PREVALENCE OF COMMON HEMOPROTOZOAN DISEASES OF CATTLE IN PABNA DISTRICT OF BANGLADESH

MD. RAKIB MAHMUD MASUM

MASTER OF SCIENCE IN MEDICINE

DEPARTMENT OF MEIDICINE AND PUBLIC HEALTH

SHER-E-BANGLA AGRICULTURAL UNIVERSITY, DHAKA-1207

DECEMBER, 2021

PREVALENCE OF COMMON HEMOPROTOZOAN DISEASES OF CATTLE IN PABNA DISTRICT OF BANGLADESH

BY

MD. RAKIB MAHMUD MASUM

REGISTRATION NO. 14-06008

A Thesis
Submitted to Faculty of Animal Science and Veterinary Medicine
Sher-e-Bangla Agricultural University, Dhaka-1207
A partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
IN
MEDICINE

SEMESTER: JULY-DECEMBER, 2021

Approved By:

Associate Prof. Dr. Md. Rashedul Islam
Supervisor
Department of Surgery and Theriogenology
Sher-e-Bangla Agricultural University

Prof. Dr. K. B. M. Saiful Islam
Co-Supervisor
Department of Medicine and Public Health
Sher-e-Bangla Agricultural University

Prof. Dr. K. B. M. Saiful Isalm
Chairman
Department of Medicine and Public Health
Sher-e-Bangla Agricultural University

DEPARTMENT OF MEDICINE & PUBLIC HEALTH

Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

CERTIFICATE

This is to certify that the thesis entitled **"PREVALENCE OF HEMOPROTOZOAN DISEASES OF CATTLE IN PABNA DISTRICT OF BANGLADESH"** submitted to the Department of Medicine & Public Health, Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M.S.)** in **MEDICINE**, embodies the result of a piece of bonafide research work carried out by **MD. RAKIB MAHMUD MASUM**, Registration No. **14-06008** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

December, 2021
Dhaka, Bangladesh

(Associate Prof. Dr. Md. Rashedul Islam)
Department of Surgery and Theriogenology
Sher-e-Bangla Agricultural University, Dhaka

DEDICATED TO
MY
BELOVED PARENTS

DECLARATION

I declare that the thesis hereby submitted by me for the MS degree at the Sher-e-Bangla Agricultural University is my own independent work and has not previously been submitted by me at another university/faculty for any degree.

Date: DECEMBER, 2021 **MD. RAKIB MAHMUD MASUM**

ACKNOWLEDGEMENTS

*The author seems it a much privilege to express his enormous sense of gratitude to the **Almighty Allah** for there ever ending blessings for the successful completion of the research work.*

*The author wishes to express his gratitude and best regards to his respected Supervisor, **Associate Prof. Dr. Md. Rashedul Islam**, Department of Surgery and Theriogenology, Sher-e-Bangla Agricultural University, Dhaka, for his continuous direction, constructive criticism, encouragement and valuable suggestions in carrying out the research work and preparation of this thesis.*

*The author wishes to express his earnest respect, sincere appreciation and enormous indebtedness to his reverend Co-supervisor **Prof. Dr. K. B. M. Saiful Islam** for his scholastic supervision, helpful commentary and unvarying inspiration throughout the research work and preparation of the thesis.*

The author feels to express his heartfelt thanks to all the teachers and staffs of the Department of Medicine & Public Health, Sher-e-Bangla Agricultural University, Dhaka, for their co-operation during the period of the study.

The author feels proud to express his deepest and endless gratitude to all of his course mates and friends to cooperate and help him during taking data from the field and preparation of the thesis. The author is very much thankful to Sher-e-Bangla Agricultural University Research System (SAURES) to provide the fund of this research.

The author expresses his heartfelt thanks to his beloved parents and Brothers and all other family members for their prayers, encouragement, constant inspiration and moral support for his higher study. May Almighty bless and protect them all.

The Author

CONTENTS

CONTENT (CONT'D)

LIST OF FIGURES

LIST OF TABLES

ACRONYMS AND ABBREVIATIONS

ABBREVIATIONS	FULL WORDS
et al.	And others/Associates
MS	Master of Science
>	Greater than
<	Less than
°C	Degree Celsius
%	Percentage
etc.	Etcetera
GDP	Gross Domestic Product
ml	Milliliter
DLS	Department of Livestock Services
EDTA	Ethylenediamine tetraacetic acid
BBS	Bangladesh Bureau of Statistics
mm	Millimeter
PCR	Polymerase chain reaction
IFA	Immunofluorescence assay
IFAT	Indirect fluorescent antibody test.
CBC	Complete blood count
PCR-RFLP	PCR-restriction fragment length polymorphism
ELISA	Enzyme-linked Immune Sorbent Assay
CMA	Chittagong Metropolitan Area
VSG	Variable surface glycoproteins
LAMP	Loop mediated isothermal amplification
TBD	Tick borne diseases

ABSTRACT

An epidemiological investigation was conducted to ascertain the prevalence of hemoprotozoan diseases of cattle in Pabna district of Bangladesh, during the period of January 2021 to December 2021.During study period a total of 60 cattle were examined from three upazilas (Bera, Santhia and Faridpur) of Pabna district. The investigation was carried out in three visits on three seasons (summer, rainy and winter). On Geimsa stained blood smear examination, 14 were found to be infected with hemoprotoza diseases. Overall prevalence of *Anaplasma marginale, Anaplasma centrale*, combined infection of *A. marginale* and *A. centrale* and *Babesia* spp. were detected 5(8.33%), 2(3.33%), 3(5%), 4(6.66%) in cattle, respectively. The effect of age, sex, breed and seasons were observed in cattle during this study. The highest prevalence of *Anaplasma* spp. and *Babesia* spp. (25% and 10% respectively) was reported in the older cattle (> 3 years of age) and the prevalence of *Anaplasma* spp. and *Babesia* spp. was higher in female (20% and 10% respectively) than male (13.33% and 3.33% respectively). All crossbred cattle showed higher prevalence of *Anaplasma* spp. and *Babesia* spp. (23.33% and 10% respectively) than local cattle (10% and 3.33% respectively). The prevalence of of *Anaplasma* spp. and *Babesia* spp. was noticed as the highest in the rainy season (25% and 10% respectively) in relation to summer (15% and 5% respectively) and winter (10% and 5% respectively) season. From the study it was evident that cattle were infected with hemoprotozoan diseases and caused a heavy economic loss which will assist to take necessary preventive measures.

CHAPTER I

INTRODUCTION

Bangladesh's rice-based agricultural production system is acknowledged to include livestock as an essential element. The nation's economy is heavily reliant on agriculture. One of agriculture's four components, livestock, plays a crucial part in the economy of the country (Baset *et al.* 2002). In 2018–19, the livestock subsector contributed 1.47% of the country's GDP, or 13.46% of the agricultural GDP, with a GDP growth rate of 3.47% for livestock (at constant prices) (DLS, 2019). Additionally, it contributes 13% of foreign currency to our national economy and employs 50% of rural residents part-time and 20% of them full-time (Khokon *et al.* 2017). The estimated number of livestock in Bangladesh is 4122.44 lakh (DLS, 2020).

Cattle play an important role in improving the livelihoods of Bangladesh's poor and marginalized people. Bangladesh has 24.5 million cattle, 80 percent of which are nondescript indigenous cattle kept by rural farmers (Habib and Bhuiyan, 2021). Bangladesh's total cattle population is estimated to be 243.91 lakh (DLS, 2020). Even if the cattle population per unit land area is high, their output is too low due to insufficient feed supply, poor genetic makeup, inadequate veterinary care provision, and a lack of scientific awareness in housing and management (Hossain, 2019). Among the various constraints of cattle farming, the outbreak of several debilitating diseases is the most significant constraint, causing economic loss and discouraging cattle farming in this country.

One of the main obstacles to livestock production in Bangladesh is parasitic infestation, and the country's hot, humid climate highly favors the growth and survival of ecto and endo parasites, which results in the violence of parasitism and is known as an endemic disease (Alim *et al.* 2012). Because of increased humidity and heat inside animal sheds brought on by stale air, crowding, animal waste, and a lack of ventilation during rainy seasons, both bacteria and parasites thrive there (Mohanta *et al.* 2011). Tick development and survival are highly favored in hot and humid climates (Kohli *et al.* 2014), which is similar to the geo-climatic conditions of Bangladesh during the rainy season (Mohanta *et al.* 2011). Tick-borne hemoprotozoan diseases cause a drastic decline in cattle health, reduced milk production and draught

performance, and even mortality, resulting in massive economic losses for the farmer (Ananda *et al.* 2009).

Protozoan parasites are responsible for severe infections in both humans and animals around the world. The infection is spread primarily through arthropod vectors or through blood transfusion (Salih *et al.* 2015). Trypanosomosis, theileriosis, babesiosis, and anaplasmosis are important veterinary hemoprotozoan diseases caused by several species of *Trypanosoma* (Tewari *et al.* 2001), *Theileria*, *Babesia* (Singh et al. 2009), and *Anaplasma* in various livestock species. *Anaplasma* spp is a gram negative rickettsial protozoan whereas *Babesia* spp, and *Theileria* spp are apicomplexan parasite which infects red blood cell (RBC), indeed transmission occurs to animal through vector bite, notably Ixodes and usually know as tick borne diseases (TBDs) (Karim *et. al.* 2012) and also worldwide distributed (Vieira *et al.* 2019; Kundave *et al.* 2018).

The northern regions of Bangladesh, particularly the Pabna district, provide almost half of Bangladesh's total milk production (Hemme *et al.* 2004). The majority of the cattle in this area are cross-bred (Bosu, 2021). Cross-bred cattle were more susceptible than indigenous cattle, and the summer season was the most prevalent for blood protozoa in tropical and subtropical countries, followed by winter and the rainy season (Hassan *et al.* 2019). Adult and female were more susceptible than young and male (Aouadi *et al.* 2017). TBDs affect approximately 80% of the world's cattle population (Ghosh *et al.* 2007). Among 867 tick species, approximately 10% of them currently known act as vectors (Jongejan *et al.* 2004). High temperatures and humidity exacerbate epidemics of TBDS in Bangladesh's mountainous and forested areas, and pure or mixed animal breeds are more susceptible to infection (Bhat *et al.* 2017). A TBD outbreak is brought on by tick and blood protozoa that proliferate, multiply, and survive in humid and hot climate conditions (Lee *et al.* 2018). The first sickness spread by an arthropod was ticks fever (Bock *et al.* 2004). In Bangladesh, *Anaplasma marginale, Anaplasma centrale*, *Babesia* spp. (Bosu, 2021), *Theileria annulata, Babesia ovis, Babesia motasi* (Hassan *et al.* 2019) were detected in cattle. Clinical symptoms included a high fever (105–107°F), anemia, frequent diarrhea, ascites, occasionally bloody diarrhea, coffee-colored urine in the final stages of babesiosis, and enlargement of the superficial lymph nodes, fever, congested mucous membranes, corneal opacity, and emaciation in cases of theileriosis (Alim *et al.*

2011). Hematological findings revealed that cattle suffered from theileriosis showed normocytic hypochromic anemia, while those suffered from babesiosis showed normocytic normochromic anemia (Hussein *et al.* 2007). TBDs increase mortality, exacerbate anemia, disguise damage, decrease milk production, have a negative impact on reproductive health, and generate global economic losses worth an estimated US$18.7 billion (Ananda and Adeppa, 2016). *Babesia* spp. resemble short and long loop formation in RBC (Piroplasmosis) in blood smear microscopy, while *Anaplasma marginale* and *Anaplasma centrale* resemble pointed round dots at the periphery and inside of RBC, respectively. RBC was identified in *Theileria spp.* to be spherical, dot-shaped, and annular in shape (Song *et al.* 2018). Due to the hot, humid atmosphere, biting flies spread disease, and their multiplication is boosted during the sexual stages (Ghosh and Nagar, 2014). The animal that was recovered acts as a carrier and, more importantly, as a possible source of infection (Zhou *et al.* 2017). To stop death and productivity losses, early detection, targeted treatment, and vector control are essential (Bary *et al.* 2018). Because there are so few parasites in peripheral blood, haemoprotozoan infections in cattle are difficult to diagnose. Therefore, the identification of low levels of parasite infection is crucial for epidemiological research (Fahrimal *et al.* 1992). The key component in controlling anaplasmosis and babesiosis in Bangladesh is epidemiological surveillance. Unfortunately, there is no much inclusive research work on hemoprotozoa in these areas.

Due to the significance of hemoprotozoan diseases, a study of hemoprotozoan diseases in cattle in the Pabna region of Bangladesh was conducted. This study may make a significant contribution to the management of hemoprotozoan infections in cattle in the Pabna region of Bangladesh.

Objectives:

- To identify hemoprotozoa in blood samples of cattle in Pabna region.
- To illuminate the prevalence and risk factors of hemoprotozoa diseases of cattle in Pabna region.

CHAPTER II

REVIEW OF LITERATURE

2.1. Prevalence of hemoprotozoan diseases of cattle in Bangladesh

Bosu, (2021) conducted a study to investigate the prevalence of blood protozoan diseases in cattle in 'Bathan' areas of Bangladesh which are located in Sirajgonj and Pabna districts during the period of September, 2018 to August, 2019. Among 100 cattle examined, *Anaplasma marginale, Anaplasma centrale*, mixed infection of *A. marginale* and *A. centrale* and *Babesia* spp. were detected 12(12%), 3(3%), 4(4%), 8(8%) in cattle, respectively. The effect of age and seasons in prevalence was observed in cattle during this study. The highest prevalence of anaplasmosis (25.80%) and babesiosis (10.34%) was reported in respectively more than 3 years and 2-3 years age group. The prevalence of anaplasmosis was noticed as the highest in the rainy season (33.33%), in relation to summer (14.29%) and winter (11.43%) season. The prevalence of babesiosis was ranked the highest in rainy season (13.33%) followed in summer (8.57%) and winter ((2.86%) season.

Hosen *et al.* (2020) aimed to conduct a cross-sectional study to investigate the prevalence of haemoprotozoan diseases in Sylhet district of Bangladesh. A one year (January to December 2018) study on hemoprotozoan diseases was conducted in crossbred and indigenous cattle. Blood samples were collected randomly from 81 crossbred and from 19 indigenous cattle from four representative areas in three seasons. Blood samples were examined by Giemsa's stained thin blood smear method. The effect of breed, sex, age and season was observed in cattle during this study. The overall prevalence of haemoprotozoan diseases in Sylhet district was 52%. Three (3) types of haemoprotozoan diseases have been identified (Anaplasmosis, Babesiosis, Mixed) among them prevalence of Anaplasmosis was 28%, babesiosis was 08% and mixed infection was 15%. The prevalence of haemoprotozoan diseases was not significant ($P>0.05$) in relation to breed but the highest prevalence found in crossbreed cattle was Anaplasmosis (29.63%). Sex-wise prevalence was also not significant ($P<0.05$) in each of the diseases and here, the highest prevalence was found in male (31.48%) in case of Anaplasmosis. In relation to age, only mixed infected cattle were differ significantly ($P<0.05$) where the highest prevalence was observed (30.43%) in case of Anaplasmosis. Hemoprotozoan diseases were

predominant in summer (36.11%) season followed by rainy (29.41%) and winter (16.67%) season. In case of mixed infection, adult cattle had significantly higher prevalence which was statistically significant ($P<0.05$). Study results revealed that burden of haemoprotozoan diseases are apparently high in Sylhet district regardless of the age, sex, breed and season.

Mostari *et al.* (2020) conducted a study to find out the status of cattle diseases in relation to age, sex and season at Debidar, Comilla, Bangladesh. A total of 1145 sick animals were examined and 45 types of diseases were identified during this study period. The overall prevalence of parasitic diseases in cattle was (8.91%). The highest prevalence of parasitic disease was observed in tick (3.93%) and during summer the prevalence of tick infestation was 5.96%.

Hassan *et al.* (2019) conducted a study in cattle and sheep in a different area of Bangladesh to identify vector borne blood protozoa. They collected a total number of 1150 blood samples randomly from Dhaka, Sirajganj and Nikhangsori for blood smear microscopy. From the clinically positive sample PCR was done followed by gel electrophoresis. They reported that prevalence of blood protozoa were 100% (55), 80% (n=320), 30% (n=120), 22% (n=44), 31% (n=22), 65% (n=16) in exotic sheep, intensive farming, milk-vita area, local cattle, hill tracts and native sheep respectively. The overall prevalence was 50.17% (n=577). They also reported that among the protozoa, *Anaplasma* spp. was 43%, *Babesia* spp. 19%, *Anaplasma* spp. with *Babesia* spp. 33%, *Theileria* spp 4% and *Anaplasma* spp. with *Babesia* and *Theileria* spp was 1%. The prevalence of blood protozoa in local breed ≥50%, up to 75% and above 75% cross or pure breed were 17.58% (n= 103), 31.91% (n=187) and 50.51% (n=296) respectively. Prevalence of blood protozoa during October to March was 16.041% (n= 94) and April to September was 83.959% (n=492). In PCR *Anaplasma marginale* showed positive band as 265 bp, *Babesia bovis* in 166 bp, and *Theileria annulata* in 312 bp, *Babesia ovis* in 422bp and *Babesia motasi* in 518bp respectively. They also concluded that the tick is act as vector and high humidity and temperature is the main risk factor for vector borne diseases.

Bary *et al.* (2018) conducted an experiment to find out the prevalence and molecular identification of haemoprotozoan diseases in Bangladesh. A total 300 blood samples were randomly collected (150 crossbred and 150 local cattle) in three consecutive

seasons (summer, rainy and winter) from four selected areas, namely Nasirabad, Patia, Bayezid and Jointika under Chittagong district of Bangladesh. The effects of topography, season, age and gender were tested in both crossbred and local cattle. The PCR was performed after consequence screening by light microscopy, which exhibited that 22 samples (14 Anaplasma spp., 6 *Babesia* spp. and 2 for mixed infections) were positive. The overall prevalence of haemoprotozoan diseases were 9.33% in crossbred and 5.33% in local cattle, among these babesiosis, anaplasmosis were recorded 2.66% and 6.00% in crossbred cattle and 1.33% and 3.33% in local cattle, respectively. The highest prevalence of anaplasmosis was found in Patia (9.33%) followed by Bayezid (4.00%), Nasirabad (2.67%) and Jointika (2.66%) and babesiosis was recorded in Bayezid (4.00%) followed by Jointika (2.66%) and Patia (1.33%). Among three seasons the highest prevalence of anaplasmosis was recorded 12.00% in crossbred cattle followed by 6.00% in local cattle in summer whereas babesiosis was highest in summer (4.00%) in crossbred cattle followed by 2.00% in local cattle. Prevalence of anaplasmosis increased significantly ($P<0.05$) with the increase of age in crossbred cattle. The highest prevalence of anaplasmosis was 13.72% and 6.94% in adult crossbred and local cattle, respectively. Occurrence of babesiosis was the highest in adult (5.88%) in crossbred than young (2.78%) in local cattle, respectively. It was revealed that haemoprotozoan diseases were more common in female cattle, among these highest prevalence of anaplasmosis was recorded (6.11%) in female crossbred cattle and (4.00%) in local cattle, respectively. Positive samples were analyzed by PCR, where 9 samples were amplified among these 4 samples (1.33%) of *Babesia* spp and 5 samples (1.67%) of *Anaplasma* spp.

Mahamud *et al.* (2015) conducted a research to investigate the prevalence of babesiosis in cattle in Sirajganj district of Bangladesh during the period of December 2013 to November 2014. During the research period they were examined a total of 395 cattle. They reported that the overall prevalence of theileriosis and babesiosis in cattle were recorded as 5.82% and 2.27% respectively. The effect of age, sex, breed and season was observed in cattle during this study. The highest prevalence of theileriosis (7.25%) and babesiosis (3.10%) was reported in the older cattle (>3 years of age) and the higher prevalence was observed in female (6.66% and 2.59%, respectively) than male (4.0% and 1.60% respectively). All crossbred cattle was showed higher prevalence than local cattle. The prevalence of theileriosis was noticed

as the highest in the rainy season (6.25%) in relation to summer (5.83%) and winter (5.05%) season. But the prevalence of babesiosis was ranked the highest in summer season (2.50%) followed in rainy (2.27%) and winter (2.02%) season that was insignificant.

Belal *et al.* (2014) conducted an epidemiological investigation to ascertain the prevalence of anaplasmosis in cattle in Sirajganj district of Bangladesh, during the period of December 2013 to November 2014. During one year study period a total of 395 cattle were examined, and where 102 were found to be infected with *Anaplasma* spp. On Geimsa stained blood smear examination, it was observed that the overall prevalence of anaplasmosis in cattle was recorded as 25.82%. The effect of area, age, sex, breed and season was observed in cattle during this study. The highest prevalence (34.19%) was reported in the older cattle (> 3 years of age) and the prevalence was higher in female (28.88%) than male (19.20%). All crossbred cattle was showed higher prevalence than local cattle. The prevalence of anaplasmosis infection was noticed as the highest in the rainy season (30.68%) in relation to summer (27.50%) and winter (15.15%) season.

Mohanta *et al.* (2011) conducted an investigation to study the tick and tick borne protozoan diseases of livestock in the hilly areas of Bangladesh. Results concluded that the prevalence of two species of ticks namely, *Boophilus microplus* (92%) and *Amblyomma testudinarium* (21.6%) and two species of blood protozoa like *Babesia bigemina* (16.63%) and *Anaplasma marginale* (14.94%). Seasonal prevalence of ticks was highest in summer (97%) in comparison to rainy (95%) and winter (86%) season. On the other hand, the seasonal prevalence of blood protozoa was highest in rainy season (45.45%) in comparison to summer (27.87%) and winter (16.55%). Animals aged more than 2 (two) years of age (52%) found to be more susceptible to blood protozoan diseases than animals aged between 1-2 years of age (33.97%). But none of the animals under one year of age were found to be infected with blood protozoan diseases.

Rony *et al.* (2010) carried out an epidemiological investigation to determine the prevalence of ectoparasitic infestation of cattle in and around the Bhawal forest area in Gazipur district in Bangladesh, during the period from November 2008 to October 2009. Of 206 cattle examined, 132 (64.07%) were found to be infested with several

species of ticks and lice. The prevalence rate was highest in case of *Boophilus microplus* (45.63%) followed by *Rhipicephalus sanguineus* (36.89%), *Linognathus vituli* (23.30%), *Haematopinus euysternus* (17.96%), *Hemaphysalis bispinosa* (16.50%), and *Damalinia bovis* (8.25%). Results revealed that, older cattle aged > 8 years are more (71.11%) susceptible than that of adults aged > 2-8 years (67.74%), and young aged ≤2 years (47.05%).

Chowdhury *et al.* (2006) carried out an epidemiological investigation on common blood parasites (protozoa and rickettsia) in clinically suspected (febrile, anorectic, non-responsive to antibiotics therapy) cattle attended at District veterinary hospital of Sirajgong. Total 60 cattle were examined for blood parasite (protozoa and rickettsia) infection. Giemsa's stained were used on peripheral blood smears of suspected cattle. These slides were examined microscopically during September to October, 2004, of which 42 cattle (70% of the total examined cattle) had *Anaplasma* spp. infection (either *Anaplasma marginale* or *Anaplasma centrale* or both) and 2 cattle (3.3% of the total cattle) had *Babesia* spp. infection but negative for other blood protozoal infection.

2.2. Prevalence of hemoprotozoan diseases in cattle in world

Abas *et al.* (2021) conducted a study to assess the prevalence of tick-borne haemoparasites and their perceived co-occurrences with viral outbreaks of FMD and LSD and their associated factors. To investigate this correlation, a total of 670 samples from cattle and buffalo were collected during the summers of 2017 and 2018 distributed throughout ranches and smallholders in two geographical locations in Egypt. Study results confirmed significantly higher prevalence rates for the TBPs in LSD-positive than LSD-negative animals, while no significant difference could be detected for the prevalence rate of the TBPs in the FMD positive and negative groups. The prevalence of *Babesia* and *Theileria* was significantly ($P < 0.05$) higher in crossbreeds than native cattle. Infections with *Anaplasma* and co-infections with *Babesia-Anaplasma* and *Theileria-Anaplasma* were significantly higher in native than cross-breeds cattle.

Mushahary *et al.* (2021) conducted a study to confirm the haemoparasites species of cattle recorded in 4 districts of Assam along the border. PCR analysis amplified *Theileria orientalis* DNA both from blood and eggs of *Rhipicephalus* (*Boophilus*).

microplus tick and *Babesia bigemina* and *Anaplasma marginale* from blood. Phylogenetic analysis revealed *T. orientalis* isolate having 98.83% and 99.00% similarity with isolates reported from Vietnam and Myanmar, a new *B. bigemina* isolate and *A. marginale* isolate bearing 93.46% similarity with isolates from USA and Mexico.

Subapriya *et al.* (2021) administrated a study to find out the prevalence of haemoprotozoans in cattle. A total of 6000 blood samples were examined for the presence of different haemoprotozoan parasites to the Large Animal Medicine-Out Patient ward of Madras Veterinary College Teaching Hospital (MVCTH), Chennai, Tamilnadu, India during the period 2014-2020 by LeishmanGiemsa staining, out of which 1899 cattle were found positive for either one or more haemoprotozoans accounting to a prevalence rate of 31.65%. *Anaplasma marginale* was found to be most predominant haemoprotozoan 1298(68.35%) followed by *Theileria annulata* 516 (27.17%), *Babesia bigemina* 77 (4.05%), *Trypanosoma evansi* 28 (1.47%), *Microfilaria* 12 (0.63%), *Ehrlichia bovis* 8(0.42%) and *Babesia bovis* 4 (0.21%). Mixed infection was observed in 44 (2.31%) cattle. The highest number of cases were recorded in Jersey cross 1249 (65.77%) followed by Holstein Friesian cross 321 (16.90%) and indigenous cattle 329 (17.32%). Of the cases recorded, 56 (2.95%) were males and 1843 (97.05%) were females. The highest prevalence of haemoprotozoans was observed in female cattle were 9.22% in cattle of 1^{st} parity, 25.17% in 2^{nd} parity, 27.54% in 3^{rd} parity, 17.06% in 4^{th} parity, 10.16% in 5^{th} parity, 2.16% in aged cattle more than 5th parity and 5.74% in cattle aged less than 1 year. In males, the highest incidence was observed in cattle belonging to the age group of 1.95% in young male cattle less than one year, 0.47% in 1 to 3 years age group, 0, 21% in 3-5 years and 0.16% each in 5-8 years and more than 8 years old male cattle. Season wise, highest prevalence was observed in monsoon season 791 (41.65%), followed by summer 576 (30.33%) and post monsoon 532 (28.01%) season.

Kaur *et al.* (2021) conducted a study aimed at establishing the prevalence, epidemiology and molecular characterization of major haemoprotozoons (*Babesia* and *Theileria*) and rickettsia (Anaplasma) of cattle in Jammu region (North India) using microscopy and Polymerase Chain Reaction (PCR). Hematology, microscopy and PCR based prevalence studies were undertaken with 278 whole blood samples from cattle. The prevalence based on microscopy was 12.9% (36/278) whereas PCR

recorded 30.22% (84/278) animals positive for haemoparasitic infections. All the samples found positive by microscopy were also recorded positive by PCR. Thus the study revealed prevalence of *Babesia bigemina*, *Anaplasma marginale* and *Theileria annulata* to be 9.7, 16.5 and 0.7% respectively.

Rajkumar *et al.* (2020) conducted a study to assess the current epidemiological status of bovine tick-borne diseases (TBDs) in and around Chennai by conventional staining method and polymerase chain reaction (PCR). A total of 154 blood smear and whole blood samples were screened by Leishman staining and PCR respectively. PCR assay (50.65%) revealed significantly higher sensitivity in detection of TBDs in clinically suspected cattle than microscopic examination (35.06%). Results showed that *Anaplasma* spp. (20.78%) was the most prevalent parasite of cattle followed by *Theileria* spp. (11.69%) and *Babesia* spp. (2.6%) in Chennai. The infection of *Anaplasma* spp. (27.77%), *Babesia* spp. (5.55%) and *Theileria* spp. (11.11%) were higher in less than 2 year of age group. The prevalence of anaplasmosis was relatively high (21.32%) in cross-bred cattle. However, higher prevalence of babesiosis (5.55%) and theileriosis (22.22%) was noticed in non-descript animals.

Jayalakshmi *et al.* (2019) carried out a study to determine the prevalence of haemoparasites in cattle in Cauvery delta region over a period of one year. A total of 228 giemsa stained blood smears were screened for the presence of haemoprotozoa, out of which 34 animals were found to be positive. An overall prevalence of haemoparasites in the sampled cattle were 14.9%, among this *Anaplasma* sp (8.3%), *Babesia* sp (3.95%), *Theileria* sp (2.19%) and *Trypanosoma* sp (0.44%) as single or mixed blood parasitic infections. In this study Anaplasmosis (14%) was highly prevalent during the winter season and Babesiosis (13.73%) was highly prevalent during summer months followed by Anaplasmosis (9.8%) and Theileriosis (7.8%), the lowest prevalence of Trypanosomiasis was observed during the rainy season. They also reported that the seasonal variation in prevalence of haemoprotozoan disease might be due to influence of climatic factors on density of vector population in that geographical area.

Anwar, (2018) carried out a study to determine the presence and distribution of tick-borne haemoprotozoan parasites (*Theileria, Babesia and Anaplasma*) in apparently healthy ruminants in Peswar, Pakistan. In the present study, a total of 1101 blood

samples (690 cattle, 243 Buffaloes, 108 sheep, and 60 goats) were examined for blood parasites from June 2013 to June 2016. Microscopic examination of Giemsa-stained peripheral blood smears exhibited an overall prevalence of theileriosis in three years to be 67.39, 15.63, 15.74, and 0%, babesiosis 15.79, 51.44, 14.81 and 41.66%, anaplasmosis 2.9, 13.99, 29.63, and 1.66% in cattle, buffaloes, sheep and goats respectively. The prevalence of theileriosis and babesiosis was significantly higher in cattle, buffaloes and sheep while goats showed low prevalence percentage of anplasmosis and 0 % of theileriosis.

Kala and Deo, (2018) carried out a study to find out the prevalence of haemoprotozoan disease in cattle during rainy season in Bihar, India. A tota 803 blood smears of suspected cases, received on the reference of field veterinarians from July 2016 to November 2016 for this study. Out of which, 598(74.47%) cases were found positive for haemoprotozoan disease by using Giemsa staining technique. Among different haemoprotozoan diseases, the highest prevalence of theileriosis (42.59%) was recorded followed by anaplasmosis (7.97%), trypanosomosis (1.62%) and babesiosis (1%). However, 21.29% of the cases were found positive for mixed infection. Most of the cases of mixed infection were observed concomitant infection of theileriosis and anaplasmosis. Such type of cases was found more severe than the cases with single infection.

Patel *et al.* (2017) conducted a study to record the incidence, risk factors and haematological changes during haemoprotozoan infections of bovines over a period from January, 2014 to December, 2016. A total of 193 suspected cases (141 cattle and 52 buffaloes) presented at Teaching Veterinary Clinical Complex and Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat, were examined for the presence of haemoprotozoan parasites by Giemsa staining technique. Total 46 (23.83%) cases (23 each of cattle and buffaloes) were found positive in this study for presence of haemoparasite infection. The effect of source of sample, season and species on incidence of haemoprasitic infections was significant whereas effect of year was non-significant. They also reported that higher incidence was observed in rainy (34.92%) followed by winter (25.42%) and summer (12.68%) season. Significantly higher incidence of Anaplasmosis (8.29%) was observed followed by Babesiosis (6.74%), Theileriosis (3.63%) and Trypanosomosis and Ehrlichiosis (2.59% each).

Vetrivel *et al.* (2017) conducted a experiment to assess the prevalence of babesiosis in cattle farms. The data were collected from 120 sample cattle owners selected from 6 blocks in 3 districts of North east zone of Tamil Nadu *viz.* Kancheepuram, Tiruvannamalai and Vellore districts. The results of the study indicated that the overall prevalence of babesiosis was 20.27 percent. The prevalence of babesiosis in Kancheepuram, Tiruvannamalai and Vellore districts was 18.52, 23.26 and 19.61 percent, respectively.

Murthy *et al.* (2016) carried out a study to record the prevalence of Haemoprotozoan infections in bovines of Shimoga region of Karnataka state, India for a period of 1 year from April 2012 to March 2013. A total of 300 blood samples were examined for the presence of haemoprotozoan parasites, of which 215 from cattle and 85 from buffaloes were examined by Giemsa staining technique. Out of 300 blood samples examined, 130 (43.3%) were found positive for Haemoprotozoan infections. Out of 215 cattle blood samples examined, 62 (28.8 %) were positive for *Theileria annulata*, 27 (12.5%) were harbored *Babesia bigemina*, 15 (6.9%) were found positive for *Trypanosoma evansi* and 06 (2.7%) samples showed *Anaplasma marginale*. Among 85 buffalo samples examined, 11 (12.9%) were showed *Theileria* spp, 04 (4.7%) found positive for *B. bigemina*, 03 (3.5%) were found positive for *T. evansi* and 02 (2.3%) were positive for *A. marginale*. Among haemoprotozoan parasites, the highest prevalence was observed with *T. annulata* followed by *B. bigemina* and *T. evansi* infection. The lowest prevalence was observed with *A. marginale* infection.

Bhatnagar *et al.* (2015) conducted a study aimed to investigate the occurrence of blood parasites (Protozoa and Rickettsia) infecting cattle in Southern Rajasthan from April 2005 to March 2014 in clinically suspected (febrile, anorectic,) cattle by examining 5257 blood samples. Out of these, 473 (9%) were found positive for blood parasites. Among the positive samples Theileriosis recorded in 42.28% samples, Anaplasmosis recorded in 42.07% samples, and Babasiosis was recorded in 15.65% samples. They also reported that highest incidence were recorded in rainy season (from June to September) (47.99%) followed by summer (26.01%) and winter season (26.0%).

Kakati *et al. (*2015) conducted a study to investigate the presence of *Theileria* in blood samples of crossbred and indigenous adult cows raised under unorganized small

scale farming system in a *Babesia* and *Anaplasma* endemic geographical area from Assam, India and to see its transmission through *Rhipicephalus* (*Boophilus*) *microplus* ticks. 57 clinical cases of cattle suspected to be of hemoparasitic infections were taken into consideration in this study. The parasites were identified based on morphology in giemsa stained blood smear followed by polymerase chain reaction (PCR). Study results concluded the prevalence of *Babesis bigemina* (64.91%), *Thileria orientalis* (21.05%) and *Anaplasma marginale* (14.03%).

Jassem and Agaar, (2015) designed a study to investigate the prevalence of bovine anaplasmosis among cattle from various areas in Wassit province of Iraq. The investigation was performed on 184 blood samples collected from suspected cattle suffering from fever (41°C), severe anemia, pale mucus membrane, progressive emaciation and drop in milk yield, including 85 male and 99 female cattle, aged from < 1 year to > 2 years. The samples were collected during the period of October 2012 - April 2013 from AL-Kut, AL-hayy, AL-Bashair, AL-Moufaqia and AL-Noamania areas. The results showed that the rate of infection was 13.04%, the rate of infection was different between age groups and were 8, 11.25 and 16.45% in ages < 1, 1-2 and 2 - 3 years, respectively. The study revealed that females were given higher percent of infection 14.14% than males 11.7%, there is no significant differences under $p > 0.05$ according to age groups and sex. The highest rate of infection was recorded in AL-Kut, 17.14% followed by AL-hayy, 14% and AL-Bashair, 10% and the lowest rate was recorded in AL-Noamania, 8.33% and AL-Moufaqia, 5%; the study showed significant differences in incidence of disease between study districts and area in Wassit governorate at $p > 0.05$.

Maharana *et al.* (2015) carried out an experiment to find out the prevalence and risk factors for haemoprotozoan infections in cattle and buffaloes of South-west Gujarat, India. A total of 480 suspected blood samples were collected from cattle (n=254) and buffaloes (n=226) during the study period from September 2011 to December 2014. The conventional optical microscopy of Giemsa stained blood smears revealed that 37% of cattle and 38.93% of buffaloes were infected with haemoprotozoan parasites including *Anaplasma marginale, Babesia bigemina, Trypanosoma evansi* and *Theileria annulata*.

Velusamy *et al.* (2014) conducted an experiment to assess the prevalence of haemoprotozoan diseases in cross-bred and indigenous cattle in relation to season, age and breed in Western part of Tamil Nadu, India. A total of 2637 blood smears were screened for haemoprotozoan diseases and samples were received from the college hospital and veterinary dispensaries in Western part of Tamil Nadu, India. Blood smears were stained using Giemsa's technique and examined under oil immersion. Microscopic examination of blood smears revealed an overall prevalence of 16.64 %; of which theileriosis was 13 %, followed by anaplasmosis 2.64% and then babesiosis 1.0 %. Among the haemoprotozoan diseases, the prevalence of theileriosis was significantly ($p<0.05$) high during summer (14.4%), followed by moderate in monsoon (13.8%) and less in fair (11.5%) seasons. However, there was no significant seasonal influence on the prevalence of babesiosis and anaplasmosis. The data on influence of breed revealed that there was a significantly ($p<0.05$) high prevalence of haemoprotozoan diseases in Holstein Friesian (HF) and Jersey cross breeds than indigenous breed and the occurrence of these haemoprotozoan diseases was found to be high among the age groups of 2-7 years in cross-bred animals and below 2 years in indigenous animals.

Chaudhri *et al*. (2013) conducted a study to assess the prevalence of haemoprotozoan infections in pyretic dairy animals of Eastern Haryana. The examination of stained blood smears from pyretic cross bred cows (3041) and buffaloes (3122) of Eastern Haryana from July, 2003 to June 2010 revealed significantly higher infection in cows (27.88%) than buffaloes (0.6%). The pyretic cross-bred cows had *Theileria annulata* (22.88%), *Trypanosoma evansi* (0.33%), *Babesia bigemina* (3.22%) and *Anaplasma marginale* (1.45%) whereas buffaloes (3122) had *T.evansi* (0.32%) and *B. bigemina* (0.32%) only. Percentage of pyretic cows detected positive for *T.annulata, B.bigemina* and *A.marginale* was high from 2006-09 (27.6 to 32.8%), 2007 – 09 (3.91 to 5.60%) and 2006-10 (0.87 to 2.7s0%), respectively. Seasonwise *T.annulata* and *B.bigemina* were recorded in higher percentage of cross-bred cows during summer (9.7 and 1.6%) and rainy season (9.2 and 1.1%).

Kamani *et al.* (2010) conducted an experiment to find out the prevalence and significance of haemoparasitic infections of cattle in North Central, Nigeria. A total of 637 blood samples from cattle from four states (Plateau, Bauchi, Nasarawa and Kaduna) of Nigeria in anticoagulant were submitted to the laboratory for

parasitological diagnosis. An overall prevalence of 25.7% was recorded for all samples examined. *Babesia bigemina* and *B.bovis* accounted for 16.0%, followed by *Theileria mutans* (3.1%), *Trypanosoma* spp (*T.vivax* and *T. congolense*) (2.8%), *Anaplasma marginale* (1.9%), *Microfilaria* (1.4%). They also recorded comparatively higher prevalence in female than male cattle and concluded that higher prevalence in female cattle might be due to stress condition as they were kept longer period for breeding and milk production purpose or supplied imbalance diet against their high demand.

Ananda *et al.* (2009) studied the prevalence of haemoprotozoan disease in crossbred cattle in Bangalore north by screening 132 clinically suspected blood samples by Geimsa's stained blood smear method. Study results concluded that among 132 crossbred cattle screened, 57 animals were found positive for haemoprotozoan parasites. Out of 57 positive ases, 41 were found positive for *Theileria annulata* and the remaining 16 were positive for *Babesia bibemina*. Study results also concluded that the highest prevalence was found in 4-6 year age group and in monsoon months.

CHAPTER III

MATERIALS AND METHODS

3.1. Area under study

To determine the prevalence of hemoprotozoa infection in cattle, the data were collected from three upazilas (Bera, Santhia and Faridpur) of Pabna district (Figure 1).

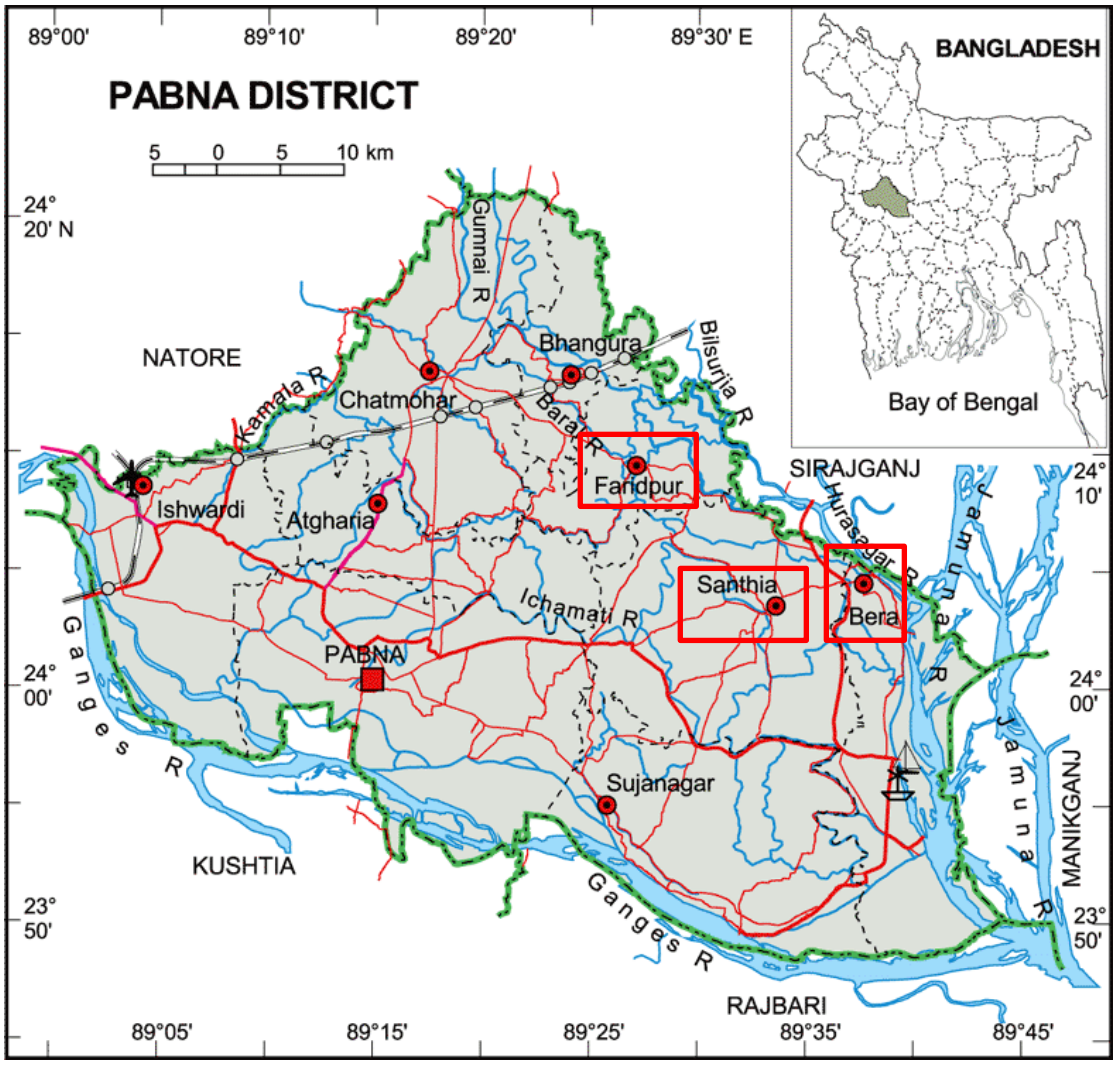


Figure 1. Study area

3.2. Study period

The samples were collected over a period of one year from January, 2021 to December, 2021 to cover all three prominent seasons in Bangladesh, namely, the winter (November to February), the summer (March to May) and the rainy season (June to October).

3.3. Geo-climatic condition of study area

The study was conducted in three upazilas of Pabna districts. The surrounding area lies within the triangular region formed by the confluence of the Padma and Jamuna (the name of the Brahmaputra River in Bangladesh) rivers. A wide alluvial plain is intersected by a network of streams, and many villages are accessible only by boat during the rainy season. There are hectors of grazing yards for herbivores. During rainy season (June-October) farmers takes their cattle back from grazing yards to households. The climatic conditions of the selected study areas were more or less similar to the average condition of the country. The climate of this area is moderate in nature. Highest temperature is 36.7 °C in April and 12.9 °C in December. Average precipitation is 101.7 mm which is highest (360 mm) in June. On the other hand, the average humidity is 77% (Bosu, 2021).

A B

Figure 2: Cattle shed. **A.** Cattle shed in winter when animal are taken in outside. **B.** Cattle shed in rainy season when animal are kept in farmers' house.

3.4. Blood sample collection

The investigation was carried out in three visits on three seasons (Summer: March-May; Rainy: June-October and Winter: November-February). A total of 60 blood samples from 60 cattle of different ages were collected randomly from the study areas suspected for haemoprotozoan disease. Blood samples were aseptically collected by needle from the jugular vein of the animals, and kept in Ethylene di-amine tetra acetate (EDTA) containing vials with leveled information for further smear preparation and staining.

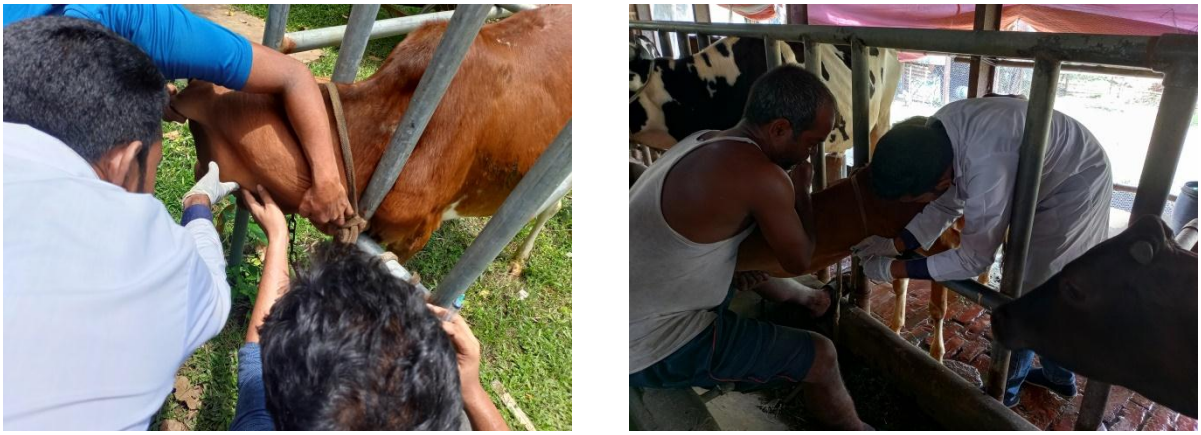


Figure 3: Blood sample collection from ear & jugular vein of cattle.

3.5. Preservation of collected samples

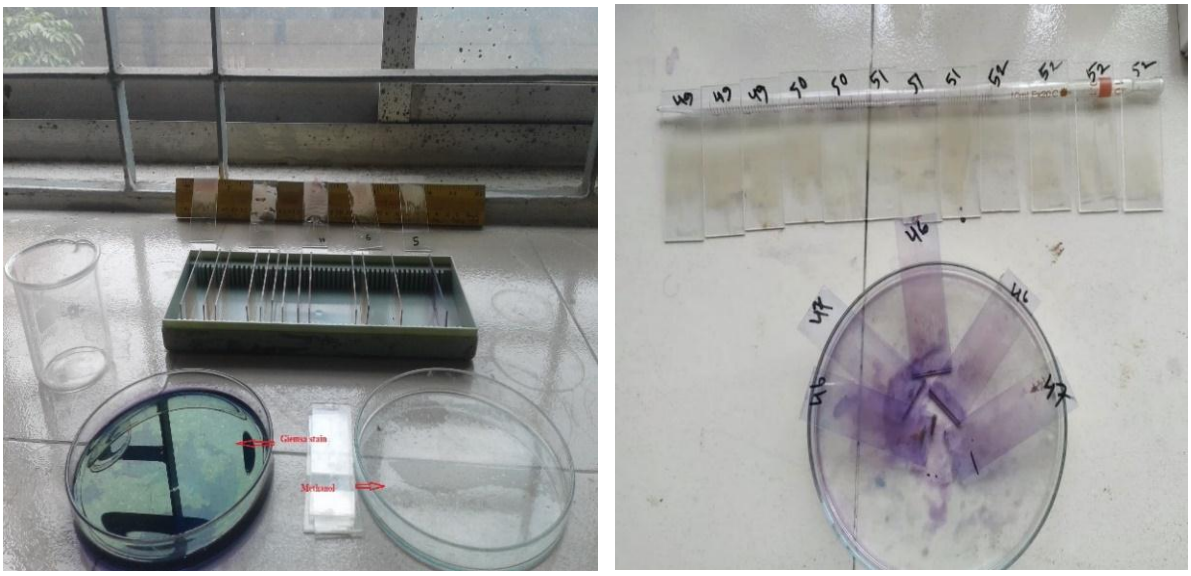
The collected blood samples were brought to the Medicine and Public Health Laboratory, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University for the detection of haemoprotozoan parasites by Giemsa's stained blood smear examination.

3.6. Blood smears preparation:

For thin smears, one drop blood was placed near one end of clean, dry and grease-free glass slide. Spreader slide inclined about 45º was pushed horizontally from the one end to another to get thin smear with clear tail. The more acute the angle between the slides, and the more slowly the spreader slide was moved, the thinner the film would be prepared. The resulting film was dried rapidly by waving it in the air, and was fixed with methanol for 2 minutes.

3.7. Preparation of Giemsa working solution:

For staining, 10% Giemsa working solution (1:9) was prepared. 90 ml of buffered distilled water (pH 7.2) was poured into a 100 ml graduated cylinder. Then, 10 ml of Giemsa stock solution was added by using a pasture pipette. Two of these solutions were mixed properly by gentle shaking or by using a glass bid. Prepared working solution was taken in a coplin jar for further staining. Giemsa working solution must be discarded after 24 hours and a fresh working solution should be prepared.



A B

Figure 4: Staining. **A.** Staining of blood samples with Giemsa stain. **B.** Slides after staining and labeling.

3.8. Examination of blood smears

A thin blood smear was prepared and stained with Giemsa's stain as per the standard protocol (Benjamin Maxine, 2005).The slides were allowed to dry in air and then examined by using built in illuminated compound microscope under oil immersion. Giemsa staining technique is the traditional method that involves microscopic examination of piroplasm in blood smear as well as in lymph node smears and is differentiated from other parasites by morphological properties (Aktas *et al.* 2006; Gul *et al.* 2015). This method is frequently used for detection of parasites as it is comparatively inexpensive. Starting from tail end of the slides to the whole field, all the hemoprotozoa encountered were studied and focused for photograph. The blood samples were examined and the season, breed and species wise prevalence was recorded. The species of haemoprotozoan parasites were identified on the basis of morphology (Soulsby, 1982; Bowmann, 2009). Blood smears were examined carefully and even the presence of few piroplasms was considered positive for haemoprotozoan diseases. However, expertise in microscopic detection of piroplasm is required in subclinical or chronic infection because parasitemia is often extremely low and may otherwise miss (Maharana *et al.,* 2016). Low parasitemia recorded in positive cases indicated the chronic form of diseases.

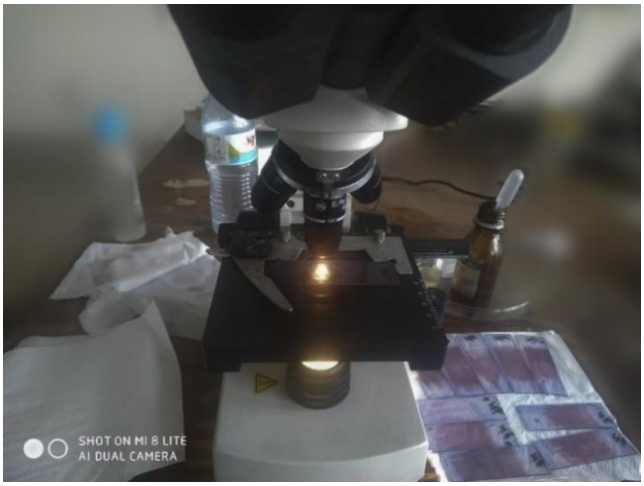


Figure 5: Microscopic examination of blood smears

CHAPTER IV

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1. Morphological identification of hemoprotozoa

On the basis of morphology, babesias are divided into two groups – small babesias (1.0–2.5 μm long) which included *Babesia bovis*, *B. gibsoni*, *B. microti*, *B. rodhaini*, etc., and large babesias (2.5–5.0 μm long) which included *Babesia bigemina*, *B. caballi*, *B. Canis*, etc., The orientation of the parasite in the red blood cells (RBCs) depends on its size because large pyriform parasites meet at their pointed ends at an acute angle to each other and small forms make an obtuse angle to each other (Ruprah. 1985). *B. bigemina* characteristically pear shaped, round (2–3 μm in diameter) oval or irregularly shaped form may also be found. *B. bovis* vacuolated signet ring forms are particularly common (Soulsby, 1982). *B. divergens* generally remained as paired form, superficially lie on the RBC, stout and pyriform or circular forms may be found. *B. major* pyriform bodies, the angle between the organism is <90°. Round forms with a diameter of about 1.8 μm are also available (Laha *et al.* 2015). *Anaplasma* spp. occur intracelularly in two morphologically distinct ultrastructural forms, dense-cored and reticulate cells in blood RBC (Soulbsy, 1982). In case of Ana*plasma marginale* pointed round dot at periphery of RBC and in Ana*plasma centrale* pointed round dot inside of RBC (*Hassan et al.* 2019).

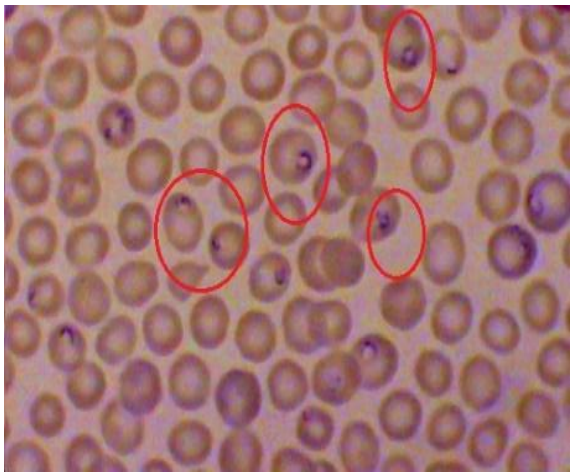
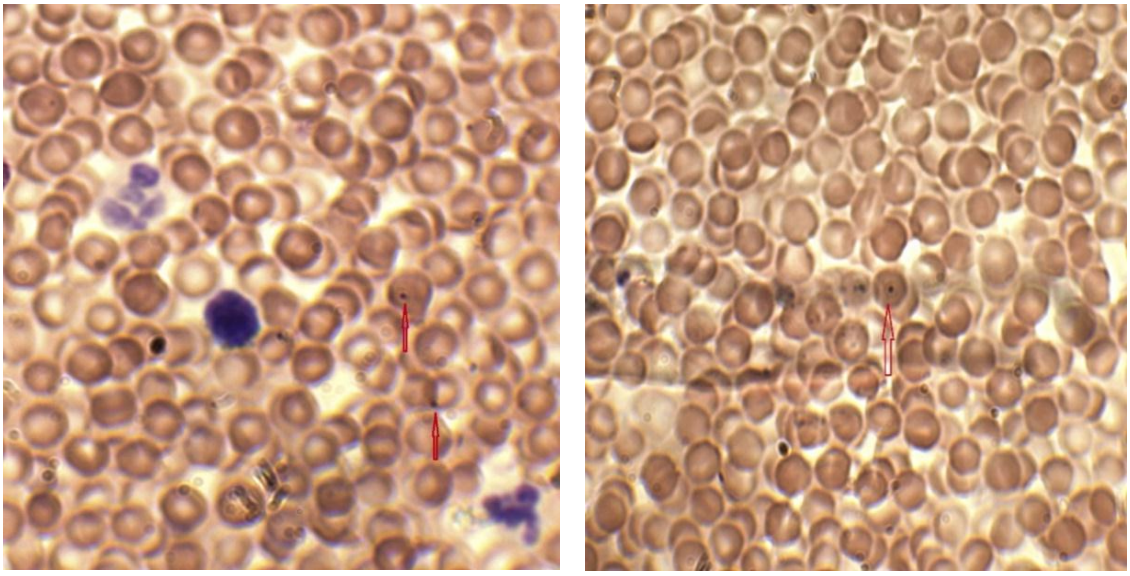


Figure 6: Microscopic examination of *Babesia* spp**.** (100x). Red circle shows pear-shaped, located in pairs, round, oval or irregular depending on the stage of development of the parasite in erythrocytes.



(A) **(B)**

Figure 7: Microscopic examination of *Anamlasma* spp. (100x). A. *Anamlasma marginale*; arrow shows morphologically distinct ultrastructural forms dense-cored cells at the margin and reticulate cells in RBC. B. *Anaplasma centrale*; arrow shows morphologically distinct ultrastructure forms dense-cored cells at the central and reticulate cells in RBC.

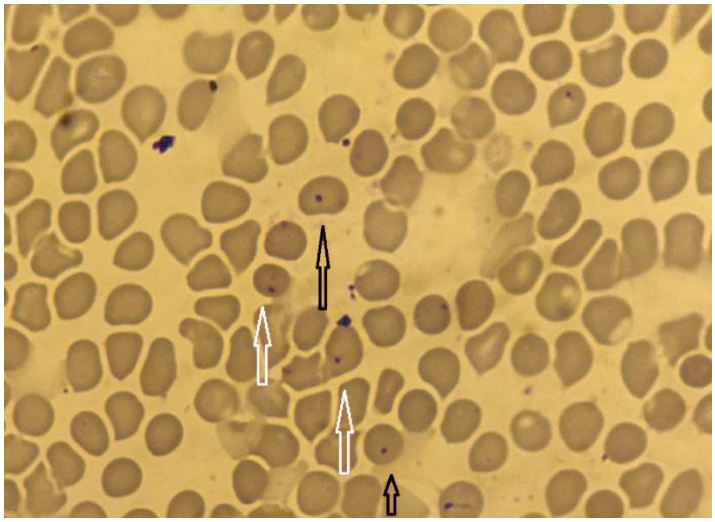


Figure 8: Co-infection of *A. marginale* and *A. centrale* (100x). White arrow shows morphologically distinct ultrastructure forms dense-cored cells at the margin. Dark arrow shows center region and reticulate cells in RBC.

4.1.2. Overall prevalence of hemoprotoza in Pabna district

In blood smear microscopy the overall prevalence of hemoprotozoa was 23.33% in study area. Among the 60 cattle examined, 14 were positive for hemoprotozoa presence. The microscopic identification showed three species of blood parasites, namely: *Anaplasma. marginale*, *Anaplasma. centrale* and *Babesia* spps

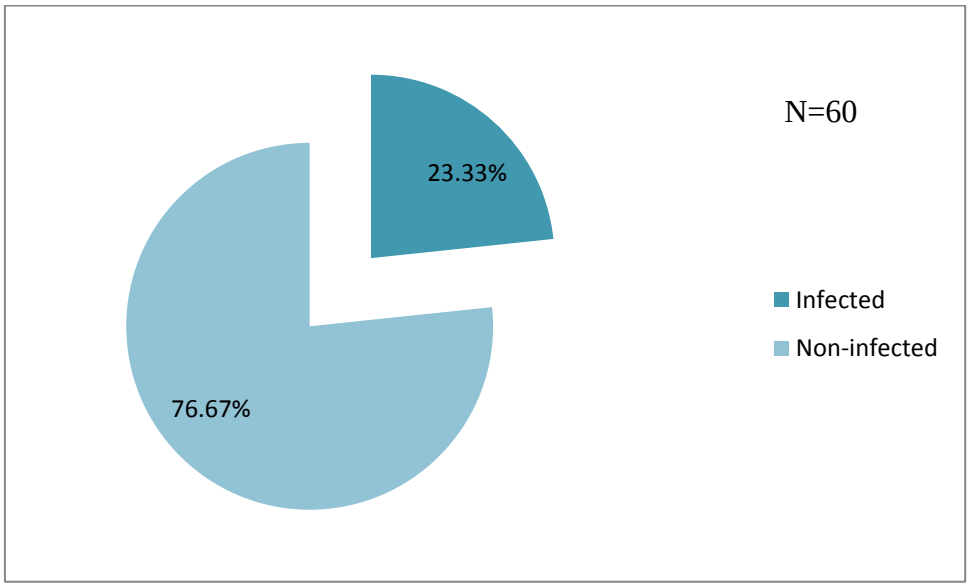


Figure 9: Overall prevalence of hemoprotozoa diseases of cattle in Pabna district

4.1.3. Species wise prevalence of hemoprotoza in Pabna district

Species wise prevalence of hemoprotozoa diseases of cattle in the study area is shown in table 1. Among positive 14 cattle, two *Anaplasma* spp. and one *Babesia* spp infection were detected. *A. marginale*, *A. centrale* and *Babesia* spp. were detected 5 (8.33%), 2 (3.33%), 4 (6.66%) in cattle, respectively. 3 samples had a mixed infection with *A. marginale* and *A. centrale***.** Single infections were more frequent than mixed infections.

4.1.4. Age wise prevalence of hemoprotoza in Pabna district

Age wise prevalence of hemoprotozoa in cattle is shown in Table 2. The highest prevalence of *Anaplasma* and *Babesia* was found in the age of above 3 years (25% and 10% respectively), followed in >2-3 years (20% and 6.66% respectively) and 6 months – 2 years of age (8% and 4% respectively).

Table 1: Species wise prevalence of hemoprotozoa of cattle in Pabna district

Hemoprotozoa		Positive cases	Prevalence (%)
Anaplasma spp.	*Anaplasma marginale*	5	8.33 %
	Anaplasma centrale	2	3.33 %
	Combined infection of *A. marginale* and *A. centrale*	3	5 %
Babesia spp.		4	6.66 %
Total		**14**	**23.33 %**

Table 2: Age wise prevalence of hemoprotozoa of cattle in Pabna district

Age	Sample tested	Prevalence (%)	
		***Anaplasma* spp.**	***Babesia* spp.**
6 months-2 years	25	8% (N=2)	4% (N=1)
2-3 years	15	20% (N=3)	6.66% (N=1)
> 3 years	20	25% (N=5)	10% (N=2)
Total	**60**	**23.33 % (N=14)**	

4.1.5. Sex wise prevalence of hemoprotoza in Pabna district

Sex wise prevalence of hemoprotozoa diseases in cattle is shown in Table 3. In case of both *Anaplasma spp* and *Babesia* spp., the higher prevalence was observed in female (20.00 % and 10 % respectively) than male (13.33% and 3.33 % respectively).

Table 3: Sex wise prevalence of hemoprotozoa of cattle in Pabna district

Sex group	No. of Sample tested	Prevalence (%)	
		***Anaplasma* spp.**	***Babesia* spp.**
Male	30	13.33% (N=4)	3.33% (N=1)
Female	30	20.00% (N=6)	10.00% (N=3)
Total	**60**	**23.33 % (N=14)**	

4.1.6. Breed wise prevalence of hemoprotoza in Pabna district

Breed wise prevalence of hemoprotozoa diseases in cattle is shown in Table 4. In case of both *Anaplasma spp* and *Babesia* spp. highest prevalence was recorded in crossbreed (23.33% and 10% respectively) in relation to local breed (10 % and 3.33% respectively).

Table 4: Breed wise prevalence of hemoprotozoa of cattle in Pabna district

Breed	**No. of Sample tested**	**Prevalence (%)**	
		***Anaplasma* spp.**	***Babesia* spp.**
Local	30	10.00%(N=3)	3.33% (N=1)
Cross breed	30	23.33% (N=7)	10.00% (N=3)
Total	**60**	**23.33 % (N=14)**	

4.1.7. Season wise prevalence of hemoprotoza in Pabna district

Season wise prevalence of hemoprotozoa diseases in cattle is shown in Table 5. In case of both *Anaplasma spp* and *Babesia* spp. highest prevalence was recorded in rainy season (25% and 10% respectively) in relation to summer (15% and 5% respectively) and winter season (10% and 5% respectively).

Table 5: Season wise prevalence of hemoprotozoa of cattle in Pabna district

Season	**No. of Sample tested**	**Prevalence (%)**	
		***Anaplasma* spp.**	***Babesia* spp.**
Summer	20	15% (N=3)	5% (N=1)
Rainy	20	25% (N=5)	10% (N=2)
Winter	20	10% (N= 2)	5% (N=1)
Total	60	23.33% (N=14)	

4.2. DISCUSSION

In developing countries like Bangladesh considerable economic losses occurs in large and small holding livestock productivity farming system due to hemoprotozoa (Belal *et al.* 2014). Prevalence studies are of immense importance for disease mapping and investigating the epidemiological triad. The eco-climatic conditions of the Pabna district are highly favorable for growth and multiplication of ticks which act as natural vectors of different hemoprotozoa like *Anaplasma* spp. and *Babesia* spp. In the present study, a total of 60 cattle blood smears were examined, out of which 14 cattle were found to be positive for hemoprotozoa. The overall prevalence of hemoprotozoan diseases was 23.33%. This finding was slightly contradicted with the reports of Bosu, (2021), who reported an overall prevalence of 27% in cattle of Pabna (Bera and Sathia upazilla) and Sirajganj (Raigonj, Chowhali and Shahjadpur upazilla) districts and Hosen *et al.* (2020) in Sylhet (52%). These variations are might be due to different geographical distribution. However, variation in geo-climatic condition, breed, and exposure of vectors and age of the animals might contribute to variable prevalence of haemoprotozoan diseases in the study areas (Muhanguzi *et al.* 2010).

Among different hemoprotozoa, the prevalence of *Anplasma* spp. was highest (16.67%) followed by *Babesia* spp. (6.66%) in this study. *Anaplasma marginale* showed highest prevalence (8.33%) followed by *Anaplasma centrale* (3.33%). In the present study mixed infection of *A. marginale* and *A. centrale* was 5%. Mixed infections were found more severe than the cases with single infection. Jayalakshmi *et al.* (2019) reported the highest prevalence of *Anaplasm*a spp (3.95%) was observed followed by *Babesia* spp (2.19%) and *Theileria* spp (0.44%) with the lowest prevalence of *Trypanosoma* spp. in cattle of Tamil Nadu, India, which supported the findings of present study. The observations of this study was in contradictory with the result of Velusamy *et al*. (2014) and Bhatnagar *et al*.(2015), who reported higher prevalence of bovine theileriosis followed by anaplasmosis and babesiosis in cattle. This study is in agreement with the findings of Khan *et al.* (2004) and Bosu, (2021), who observed highest prevalence rate of *Anaplasma marginale* compared to other hemoprotozoa in cattle. Belal *et al.* (2014) and Mahmud *et al.* (2015), who reported that the overall prevalence of anaplasmosis in cattle was recorded as 25.82%, Babesiosis was 2.27% respectively in Sirajganj district of Bangladesh, which closely

supports the findings of this experiment. Similar results of this present study obtained by Bosu, (2021) who reported that the prevalence of *A. marginale*, *A. centrale*, combined infection of *A. marginale* and *A. centrale* and *Babesia* spp. was 12%, 3%, 4%, 8% respectively in Pabna and Sirajganj district. In Bangladesh, Alim *et al.* (2012) reported that the prevalence of babesiosis was 1.85% in Noakhali district, and 2.78% respectively in Khagrachori district, which are lower than the findings of this study. Samad *et al.* (1989) and Chowdhury *et al.* (2006) recorded a comparatively lower (3.28% and 3.30% respectively) prevalence of *Babesia bigemina* infection in cattle of the selected Milk vita project areas of Bangladesh and Sirajganj sadar area of Bangladesh respectively. But Hassan *et al.* (2019) detected very high prevalence of *Anaplasma* spp. (43%) and *Babesia* spp. (19%) in cattle and sheep of Bangladesh. The lower prevalence of *Babesia* spp. is might be due to lower occurrence of vector *Rhipicephalus microplus* or might be due to easily diagnose by field veterinarians on the basis of their peculiar symptoms of haemoglobinurea unlike other haemoprotozoan disease. The prevalence depends largely on the distribution and density of the reservoir hosts, season, and vectors (Singh *et al.* 2000; Ogden *et al.* 2002). These variations are might be due to different geo climatic conditions of study area, exposure to vectors and age of examined animals.

The present study revealed that age also influences the occurrence of the infections of hemoprotozoa. The highest prevalence of *Anaplasma* spp. and *Babesia* spp. were found in the age of above 3 years (25% and 10% respectively), followed in 2-3 years (20% and 6.66% respectively) and 6 months-2 years of age (8% and 4% respectively). In similar works on anaplasmosis implemented in Sirajganj district by Belal *et al.* (2014), higher prevalence was found in adult cattle (34.19%) followed by young cattle (20.51%), and calf (14.11%). Similar results of this study were found by Kamani *et al.* (2010) who observed higher prevalence in adult than young cattle. The results of present study agree with Islam *et al.* (2009) who found that prevalence of tick infestation was higher in old cattle than young. Observation of this study also supported by the findings of of Ananda *et al.* (2009) who reported higher prevalence in animals aged more than 3 years followed by the lower prevalence in 1-2 years of age. Findings of hemoprotozoa infection in this investigation were supported by the observation of Urquhart *et al.* (1996) and Annetta *et al.* (2005) who reported an inverse age resistance of the disease where adult showed more susceptibility than

calves. This might be due to rapid immune responses to primary infection by the calves through a complex immune mechanism (Annetta *et al.* 2005). *K*han *et al.* (2004) also supported the findings of this experiment and reported that the highest prevalence of hemoprotozoa in animals aged more than 3 years followed by the lowest prevalence in less than 1 year of age. Endemic instability of the study areas might responsible for frequent infections in adult cattle where newborn calves were protected by colostral immunity (Cynthia *et al.* 2011). The earlier researchers reported that calves up to 9 months or even 1 year of age usually showed no clinical illness whereas cattle 1 to 2 years or above 3 years of age may develop acute or fatal form of disease. On the contrary, earlier observation was in contrast with the observation of Muhanguzi *et al.* (2010) who found higher prevalence of anaplasmosis in calves and lowest in young cattle in Uganda and the difference was explained by dominant immune responses to *Anaplasma* spp. infection. Age resistance, perhaps in combination in some cases with maternal antibodies, is reflected in the reduced number of clinical outbreaks in young animals (Hosen *et al.* 2020).

Femininity of animals also has influences in the occurrence of haemoprotozoan diseases. In current study highest prevalence of *Anaplasma* spp and *Babesia* spp was observed in Female cattle (20% and 10% respectively) compared to male cattle (13.33% and 3.33% respectively). The findings of this study are in agreement with the findings of Belal *et al.* (2014) who also reported higher prevalence of anaplasmosis in female cattle (2.59%) than male cattle (1.60%). Similar findings were found by Mahmud *et al.* (2015) and Bary *et al.* (2018) where they separately observed that the prevalence of hemoprotozoa was higher in female than male. This result also agreed with the report of Sarkar, (2007) who reported the prevalence of ectoparasites were significantly higher in female than male. This study showed consistency with the observation of Kamani *et al.* (2010), who recorded comparatively higher prevalence in female than male cattle in Nigeria. Higher prevalence in female cattle might be due to stress condition as they were kept longer period for breeding and milk production purpose or supplied imbalance diet against their high demand (Kamani *et al.* 2010).

In this study highest prevalence of *Anaplasma* spp and *Babesia* spp was observed in cross breed cattle (23.33% and 10% respectively) compared to local cattle (10% and

3.33% respectively). This study is in line with the findings of Hosen *et al.* (2020), who reported that, the prevalence of Anaplasmosis and babesiosis was highest in crossbreed cattle (29.63% and 9.88% respectively) compared to local indigenous breed (21.05% and 5.26% respectively). In the study area it was observed that the indigenous cattle were moved freely in pasture land whereas crossbreed cattle were confined to farms. The higher prevalence of babesiosis in crossbreed cattle in our study is in line with the findings of Alim *et al.* (2012) and Mahmud *et al.* (2015). In case of anaplasmosis our findings are in correspondence with the findings of Chowdhury *et al.* (2006) who recorded prevalence of anaplasmosis in cross breed cattle (58.33%) and indigenous cattle (11.63%) in Sirajganj district. Breed differences are also important in the susceptibility of cattle to tick borne diseases where Cattle of European origin like Holstein are usually highly susceptible (Tabor *et al.* 2017). Breed susceptibility of Babesiosis and anaplasmosis recorded in this study was supported by the report of Mahmud *et al.* (2015) and Belal *et al.* (2014). Variation in geo-climatic condition, breed, and exposure of vectors and age of the animals might contribute to variable prevalence of hemoprotozoan diseases in the study areas (Muhanguzi *et al.* 2010). Constant exposure of infections and development of immunity against such infections might be responsible for lower prevalence in indigenous cattle (Siddiki *et al.* 2010). On the contrary, more attention in the management of HF crossbred cattle might give less chance of pre exposure of vectors and develop no or less immunity, resulting frequent occurrence of such diseases (Siddiki *et al.* 2010).

There is a considerable seasonal variation with occurrence of haemoprotozoa infection. . In this current study both *Anaplasma spp* and *Babesia* spp. highest prevalence was recorded in rainy season (25% and 10% respectively) in relation to summer (15 % and 5% respectively) and winter season (10% and 5% respectively). In case of anaplasmosis, the findings of this study supported by Belal *et al.* (2014) who reported that anaplasmosis ranked the highest in rainy season (30.68%) in relation to summer (27.50%) and winter (15.15%) season. Mohanta *et al.* (2011) also supported the findings of this study and reported that prevalence of hemoprotozoan diseases was higher in rainy season (45.45%) than that in the summer (27.87%) and winter (16.55%) in livestock of three hilly areas of Bangladesh. The prevalence of the tick was higher in the dry season than a rainy season (Eyo *et al.* 2014). The incidence of

haemoprotozoan diseases varies with geographic area; it depends on climatic conditions such as temperature, humidity and rainfall (Radostits *et al.* 2000), which might be accounted for higher prevalence of such infections in the rainy season of the study. It is assumed that, due to the availability of green grasses in the rainy season, the animal maintain a good health and remain carrier state in endemic areas. On the other hand, tick population is high (95%) at this time. This increased tick population may take part in disseminating the infection to the unexposed animal rendering high prevalence of hemoprotozoan diseases in the rainy season. Lower temperature and humidity of winter months were less favorable for the growth and multiplication of tick vectors which might contribute to lower frequency of such diseases in the study population (Zahid *et al.* 2005). This is contradictory to the observation made by Krishnamurthy *et al.* (2016), who have reported the highest prevalence of haemoprotozoan infection (66.6%) during monsoon season in cattle in Shimoga region of Karnataka, India. This variation might be due to changing climatic conditions.

CHAPTER VI

SUMMARY AND CONCLUSION

The present study concluded that the Pabna district of Bangladesh was highly endemic for hemoprotozoa diseases. Prevalence of *Anaplasma* spp. was higher than *Babesia* spp. Hemoprotozoa diseases was higher in aged, female and crossbreed cattle compared to young, male and local indigenous cattle. During summer season prevalence of hemoprotozoa diseases was higher compared to rainy and winter season due to high prevalence of tick population in hot and humid condition of Bangladesh. This study could be useful to forecast the diseases based on age, sex, breed of cattle and season of study area. Screening of carrier status is important for early diagnosis and implementation of tick control measures to prevent economic losses in cattle. DLS (Department of Livestock Services) need to be strengthening with well-equipped laboratory facilities in order to diagnosis hemoprotozoa diseases and DLS needs to organize hemoprotozoa vector control program regularly.

REFERENCES

Abas, O., Abd-Elrahman, A., Saleh, A. and Bessat, M. (2021). Prevalence of tick-borne haemoparasites and their perceived co-occurrences with viral outbreaks of FMD and LSD and their associated factors. *Heliyon*. **7**(e06479): 1-6.

Aktas, M., Altay, K., and Dumanli, N. (2006). A molecular survey of bovine *Theileria* parasites among apparently healthy cattle and with a note on the distribution of ticks in eastern Turkey. *Vet. Parasitol.* **138**: 179-185.

Alim, M. A., Das, S., Roy, K., Masuduzzaman, M., Sikder, S., *et al.* (2012) Prevalence of haemoprotozoan diseases in cattle population of Chittagong division, Bangladesh. *Pak. Vet. J.* **32**: 221-224.

Ananda, K. J. and Adeppa, J. (2016) Prevalence of Haemoprotozoan infections in bovines of Shimoga region of Karnataka state. *J Parasit Dis*. **40**: 890-892.

Ananda, K. J., D'Souza, P. E. and Puttalakshamma, G. C. (2009). Prevalence of Haemoprotozoan diseases in crossbred cattle in Bangalore north. *Veterinary world.* **2**(1): 15-16.

Annetta, Z. S., Jeremy, Gray, Helen, E., Skerrett and Mulcahy, G. (2005). Possible mechanisms underlying age-related resistance to Bovine babesiosis. *Parasite Immunology*. **27**: 115-120.

Anwar, K. (2018). Epidemiology of tick-borne infection in ruminants in Peshawar. *J. Adv. Parasitol.* **5**(1): 6-10.

Aouadi, A., Leulmi, H., Boucheikhchoukh, M., Benakhla, A., Raoult, D., *et al.* (2017). Molecular evidence of tick-borne hemoprotozoan-parasites (*Theileria ovis* and *Babesia ovis*) and bacteria in ticks and blood from small ruminants in Northern Algeria. *Comp. Immunol. Microbiol. Infect. Dis.* **50**: 34–39.

Bary, M. A., Ali, M. Z., Chowdhury, S., Mannan, A., Nur e Azam, Hossain, M. A. *et al.* (2018). Prevalence and molecular identification of haemoprotozoan diseases of cattle in Bangladesh. *Adv. Anim. Vet. Sci*. **6**: 176-182.

Baset, M. A., Rahman, M. M., Islam, M. S., Das, G. B. and Ara, A. (2002). Beef cattle production in Bangladesh- a review. *Online Journal of Biological Sciences.* **2**(6): 429-435.

Belal, S. M. S. H., Mahmud, M. A. A. and Ferdous, M. J. (2014). Prevalence of anaplasmosis in cattle in Sirajganj district of Bangladesh. *Res. Agric., Livest. Fish.* **1**(1): 97-103.

Benjamin Maxine, M. (2005). Outline of veterinary clinical pathology. III edn. Kalyani Publishers, New Delhi.

Bhat, S. A., Singh, N. K., Singh, H. and Rath, S. S. (2017). Molecular prevalence of *Babesia bigemina* in *Rhipicephalus microplus* ticks infesting cross-bred cattle of Punjab, India. *Parasit Epidemiol Control*. **2**: 85-90.

Bhatnagar, C. S., Bhardawaj, B., Sharma, D. K. and Meena, S. K. (2015). Incidence of haemoprotozoan diseases in cattle in Southern Rajasthan. India. *Int. J. Curr. Microbiol. App. Sci.* **4**(3): 509-514.

Bock, R., Jackson, L. and Jorgensen, W. (2004). Babesiosis of cattle. *Parasitology.* **129**(1): 247–269.

Bosu, A. K. (2021). Prevalence of blood protozoan diseases of cattle in 'Bathan' areas in Bangladesh. MS thesis, Department of Microbilogy and Parasitology, Sher-e-Bangla Agricultural University, Dhaka.

Bowmann, D. D. (2009). George's parasitology for veterinarians, 9th edn. Saunders Elsevier, St, Louis.

Chaudhri, S.S., Bisla, R.S., Bhanot, V. and Singh, H. (2013). Prevalence of haemoprotozoan infections in pyretic dairy animals of Eastern Haryana. *Indian J. Anim. Res.* **47**(4): 344-347.

Chowdhury, S., Hossain, M. A., Barua, S. R. and Islam, S. (2006). Occurrence of common blood parasites of cattle in Sirajgonj sadar area of Bangladesh. *Bangl. J. Vet. Med.* **4**(2): 143–145.

Cynthia, M., Kahn, M. A., Line, S., Susan, E. and Aiello, B. S. (2011). Marck Veterinary Manual, Online Ed. Merck Sharp & Dohme Corp, a subsidiary of Merck & Co., Inc. Whitehouse Station, NJ, USA.

DLS, (2021). Livestock Economy at a Glance 2021-22. Department of Livestock Services, Farmgate, Dhaka.

Eyo, J. E., Ekeh, F. N., Ivoke, N., Atama, C. I., Onah, I. E., Ezenwaji, N. E., Ikele, C. B. (2014). Survey of tick infestation of cattle at four selected grazing sites in the tropics. *Glob Vet*. **12**(4):479–486

Fahrimal, Y., Goff, W. L. and Jasmer, D. P.(1992). Detection of *Babesia bovis* carrier cattle by using polymerase chain reaction amplification of parasite DNA. *J. Clin. MicrobioL.* **30**(6): 1374-1379.

Ghosh, S. and Nagar, G. (2014). Problem of ticks and tick-borne diseases in India with special emphasis on progress in tick control research: a review. *J Vector Borne Dis.* **51**: 259-270.

Ghosh, S., Azhahianambia, P. and Yadavb, M. P. (2007). Upcoming and future strategies of tick control: a review. *J Vector Borne Dis*. **44**: 79–89.

Gul, N., Ayaz, S., Gul, I., Adnan, M., Shams, S. and Akbar, N. (2015). Tropical theileriosis and east coast fever in cattle: present, past and future perspective. *Int. J. Microbio. App. Sci*. **4**(8): 1000-1080.

Habib, M. A., Bhuiyan, A. K. F. H., Bhuiyan, M. S. A. and Khan, A. A. (2003). The Red Chittagong Cattle: An animal genetic resource of Bangladesh. *Bang. J Anim. Sci.* **32**: 101-108.

Hassan, M. Z., Giasuddin, M., Rahman, M. M., Ershaduzzaman, M., Hasan, M., *et al*. (2019) Identification of vector borne blood protozoa in cattle and sheep in Bangladesh. *J Virol* Antivira. **2**(4): 1-6.

Hemme, T., Garcia, O., and Khan, A. R. (2004), A review of milk production in Bangladesh with particular emphasis on small-scale producers, FAO-Pro-Poor Livestock Policy Initiatives. FAO, Rome. pp. 7.

Hosen, M. M., Jewel, A. B., Chowdhury, M. S. R., Uddin, M. B., Hossain, M. M., Rahman, M. M., Rahman, M. M. (2020). Prevalence of haemoprotozoan diseases in cattle population in Sylhet District of Bangladesh. *Adv. Anim. Vet. Sci*. **8**(7): 748-752.

Hossain, M. U. (2019). Present scenario of using feed additives during cattle fattening in Dhaka district of Bangladesh. MS thesis, Department of Animal Production and Managemnt, Sher-e-Bangla Agricultural University, Dhaka.

Hussein, A. H., Mohammed, N. A. E. S. and Mohammed, H. K. (2007). Theileriosis and babesiosis in cattle: haemogram and some biochemical parameters. In Proceedings of XIII International Congress of International Society of Animal Hygiene, June,Tartu, Estonia, pp. 143–150

Islam, M. S., Rahman, S. A., Sarker, P., Anisuzzaman and Mondal, M. M. H. (2009). Prevalence and population density of ectoparasitic infestation in cattle in Sirajgonj district, Bangladesh. *Bangladesh Research Publications Journal*. **2**: 332-339.

Jassem, G. A. and Agaar, O. A. (2015). Molecular and biochemical study of *Anaplasma marginale* in cattle in Wassit Province of Iraq. *J. Bacteriol. Res.* **7**(4): 36-41.

Jayalakshmi, K., Sasikala, M., Veeraselvam, M., Venkatesan, M., Yogeshpriya, S., Ramkumar, P. K., Selvaraj, P. and Vijayasarathi, M. K. (2019). Prevalence of haemoprotozoan diseases in cattle of Cauvery delta region of Tamil Nadu. *J Parasit Dis.* **43**(2):308-312.

Jongejan, F. and Uilenberg, G. (2004). The global importance of ticks. *Parasitology*. **129**(1): 3-14.

Kakati, P., Sarmah, P. C., Ray, D., Bhattacharjee, K., Sharma, R. K., Barkalita, L. M., Sarma, D. K., Baishya, B. C., Borah, P. and Stanley, B. (2015). Emergence of oriental theileriosis in cattle and its transmission through *Rhipicephalus (Boophilus) microplus* in Assam, India. *Veterinary World.* **8**(9): 1099-1104.

Kala, S. and Deo, B. G. (2018). Prevalence of haemoprotozoan disease in cattle in rainy season. *Int.J.Curr.Microbiol.App.Sci.* **7**(7): 2693-2699.

Kamani, J., Sannusi, A., Egwu, O. K., Dogo, G. I., Tanko, T. J., Kemza, S., Tafarki, A. E. and Gbise, D. S. (2010). Prevalence and significance of haemoparasitic infections of cattle in NorthCentral, Nigeria. *Vet. World*. **3**(10): 445-448.

Kaur, R., Yadav , A., Rafiqi, S. I., Godara, R., Sudan, V., Chakraborty, D. and Katoch, R. (2021). Epidemiology, haematology and molecular characterization of haemoprotozoon and rickettsial organisms causing infections in cattle of Jammu region, North India. *BMC Vet Res*. **17**(219): 1-12.

Khan, M. Q., Zahoor, A., Jahangir, M. and Mirza, M. A. (2004). Prevalence of blood parasites in cattle and buffaloes. *Pakistan Veterinary Journal*. **24**(4): 193-195.

Khokon, M. S. I., Azizunnesa, M., Islam, M. M., Chowdhury, K. B., Rahman, M. L. and Ali, M. Z. (2017). Effect of mastitis on post-partum conception of cross bred dairy cows in Chittagong district of Bangladesh. *J. Adv. Vet. Anim. Res.* **4**(2):155-160.

Kohli, S., Atheya, U. K., Thapliyal, A. (2014). Prevalence of theileriosis in cross-bred cattle: its detection through blood smear examination and polymerase chain reaction in Dehradun district, Uttarakhand, India. *Vet. World.* **7**: 169-171.

Krishnamurthy, C. M., Ananda, K. J., Adeppa, J. (2016). Prevalence of Haemoprotozoan infections in bovines of Shimoga region of Karnataka state. *J Parasit Dis.* **40**(3):890–892.

Kundave, V. R., Ram, H., Banerjee, P. S., Garg, R., Mahendran, K., *et al.* (2018). Development of multiplex PCR assay for concurrent detection of tick borne haemoparasitic infections in bovines. *Acta parasitological*. **63**: 759- 765.

Laha, R., Das, M. and Sen, A. (2015). Morphology, epidemiology, and phylogeny of *Babesia*: An overview. *Trop Parasitol*. **5**(2):94-100.

Lee, S. H., Mossaad, E., Ibrahim, A. M., Ismail, A. A., Moumouni, P. F. (2018). Detection and molecular characterization of tick-borne pathogens infecting sheep and goats in Blue Nile and West Kordofan states in Sudan. *Ticks Tick Borne Dis.* **9**(3): 598-604.

Maharana, B. R., Tewari, A. K., Saravanan, B. C., Sudhakar, N. R. (2016). Important hemoprotozoan diseases of livestock: Challenges in current diagnostics and therapeutics: An update. *Veterinary World.* **9**(5): 487- 495.

Maharana, B.R., Kumar, B., Prasad, A., Patbandha, T. K., Sudhakar, N. R., Joseph, J. P. and Patel, B. R. (2015). Prevalence and assessment of risk factors for haemoprotozoan infections in cattle and buffaloes of South-West Gujarat, India. *Indian J. Anim. Res.* **50**(5): 733- 739.

Mahmud, M. A. A., Belal, S. M. S. H. and Hossain, M. A. (2015). Prevalence of theileriosis and babesiosis in cattle in Sirajganj district of Bangladesh. *Res. Agric. Livest. Fish.* **2**(1): 79-86.

Mohanta, U. K., Anisuzzaman and Mondal, M. M. H. (2011). Tick and tick borne protozoan diseases of livestock in the selected hilly areas of Bangladesh. *Int. J. Agril. Res. Innov. & Tech.* **1**(1&2): 60-63.

Mostari, K. D., Al-Noman, K. M., Harun-ur-Rashid, S. M., Haque, M. A. and Azam, M. G. (2020). Study on status of cattle diseases in relation to age, sex and season at Debidwar, Bangladesh. *IOSR Journal of Agriculture and Veterinary Science (IOSRJAVS).* **13**(1): 55-65.

Muhanguzi, D., Ikwap, K., Picozzi, K. and Waiswa, C. (2010). Molecular characterization of *anaplasma* and *ehrlichia* species in different cattle breeds and age groups in Mbarara district (Western Uganda). *International Journal of Animal and Veterinary Advances*. **2**: 76-88.

Murthy, C. M. K., Ananda, K. J. and Adeppa, J. (2016). Prevalence of haemoprotozoan infections in bovines of Shimoga region of Karnataka state. *J Parasit Dis*. **40**(3): 890–892.

Mushahary, D., Bhattacharjee, K., Sarmah, P., Deka, D., Tamuly, S., and Bora, S. (2021). Molecular detection and characterization of haemoprotozoan and haemorickettsial parasites of cattle in the Indo-Bhutan Border districts of Assam, India. *International Journal of Livestock Research*. **11**(3): 83-91.

Ogden, N. H., Casey, A. N. J., French, N. P., Adams, J. D. W. and Woldehiwet, Z. (2002). Field evidence for densitydependent facilitation amongst *Ixodes ricinus* ticks feeding on sheep. *Parasitol*. **124**: 117–125.

Patel, M. D., Kumar, N., Rathod, P. H., Tyagi, K. K. and Sorathiya, L. M. (2017). Incidence and Haematological changes in haemoprotozoan infections in bovines of South Gujarat. *Indian J. Vet Sci. Biotech*. **13**(2): 62-66.

Radostits, O. M., Blood, D. C., Gay, C. C. and Hinchcliff, K. W. (2000). Veterinary medicine—a text book of the disease, sheep, goats, pigs and horse, 9th edn. ELBS, Baillier, London. pp: 1172-1173, 1289-1290.

Rajkumar, R., Vijaya Bharathi, M., Selvaraju, G. and Senthil Kumar, A. (2020). Epidemiological studies on bovine tick-borne haemoparasitic diseases in Chennai. *International Journal of Livestock Researc*h. **10**(2): 36-45.

Rony, S. A., Mondal, M. M. H., Begum, N., Islam, M. A. and Affroze, S. (2010). Epidemiology of ectoparasitic infestations in cattle at Bhawal forest area, Gazipur. *Bangl. J. Vet. Med.* **8**(1): 27 – 33.

Ruprah, N. S. (1985): Text book of Clinical Protozoology, Oxanian Press Pvt. Ltd. New Delhi, pp. 286.

Salih, D. A., El Hussein, A..M. and Singla, L. D. (2015). Diagnostic approaches for tick borne haemoparasitic diseases in livestock. *J. Vet. Med. Anim. Health.* **7**(2): 45–56.

Samad, M. A., Bashar, S. A., Shahidullah, M. and Ahmed, M. U. (1989). Prevalence of haemoprotozoan parasites in cattle of Bangladesh. *Indian Journal of Veterinary Medicine*. **13**: 50-51.

Sarkar, M. (2007). Epidemiology and pathology of ectoparasitic infestation in Black Bengal Goats in Bangladesh. M.Sc. thesis. Department of Parasitology, Bangladesh Agricultural University, Mymensingh.

Siddiki, A. Z., Uddin, M. B., Hasan, Hossain, M. F., Rahman, M. M., Das, B. C., Sarker, M.S. and Hossain, M. A. (2010). Coproscopic and haematological approaches to determine the prevalence of Helminthiasis and protozoan

diseases of Red Chittagong Cattle (RCC) breed in Bangladesh. *Pakistan Veterinary Journal*. **30**: 1-6.

Singh, A. P., Singla, L. D. and Singh, A. (2000). A study on the effects of macroclimatic factors on the seasonal population dynamics of *Boophilus micropus* (Canes, 1888) infesting the cross-bred cattle of Ludhiana district. *Inter. J. Anim. Sci.* **15**: 29–31.

Singh, H., Mishra, A. K., Rao, J. R. and Tewari, A. K. (2009). Comparison of indirect fluorescent antibody test (IFAT) and slide enzyme linked immunosorbent assay (SELISA) for diagnosis of *Babesia bigemina* infection in bovines. *Trop. Anim. Health Prod.* **41**(2): 153–159.

Song, R.,Wang, Q., Guo, F., Liu, X., Song, S. *et al.* (2018). Detection of *Babesia* spp., *Theileria* spp. and *Anaplasma ovis* in border regions, northwestern China. *Transbound Emerg dis*. **65**: 1537-1544.

Soulsby, E. J. L. (1982). Helminths, arthropods & protozoa of domesticated animals. 7th edn. ELBS, Bailliere Tindall, London.

Subapriya, S., Senthil, N. R., Gowri, B., Chandrasekaran, D., Gopalakrishnan, A., Arunaman, C. S., Selvaraj, P. and Vairamuthu, S. (2021). Prevalence of haemoprotozoal diseases in cattle: A review of 6000 cases. *The Pharma Innovation Journal.* **10**(8): 535-541.

Tabor, A. E., Ali, A., Rehman, G., Garcia, G. R., Zangirolamo, A. F., Malardo, T., Jonsson, N. N. (2017). Cattle Tick *Rhipicephalusmicroplus*-Host Interface: A Review of Resistant and Susceptible Host Responses. *Front. Cell. Infect. Microbiol*. **7**: 506.

Tewari, A. K., Ray, D., Mishra, A. K. and Bansal, G. C. (2001). Identification of immuno dominant polypeptides common between *Babesia bigemina* and *Theileria annulata*. *Indian J. Anim. Sci.* **71**: 679–680.

Urquhart, G. M., Armour, J., Duncan, J. L., Jennings, F. W. (1996). Veterinary Parasitology. 2nd Ed, Black well Science Ltd, Oxford, UK, pp. 213, 242-251.

Velusamy, R., Rani, N., Ponnudurai, G., Harikrishnan, T. J., Anna, T., Arunachalam, K., Senthilvel, K. and Anbarasi, P. (2014). Influence of season, age and breed on prevalence of haemoprotozoan diseases in cattle of Tamil Nadu, India. *Veterinary World.* **7**(8): 574-578.

Vetrivel, D., Serma, A., Pandian, S., Selvakumar, K. N. and Shree, J.S. (2017). A study on the prevalence of Babesiosis in north eastern agro-climatic zone of Tamil Nadu, India. *Journal of Entomology and Zoology Studies*. **5**(5): 1271-1273.

Vieira, L. L., Canever, M. F., Cardozo, L. L., Cardoso, C. P., Herkenhoff, M. E. *et al.* (2019). Prevalence of *Anaplasma marginale*, *Babesia bovis*, and *Babesia bigemina* in cattle in the Campos de Lages region, Santa Catarina state, Brazil, estimated by multiplex-PCR. *Parasite Epidemiol Control*. **6**: e00114.

Zahid, I. A., Latif, M. and Baloch, K. B. (2005). Incidence and treatment of theileriasis and babesiasis. *Pakistan Veterinary Journal*. **25(**3): 137-139.

Zhou, M., Cao, S., Sevinc, F., Sevinc, M. and Ceylan, O. (2017), Molecular detection and genetic characterization of *Babesia*, *Theileria* and *Anaplasma* amongst apparently healthy sheep and goats in the central region of Turkey. *Ticks Tick Borne Dis*. **8**: 246-252.