PREVALENCE OF BLOOD PROTOZOANS OF MURINE RODENTS IN DHAKA CITY, BANGLADESH

A thesis

By

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DHAKA-1207

JUNE, 2022

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REGISTRATION NUMBER: 20-11132

A Thesis

Submitted to Department of Microbiology and Parasitology

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

Requirements for the degree of

MASTERS OF SCIENCE (MS) IN PARASITOLOGY

Semester: January-June/2022

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CERTIFICATE

This is to certify that the thesis entitled ***"PREVALENCE OF BLOOD PROTOZOANS OF MURINE RODENTS IN DHAKA CITY, BANGLADESH"*** *submitted to the Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* ***Master of Science in Parasitology,*** *embodies the result of a piece of bona fide research work carried out by* ***Nitol Chandra Das*** *Registration No.* ***20-11132*** *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.

Dated:

Place: Dhaka, Bangladesh.

Professor Dr. Uday Kumar Mohanta
Supervisor
Department of Microbiology and Parasitology
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Dedicated

To

My Beloved Parents

ACKNOWLEDGEMENTS

At the beginning, the author bows the grace and mercy of the "Almighty God" the omnipresent, omnipotent and omniscient, who enabled him to successfully complete the research work and the thesis leading to Master of Science in Parasitology under the department of Microbiology and Parasitology.

The author would like to express her heartfelt gratitude and sincerest appreciation to her research Supervisor ***Prof. Dr. Uday Kumar Mohanta****, Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University, Dhaka, for his valuable guidance, advice, immense help, encouragement and support throughout the study. Likewise, grateful appreciation is conveyed to Co-supervisor* ***S.M. Abdullah,*** *Assistant Professor, Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University, Dhaka, for his constant encouragement, cordial suggestions, constructive criticisms and valuable advice to complete the thesis along with faculties of the Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University for their rendered novel services towards her as their student.*

The author would like to express her deepest respect and boundless gratitude to all the respected teachers of the Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University, Dhaka, for their valuable teaching, sympathetic co-operation, and inspiration throughout the course of this study and research work.

The author is deeply indebted and grateful to her parents and family members without whose love, affection, inspiration and sacrifice this work would have not been completed.

The author appreciates the assistance rendered by the staff members of the Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University, who have helped her during the period of study. The author also expresses her cordial gratitude to all MS students, Department of Microbiology and Parasitology for their kind cooperation throughout the research period. The author wishes to extend her special thanks to her classmates and seniors for their keen help as well as the heartiest co-operation and encouragement during research.

The Author

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ACRONYMS AND ABBREVIATIONS

ABBREVIATION		FULL MEANING
MS	=	Master of Science
et al.	=	And others/Associates
etc.	=	et cetera

PREVALENCE OF BLOOD PROTOZOANS OF MURINE RODENTS IN DHAKA CITY, BANGLADESH

ABSTRACT

Rodents are small mammals which are responsible for the transmission of various deadly pathogens with zoonotic significance. Blood borne protozoans play a crucial role in this regards. The present study was performed to determine the prevalence of blood protozoan parasites of murine rodents in Dhaka city, Bangladesh. A total of 80 rodents with four rodent species were captured, namely, *Rattus norvegicus* (n=28), *Rattus rattus* (n=14), *Bandicota bengalensis* (n=30) and *Mus musculus* (n=8). The rodents were live captured by using traps from stationary/grocery shops (n=25), local rent houses (n=20), houses from slum areas (n=15) and fish markets (n=20). The overall prevalence of this study was 50%. The highest infection rate was found in *R. norvegicus* (57.14%) followed by *B. bengalensis* (53.33%), *M. musculus* (37.5%) and *R. rattus* (35.71%). Among the different areas, the highest prevalence was recorded in slum areas (66.67%) followed by fish markets (50%), stationary/grocery shops (48%), and local rent houses (40%). Five blood protozoa detected from the rodents which were *Plasmodium* spp. (31.25%), *Anaplasma marginale* (21.25%), *Anaplasma centrale* (17.5%), *Babesia* spp. (10%), and *Trypanosoma* spp. (3.75%). The ratio of single and mixed infection was equal (50%). Among the observed blood protozoa, *Trypanosoma* spp. and *Babesia* spp. have a great public health significance. Therefore, proper attention is recommended to prevent rodent born protozoal zoonosis through integrated control program.

Keywords: Blood protozoa, Prevalence, Rodents, Dhaka city.

CHAPTER I

INTRODUCTION

Bangladesh is the eighth-most densely populated country in the world, with a population exceeding 163 million people, in an area of 148,460 square kilometers (57,320 sq. mi). The country is divided in three regions. Most of the area is dominated by the largest river delta in the world, Ganges Delta. The northwest and central parts of the country are formed by Madhupur and Barind plateaus and northeast and southeast part comprises hill ranges (Geography, 2022). Bangladesh is predominantly rich fertile flat land and most of it is less than 12 m (39 ft.) above sea level. 17% of the country is covered by forests and 12% is covered by hill tracts (Ali, 1996).

Rodents, belonging to the order *Rodentia*, are the largest groups of mammals in earth. It comprises approximately 42% of global mammalian population which occupies almost 2,277 known species in 33 families (Wilson *et al*., 2005). Most of the species belongs to three families including, Muridae, Microtidae, and Sigmodontidae respectively, of which, Muridae is the largest. The Muridae family comprises more than 1,383 species of the mice and rats. The members of this family are often collectively called murids, or muroid rats, which are nocturnal and omnivorous (Britannica, 2020).

In Bangladesh, about 20 species of rats are available under four families. These include Sciuridae (9 species), Muridae (8 species), Spalacidae (1 species) and Hystricidae (2 species) (Ahmed *et al.*, 2009). Common Muridae species that are found in Bangladesh are the *Rattus rattus* (House mouse, common house mouse, indur), *Bandicoota indica* (Bandicoot rat, Dhadi indur), *Bandicoota bengalensis* (Lesser Bandicoot rat, Metho indur), *Rattus Norvegicus* (Norway rat, Badami indur), *Cannomys badius* (Bamboo rat, small mountain rat)), *Suncus murinus Linnaeus* (Shrew,chika,c huchoo) etc. (Ahmed, 2009; Burgin,2018).

The economic and public health significance of rodent is vast. They can cause significant economic losses through feeding on stored food, destruction of crops and increase health

risk by transmitting various infectious agents to humans (Khaghani, 2007). In the form of transmission, rodent-borne diseases can be divided into two main categories: directly and indirectly transmitted diseases. In the first category, diseases are transmitted by being bitten or by inhalation of the germ in feces of rodents, whereas in the second category, humans are infected as the result of consuming food and water contaminated by rodent feces or urine. On the contrary, rodents could act as amplifier hosts in the case of diseases transmitted by arthropod vectors from rodents to humans. Moreover, dead rodents accidentally taken by livestock could mediate disease transmission to humans if products of these livestock were not treated properly prior to consumption (Meerburg *et al.,* 2009).

The zoonotic significance of rodent is enormous. An increasing number of cases associated to parasitic zoonosis were recorded in some parts of the world (Han *et al*., 2015). The factors behind this accelerated rate include, habitat modification, overpopulation, and mass migration (Chomel *et al*., 2007). Residential areas, especially urban cities like Dhaka, having a great concern considering the emergence of zoonotic diseases. These areas provides a favorable habitats to certain species of wild animals which have a regular and increased contact with humans (Luniak, 2004). In urban areas, house mice (*M. musculus*) and wild rats (*R. rattus*, *R. norvegicus*) occupies various habitats than the other species, and cause considerable problem due to their high reproductive capacity, zoonotic potential, and engagement towards close association with humans (Battersby *et al*., 2002; Clinton, 1969).

Zoonotic diseases harbored by rodents are caused by protozoa (e.g. toxoplasmosis, babesiosis, trypanosomiasis, leishmaniasis), helminths (e.g. hymenolepiasis, trichinellosis, echinococcosis, and capillariasis), viruses (e.g.Lassa fever, Hantavirus diseases, tick-borne encephalitis, as well as Argentine and Bolivian hemorrhagic fever), and bacteria (e.g., plague, leptospirosis, lyme disease, and relapsing fevers) (Rohollah *et al*., 2012; Asante *et al*., 2019). In case of protozoan diseases, blood borne protozoan have great importance. Important rodent borne blood protozoan diseases are leishmaniasis (*Leishmania major*, *L. infantum*); trypanosomiasis (*Trypanosoma lewsi*); babesiosis (*Babesia microti*); malaria (*Plasmodium berghei*), anaplasmosis (*Anaplasma marginale, A. centrale*) (Helmy *et al*., 2017; Seifollahi and Sarkeri, 2016; Killick, 2009).

Wild rodents constitute a large portion of potential reservoirs for *Leishmania* sp. (Pourmohammadi *et al*., 2008). In a study at Spain, *L. infantum* DNA was found in 27 % (10 out of 37) of the rodents analyzed. The infection was found in the three investigated species with prevalences of 33.3 % in *R. rattus*, 20.8 % in *A. sylvaticus*, and 50.0 % in *M. musculus* (Ashford, 1996). *Plasmodium berghei*, a species in the genus *Plasmodium* and subgenus *Vinckeia*, is a protozoan parasite that cause malaria in certain rodents. Isolated from rats in central Africa, *P.berghei* is one of four *Plasmodium* species that have been described in African murine rodents. The others are *Plasmodium chabaudi*, *Plasmodium vinckei*, and *Plasmodium yoelii*. Due to its ability to infect rodents, *P. berghei* is a popular model organism for the study of human malaria (Navea *et al*., 2015; Vincke and Lips, 1948). Trypanosomiasis in rodent mainly caused by *T.Lewsi.* In Iran, 6 (11.54%) rodents were infected with *Trypanosoma lewisi*. Anti-*Leishmania* antibodies were detected in the sera of 8 (15.34%) of the rodents (Craig *et al*., 2012). Human babesiosis is a zoonotic disease transmitted via rodent usually caused by *B. microti.* In United States of America, there are found human babesiosis caused by *B.microti* (Seifollahi and Sarkeri, 2016; Gorenflot *et al*., 1998). The first human babesiosis case in Japan was identified in Kobe in 1999. The study stated that, the patient was infected through blood transfusion from an asymptomatic donor infected with a variant of *B. microti* (Homer *et al*., 2000; Saitoito *et al*., 1999). In a study of Bangladesh, there found a lower anaplama and babesia infection at 7.5% and 4.7% respectively (Islam *et al*., 2020).

Rodent borne blood protozoan diseases have great public health significance throughout the world. But unfortunately, a very few or no study was conducted regarding this aspect in Bangladesh. Keeping all the points mentioned above, the aim of this present study was undertaken with the following objectives.

- Morphological identification of blood protozoan parasites in rodents.
- Prevalence of blood protozoan in murine rodents in Dhaka city.

CHAPTER II

REVIEW OF LITERATURE

Rat and mice are worldwide distributed and are the most common rodents found in the city and its surroundings area. Four species of rodents namely black rats (*Rattus rattus*), brown rats/Norwegian rats (*Rattus norvegicus*), lesser bandicoot rat (*Bandicota bengalensis*) and house mouse (*Mus musculus*) are very common around human habitats in tropical and sub-tropical regions. The breeding of rodents has been increased rapidly in the recent years because of the abundance of food resources and lack of environmental hygiene in urban areas (Arfa, 1987; Abdel and Eisha, 1997). Rodents impose economic damages, and involve significant impact on public health system. They can cause destruction of food stuffs, electrical equipment and buildings by contamination or gnawing with excreta resulting in significant economic losses (Coomansingh *et al.*, 2009).

Wild rodents are reservoirs of various zoonotic diseases, such as toxoplasmosis, babesiosis, and leishmaniasis. In a study at Boyer-Ahmad district, southwestern Iran, a total of 52 rodents were collected from different parts of Boyer-Ahmad district, in Kohgiluyeh and Boyer-Ahmad province, using Sherman live traps. 37 (71.1%) of them were infected with at least one protozoan parasite and 6 (11.54%) were infected with *Trypanosoma lewisi*. Some of them might be potential risks to residents and domestic animals in the region (Seifollahi *et al*., 2016).

Several blood protozoan parasites were examined. In this study, 82(63.08%) were positive, out of 130 captured rodents. Protozoans that were found are *Plasmodium* 63(48.46%), *Trypanosoma* 4(3.08%), *Toxoplasma* 6(4.62%), *Babesia* 7(5.38 %) and *Anaplasma* 2(1.54%). The distribution of the hemoparasites in the nine different species of rodents were 81.82% each in *Steatomys pratensis* (fat mouse) and *Thryonomys swinderianus* (cane rat), 80.77% in *Thamnomys rutilans* (thicket rat), 75.00% in *Rattus*

rattus (black rat), 72.22% in *Mus musculus* (house mouse), 36.36% in *Hystrix cristata* (porcupine) and 50.00% in *Cricetomys gambianus* (Gambian giant rat). Thirty-one (51.67%) of the 60 male rodents and 51(72.86%) of the 70 female rodents were found to be parasitized (Ajayi *et al*., 2007).

Isaac *et al*., (2018), stated that rodents harbor a number of parasites that could be of public health importance. He profiled that the helminth and protozoan parasites in trapped rodents are in six genera, *Apodemus* sp., *Crocidura* sp., *Mastomys natalensis*, *Mus musculus*, *Rattus* sp., and *Sorex* sp. They were identified from 502 trapped small mammals. Overall, *M. musculus* (71.9%) and *Rattus rattus* (20.1%) were the most frequently captured. In an examination of blood, gastrointestinal contents, and brain tissues, six protozoan parasites (*Babesia* sp., *Trypanosoma lewisi*, *Plasmodium* sp., *Eimeria* sp., and *Toxoplasma gondii*) were isolated where 3 of them were blood protozoan. The presence of *Plasmodium* sp. in more than a half of the number of rodents examined and the detection of *Babesia* sp. in all surveyed sites. *T. lewisi* was also found.

A study was conducted In Senegal to determine the link between the distribution and spread of two parasite species (*Leishmania* spp. and *Trypanosoma lewisi*) and the progressive invasion by two commensal rodent species (The house mouse; *Mus musculus domesticus* and the black rat, *Rattus rattus*). The findings were 17.5% of *R. rattus* were infected by *L. major* and 27.8% by *T. lewisi*. Study also stated that, *R. rattus* also act as a potential reservoir for *Leishmania major* and *T. lewisi* in the southern part of Senegal. The presence of these two pathogens in *R. rattus* may be of different origins. *R. rattus* could have been locally contaminated with *L. major*. Conversely, *T. lewisi* infection could have been introduced in Senegal by *R. rattus*. Altogether, these data show that *R. rattus* is a carrier of both parasites (Cassan *et al*., 2018).

Five rat species were recovered with *Rattus rattus* being the most dominant species, followed by *Rattus norvegicus*, *Rattus exulans*, *Rattus annandalei* and *Rattus argentiventer*. Two blood protozoan species were found infecting the rodent population namely, *Plasmodium* sp. (42.1%) and *Trypanosoma lewisi* (25.0%). This study reports the presence of *Plasmodium* sp. for the first time in the rodent population in Malaysia. Two

main intrinsic factors were identified affecting the parasitic infections. *Trypanosoma lewisi* infections were influenced by host age and sex with infections observed higher in male and juvenile rats meanwhile *Plasmodium* sp. infections were observed almost similar in both sexes (Alias *et al*., 2014).

Makokha *et al*., (2011), conducted a study to determine the prevalence, intensity and morphometric parameters of haemoparasites of small rodents inhabiting Kakamega forest of Western Kenya. *Trypanosoma* and *Plasmodium* species were found in *P. jacksoni* and *Mastomys* rats. Trypanosoma having a prevalence of 20.34% and 40.74% whereas, that of Plasmodium was 6.78% and 3.70% in *P. jacksoni* and *Mastomys,* respectively. The mean *Trypanosoma* sp. and *Plasmodium* sp. intensity was 0.063% and 0.067% in *P. jacksoni,* and 0.47% and 0.01% in *Mastomys,* respectively. Pleomorphic trypomastigotes were the main blood stage parasite forms in the two rodent species. Morphometric data showed that trypanosomes from the two rodent species differed significantly ($P<0.05$) in the means of their Nuclear (0.87- *P. jacksoni* vs 1.05-*Mastomys* sp) and Kinetoplast (1.36-*P. jacksoni* vs 1.52-*Mastomys* sp) indices. Ring-stage *Plasmodium* trophozoites were observed in both the rodent species. Most trophozoites had single chromatin dots.

In Arbil province of Iraq, a survey was conducted where blood and tissue parasites from 233 mice and rats was observed. The specimens consist of 105 number of house mice (*Mus musculus*), 99 black rats (*Rattus rattus*), and 29 Norway rats (*Rattus norvegicus*), collected from different localities. *Trypanosoma lewisi* was found in 7 (7.07%) of 99 *R. rattus* and in 5 (17.86%) of 29 *R. norvegicus,* while *Trypanosoma musculi* was found in 4 (3.80%) of 105 *M. musculus*. This is the first record of this trypanosome from rodents in Iraq (Molan and Hussein, 1988).

Karbowiaki and Wita (2001), reported that, two out of three brown rats (*Rattus norvegicus*) were infected with *Trypanosoma lewisi*. The morphometric features of trypanosomes were in accordance with the features. This is first note about infection of free living rats with T. lewisi in Poland.

Dobigny *et al*., (2011), observed that, invading rodent species can harbor parasites with potential transmission to native rodents and/or humans. To investigate trypanosomes prevalence in rodents, the spleen of 76 rodents from Niger identified by their karyotype was used as a DNA source for Trypanosoma detection using a newly developed qPCR assay. Of the invasive black rat, *Rattus rattus*, 71% (10/14) were PCR positive as well as 6% (4/62) of native African rodents. *Trypanosoma lewisi* was present in all positive black rats and the sequences displayed 100% similarity with *T. lewisi*-infected humans in Senegal. *T. lewisi* was also detected in one *Acomys johannis*, suggesting a possible transmission to native species.

Trypanosoma lewisi prevalence of 93 (21.7%) was found in 429 *Rattus norvegicus* was stated. The infection rate was different in both sexes, 24.9% (68/273) being detected for males and 16% (25/156) for females (Linardi and Botelho, 2002).

Jittapalapong *et al*., (2008), conducted a molecular technique for *Trypanosoma* sp. identification in rodents in Thailand. Blood was collected from a total of 276 rodents trapped from urban and rural areas of three Thai province. These samples were processed for DNA isolation and tested with a PCR assay universal for the genus Trypanosoma, followed by internal transcribed spacer 1 (ITS-1) sequence analysis to identify infections in positive samples. Herpetosoma known as *T. lewisi*-like trypanosomes were present among *Rattus* sp. (14.3%) and *Bandicota* sp. (18.0%) and salivarian trypanosomes closely related to *T. evansi* were detected in *Leopoldamys* (20%) and *Rattus* (2.0%) species.

The presence of *Trypanosoma lewisi* was detected in Sri-Lanka in *R. norvegicus*. Out of a total of 245 rats investigated, 54 rats (26 adults and 28 sub-adults; sex ratio 2 (male): 3 (female) were blood positive for *T. lewisi* as detectecd by screening both thin blood films and wet mounts. In wet mounts, the presence of *T. lewisi* parasites were detected by their wriggling movement among the blood cells (Sannasuriya *et al*., 1999).

Young *et al*. (2019), stated that the Babesia species including *Babesia microti*, *Babesia divergens*, B. divergens-like, *Babesia venatorum* and *Babesia duncani* are responsible for

human infection. In Europe, Asia and North America respectively, the main vectors of zoonotic Babesia species are *Ixodes ricinus, Ixodes persulcatus* and *Ixodes scapularis* ticks. B.microti is the main causative agent of human babesiosis, especially in North America (Westblade *et al.* 2017).

CHAPTER III

Materials and Methods

3.1. The Study Area:

The area of the research work was located in Dhaka, the capital and the largest city of Bangladesh, located at 23°42′N ,90°22′E, on the eastern banks of the Buriganga River. The city has a distinct monsoonal season, with an annual average temperature of 26 °C (79 °F). It is one of the most densely populated cities in the world. The city is full of multistoried building, skyscrapers as well as slums. Considering all the demographic, topographic and climatic aspects, it makes Dhaka as a suitable habitation for rodents. All the samples were collected from different grocery shops, vegetable and fish markets (Kacha Bazar), general households and slum areas located at Agargaon and West Kafrul (Taltola) region of Dhaka North City Corporation

3.2. Rodent Catching and Collection:

A total of 80 rats were trapped from different places using iron/steel stick traps. Traps were set before sunset at the desired locations cautiously with bait inside, and were collected in the next morning. Then, all the captured rodents were brought to the Microbiology & Parasitology Laboratory, Sher-e-Bangla Agricultural University, Dhaka for further studies.

(A)

(B)

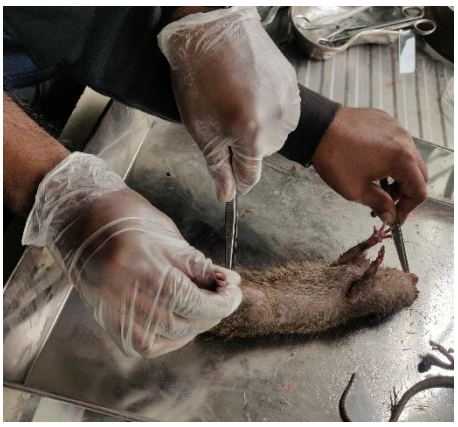
(C)

(D)

Figure 1: Rodent capturing and collection.

3.3. Rodent Identification:

Each of the rat was put separately in a glass flask and anesthetized with a cotton plug soaked in chloroform. Then, the rats were identified, classified and categorized based on their species, sex, site of collection and date. Species was identified by observing their coat color, size, shape, appearance, measuring their head-body length and specific tail length. Sex was identified by observing distinct sex organs (Testicles and Ovaries). Then all the data were registered in a tabulating sheet.



(A)

(B)

Figure 2: Rodent species and sex identification. A: sexing; B: length measurement.

3.4. Blood Collection and Preservation:

Scientifically, blood of rodents can be collected from tail vein or orbital sinus or jugular vein or temporary cannula or cardiac puncture. For this study, blood was collected by cardiac puncture. After locating the heart, a 1ml/3ml syringe (needle size 22-25 gauge) was used for puncturing. Then, collected blood was preserved in an EDTA containing collecting tube (purple tip) for further studies.

(A) (B)

(C) (D)

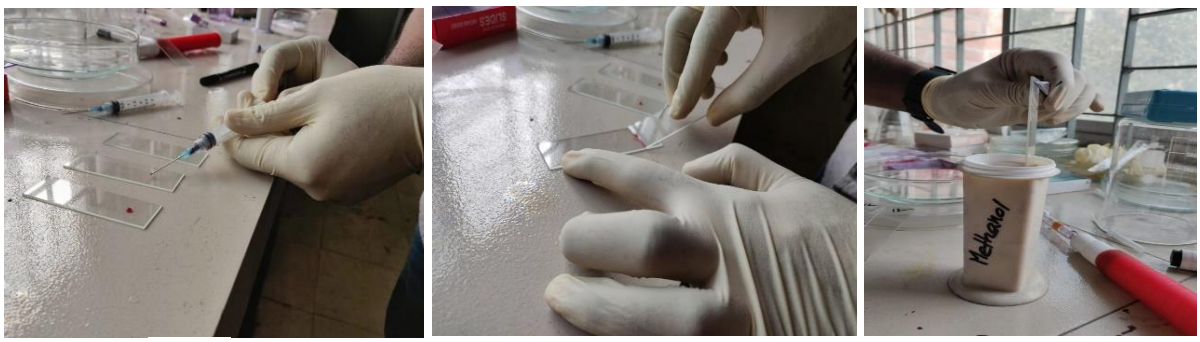
Figure 3: Blood collection and preservation. A and B: Heart puncture for blood Collection; C and D: Blood preservation.

3.5. Blood Smear (Thin) Preparation and Giemsa Staining: For observing the blood protozoa, 3 procedures are required which are given below.

1. Preparation of a thin blood smear on a glass slide.
2. Preparation of Giemsa working solution.
3. Staining the smear with Giemsa solution and Microscopic Examination.

3.5.1. Preparation of thin blood smear:

A thin blood smear was prepared for the study. In a thin blood smear, thickness decreases progressively towards the feathered edge. At first, a small drop of blood was placed on a clean, grease-free, pre-labeled glass slide. Another glass slide was set at 30-45º angle up to the drop, allowing the drop to spread along the contact line. After spreading, quickly pushed the upper slide (Spreader) toward the end of the lower slide. At three slides were prepared for each sample. A correctly done smear had a good feathered edge, where blood cell should be in monolayer, not touching one another. The smear was set to dry for few minutes and then fixed by dipping in absolute methanol for 2-3 minutes using a coplin jar.

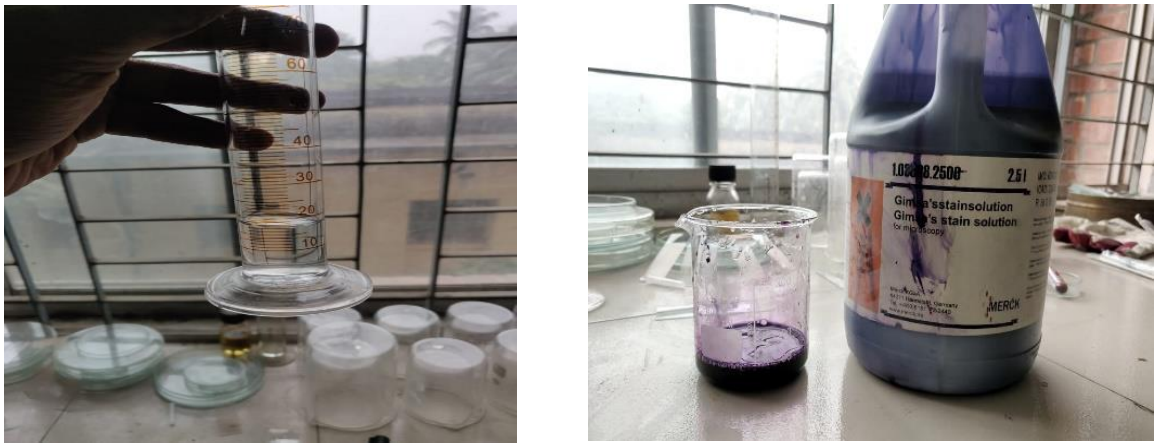


(A) (B) (C)

Figure 4: Thin blood smear preparation. A and B: smear preparation; C. Fixation in methanol.

3.5.2. Preparation of Giemsa working solution: For staining, 10% Giemsa working solution (1:9 dilution) was prepared. 90 ml of buffered distilled water (p^H 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 solution was 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 was discarded after 24 hours, and a fresh working solution was prepared for preparing new slides.

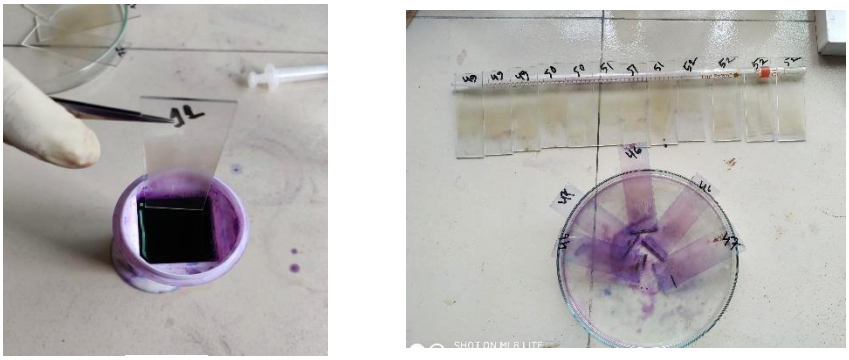


(A) (B)

Figure 5: Giemsa working solution preparation. A: measuring distilled water; B: Working solution.

3.5.3. Staining the smear with Giemsa working solution:

A fixed thin smeared slide was taken. The slide was flooded with 10% Giemsa working solution by using a coplin jar. For more than one sample, bulk staining was done. Then the slides were kept in rest for 35-40 minutes. After staining, slides were taken out of the jar and washed with running tap water and left to air dry.



(A) (B)

Figure 6: A. Staining with Giemsa solution and B. Air dried.

3.5.4. Microscopic observation:

The prepared slides were the observed under microscope at 100X using immersion oil. The morphological identification of blood protozoa done accordingly to the keys and description of two books of Soulsby and M.A Taylor.

(C)

Figure 7: Microscopic observation of prepared slides.

3.6. Statistical analysis:

The data comprised locations, rat species and sex, different niches, parasite species and infection nature (single and mixed) were considered independent variables. Data for low detection rates were excluded from the analyses. Pearson's chi-square and Fisher's exact test were used to assess the association of parasite detection rates in different study locations, species, sex and niches of the rats and species of parasite and nature of infection in GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). A *p* value considered significant when it was <0.05.

CHAPTER IV

RESULTS

4.1. Protozoan microscopic and morphological identification

4.1.1. Identification of *Trypanosoma* spp.

Through the microscopic observation of Giemsa stained blood smears, *T. lewsi* appeared as classical slender form. It has a long posterior end with a sub-terminal oval kinetoplas. The nucleus in located in the anterior part of the body and flagellum is free (Figure: 8).

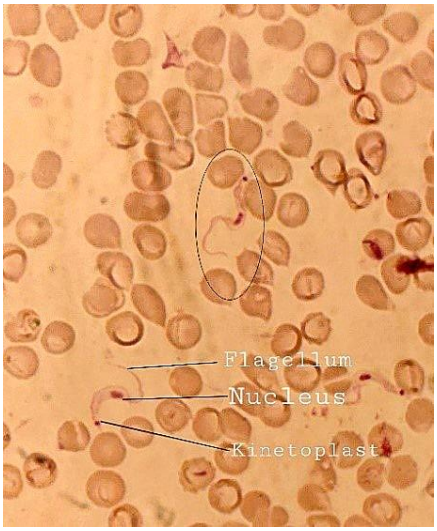
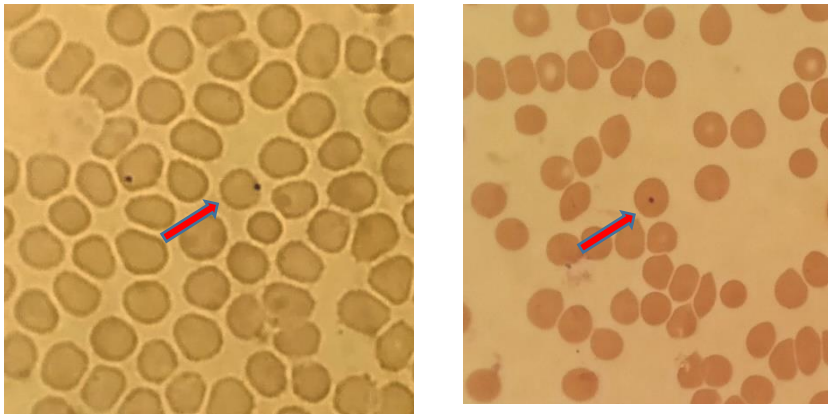


Figure 8: Different parts of *Trypanosoma lewsi*, containing kinetoplast, nucleus and flagellum.

4.1.2. Identification of *A. marginale* and *A.centrale*:

A. marginale seen in rodent blood, giemsa staining with 100X oil immersion. It is an intracellular organisms appear as basophilic, spherical inclusions generally located near the margin of erythrocytes. In case of *A.centrale*, round inclusion body was seen in the central part of the erythrocyte (Figure: 9).

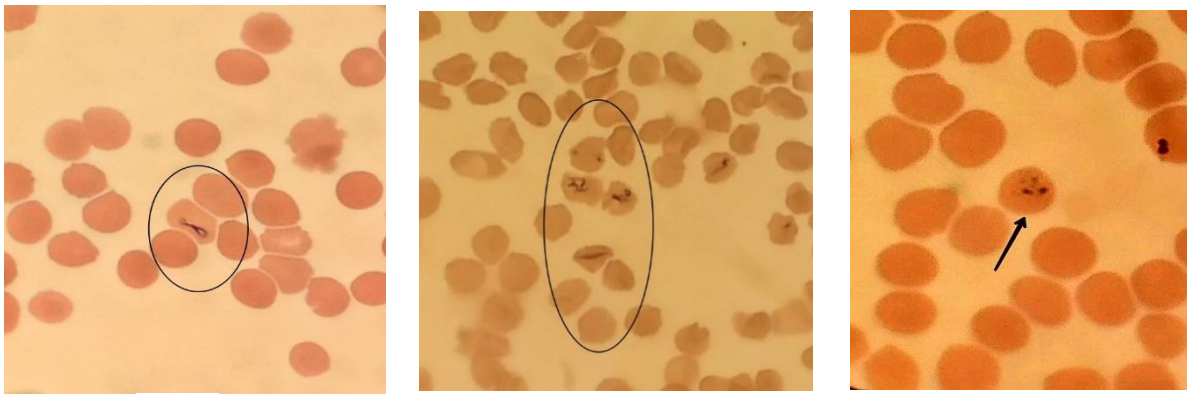


(A) (B)

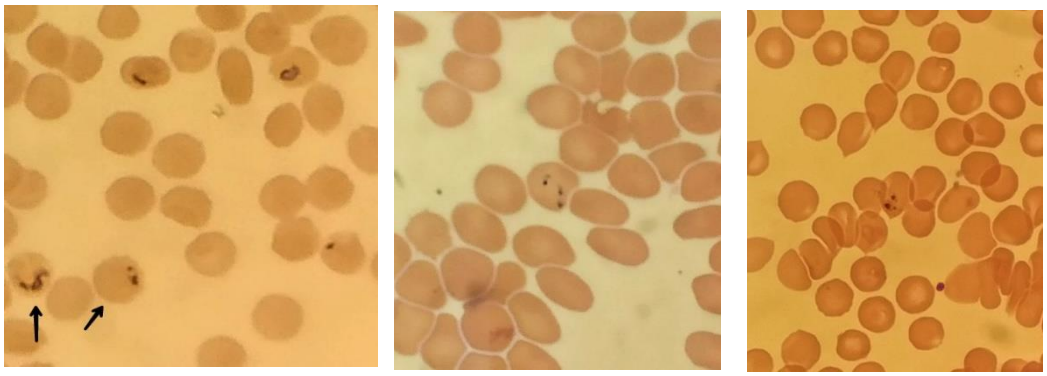
Figure 9: A. *A. marginale* and B. *A.centrale*

4.1.3. Identification of *Babesia* spp.

In rodent blood, *B. microti* is commonly found. In *Babesia* infection, infected red blood cells (RBCs) are normal in size. Sometimes rings are seen, and they may be vacuolated, pleomorphic or pyriform. Occasional classic tetrad-forms (Maltese cross) or extracellular rings can be present (Figure: 10).



(A) (B) (C)

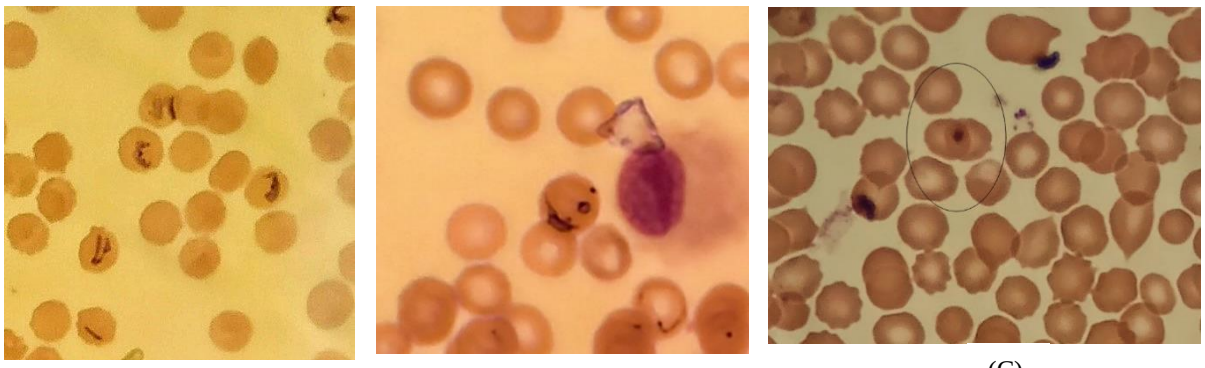


(D) (E) (F)

Figure 10: *Babesia* spp. showing different shape and size as pleomorphic, comma, pyriform and tetrad shape.

4.1.4. Identification of *Plasmodium* spp.

Plasmodium berghei is a species in the genus *Plasmodium* subgenus *Vinckeia*. Like other malarial species, it has 4 basic stages as ring stage, trophozoite, schizonts and gametocytes. Malariae trophozoites have compact cytoplasm and a large chromatin dot (Figure: 11B). Occasional band forms and/or "basket" forms with coarse, dark-brown pigment is seen. The ring has large chromatin dot inside the cytoplasm. Sometimes, compact or amoeboid forms is seen in smears.

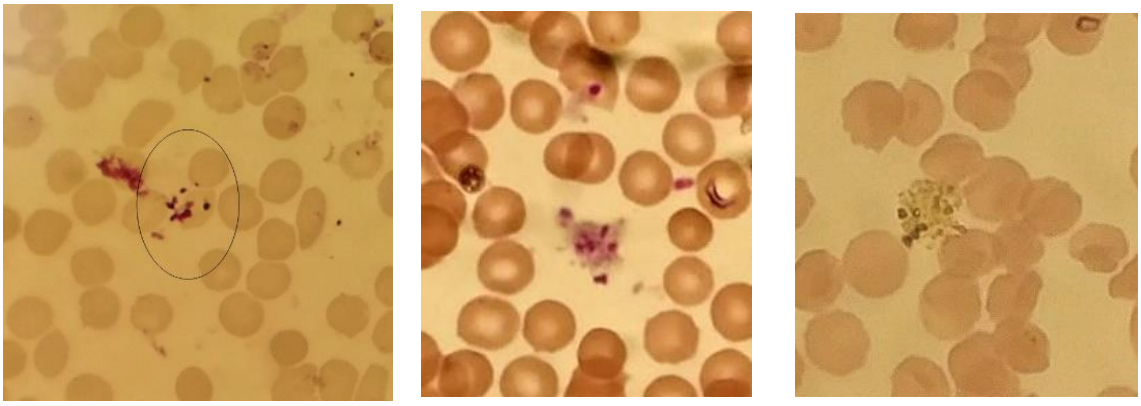


(A) (B) (C)

Figure 11: A, B. Trophozoite stage of *Plasmodium* spp. C. Ring form.

Malariae trophozoites have compact cytoplasm and a large chromatin dot. Occasionally forms “basket” forms with coarse, dark-brown pigmentation. The ring has large chromatin dot inside the cytoplasm (Figure: 11C). Sometimes, compact or amoeboid forms is seen in smears.

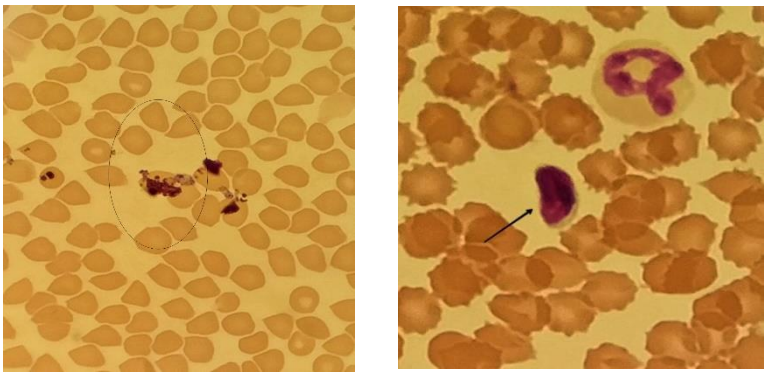
In case of schizont form, schizonts have 6 to 12 merozoites with large nuclei, clustered around a mass of coarse, dark-brown pigment (Figure 12). Merozoites can occasionally be arranged as a rosette pattern.



(A) (B) (C)

Figure 12: Schizont stage of *Plasmodium* spp.

The gametocytes are round to oval with scattered brown pigment; they may almost fill the infected red blood cell. The shape varies from crescent shape (banana-shape), bean shape to sausage shape (Figure: 13A and 13B).



(A) (B)

Figure 13: A. Gametocytes *of Plasmodium* spp. B. Cropped and zoomed photo of gametocyte.

4.2. PREVALENCE OF BLOOD PROTOZOAN OF RODENTS

4.2.1. Overall prevalence

The study was carried out in a total of 80 rodents which were collected from different places of Dhaka city, Bangladesh. Out of 80 rodents, 40 (50%) rodents were found infected with different blood protozoan parasites (Table. 1).

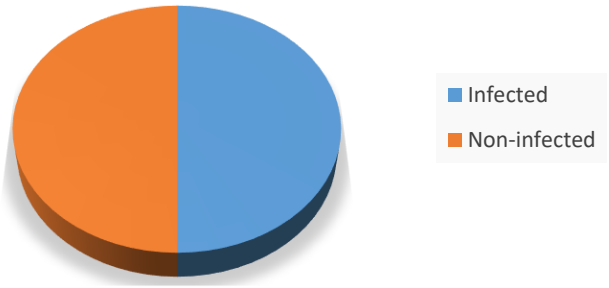


Figure 14: Overall prevalence of blood protozoan infection in rodents.

4.2.2. Prevalence of blood protozoan infection according to rodent species

Four types of rodent species were identified among the captured rodents. *Bandicota bengalensis* (30) contributed the highest numbers followed by *R. norvegicus* (28), *R. rattus* (14), and *M. musculus* (08). Among four species of rodents, *R. norvegicus* had the highest protozoan infection 16 (57.14%), followed by *B. bengalensis* 16 (53.33%), *M. musculus* (37.5%) and *R. rattus* 5 (35.71%) (Table.1).

Table 1: Prevalence of Protozoan infection according to rodent species

Rodent Species	No. of rodent examined	No. of infected rat (prevalence)	p-value
R. norvegicus	28	16 (57.14%)	
B. bengalensis	30	16 (53.33%)	0.5035
M. musculus	08	3 (37.50%)	
R. rattus	14	5 (35.71%)	
Total	80	40 (50%)	

4.2.3. Prevalence of blood protozoan infection according to sex of rodents

Among four rodent species, both male and female rodents were found infected. The prevalence of infection was greater in the female counterpart for every species. In case of *R. norvegicus,* the prevalence in male and female was found 50% and 66.67% respectively (Table 2). Similar observations were noted in *B.bengalensis* (Male: 52.38%, Female: 55.55%), *R.rattus* (Male: 33.33%, Female: 40%), and *M.musculus* (Male: 28.57%, Female: 100%).

Table 2: Prevalence of protozoan infection according to sex of rodents.

Rodent species	Sex of rodent	No. of rodent	No. of infected rodents (%)	p-value
R.norvegicus	Male	16	8 (50.00%)	0.4589
	Female	12	8 (66.67%)	
	Total	28	16 (57.14%)	
B.bengalensis	Male	21	11 (52.38%)	>0.9999
	Female	9	5 (55.55%)	
	Total	30	16 (53.33%)	
R.rattus	Male	9	3 (33.33%)	>0.9999
	Female	5	2 (40.00%)	
	Total	14	5 (35.71%)	
M.musculus	Male	7	2 (28.57%)	>0.9999
	Female	1	1 (100%)	
	Total	8	3 (37.50%)	
Total		80	40 (50.00%)	

4.2.4. Prevalence of blood protozoan infection according to protozoan species in male and female rodents

Five species of blood protozoa were identified from the study sample. Among them, *Plasmodium* spp. contributes the highest prevalence (31.25%) followed by *A. marginale* (21.25%), *A. centrale* (17.50%), *Babesia spp*. (10.00%) and *Trypanosoma* spp. (3.75%). The prevalence of *A. marginale* (21.25%) is higher than that of the *A. centrale* (17.50%). *Anaplasma* spp. was higher in female (44.45%) than that of male (24.52%) as *Plasmodium* spp. On the other side, *Babesia* spp. and *Trypanosoma* spp. had higher prevalence in male than that of female (Table: 3).

Table 3: Prevalence of blood protozoan species in male and female rodents.

Protozoan species	No. of infected rodents (%)	No. of male rodents (%)	No. of female rodents (%)
Plasmodium spp.	25 (31.25%)	13 (24.52%)	12 (44.45%)
A. marginale	17 (21.25%)	10 (18.86%)	7 (25.93%)
A. centrale	14 (17.5%)	9 (16.98%)	5 (18.51%)
Babesia spp.	8 (10%)	6 (11.32%)	2 (7.41%)
Trypanosoma spp.	3 (3.75%)	2 (3.771%)	1 (3.70%)
**p<0.0001*			

4.2.5. Prevalence of blood protozoan infection in rodents captured from different niches:

The highest number of rodents were captured from stationary/grocery shops (25) followed by local rent houses (20), fish markets (20) and slum areas (15) respectively. The highest infection was observed in the slum areas (66.67%) followed by fish market (50%), grocery shops (48%) and rent houses (40%) (Table.4).

Table 4: Prevalence of blood protozoan infection in different niches.

Niches	No. of trapped rodents	No. of infected rodents (%)	p-value
Stationary/Grocery shops	25 (31.25%)	12 (48%)	
Local Rent House	20 (25%)	8 (40%)	0.4741
Slum areas	15(18.75%)	10 (66.67%)	
Fish/Meat market	20 (25%)	10 (50%)	
Total	80 (100%)	40 (50%)	

4.2.6. Prevalence of single and mixed (co-infection) infection in examined male and female rodents

Both single and mixed infections with blood protozoan were found. Analysis showed that the ratio of single and mixed infection is equal (50%).

Table 5: Prevalence of single and mixed infection within male and female rodents.

Sex	Type of infection				Total
	Single	p-value	Mixed	p-value	
Males	12 (50%)		12 (50%)		24
Females	8 (50%)	0.5874	8 (50%)	0.5874	16
Total	20 (50%)		20 (50%)		40

Chapter V

Discussion

In this present study, all the trapped rodents were belonged to four different rodent species, *R. norvegicus, R. rattus, B. bengalensis and M. musculus*. These rodents are peri-domestic, omnivorous, highly prolific. The prime objective of this study was to give an overview of blood protozoan parasites of rodents in Dhaka city. Five species of blood protozoan parasites were found, namely, *Plasmodium* spp., *A. marginale*, *A. centrale*, *Babesia* spp., and *Trypanosoma lewsi.*

The overall prevalence of this study was 50%, where 40 rodents out of 80 captured rodents were infected by one or more blood protozoan parasites. Most of the above parasites that were found in rodents in this study were also reported in Bangladesh as well as other parts of the world. Three similar blood protozoan parasites were found in a study in Bangladesh in rodents and shrews (Islam *et al*., 2020). Unfortunately, no other study was found on rodent blood borne parasites except this study to our best of knowledge. Many authors reported similar blood parasites of rodents in different parts of the world (Alias *et al*., 2014, Ajayi *et al*., 2007, Kreier *et al*., 1972; Makokha *et al*., 2011).

In this study, *Plasmodium* spp. was the most abundant blood protozoan. The species of *Plasmodium* that were found in rodents were *P. berghei*, *P. chabaudi*, *P. vinckei*, and *P. yoelii.* (Déchamps *e*t *al*., 2010). The presence of *Plasmodium* spp. in rodents was also reported in other studies (Kreier *et al*., 1972; Makokha *et al*., 2011; Garnham and Duggan, 1986). The prevalence of *Plasmodium* spp. in this study was 31.25%, where 25 out of 80 rodents were infected with this protozoan. A lower prevalence of *Plasmodium* spp. was reported with 6.8% infection rate (Makokha *et al*., 2011). Higher infection with *Plasmodium* was also seen in some studies with an infection rate of 42.1% and 48.46%

respectively by Ajayi *et al*., 2007 and Alias *et al*., 2014. The higher number of *Plasmodium* infection in Bangladesh because of there is high concentration of mosquito population as well as very high malarial disease prevalence in this region. The variation in prevalence was might be due to the variation of sample size, geography, rodent species etc.

A study of Ramakrishnan and Prakash (1950) stated that, *Plasmodium berghei* infecting *R. norvegicus* and *R. rattus* rodent species. In this present study, both of these rodent species were also infected by *Plasmodium* spp. *Plasmodium* spp. infections were closely similar between both sexes (Alias *et al*., 2014). But, in this present study, female rodents were more affected than the male rodents (24.52% in male and 44.45% in female).

T. lewisi was found in this study. It is a widely distributed protozoan that can harbored within rodent blood. *Trypanosoma* infection in rodents had been recorded throughout the world in many studies (Sannasuriya *et al*., 1999; Jittapalapong *et al*., 2008; Linardi and Botelho, 2002; Dobigny *et al*., 2011; Karbowiak and Wita, 2001; Makokha *et al*., 2011). A lower infection of 3.75% was found in this study, which was consistent with earlier results by Shafiyyah *et al.,* 2012; Kia *et al.,* 2001; Molan and Hussein (1988). A higher prevalence was found in a study of Brazil having 21.7% infection rate (Linardi and Botelho, 2002) reason why brazil have more fly population and located in a tropical region. Some studies showed that *R. norvegicus, Bandicota Bengalensis*, *R.rattus* rodent species were found positive for *Trypanosoma infection (*Sannasuriya *et al*., 1999; Jittapalapong *et al*., 2008). Higher *T. lewisi* infections in male compared to female rats was observed in some studies (Linardi and Botelho, 1985). Which is supported by the present study. Where, male rodents had slightly higher infection rate than the female (3.771% in male and 3.70% in female). This could be due males are more active and having larger territories.

Some studies stated that, rodents that were trapped and living near human settlements, were more likely to infect by *Trypanosoma* species (Pumhom *et al*., 2015). In case of salivarian trypanosomiasis, it is transmitted by Tsetse fly (*Glossina* spp), which mainly found in tropical Africa. These are biological vector for trypanosomes, which cause sleeping sickness in human and animal trypanosomiasis mainly in sub-African region. But in

Bangladesh, there found no tsetse fly or any recorded studies. But nonpathogenic or stercorarian trypanosome are found that are transmitted via kissing bug, sheep bed or by tabanid flies. This could be a reason for a lower infection rate of *Trypanosoma* spp. in this study.

Another blood protozoan found in this study was *Babesia* spp., having a prevalence of 10%. Where, male rodents had the higher infection rate (11.32%) than that of female rodents (7.41%). A lower Babesia infection rate was reported in some studies (Mardosait ˙ e-Busaitien *et al*., 2021; Selma Usluca *et al*., 2019). In Bangladesh, Babesia infection at a rate of 4.7% was reported by Islam *et al*., 2020, which is lower than our present study. Hot and humid condition as well as greater population density is favorable for tick population and this could be a reason for higher *Babesia* infection in our study. There found some case of human babesiosis in accordance with rodents. In US, hundreds of human babesiosis case had been recorded associated with *Babesia microti*, the rodent babesia transmitted by *Ixodes dammini* tick (Persing *et al*., 1995; Quick *et al*., 1993). In Taiwan and Japan, there also found human babesiosis cases accordance with *Bandicoot* rat (Saito-ito *et al*., 1999).

In this present study, *Anaplasma* spp. was also found in trapped rodents. The prevalence of *Anaplasma spp*. infection was 21.25% for *Anaplasma marginale* and 17.5% for *A.centrale* respectively. In a study of Bangladesh, there found a lower *Anaplasma* prevalence at 7.5% infection rate (Islam *et al*., 2020). This might be due to lower sample size and geographical variation of our study. Factors like abundance and population size of the tick vector, the climatic and ecological features including sample periods might be considered for the variation of parasitism (Matei *et al.*, 2018; Mihalca and Sándor, 2013). Anaplasmosis is a common vector borne rickettsial disease of humans and animals. It is transmitted by tick species including *Argus persicus*, *Boophilus annulatas*, *B.microplus*, *Dermacentor albipictus*, *Ixodes ricinus* etc (Marchette and Stiller, 1982). In Central and Eastern Europe, *I. ricinus* is the dominant tick on humans, birds and rodents (Briciu *et al.*, 2011; Nosek and Sixl, 1972).

Rodents were found to be infected on both sexes. In every species of rodent, the females had higher infection rates than their male counterpart. For *R. norvegicus,* 66.67% female was infected with blood protozoan where male prevalence was 50%. Similar scenario was seen in other rodents as in *B.bengalesnis,* Male: 52.38%, Female: 55.55%; in *R.rattus*, Male: 33.33%, Female: 40% and in *M.musculus,* Male: 28.57%, Female: 37.50% respectively. Which is not supported with the study of Alias *et al.* (2014), where male rats were more infected with blood or tissue protozoa because male rats can explore a larger area than female rats. All of the rodents in our study were caught between late winter and early spring (February to March), which is regarded as rodent breeding season. Significant numbers of pregnant female rodents were also identified, and it was found that 100% of the pregnant rodents had an infection. That might be the reason why females in our study had higher rates of protozoan infection.

In this study, rodents were captured from different habitats of Dhaka city. The results of the study have revealed that *R.norvegicus* had highest infection rate (57.14%) followed by *B.bengalensis, R.rattus* and *M.musculus*. Among 80 rodents captured, majority were collected from different grocery shops/ stationary shops (25, 31.5%) followed by local rent houses (20, 25%), slum areas (15, 18.75%) and fish markets (20, 25%). A similar study was conducted in Argentina, where, 74% were commensal rodents (*Mus domesticus*, *Rattus rattus* and *Rattus norvergicus*) (Castillo *et al*., 2003). The present study reported that the highest number of infected rodents found from the houses in the slum areas (66.67%), followed by Fish markets (50%), stationary shops (48%) and local rent houses (40%). In Bangladesh, slum areas were responsible for various disease transmission. Lack of access to proper health services, poor housing and sanitation condition in slum communities, increase parasitic infection by rodents and assist the epidemic transmission of infectious diseases in humans (Glass *et al*., 1989; Childs *et al*., 1991; Ko *et al*., 1999).

Chapter VI

Summary and Conclusion

This study was performed in Dhaka city aimed to find out the prevalence and morphological identification of blood protozoan parasites of murine rodents. Four type of rodent species was found such as *R. norvegicus, R. rattus, B. bengalensis and M. musculus* which are most commonly found in city and suburbs. A total of 80 rodents were captured by trap and result showed overall prevalence of 50% infection rate by one or more blood protozoan parasites. *R.norvegicus* had highest infection rate of 57.14% followed by *B.bengalensis, R.rattus* and *M.musculus*.

Both male and female rodents were infected with blood protozoan. The highest number of infected rodents found from the houses in the slum areas (66.67%), followed by Fish markets (50%), stationary shops (48%) and local rent houses (40%). The result showed the prevalence of *Plasmodium* spp. (31.25%) is the highest followed by *A. marginale* (21.25%), *A. centrale* (17.5%), *Babesia* spp. (10%), and *Trypanosoma* spp. (3.75%). Most of them have public health significance. Rodents are living alongside humans in city areas and play important role in epidemiology of zoonotic diseases. The information that were achieved from this study will help us to understand about major blood protozoan infection that rodent harbor and possibly transmit disease to humans. In order to avoid such a condition, rodent population control measures should be implemented in Dhaka city and other parts of Bangladesh. Further research activities should be conducted in relation to present study.

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