and symptoms such as fever, anaemia, weight loss etc. Recent investigation has revealed that the hyperthermic dog have higher prevalence of blood parasites. In Nepal, there was little study on prevalence of blood parasites in dogs, so this study was conducted to determine the prevalence of blood parasites in dogs.

CHAPTER III

MATERIALS AND METHODS

3.1 Study area

Study site was Bhaktapur district which is located at Bagmati province and is one of the seventy-seven districts of a landlocked country Nepal. It covers an area of about 123.1 Km^2. It's headquarter is Bhaktapur. It lies on the elevation of 27.672222°N and 85.427778°E. Bhaktapur municipality is one of municipality of Bhaktapur district which is administratively divided into 10 wards. It covers an area of 16.89 Km^2. It lies on elevation of 27°40'20″N 85°24'40″E and in an altitude of 1401 meter above the sea level. It is surrounded by Changunarayan municipality in east and north, Madhyapur Municipality in west and Suryabinayak municipality in south. According to census 2021, the total populations of Bhaktapur municipality are 77,136.

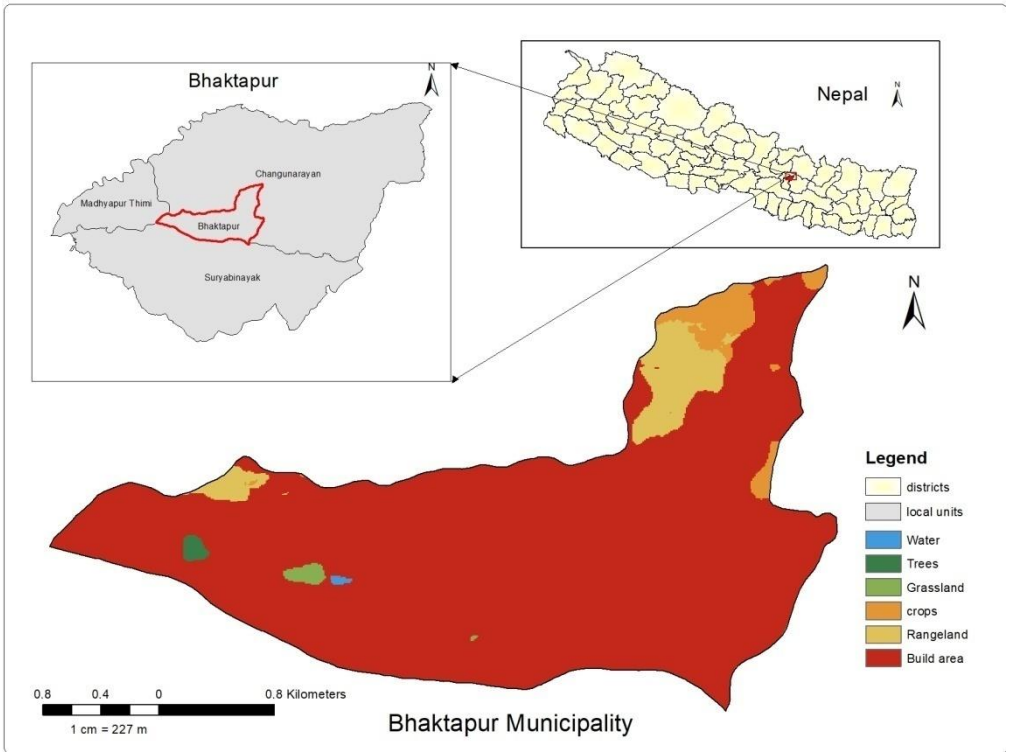

Fig. 1: Map showing study area

3.2 Materials

The materials that were used during study have been listed below:

3.2.1 For lab

i. Slides, slides box
ii. Forceps
iii. Micropipette, Micropipette tip
iv. Copling jar
v. Microscope
vi. Cotton

3.2.2 For field

i. Gloves
ii. Syringe
iii. Blood collecting tube

3.2.3 Chemicals required

i. Giemsa's stain
ii. Immersion oil
iii. Methanol
iv. Distilled water

3.3 Blood sample collection

Blood sample was collected from different area of Bhaktapur municipality irrespective of age, sex and breed from September 2022 - February 2023. Altogether 150 samples were collected, 100 samples were from stray dogs and 50 samples from pet dogs. With the help of veterinary assistant, from each dog, 3ml of blood was collected aseptically with the help of disposable syringe from the saphenous or cephalic vein of dogs. The collected samples were placed in a clean Ethylene Diamine Tetraacetic Acid (EDTA) tube which was used as anticoagulant and labeled with sample number. All the information like owners name, address, sex, age and breed were recorded from pet dogs whereas address, sex, age and breed were recorded from stray dogs. Dogs was classified into three age groups as puppies (zero - six months), young dogs (>six month to 12 months) and older (>12 months) as described by Bone, (1988). The age of the stray dogs was identified with

the help of local people and veterinary assistant. Similarly, health condition and presence or absent of ticks were also recorded.

3.4 Preservation of samples

The collected blood samples were immediately preserve in cool box after sample collections and were carried to lab of Central Department of Zoology, Kirtipur for immediate examination. If the lab work was delay, then samples were preserved in refrigerator at 4°C.

3.5 Preparation of slides and staining

Each collected blood samples were examined microscopically by using thin blood smear method staining with Giemsa's stain (Chatterjee, 2019). At first, a drop of blood was taken in a glass slide with the help of micropipette at a distance of about half an inch from the right end. A smear was prepared by using another slide tilted at an angle of 45° in contact with the drop of blood and pushed gently to the left till blood was exhausted and was air dried immediately. Then, the smear was fixed in methanol for three to five minutes by adding two to three drops. This fixed slide was then stained in working solution (5%) of Giemsa stain in coplin jar for 40-45 minutes. After that, the slide was washed slowly after removing from Giemsa stain and let it to dry and examined under microscope using oil immersion.

3.6 Microscopic examination of blood parasite

The stained slide was examined under oil immersion by using light microscope under high power magnification (100x) which is considered as best (Njunda *et al.*, 2013). The slides was examined from tail end to whole field parasites and focused and took photographs of parasites that were seen. The parasites were identified with the helps of experts and from different literatures following their morphological characters. *Babesia* was 1.5 µm – 5 µm long according to their species. Have or lack pear shape, pointed one end and round other, oval, round, signet ring form, multiple infection that is more than one organism upto 16 may be found in single RBC, depending on the stage of development of the parasites in RBC (Laha *et al.*, 2015). *Ehrlichia canis* is 0.5 – 0.9 µm in diameter and in microscopic examination of Giemsa stained blood smear, it were identified by the presence of morulae in mononuclear cell (Hildebrendt *et al.*, 1973). *Anaplasma* occurs intracellularly as solid dots on the margin or on center whereas some

species occurs predominantly in neutrophils where it forms host membrane enclosed morulae (Atif *et al.*, 2021) whereas *Hepatozoon* was frequently diagnosed on the basis of detection of ellipsoidal shaped gamonts in neutrophills in stained blood smear (Baneth and Shkap, 2003).

3.7 Statistical data analysis

The data were entered in the Microsoft Excell 2007. Data were coded and statistical analysis was done through SPSS V25. The association between outcomes and individual explanatory variables was assessed by using Pearson's Chi Square test. Level of confident was set at 95% and a value of $P< 0.05$ was considered significant.

3.8 Ethical, Legal and social implication

A prior permission was taken from Veterinary hospital and Livestock Expert Service Center (VHLESC), Bhaktapur and Bhaktapur municipality before conducting this study.

CHAPTER IV

RESULTS

4.1 Prevalence of blood parasites in dogs

A total of 150 blood samples (100 from stray dogs and 50 from pet dogs) were collected from the study area and examined. Among them, 41 (27.33%) blood samples were found positive with various types of blood parasites (Fig. 1).

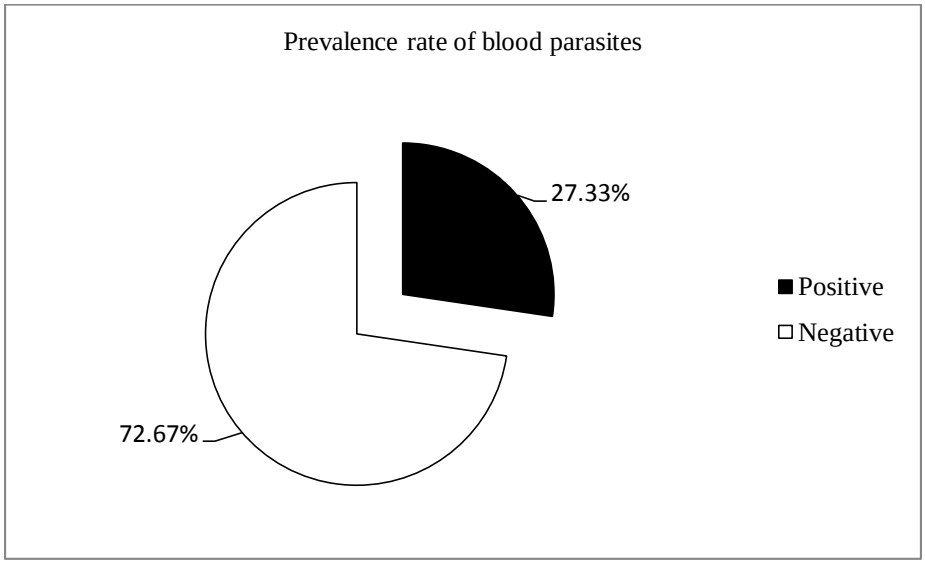

Fig. 2. Prevalence of blood parasites in dogs

4.2 Prevalence of blood parasites in pet and stray dogs

Among a total of 50 blood samples of pet dogs, 10 that are 20% of samples were found positive. And out of 100 blood samples of stray dogs, 31 (31%) were found to be infected with various types of blood parasites (Fig. 2) which was found statistically insignificant (χ^2= 2.03, d.f.= 1, P value= 0.154).

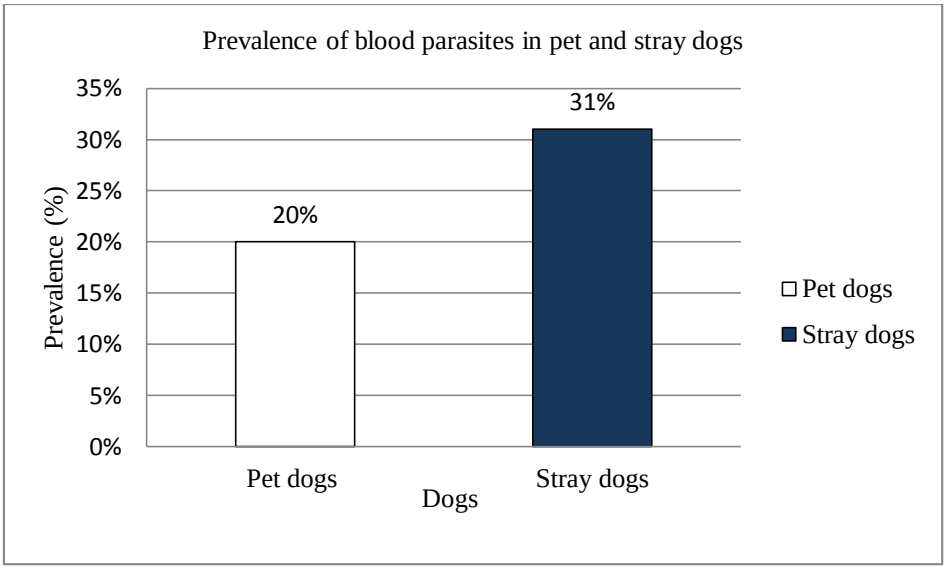

Fig. 3. Prevalence of blood parasites in pet and stray dogs

4.3 Prevalence of specific blood parasites

In both pet and stray dog, altogether 4 species of blood parasites, *Anaplasma* spp., *Babesia* sp., *Ehrlichia* sp and *Hepatozoon* sp. were found. Among them, *Anaplasma* spp. was found as highest prevalence rate (18.67%) followed by *Babesia* sp. (6.67%), *Ehrlichia* sp. (2.67%) and *Hepatozoon* sp. (1.33%) as shown in Table 1.

Table 1. Prevalence of specific blood parasites

S.N.	Genera of blood parasites	Total samples	Total positive samples	Prevalence rate
1.	*Anaplasma* spp.	150	28	18.67%
2.	*Ehrlichia* sp.		4	2.67%
3.	*Hepatozoon* sp.		2	1.33%
4.	*Babesia* sp.		10	6.67%

4.4 Prevalence of specific blood parasites in pet and stray dogs

The analysis done between pet and stray dogs showed that the stray dogs have higher prevalence rate of blood parasites (*Anaplasma* spp.=20%) as compared to that of pet dogs and lower prevalence of blood parasites *Ehrlichia* sp. in pet dogs (2%) and *Hepatozoon* sp. (2%) in stray dogs (Table 2). There was a significant difference between specific blood parasites and prevalence of blood parasites in stray and pet dogs.

Table 2. Prevalence of specific blood parasites in pet and stray dogs

Genera of blood parasites	Parasitic prevalence in pet dogs (n=50)	Parasitic prevalence in stray dogs (n=100)	Chi square value	P value
Anaplasma spp.	8 (16%)	20 (20%)	χ^2= 150	0.001
Ehrlichia sp.	1 (2%)	3 (3%)		
Hepatozoon sp.	-	2 (2%)		
Babesia sp.	3 (6%)	7 (7%)		

4.5 Age wise prevalence of blood parasites

A total of nine samples from puppy (zero to six month), 16 samples from young (>six months to 12 months) and 125 samples from older (>12 months) were collected during the study period. The overall prevalence of different age group of dogs showed that sample collected from young dogs recorded higher infection rate (31.25%), followed by older dogs (27.2%) and lower in puppy (22.22%) which is shown in Fig. 3.

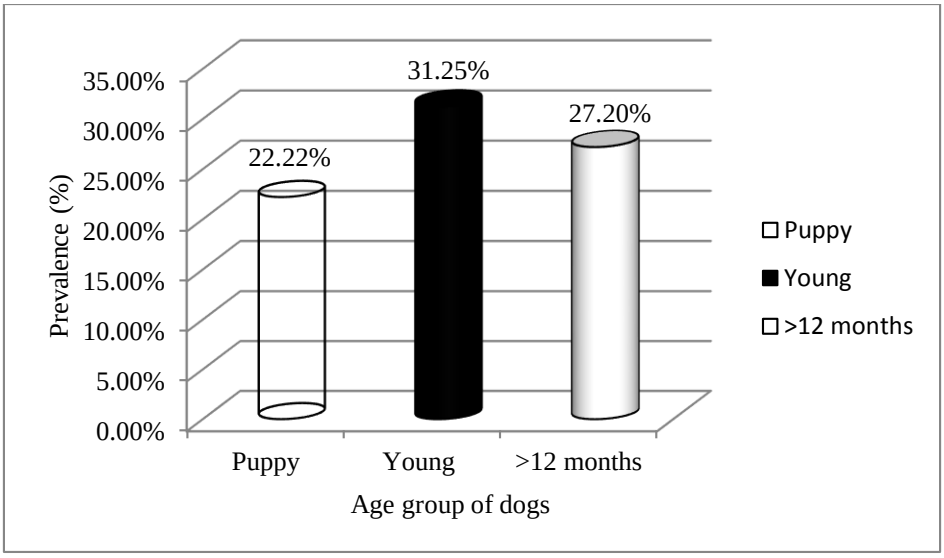

Fig. 4. Age wise prevalence of blood parasites

A total of four, seven and thirty nine samples were collected from puppy, young, and older (>12 month) age group of pet dogs. Among them, one (25%), two (28.57%) and seven (17.94%) of samples were found to be infected with different species of blood parasites. Similarly, in stray dogs there were five, nine and eighty six samples collected from each age group which found one (20%), three (33.33%) and 27 (31.39%) samples infected. Age wise prevalence analysis, showed that higher prevalence was found in stray dogs of age group six months to 12 months and lower in pet dogs of older age group (Fig. 4). There was no significant relationship between age of both pet and stray dogs with prevalence (Pet: χ^2= 0.486, d.f.=2, P= 0.784) (Stray: χ2= 0.311, d.f.= 2, P= 0.856).

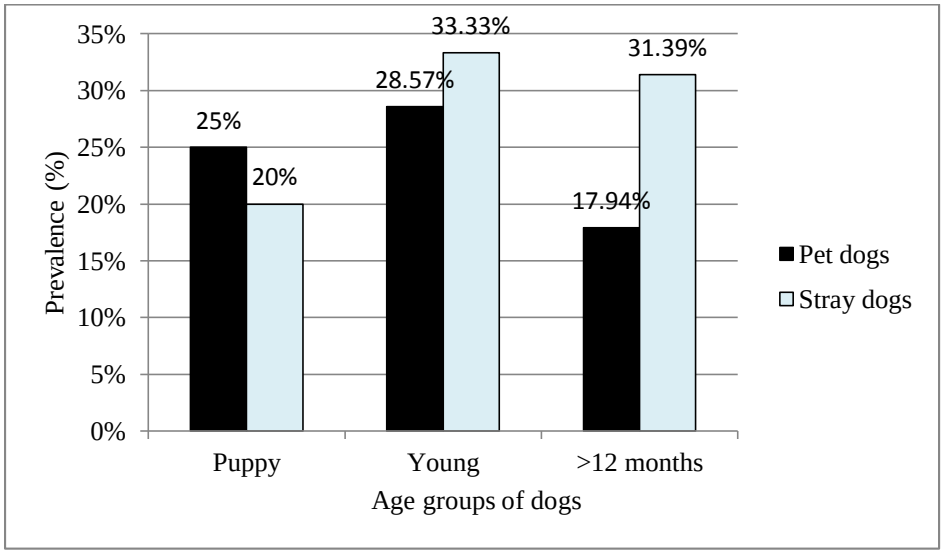

Fig. 5. Age wise prevalence of blood parasites in pet and stray dogs

4.6 Sex wise prevalence of blood parasites

Out of 103 blood samples collected from male dogs, 28 (27.18%) samples were found to be infected and out of 47 samples from female dogs, 13 (27.65%) samples of blood sample were found to be infected for different blood parasites (Fig. 5).

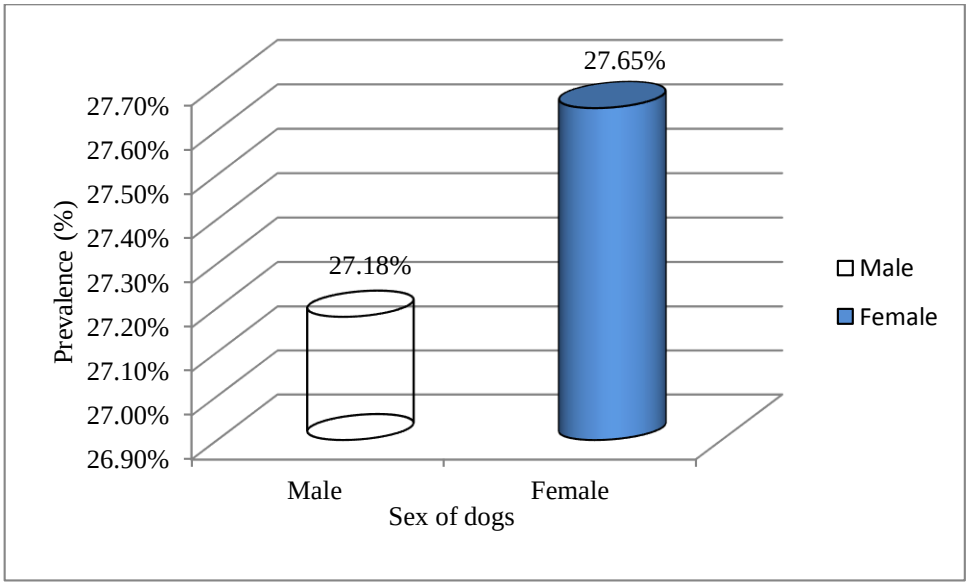

Fig. 6. Sex wise prevalence of blood parasites

During the study, 72 from male, 28 from female blood samples were collected from stray dogs and 31 and 19 samples from pet dogs. During analysis, female of stray dogs showed higher prevalence (39.28%) among both pet and stray dogs, whereas in pet dogs, male showed higher infections (25.80%) as compared to that of female (10.58%) which is shown in fig. 6. However there was no significant relationship seen in between prevalence of blood parasites and sex of pet and stray dogs (Pet: P= 0.190, Stray: P= 0.264).

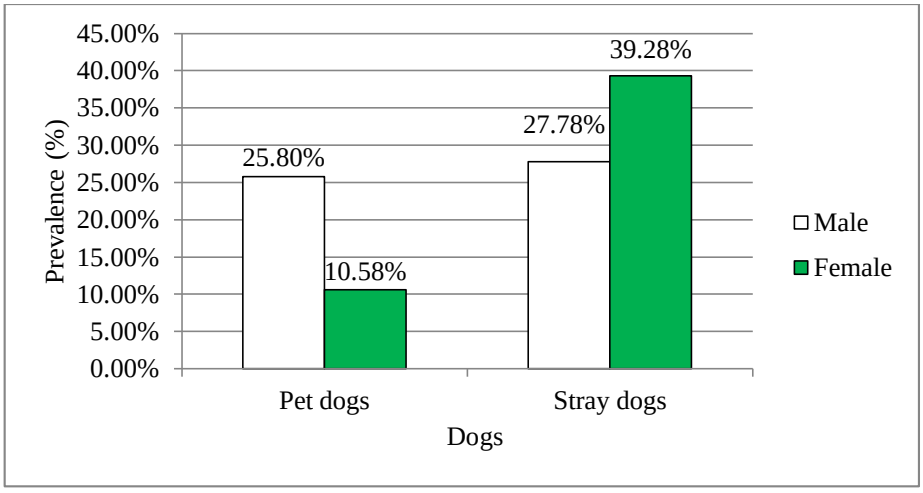

Fig. 7. Sex wise prevalence of pet and stray dogs

4.7 Breed wise prevalence of blood parasites in dogs

In pet dogs, altogether seven breeds of dogs were found whereas in stray dogs, only local breed of dogs were recorded. Higher prevalence of blood parasites was recorded in local dogs (21.33%) among both pet and stray dogs, whereas in pets dogs only, Japanese breed have higher (6%) prevalence of parasites. But there was no significant association between breed of dogs and prevalence of parasites (χ^2= 3.101, d.f.= 6, P= 0.796).

Table 3. Breed wise prevalence of blood parasites in dogs

S.N.	Breed	Pet dogs (n=50)	Stray dogs (n= 100)	Total (150)
1.	Cross breed (n=10)	2 (4%)	-	2 (1.33%)
2.	Golden retriever (n=8)	2 (4%)	-	2 (1.33%)
3.	Japanese breed (n=17)	3 (6%)	-	3 (2.0%)
4.	Cocker spaniel (n=7)	1 (2%)	-	1 (0.67%)
5.	Bilati (n=1)	0 (0%)	-	0 (0%)
6.	Local (n=105)	1 (2%)	31 (31%)	32 (21.33%)
7.	Pop (n=2)	1 (2%)	-	1 (0.67%)

4.8 Prevalence of blood parasites in tick infestation and non-tick infestation dogs

Out of 150 dogs that were chosen for sample collection, 21 dogs were infected by tick and 129 dogs were non-infected. Both types of dogs that are infected by ticks as well as non-infected by ticks found infected with blood parasites. The samples collected from 21 dogs infected by tick's results 11 (52.3%) samples positive whereas 129 samples taken from non-infected dogs results 30 (23.26%) sample infected with blood parasites (Fig. 7).

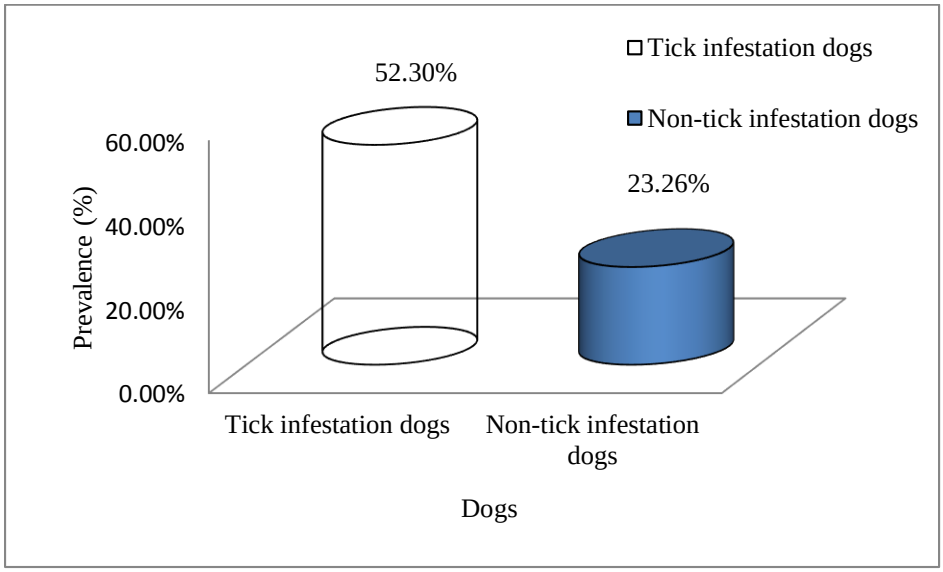

Fig. 8. Prevalence of blood parasites in tick infestation and non-tick infestation dogs

In both pet dogs and stray dogs, sample collected from the dogs that were infestation by ticks showed higher prevalence of blood parasites (66.67% and 50%) as compared to non-tick infestation dogs (Table 4) which was found statistically insignificant.

Table 4. Prevalence of blood parasites in tick infestation and non-tick infestation in pet and stray dogs

Dogs	Parasitic prevalence in tick infected dogs (n= 3, 18)	Parasitic prevalence in tick non-infected dogs (n= 47, 82)	Chi square value	P value
Pet dogs	2 (66.67%)	8 (17.02%)	4.344	0.37
Stray dogs	9 (50%)	22 (26.82%)	3.705	0.054

4.9 Occurrence of species of blood parasites in tick infestation dogs

Out of 21 blood samples collected from tick infestation dogs, 11 samples were found positive with three different species of blood parasites: *Anaplasma* spp., *Babesia* sp. and *Hepatozoon* sp. Among these blood parasites, *Anaplasma* spp. showed higher infection (33.33%) followed by *Babesia* sp. (19.05%) and *Hepatozoon* sp. (4.76%) in tick infestation dogs which are shown in figure 9.

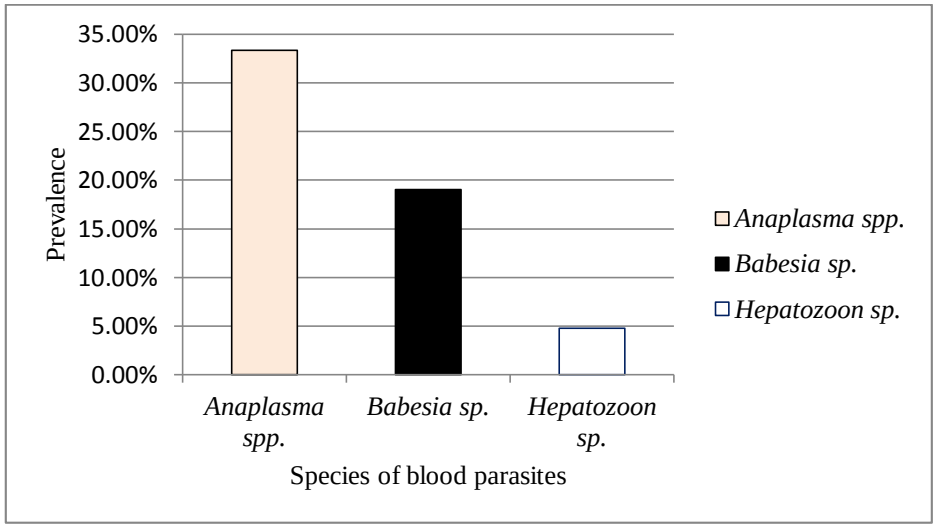

Fig. 9. Occurrence of species of blood parasites in tick infestation dogs

4.10 Concurrency of blood parasites in pets and stray dogs

Out of 150 samples, single infection was found higher (25.33%) and double infection lower (2%). In individual analysis of pet dogs and stray dogs, similar results obtained, single infections higher than double infections (Table 5) which showed significant difference ($p<0.001$).

Table 5. Concurrency of blood parasites in pets and stray dogs

Dogs	Total samples	Single infection	Double infections
Pet	50	8 (16%)	2 (4%)
Stray	100	30 (30%)	1 (1%)
Both	150	38 (25.33%)	3 (2%)

CHAPTER V

DISCUSSION

Dogs which was the first domesticated companion animals serves as reservoirs, carrier and transmission of different disease to human, livestock and wildlife. Different blood sucking arthropods vectors such as ticks, fleas, lice, triatomines, mosquitoes, tabanids and phlebotomines sand flies transmits different types blood parasites such as *Babesia* species, *Leishmania* species, *Anaplasma* species, *Hepatozoons* species, *Trypanosoma* species and many others parasites (Dantas- Torres, 2008) that causes different types of disease which results weakness, loss of weight of animals, anaemia etc. and in an extreme loads of parasites may lead to lethal of animals as well.

In the present study, it has been observed that out of 150 samples collected from pet and stray dogs, the overall prevalence of blood parasites were found to be 27.33%. This finding was similar to study done by Hii *et al.*, (2012) from Australia (29.13%), to that of Belgrade area (31.32%) by Pavlovic *et al.*, (2017), in Sir Lanka (25.3%) by Rajakaruna *et al.*, (2021). The current findings was higher than the study conducted by Kumar *et al.*, (2009) on prevalence of haemoprotozoans in canines in Chennai city which records 11.6% prevalence of parasites. Similarly study done from Nepal by Subedi and Shrestha, (2012); Phuyal *et al.*, (2017) and Bhatta *et al.*, (2018) also showed lower prevalence of parasites (14%, 12% and 14.66%) as compared to the present study. This may be due to the current study includes both stray and pet dogs whereas the prior study includes pet dogs. Whereas the study done by Bhattacharjee and Sarmah (2013); Pam *et al.*, (2013), Balakrishanan *et al.*, (2014); Guven *et al.*, (2017); Iatta *et al.*, (2021) reports higher prevalence (43% - 83%) than the present study. This may be due to variation in geographical distribution, sample size, length of time and diagnosis of parasites done by real- time PCR methods. Similarly from Nepal, study done by Diaz-Reganon *et al.*, (2020) in stray dogs' recorded 81.43% higher prevalence of parasites as compared to present study. This might be because that research conducted only in stray dogs and blood parasites was detected by real-time PCR methods but in the current study, study conducted on basis of both pet and stray dogs and microscopic examination were used to detect blood parasites. It was found that PCR is significantly more sensitive and specific diagnostic methods than microscopy method in detection of blood parasites (Jittapalapong *et al.*, 2006; Rani *et al.*, 2011 and Jain *et al.*, 2017). In present study, stray dogs have

higher (31%) prevalence of parasites as compared to pet dogs (20%). Similar findings was recorded by Gadahi *et al.*, (2008) on prevalence of blood parasites in stray and pet dogs in Hydrabad area: comparative sensitivity of different diagnostic technique for the detection of microfilaria which found higher prevalence (13%) of blood parasites in stray dogs and lower (9%) in pets dogs. In same manner, Bhattacharjee and Sarmah (2013) also support the present findings recording prevalence of blood parasites, 58.03% in pet dogs, 54.54% in working dogs and 63.64% in stray dogs.

Different species of blood parasites such as *Anaplasma* spp., *Babesis* sp., *Echrlichia* sp. and *Hepatozoon* sp. were found in the current study. The study conducted by Subedi and Shrestha (2012) recorded *Babesia* spp. and *Echrlichia* spp. Similarly, Bhatta *et al.*, (2018) founds *Babesia* sp. (2%), *Echrlichia* sp. (10.66%), *Anaplasma* sp. (1.34%) and *Trypanosoma* sp. (0.66). Similar blood parasites from stray dogs were recorded by Diaz-Reganon *et al.*, (2020) such as *Hepatozoon* sp., *Anaplasma* sp., *Echrlichia* sp., *Leishmania* sp. and *Babesia* spp. Among the four species of parasites, *Anaplasma* spp. was recorded as the highest (18.67%) prevalence blood parasites followed by *Babesia* sp. (6.67%) and the least was *Hepatozoon* sp. (1.33%). Research conducted by Sathish *et al.*, (2021) supports the current findings revealing *Anaplasma* sp. higher followed by *Babesia* spp. Similar findings were recorded by Dantas-Torres *et al.*, (2020) in two Brazilian region, Sao Joaquim da Bicas showed similar prevalence (16.5%) as the present study whereas from Goiana area recorded higher prevalence (45.1%) than the current study. This may be due to the presence of more ecto-parasites in dogs, carelessness of dog owner about the effects of ecto-parasites. In the present study, between pet and stray dogs, stray dogs have higher prevalence (20%) of *Anaplasma* spp. Konto *et al.*, (2017) conducted research on molecular and serological prevalence of *Anaplasma* and *Ehrlichia* sp. among stray dogs in east Malaysia and found 27% sample infected from from *Anaplasma* sp. Similarly, Daiz-Reganon *et al.*, (2020) also recorded *Anaplasma* sp. (31.43%), which is higher than the present study.

In age wise overall prevalence, young dogs (6 months- 12 months) showed a higher prevalence (31.25%) of parasites followed by older (>12 months) (27.2%), puppy (zero – six months) (22.22%). This finding was supported by Oduye and Dipeolu (1976) who have conducted research on blood parasites of dogs in Ibadan. Similarly, the research conducted by Sahu *et al.*, (2014) also records 17.07% prevalence in less than one year dogs and 9.73% above one year. In stray dogs as well, younger (33.33%) have higher

infections followed by older age dogs (31.39%), puppy (20%) whereas pet dogs have higher prevalence of parasites in young (28.57%), followed by puppy (25%) and then older dogs (17.94%) but it was found statistically insignificant. This may be due to vertical transmission (Fukumoto *et al.*, 2005) or maternal colostrums of dogs were either non-protective or short lived protection which leads to the weak immunity against blood parasites (Oduye and Dipeolu, 1976).

In the present study, overall sex wise prevalence showed that female were more (27.65%) infected than the male (27.18%). However there was no significant association between sex and prevalence of parasites. While carrying individual analysis of stray dogs, it was also found that female have higher (39.28%) prevalence of parasites than male (27.78%). This finding was supported by Gadahi *et al.*, (2008), recording 18.6% in female and in male 9.33%. Similarly by Shitta, (2012); El-Dakhly *et al.*, (2013) and Phuyal *et al.*, (2014). In same manner by Pam *et al.*, (2013) recording 23% in female, 20% in male, similarly by Do *et al.*, (2021). It may be due to stress arisen during reproductive activities that lead to low immunity and get easily attacked by blood parasites, due to sedentary behavior during nursing their offspring that cause more tick infestation. However in pet dogs, male showed higher infection than female. Different articles supports these findings such as Subedi and Shrestha, (2012); Bhatta *et al.*, (2018); Obeta *et al.*, (2020); Iatta *et al.*, (2021). This might be explained due to roaming nature of male dogs in search of mate that increase the exposure to ticks (Mundim *et al.*, 2008).

Out of 150 blood samples, 105 samples were from local breed, 17 from Japanese breed and so on. Overall prevalence showed that local breed have higher prevalence (21.33%) as compared to other breed. This finding was supported by Pam *et al.*, (2013) who have conducted research on parasites in dogs in Vom, Jos south local government, Plateau state which showed higher prevalence of blood parasites in local dogs. But the current study was in contrast with the study conducted by Subedi and Shrestha, (2012); Phuyal *et al.*, (2017); Obeta *et al.*, (2020) and Ezema *et al.*, (2021) but showed statistically insignificant. This may be due to the difference in sample size.

According to tick infestation and non-tick infestation, the prevalence of blood parasites was found higher (52.3%) in the tick infestation dogs than non-tick infestation (23.36%) but statistically found insignificants. The finding was supported by Diaz- Reganon *et al.*, (2020), showing 60% in tick infected dogs and 40% in non-tick infected dogs. Out of 21

blood samples collected from tick infestation dogs, 11 samples were found positive with three different species of blood parasites: *Anaplasma* spp., *Babesia* sp. and *Hepatozoon* sp. Among these blood parasites, *Anaplasma* spp. showed higher infection (33.33%) followed by *Babesia* sp. (19.05%) and *Hepatozoon* sp. (4.76%) in tick infestation dogs. In case of concurrency of prevalence of blood parasites, a single infection was found higher (25.33%) than the double infections (2%). This finding was supported by the research done by Piratae *et al.*, (2015) on molecular detection of *Ehrlichia canis*, *Hepatozoon canis* and *Babesia canis vogeli* in stray dogs in Mahasarakham which showed higher in single infections (32.91%) than co-infection of *E. canis* and *B. canis vogeli*. Similarly, the others researchers from different country, Yabsley, (2008); Hii *et al.*, (2012); Hoffman *et al.*, (2019); Alanazi *et al.*, (2021) and Do *et al.*, (2021) recorded single infection higher than co-infections with two or more pathogens.

CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The overall prevalence of blood parasites was recorded as 27.33% in pet and stray dogs. Higher infection was found in stray dogs with various types of blood parasites. Altogether, 4 species of parasites *Anaplasma* spp., *Babesia* sp., *Ehrlichia* sp. and *Hepatozoon* sp. were observed in the study. The younger dog's recorded higher infestation followed older dogs and puppy. In sex wise prevalence, female showed higher infection as compared to male. Higher infestations of parasites were recorded from local breed followed by Japanese breed. In current study, higher occurrence of parasites was found in tick infected dogs than non-tick infected dogs. From tick infestation dogs, *Anaplasma* spp., *Babesia* sp. and *Hepatozoon* sp. of blood parasites were recorded. Single infection of blood parasites was more common than double infection. There were no significant association with factor such as age, sex, breed and tick infestation with prevalence of blood parasites. However, there was a significant relationship found in species of blood parasites and concurrency of blood parasites ($P< 0.001$).

6.2 Recommendations

- In current study, the blood sample of pet and stray dogs were not equal, it should be better to take equal number of blood samples.
- The recorded parasite such as *Babesia* and *Ehrlichia* are zoonotic to human. So, care must be taken while handling the affected dogs and its sample.

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ANNEX

Photographs

Photo 1. *Anaplasma* spp.

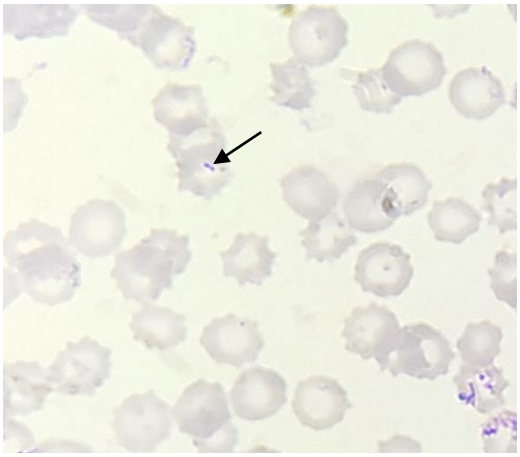

Photo 2. *Babesia* sp.

Photo 3. *Hepatozoon* sp.

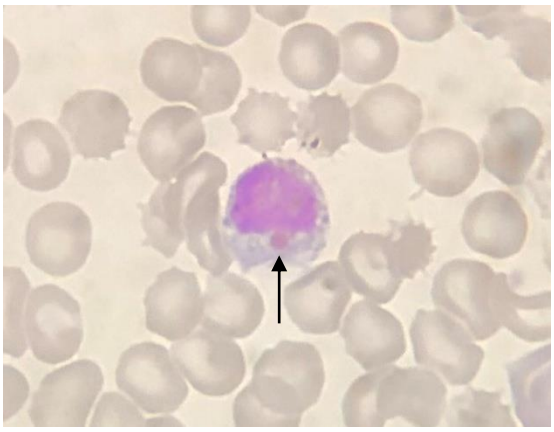

Photo 4. *Ehrlichia* sp.

Photo 5. Collecting pet dog sample

Photo 6. Collecting stray dog sample

Photo 7. Healthy dog

Photo 8. Dog infected with ticks

Photo 9. Dropping a blood in slide

Photo 10. Preparing the slide

Photo 11. Microscopic examination of samples