ACKNOWLEDGEMENT

These are few lines of acknowledgement can never substitute the deep appreciation that I have for all those without whose help, support and inspiration this dissertation would not have taken its present shape.

The author is ever grateful and indebted to the **Almighty Allah**, the creator and soul authority of universe, who enabled me to complete this work successfully.

The author express his deepest sense of gratitude, sincere appreciation and profound regards to authors reverend teacher, **Dr. Mohammad Mahmudul Hassan,** Associate Professor, Department of Physiology, Biochemistry and Pharmacology, Chittagong Veterinary and Animal Sciences University for his scholastics guidance, uncompromising principles, sympathetic supervision, valuable advice, constant inspiration, radical investigation and constructive criticism in all phases of this study and preparing the manuscript.

My sincere thanks to all of my **Friends** and well **Wishers** for their helps, encouragement and inspiration during the study period and preparations of report.

The Author

Abstract

The field study was undertaken between 2010 to 2013 by joint research team of Ecohealth Alliances and ICDDR'B and laboratory work was done during the period of June, 2013 to February, 2014 in Pharmacology laboratory of Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong, Bangladesh to find out the prevalence of haemo-protozoa in three species of Rodents in different areas of Bangladesh. A total of 160 blood smears were included in the study and the overall prevalence of *Babesia* (5.0%), *Anaplasma* (2.5%) and *Plasmodium* (2.5%) was observed but the variation was not significant ($p>0.05$). On the basis of location, the overall prevalence of Babesia was highest (11.11%) in Joypurhat, Plasmodium was highest (10.53%) in Chakaria and Anaplasma was highest (12.50%) in Lalmonir hat. In conclusion, it is important that, further study is need to find out the actual prevalence of haemoparasites and public health importance of those parasites of Rodents.

Key words: Rodents, *Babesia, Anaplasma, Plasmodium*, Prevalence

CHAPTER-I

INTRODUCTION

Livestock is one of the most potential sub-sectors of agriculture in Bangladesh which plays an indispensible role to promote human health and national economy of the country. Livestock not only assist to upgrade the financial condition but also made a substantial contribution to human nutrition. In recent years, there has been increasing recognition of the importance of livestock to the poor, with estimates indicating that at least 70% of the rural poor depend on livestock for part of their livelihoods (Livestock in Development, 1998). As such, there is an increasing demand for a better understanding of the role of livestock in poverty reduction. In the past, the focus of most livestock development projects has been on raising production levels through better disease control and the introduction of such new technologies as fodder plants, equipment or management practices. However, most interventions did not have an explicit focus on the poor (Livestock In Development, 1998) and little information has been generated on the impact of livestock-oriented projects on the rural poor. With regard to animal health, little is known about the impact of specific diseases on poor households or indeed, about the differing needs of the poor as consumers of animal healthcare (Heffernan and Misturelli, 2000). Rodents comprise the largest and most diverse group of mammals with over 1700 different species. They occupy a wide range of habitats from sub-terrestrial to terrestrial to arboreal, and vary greatly in size and appearance. The smallest rodent is the harvest mouse which weighs in at 6 grams and the largest is the capybara which can weigh over 50kilograms. Specialized anatomical features emphasize the diversity of the group and include the beaver tail, porcupine quills, and the patagium of the flying squirrel. Rats eat an amount of food equivalent to 7% of their body weight daily, i.e. a rat with a body weight of 250g will eat around 25g daily, amounting to 6.5kg of grain a year (Etkind *et al.,* 1980). Mice eat a daily amount equivalent to around 15% of their body weight, i.e. a mouse weighing 25g will eat between 3 and 4g a day, amounting to 1.4kg of grain a year. It should, however, be borne in mind that the actual losses are much higher than the amount of produce eaten by the animals, as they contaminate the stored produce with urine, faeces, hair and pathogenic agents. As it is difficult or even impossible to

remove filth produced by rodents from the stored produce, infested batches often has to be declared unfit for human consumption or written off as total losses. There are around 50 diseases which can be transferred to man by rodents, including typhoid, paratyphoid, trichinosis, scabies, plague and haemorrhagic fevers like ebola. In addition, rodents may be vectors of a large number of diseases affecting domestic animals. The problems and costs resulting from these diseases are not normally taken into account when assessing infestation by rodents. The prevalence of the hemo-parasites ranged from 0.00-83.33% and 0.00-100.00% in the male and female rodents respectively (Cullen and Levine, 1975). However, the sex-related prevalence rates were not significantly different ($p>0.05$). The public health significance of the parasites encountered is discussed. Several diseases likely to be transmitted by animals are emerging as serious threats to public health. However, the epidemiology of many zoonotic diseases is poorly understood, and even in the UK, baseline data on the prevalence of important zoonoses in wildlife are scarce (Ebert, 1998). This present study as part of a larger consideration of the issues, aimed to make, we believe for the first time, a preliminary investigation of the parasites infecting urban rats and thereby help assess the public health risks. There are many research works are done on Hemo-parasitic diseases at different region in Bangladesh at different species of animals but incase of rodents it is not available which is very important. The study was undertaken in different area of Bangladesh like Faridpur, Joypurhat, Kulaura, Chakaria, Rangamati, Sitakunda, Mirsarai, Bandarban & Rajshahi to fulfill the following objectives:

Objectives of the study:

1) To know the prevalence of hemo-parasitic diseases in different species of Rodents.

2) To know the prevalence of hemo-parasitic diseases in Rodents of different areas of Bangladesh.

CHAPTER-II

REVIEW OF LITERATURE

Rodents are largest group of mammals, representing, approximately 43 percent of all mammalian species. Families in the order Rodentia include rats,mice, porcupines, hamsters, beavers, squirrels, chipmunks, lemmings, muskrats and guinea pigs (rabbits are not rodents).These families range in size from the pygmy mice, which are 4.7 inches long (12 centimeters) and weigh 0.1 ounces (4gm),to the capybara, which is 39.4 inches long (100 centimeters) and can weigh 110 pounds (50kilograms). Most rodents are relatively small animals such as mice, rats and squirrels. In a number of countries they cause as much if not more damage than insect pests. Rodents have an exceptional ability to adapt themselves to different environmental conditions and an incredible potential for reproduction one pair of rats can theoretically have 350 million offspring within the space of three years. Estimates state that over 3.5 million rats are being born daily **(Telford** *et al.,* 1993).

2.1. Characteristic features of rodents

Rodents are characterized by their teeth. They have a pair of incisor teeth in the upper and lower jaws, separated from the molars by a large gap (diastema). The incisors are curved inwards and have an extremely hard anterior coating the softer inside layer is worn down much more rapidly than the hard, outer layer. This means that the teeth are continually kept sharp, enabling them to damage even materials such as masonry and electric cables (Telford *et al.,* 1993).

2.2. Behavior of rodents

Rats are exceptionally cautious and intelligent creatures. Changes in their environment, such as newly laid bait, are initially regarded with suspicion and only accepted hesitatingly after several days of becoming accustomed to their presence. This is known as "new object reaction". it has important consequences for control measures (Telford *et al.,* 1993).

Fig-1: *Rattus rattus*

Fig-2: *Rattus rattus*

Fig-3: *Mus musculus*

Fig-4: *Mus musculus*

Fig-5: *Vandaleuria oleracea*

Fig-6: *Vandaleuria oleracea*

2.2.1. The Black Rat *(Rattus rattus)*

The Black rat lives in loose colonies and usually in the roof area of stores, where it generally builds its nest. Therefore it is often called "Roof rat". it is an excellent climber, can jump heights of up to 1 m and squeeze through an opening of only 12 mm in diameter. Black rats rarely use established runs as Norway rats do **(**Telford *et al.,* 1993).

2.2.2. The Norway rat (*Rattus norvegicus*)

The Norway rat lives in colonies or groups outdoors, only entering stores for food. It lives in burrows which it digs near stores or beneath foundations. Norway rats are good runners and swimmers, but cannot climb as well as Black rats. They can jump about 60cm and squeeze through openings only 12mm wide. They tend to use established runs and normally return to a food source once they have accepted it (Telford *et al.,* 1993).

2.2.3. The House Mouse (*Mus musculus)*

The House mouse lives in fixed families, primarily in buildings or stores, and its preferred source of food is grain. It can survive for long periods without water, being able to make use of the grain moisture and water produced by its own metabolism.Its radius of activity is very small, not exceeding a space of 10 x 10 m. It is capable of spending its entire life within the confines of a single pile of stacks.This makes its detection particularly difficult. House mice are good runners and climbers, and can jump heights of up to 30cm. Their small size enables them to squeeze through openings only 6mm wide. House mice are very curious animals; the paths they follow are irregular, nibbling here and there, and they do not regularly return to specific food sources (Telford *et al.,* 1993).

2.2.4. The Multi-mammate Rat (*Mastomys natalensis*)

These animals live in colonies in burrows outdoors, but enter stores to obtain food. They live largely from plant substances, but do also eat insects and meat. They are excellent climbers, jumpers and swimmers. Due to the small size of these animals, they are referred to in many countries as mice **(**Telford *et al.,* 1993).

2.2.5. The Bandicoot Rat (*Bandicota bengalensis*)

The Bandicoot rats often live on their own outdoors where they dig elaborate burrows. The entrances are marked by noticeable piles of earth. The burrows comprise a number of chambers, where the rats store up to 10kg of grain. They use established runs, which may well be sprinkled with odd grains they have dropped in transporting food. They are excellent swimmers (Telford *et al.,* 1993).

2.2.6. The Pacific Rat *(Rattus exulans)*

These animals live outdoors, building their nests above ground in branches, bushes or niches in rocks. They feed largely on plant substances. They are very active and are good runners, jumpers and climbers. Occasionally they enter houses and stores (Telford *et al.,* 1993).

2.3. Senses of rodents

Rats and mice are animals which are most active at night or at dusk. Their vision is thus quite poor. They are very sensitive to light, but they cannot see clearly and are color-blind. Their poor vision is compensated for by their excellent senses of hearing, feeling, smell and taste. Rodents are able to sense ultrasound. Their sense of smell enables them to find food, identify other beings and recognize runs and territorial limits. Their excellent sense of taste makes them fastidious. This is an important fact to be borne in mind when selecting bait. Their whiskers serve as feelers **(**Foster and Cameron**,** 1970).

2.4. Reproduction

Rodents have important reproduction ability. They reach full sexual maturity very soon after birth; have a number of litters a year and a large number of offspring per litter:

Rodent species	Number of litters per year	Number of offspring per litter	Gestation period (days)
R. rattus	6 – 8	4 - 12	21 – 23
R. norvegicus	3 – 7	6 - 10	20 – 24
R. exulans	2 – 6	2 - 5	20 – 21
N. musculus	7	4 - 8	20 – 22
M. natalensis	up to 12	9- 13	23
B. bengalensis	up to 11	6 - 8	22 – 26

2.5. Haemo-parasitic diseases of rodents

Climatic condition of Bangladesh favors the tick population, which are vectors of various tick borne diseases. Prevalence of tick borne protozoan parasites such as *Babesia bigemina, Anaplasma centrale, Anaplasma marginale, Babesia bovis, Babesia gibsoni*, *Babesia canis* has been reported in animals of Bangladesh. Therefore, few important literatures related to these diseases are reviewed here.

2.5.1. Anaplasmosis

Bram, (1983) found that anaplasmosis is characterized by fever, severe anemia, jaundice, brownish urine, loss of appetite, dullness or depression, rapid deterioration of physical condition, muscular tremors, constipation, yellowing of mucous membrane and labored breathing**.** Merck Veterinary Manual, (1997) published that anaplasmosis, formerly known as Gall sickness, traditionally refers to a disease of ruminants caused by obligate intra-erythrocytic bacteria of the order Rekettsiales, family Anaplasmataceae, genus *Anaplasma*.

2.5.2. Etiology of Anaplasmosis

Bram, (1983) found that there are many Anaplasma species parasites but *Anaplasma marginale* and *Anaplasma centrale* are the most important species. Ristic and Weinman, (1968) mentioned that clinical anaplasmosis is usually caused by *Anaplasma marginale*.Rodents is also infected with *Anaplasma spp*, which generally results in mild disease.

2.5.3. Epidemiology of Anaplasmosis

2.5.3.1. Geographical occurrence

Smith, (2002) showed that anaplasmosis is seen worldwide and has been reported in at least 40 states in the USA Gautam and Banerjee, (1982) that the animals show clinical diseases under stress of certain intercurrent diseases, inclement weather, pregnancy and lactation. The exotic and to lesser extent crossbred animals are fully susceptible. The disease causes direct losses due to prolonged period of convalescence, low productivity and mortality. Blood and Henderson, (1968) found that anaplasmosis is transmitted by a diverse group of biological and mechanical vectors. Infection occurs sporadically in temperate climate areas. Lew and Jorgensen, (2005) mentioned that anaplasmosis occurs in tropical and subtropical regions worldwide (~40° N to 32° S), including Asia.

2.5.3.2. Mode of infection

Ristic and Weinman, (1968) mentioned that *Anaplasma* is one of the most important parasites transmitted by at least 20 ticks species, including *Argas persicus, Ornithodoros lahorensis*, *Boophilus annulatus, B. decoloratus, B. microplus, Dermocentor albipictus, D. andersoni, D. accidentalis, D. variabilis, Hyalomma excavatum, Ixodes ricinus, Rhipicephalus bursa, R. sanguineus and R. simus* (Marchette and Stiller, 1982) but mostly *Boophilus microplaus* causing Anaplasmosis. Various other biting arthropods have been implicated as mechanical vectors. Experimental transmission has been demonstrated with a number of species of *Tabanus* (Horse fly) and with mosquitoes of the genus *Psorophora*. Ristic, (1996) found that the experimental and epizootiological evidence incriminates horse flies (*Tabanus spp*.) as the most significant insect vector of Anaplasmosis. Transmission by flies is affected by direct transfer of blood from infected to susceptible animal and

must take place within a few minutes after feeding on an infected animal. Blood *et al.,* (1968) reported that anaplasmosis is spreaded from animal occurs chiefly by insect vectors. A variety of arthropods may act as vectors but significant natural vectors are vectors are ticks in the family Ixodidae and flies in the family Tabanidae. Of ticks, the one –host *Boophilus spp*. are major importance in tropical and subtropical regions and three-host Dermocentor spp. major importance in the Western USA. Merck Veterinary Manual, (1997) published that numerous species of tick vectors (*Boophilus, Dermocentor, Rhipicephalus, Ixodes, Hyaloma and Ornithodoros*) can transmit *Anaplasma spp.* after feeding on an infected animal, intrastadial transmission may occurs. Transplacental transmission has been reported and is usually associated with acute infection of the dam in the second or third trimester of generation. Anaplasmosis may also be spread through the use of contaminated needles or dehorning or other surgical instruments. Soulsby, (1986) found that transmission by blood sucking flies is well recognized and Tabanids deer flies, stable flies and mosquitoes are the insects chiefly concerned. Direct transfer of infected blood must take place for insect transmission and this must occur within a few minutes after feeding on an infected animal. Mechanical transmission of Anaplasmosis is well known and major and minor operation operations in cattle husbandry such as dehorning, castration, vaccination, blood sampling etc. may be responsible for the transmission of Anaplasmosis both in and out of season.

2.5.3.2. Risk factors

2.5.3.2.1. Susceptible host

Shompole *et al.,* (1989) reported that Anaplasma spp are obligate intra-erythrocytic parasites in the order Rickettsiales which infect domestic and wild ruminants transmitted biologically by certain tick species and mechanically by other blood sucking arthropods and fomites. Maas and Buening, (1981) found that *Anaplasma marginale* (the type species for cattle) also causes latent anaplasmosis in Rodents.

2.5.3.2.2. Nutritional status

Blood and Henderson, (1968) mentioned that clinical disease is less severe in animal on a low plane of nutrition. Exposure of infected, clinically normal animals to devitalizing environmental influences, particularly shortage of feed and the presence

of other diseases may result in the development of acute introduced into outbreaks among them are not uncommon 2-3 weeks after entry.

2.5.3.2.3. Season

Blood and Henderson, (1968) mentioned that in temperate climates, a seasonal occurrence of disease occurs in association with seasonal occurrence of the insect vectors. Winter outbreaks are likely associated with iatrogenic transmission or possibly the winter tick.

2.5.4. Clinical signs of Anaplasmosis

Barry and van Niekerk, (1990) reported that anaplasmosis is suspected of causing abortions in Rodents that are subjected to physical stress, such as walking long distances during the dry seasons. Therefore this parasite may be of economic importance under certain conditions. Tick paralysis caused by a toxin produced by some species of ticks, such as the Karoo paralysis tick *Ixodes rubicundus*, is the most economically important tick problem in South Africa. It affects also in Rodents. Fourie *et al.,* (1989) reported that in southwestern Orange Free State, most cases occurred in the first week in May, but the timing of outbreaks varied with latitude; it is possible that rainfall and low temperature have an influence on when outbreaks occur. Bram, (1983) mentioned that anaplasmosis is characterized by fever, severe anemia, jaundice, brownish urine, loss of appetite, dullness or depression, rapid deterioration of physical condition, muscular tremors, constipation, yellowing of mucous membrane and labored breathing. Urquhart *et al.,* (1996) reported that the clinical features include pyrexia, anorexia, labored breathing and severe drop in milk yield or abortion. Occasionally per acute cases occur, which usually die within a day of the onset of clinical signs.

2.5.5. Post-mortem findings of Anaplasmosis

Blood and Henderson, (1968) reported that the most obvious findings are emaciation, pallor of the tissues and thin watery blood. There is mild jaundice and the liver is enlarged and deep orange in color. The kidneys are congested and there may be myocardial hemorrhages. The spleen is enlarged with soft pulp. The bone marrow cavity may be reddening by increased hematopoietic tissue in acute case but there

may be serious atrophy of marrow fat in chronic case. Ristic, (1996) reported that the gross pathological changes are typical of anemia in which erythrocytes are removed by the reticulo-endothelial system. The prominent changes are icterus mucous membrane, enlarged spleen and obstructed gall bladder. Petechial hemorrhage may be observed on the epicardium and pericardium and the heart is usually pale and flabby. The liver may be mottled yellow or brown, hepatic and mediastinal lymphnodes are brown, moderately and moist on section.

2.5.6. Diagnosis of Anaplasmosis

Splitter *et al.,* (1956) and Shompole *et al.,* (1989) reported that based on the intra-erythrocytic location of inclusion bodies, as a conventional diagnostic method. Lew and Jorgensen, (2005) and Lew and Jorgensen, (2002) reported that the PCR, as a more sensitive and specific technique than other conventional methods, has been increasingly applied to diagnose anaplasmosis in blood and tick vectors. Ristic, (1996) reported that during the acute stage of anaplasmosis, the diagnosis is made on the basis of clinical symptoms, hematological changes and microscopic examination of stained peripheral blood films for intraertthrocytic inclusion bodies. Giemsa staining is the oldest and most frequently used method. Other staining methods include toluidine blue and acridine orange. In contrast to the easy with which acute form of anaplasmosis are recognized. Blood and Henderson, (1968) reported that *anaplasma* organism can be diagnosed by the blood smear prepared from the peripheral blood. Diff-Quick staining of blood smears is as accurate as Giemsa in the detection of *Anaplasma spp*. and can be completed in 15 seconds as prepared to mearly an hour for Giemsa. Ristic, (1996) reported that various soluble and corpuscular antigens extracted from the blood of infected animals has been used for serologic diagnosis of Anaplasmosis. Currently used tests are Complement Fixation (CF), Capillary Tube Agglutination (CTA) and Card Agglutination (CD). Blood and Henderson, (1968) reported that the complement fixation test is the standard test for the detection of carrier animals. It is satisfactory for use in cattle, goat, sheep and Rodents but antibody titer is highest during the active phase of the disease. A rapid card agglutination test, which tests serum or plasma for antibodies against *Anaplasma marginale*, is cheap and quick and sufficiently accurate to be used as a hard test. Other serological tests like a capillary tube agglutination test, indirect fluorescent

antibody test, a dot ELISA are also used for the detection of *Anaplasma spp*. Nucleic probe analysis can be used to detect low level of parasitemia.

2.5.7. Treatment of Anaplasmosis

Blood and Henderson, (2000) reported that treatment is with tetracyclines. Treatment of clinical disease can be with oxytetracycline, 6-10 mg/kg BW daily for three days, or a single injection of long-acting oxytetracycline at a dose of 20mg/kg intramusclularly. The convalescent period is long. Concurrent administration of estradiol cyionate (14.3mg/kg intramusclularly) appears to improve the rate of recovery by promoting parasitemia during treatment. Tetracycline will not eliminate infection and immunity will persist. Blood transfusions are indicated in animals with a PCV less than 50%. Imidocarb (3mg/kg BW) is also an effective treatment for clinical cases and does not interfere with the development of acquired immunity to *A. marginale.* Anaplasmosis is also treated with the tetracycline or oxytetracycline injection (6-10 mg/kg BW daily for 3 days) and imidocarb (3 mg/kg Bw) is also used. Oral administration of chlortetracycline (11mg/kg Bw) for 30-60 days is also effective in eliminating the carrier-state.

2.5.8. Prevention and Control of Anaplasmosis

Blood and Henderson, (2000) reported that the eradication of anaplasmosis is not a practicable procedure in most countries at the present time because of the wide range of insects which are capable of carrying the disease, the long infectivity of carrier animals, and in some areas, the presence of carriers in the wild animal population. In enzootic areas some benefits is derived from the control of ticks and others vectors weekly dipping in an acaricide is used in tropical areas to control this and other tick-borne diseases. The introduction of the disease into herds by carrier animals should be prevented by prior serological testing. Attention should be also given to prevent iatrogenic transmission with instrument used for injection or surgical operations by disinfection after use on each animal. Exposure negative animals that are to be introduced into an enzootic area should be vaccinated. Serological tests should be done and culling of reactors or treating them as outlined above to eliminate carrier state. If an outbreak does occur, affected animals should be treated vigorously and in-contact animals vaccinated and placed on a regimen of prolonged tetracycline

protection. Most control programs in enzootic areas are based on increasing the resistance of the population by immunization. In any vaccination programs particularly attention should be paid to the animals at high risk, particularly animals brought in from non-enzootic areas, those in surrounding similar areas to which infection may be spread by expansion of the vector population under the influence of suitable climatic conditions, and animals within the are which are likely to be exposed to climatic or nutritional stress. Urquhart *et al.,* (1996) mentioned that vaccination of susceptible stock with small quantities of blood containing the mildly pathogenic *A. centrale* or a relatively avirulent strain of *A. marginale* is practiced in several countries; any clinical sings in adult being controlled by drugs. Pegram *et al.,* (1993) mentioned that control of Tick-borne diseases has traditionally been based on dipping of animals using acaricides. Initiated during colonial times, government-sponsored programmes were introduced to protect exotic and crossbred animals. In many countries, dipping services were provided by the State and were backed up by laws making dipping compulsory. In areas of high infestation, treatment could be provided as often as twice a week. Taylor *et al.,* (2007) reported that the control of the disease depends on effective quarantine to prevent the introduction of the vector tick. The control of ticks by dipping or spraying animals at risk with recommended acaricides. In routine surgery, Care should be taken to prevent accidental transfer of blood from one animal to another. Widespread use of tick vaccines may also have a significant influence on the incidence of infection in cattle**.** Radostits *et al.,* (2008) also reported it.

2.5.2. Babesiosis

Merck Veterinary Manual, (1997) published that babesiosis is caused by intra-erythrocytic protozoan parasites of the genus *Babesia*. The disease, which is transmitted by ticks, affects a wide range of domestic and wild animals and occasionally humans.

2.5.2.1. Etiology of Babesiosis

Radostits *et al.,* (2008) found that babesiosis is a hemo-parasitic disease caused by protozoa of the genus *Babesia* (Phylum: Apicomplexa), which infects mainly ruminants Melendez, (2000). Infection of a vertebrate host is initiated by inoculation

of sporozoite form of parasites into the blood stream during the taking of a blood meal. Hungerford, (1962) reported that there has been much confusion in referring to the various tick fever diseases. Thus, in the literature of the world there is a tendency to the organisms as *Babesia argentiana and Babesia bigemina.*

2.5.2.2. Epidemiology

It is very important to recognize some characteristics of the ticks in order to better understand the biology of babesiosis. Globally, the geographic distribution of the tick and consequently of babesiosis, can be classified into three zones: Free zones: Locations where the tick does not occur because of weather conditions, so babesiosis is not present. Areas of enzootic instability: Locations where there is a well-defined cold season, leaving goat for long periods without tick contact. This allows the antibody levels to drop to a point where during the warmer months they are very susceptible to outbreaks of babesiosis. Endemic areas: Location where the prevalence of ticks is high enough to occur all year long, essentially reinoculating and therefore boosting the animals. Soulsby, (1986) reported that Babesia infection in Rodents is found in Southern Europe, Middle East, Soviet Union, South East Asia, also Africa and others parts of the tropics. *Babesia ovis* is distributed throughout tropical and subtropical areas, also in Southern Europe, Soviet Union.

2.5.2.2.1 Risk factors

2.5.2.2.1.1. Host factors

Urquhart *et al.,* (1996) reported that in endemic areas, particularly in adult animals, is often associated with some forms of stress, such as parturition or prevalence of another disease, such as tick-borne fever.

2.5.2.2.1.2. Age

Urquhart *et al.,* (1996) reported that in endemic areas, the young first acquires immunity passively, in the colostrums of the dam and as a result. Often suffers only transient infections with mild clinical sings. It is frequently stated that there is an inverse age resistance to *Babesia* infection in that young animals are less susceptible to babesiosis than other animals. Soulsby, (1986) mentioned that inverse age susceptibility occurs in Babesia infections, young animals being naturally resistant

while older animals are fully susceptible. Youngs in an enzootic area are free of clinical sings and have a very low parasite density. The passive transfer of maternal antibodies via the colostrums is probable responsible in part of this resistance.

2.5.2.2.1.3 Environmental factors

Blood and Henderson, (1968) reported that there is a seasonal variation in the prevalence of clinical Babesiosis and the greatest incidence found soon after the peck of the tick population. Of the climatic factors, air and temperature is the most important because of its effect on tick activity, higher temperature increases it, humidity and rainfall has little effect and even with temperature the effect is limited once a threshold of 7-10ºC minimum temperature is exceed. Urquhart *et al.,* (1996) reported that in endemic areas, where there are many infected ticks, the immunity of the host is maintained at a high level through repeated challenge and overt disease is rare. In contrast, where there are few ticks or when they are confined to limited areas, the immune status of the population is low, and the young animals receive low little, if any, colostral protection.

2.5.2.2.1.4. Other factors

Urquhart *et al.,* (1996) reported that if in endemic area, the number of ticks suddenly increases due to favourable climatic conditions of clinical cases may rise sharply. This situation is known as enzootic instability. Blood and Henderson, (1968) reported that in housed animal, the level of antibodies in the patient are at their lowest when the animal comes out of the barn in the spring and gradually increases as they are exposed to vectors ticks. In enzootic areas, the animals most commonly affected by clinical diseases are susceptible animal introduced for breeding purposes, for slaughter, or in transit, other stress like parturition, starvation or concurrent disease. Break down of immunity are likely to occur if there is a superimposed infection with different parasites especially *Anaplasma marginale* Endemic (enzootic) stability is achieved in area where all young animals are frequently exposed to the parasite while they are still protected by colostral and innate immunity and endemic instability occurs if some animal fail to become infected for prolong period after birth. Various factors such as changes in climatic condition and frequency of acaricidal treatment can influence the tick population.

2.5.2.3. Mode of transmission

Zaugg, (2009) reported that *Babesia spp*. are a various group of tick-borne, obligate, intraerythrocytic Apicomplexan parasites infecting a wide variety of animals. Ticks are most often infected transovarially. The female tick becomes infected by the ingestion of parasites during engorgement. After it drops off the host, the Babesial agents reproduce within the tick soft tissues. Some of the reproducing organisms are incorporated within developing tick embryos, and the disease agents are transmitted to new hosts by the feding of ensuing tick larvae, nymphs, or adults.

2.5.2.4. Clinical signs of babesiosis

Radostits *et al.,* (2000) reported that incubation period of babesiosis is 2-3 weeks. *B. bigemina* and *B. bovis* produce acute syndromes which are clinically indistinguishable, and are characterized by high fever (41°C), anorexia, depression, weakness, cessation of rumination, and a fall in milk yield. Hemoglobinuria can be seen, the color of urine is dark-red to brown. Respiratory and heart rates are increased, and the red conjunctivae and mucous membranes change to the extreme pallor of severe anemia and abortion might occur in pregnant animals. Subacute syndrome also occurs in young animals, but fever is mild and hemoglobinuria is absent. Rahbari *et al.,* (2008) mentioned that in case of Babesiosis develop fever and parasitemia within 2 to 4 days; the clinical signs of the disease include anorexia, listlessness, anemia, moderate jaundice and hemoglobinuria. In intact animals, hyperthermia returned to normal on the fourth day after the peak pyrexia, and parasitemia is eliminated within the course of the disease.

2.5.2.5. Necropsy findings of babesiosis

Radostits *et al.,* (2000) found that in acute cases of Babesiosis in all species, in which patient die after a brief illness and during an anemic crisis, typical lesions are jaundice, thin watery blood, pale tissues, and enlargement of the spleen which has a soft pulpy consistency and gross enlargement and dark brown discoloration of the liver. The gallbladder is distended with thick, granular bile, the kidynes are enlarged and dark, and the bladder contains red brown urine. Echymotic hemorrhages are present under the epicardium and endocardium, the pericardial sac contains an increased quantity of blood stained fluid. In sub-acute or chronic cases of fairly long

duration, the carcass is emaciated but haemoglobinuria is absent; the other cases are present but less pronounced.

2.5.2.6. Pathology of Babesiosis

Burtis and Ashwood, (1999) reported that histopathologic examination revealed focal necrosis, lymphohistiocytic, ericholangitis and cholangiohepatitis and canalicular cholestasis in the liver. Severe oedema, mild lymphocytolysis and haemorrhagic lymphadenitis were also present. Pathologic examinations of the tissues indicated that the kidneys and lungs were the organs most severely affected. Acute alveolar oedema and infiltration of neutrophils and macrophages in interstitial were present. Acute diffuse proliferative glomerulitis, congestion and stasis in glomerular capillaries and acute tubular necrosis were also present. Biochemical parameters were determined. Meyer and Harvey, (2004) reported that the values of glucose (glucose oxidase), creatinine (Jaffe), BUN (urease), AST (Carman), ALT (Ritman and Frankel), total bilirubin (Vandenberg), total protein (Biuret), fibrinogen (refractometry) and urinalysis were measured in this study. Haematologic parameters were estimated. Rahbari *et al.,* (2008) reported that data were compared between the control and the infected animals according to leukocyte count in both infected groups was significantly ($P<0.05$) decreased. Nevertheless, lymphocyte count in both groups was higher than those of normal, reached the peak on days 8 and 10 post inoculation ; neutrophil count was decreased.

2.5.2.7. Diagnosis of Babesiosis

Nagore *et al.,* (2004) and Inci *et al.,* (2010) reported that blood smears and clinical findings are useful in acute cases of Babesiosis, but are not sufficient in subclinical cases. The complement fixation test is used as a serological test. The most commonly used tests are ELISA, PCR and a DNA probe, which can detect specific parasitemias at very low levels of infection (Radostits, 2008). Recently, the reverse line blot (RLB) is a versatile technique for simultaneous detection, based on the recognition of specific gene regions by oligonucleotide probes.

CHAPTER III

MATERIALS AND METHODS

3.1. Study area

The study was carried to measure the prevalence of blood parasitic diseases in rodents of Faridpur, Joypurhat, Kulaura, Chakaria, Rangamati, Rangpur, Sitakunda and Bandarban.

3.2. Study period

The field study was undertaken between 2010 to 2013 by joint research team of Ecohealth Alliances and ICDDR'B and laboratory work was done during the period of June, 2013 to February, 2014 in Pharmacology laboratory of Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong, Bangladesh.

3.3. Source population

A total of 160 blood smears were collected randomly from three (3) different species of rodents from Faridpur, Joypurhat, Kulaura, Chakaria, Rangamati, Rangpur, Sitakunda and Bandarban during the study period.

3.4. Processing of *Mus musculus and Rattus rattus*

1. At first animals were removed from traps by placing a handling bag over the back door and coaxing animal out by running an instrument along outside of traps

2. Weight was taken of animal (grams) using by proper spring scale while the animal was in the handling bag and prior to inserting the anesthetic ball into the bag. After the animal anesthetized and removed, then weight of the empty bag taken and subtract the bag from the total

3. Then anesthetized the rodents with isoflurane using an anesthetic ball:

   a. 0.4ml of isoflurane was applied to cotton ball and put the cotton ball into a metal tea ball

   b. Then carefully placed the metal ball into the bag with the rodent.

   c. The animal was observed closely until anesthetized and breathing was quick then slow as the animal progresses under anesthesia

d. Toe pinch was performed to indicate proper depth of anesthesia.

e. After withdrawal reflex was suppressed and removed the rodent from bag for processing

f. The skin of rodents was pinched within the spine meets and the head between the handlers forefinger

4. Blood samples were collected immediately after the rodent was anesthetized

3.5. Procedure of blood collection

1. At first animal were anesthetized and placed forefinger and thumb along and below the eye. Then skin was pushed down towards to the work surface and in the posterior direction. Careful was taken not to crust the esophagus of the animal. As a result the eye was protruding at the point of retro-orbital sinus. A standard non-heparinised micro-hematocrit capillary tube was inserted under the eyeball and into the eye. Capillary tubes were pushed gently until it stops (capillary bed) and twist the tube. Blood was flown into the capillary tube. 1-3 capillary tubes were filled per animal depending upon animal size.
2. The full capillary tube was placed into a labeled 1.2 ml eppendorf and spun in a micro-centrifuge for allowed to settle overnight on an ice to let the serum separate. Then, the serum was transferred to a cryovial and frozen.
3. Eye was closed with a cotton ball and applied gentle pressure to stop bleeding.
4. The rubber blood pusher was attached to expel the blood from the capillary tube into labeled collection tube as soon as possible after collection.
5. Blood was stored in cooler immediately for short term storage.

3.6. Processing of *Vendeleuria oleracea*

For blood collection of *Vendeleuria oleracea* ventral tail vein was selected.

3.7. Procedure of blood collection

At first the vein location was identified in the center of the ventral tail. The vein was deeper then the mice. A tourniquet was applied in tail. A needle with the appropriate gauge (usually 27 gauge) was entered into the skin at a shallow angle about one third down the length of the tail. A small syringe was used to collect the blood.

3.8. Staining and examination of blood samples

The prepared blood smears were stained with the Giemsa stain (working solution) for 25 to 30 minutes. After rinsing with water of the stained blood smears, they were air dried and examined under microscope (10X100x) with immersion oil for the identification of blood parasites as described by Soulsby, (1982).

3.9. Identification of parasites

Identification of following haemo-parasites was done based on the salient characteristics found in microscopic examination:

***Babesia* spp:**

The parasites in the erythrocytes were ring shaped, oval bizarre amoeboid shapes, frequently appearing vacuolated (Fig-7). Ring or oval-shaped parasites usually had a single nucleus on their periphery, and larger parasites had 2 or 3 nuclei.

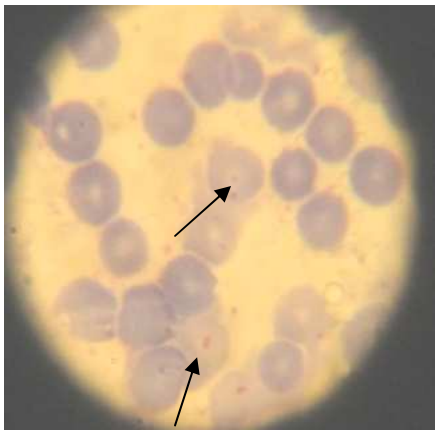

Fig-7: *Babesia* spp

Anaplasma spp.

The parasites in the erythrocytes were spot pin point organism found in the periphery (Fig-8) of the RBC.

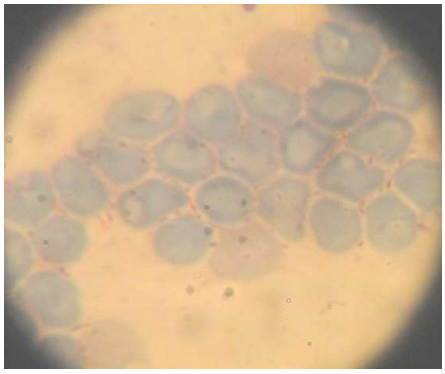

Fig-8: *Anaplasma* spp

***Plasmodium* spp.** Numerous rickettsia-like inclusion bodies were seen. They were circular or oval in section (Fig-9), oval forms, bounded by discrete walls and containing fibrous material. The bodies were found lying singly or in aggregations within the cell cytoplasm. Both single bodies and the aggregations appeared to be enclosed in a membrane of host origin.

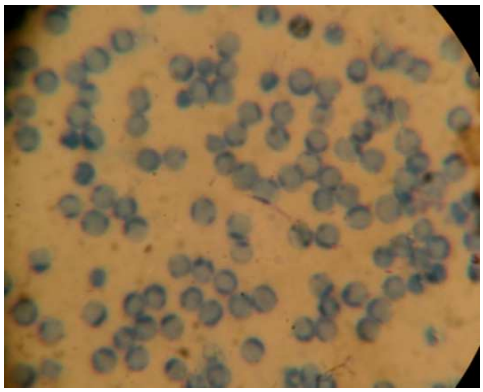

Fig-9: *Plasmodium* spp

Statistical analysis

The data obtained were imported in the Excel-2007 and transferred to the STATA/IC-11.0 software. Descriptive study was done to find out the percentages and Chi2 test was done to compare the prevalence of haemo-parasites within species and location. The significant level was anticipated when the value of $p<0.05$.

CHAPTER –IV

RESULTS AND DISCUSSION

4.1. Results:

Table-1: Descriptive analysis of species, location and haemo-parasites of Rodents

Variable	Category	Percentage (%)	SEM	95% CI
Species	*Mus musculus*	16.87	0.029	11 – 22.74
	Rattus rattus	78.12	0.032	71.65 - .84.59
	Vandeleuria oleracea	5.01	0.017	1.58 – 8.41
Location	Chakaria	11.87	0.025	6.80 - 16.94
	Bandarban	2.50	0.012	0.05 – 4.94
	Faridpur	28.12	0.035	21.08 – 35.16
	Joypur Hat	16.87	0.029	11.00 – 22.74
	Lalmonir Hat	5.0	0.017	1.58 – 8.41
	Rangamati	11.87	0.025	6.80 – 16.94
	Rangpur	8.75	0.022	4.32 – 13.17
	Sitakunda	15.0	0.028	9.40 – 20.59
Parasities	Negative	90.0	0.023	85.30 – 94.69
	Babesia spp.	5.0	0.017	1.58 – 8.41
	Plasmodium spp.	2.50	0.012	.05 – 4.94
	Anaplasma spp.	2.50	0.012	.05 – 4.94

Table-2: Comparative scenario of parasitic infection on different species and locations

Variable	Category	Percentage (Positive)			P-value
		Babesia spp.	*Plasmodium spp.*	*Anaplasma spp*.	
Species	*Mus musculus* (N=27)	1 (3.70%)	1 (3.70%)	0	0.90
	Rattus rattus (N=125)	7 (5.60%)	3 (2.40 %)	4 (3.20%)	
	Vandeleuria oleracea (N=8)	0	0	0	
Location	Chakaria (N=19)	2 (10.53%)	2 (10.53%)	0	0.15
	Bandarban (N=4)	0	0	0	
	Faridpur (N=45)	2 (4.44%)	2 (4.44%)	0	
	Joypur Hat (N=27)	3 (11.11%)	0	0	
	Lalmonir Hat (N=8)	0	0	1 (12.50%)	
	Rangamati (N=19)	1 (5.26%)	0	1 (5.26%)	
	Rangpur (N=14)	0	0	1 (7.14%)	
	Sitakunda (N=24)	0	0	1 (4.17%)	

4.1.1. Species

From total (160) of blood smears, 90% were negatives to haemoparasites. Among others, the three species of rodents the overall prevalence of haemoparasites were 5% in *Babesia*, 2.5% in both *Aaplasma* and *Plasmodium*. This study was performed in three species of Rodents such as *Rattus rattus, Mus muscullus* and *Vandeleuria oleracea.* From table-2 it is found that *Rattus rattus* species is more susceptible to Babesiosis because rate of infection is higher (5.60%) than Anaplasmosis (3.20%) and *Plasmodium* (2.40%). Rate of infection of Plasmodium (3.70%) and Babasiosis (3.70%) were equal to *Mus muscullus* species. There is no Anaplasmosis infection recorded in this study period in *Mus muscullus*. There is no positive case found in *Vandeleuria oleracea* in this study population. There were no significant ($p>0.05$) variation in overall prevalence among the haemoparasites with three species of rodents. The results were illustrates in the table-1 and 2.

4.1.2. Location

In Chakaria upazilla out of 19 suspected animals, 2 animals were *Babesia* (10.53%) positive, 2 animals were *Plasmodium* (10.53%) positive. There was no Anaplasmosis infection recorded in Chakaria upazilla in this study population. In Bandorban district out of 04 suspected animals there was no positive case of haemoparasitic infection recorded in this study population. In Faridpur district out of 45 suspected animals, 2 animals were Babesiosis (4.44%) positive and 2 animals were *Plasmodium* (4.44%) positive. There was no Anaplasmosis infection recorded in Faridpur district in this study population. In Jaypurhat district out of 27 suspected animals, 2 animals were Babesiosis (11.11%) positive. There was no *Plasmodium* and *Anaplasma* infection recorded in this study population. In Lalmonirhat district out of 08 suspected animals, 1 animal was Anaplasmosis (12.50%) positive. There was no Babesiosis and *Plasmodium* infection recorded in this study population. In Rangamati district out of 19 suspected animals, 1 animal was Babesiosis (5.26%) positive, 1 animal was Anaplasmosis (5.26%) positive. There was no Plasmodium infection recorded in this study population. In Rangpur district out of 14 suspected animals, 1 animal was Anaplasmosis (7.14%) positive and there was no Babesiosis and Plasmodium infection recorded in this study population. In sitakunda upazilla out of 24 suspected animals, 1 animal was Anaplasmosis (4.17%) positive. There was no Babesiosis and

Plasmodium infection recorded in this study population. There were no significant ($p>0.05$) variation in overall prevalence among the haemoparasites within the different location. The results were illustrates in the table-1 and 2.

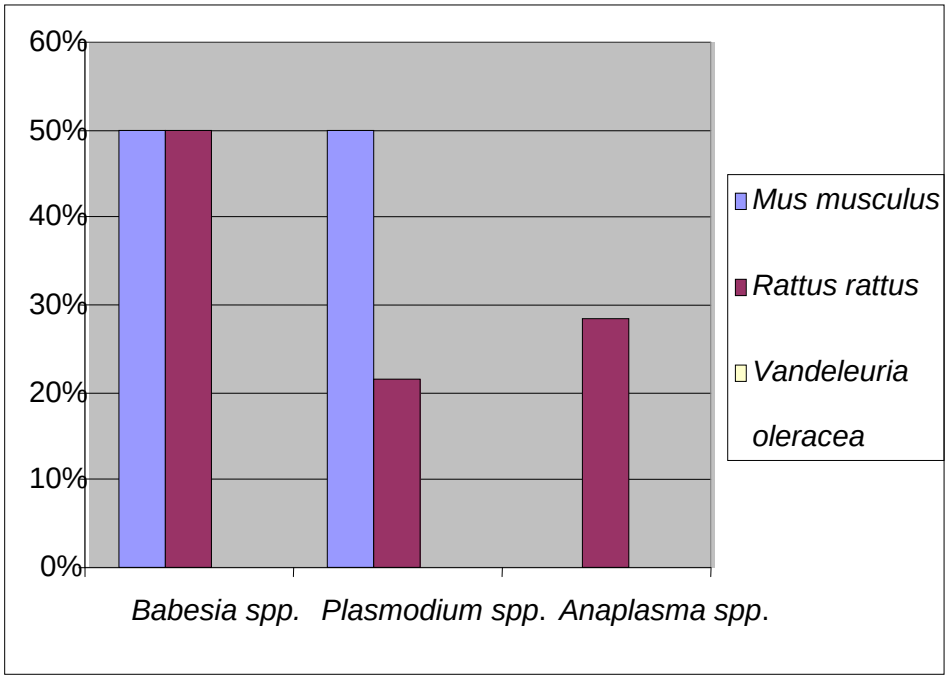

Fig-1: Haemo-parasitic infection in different rodent species

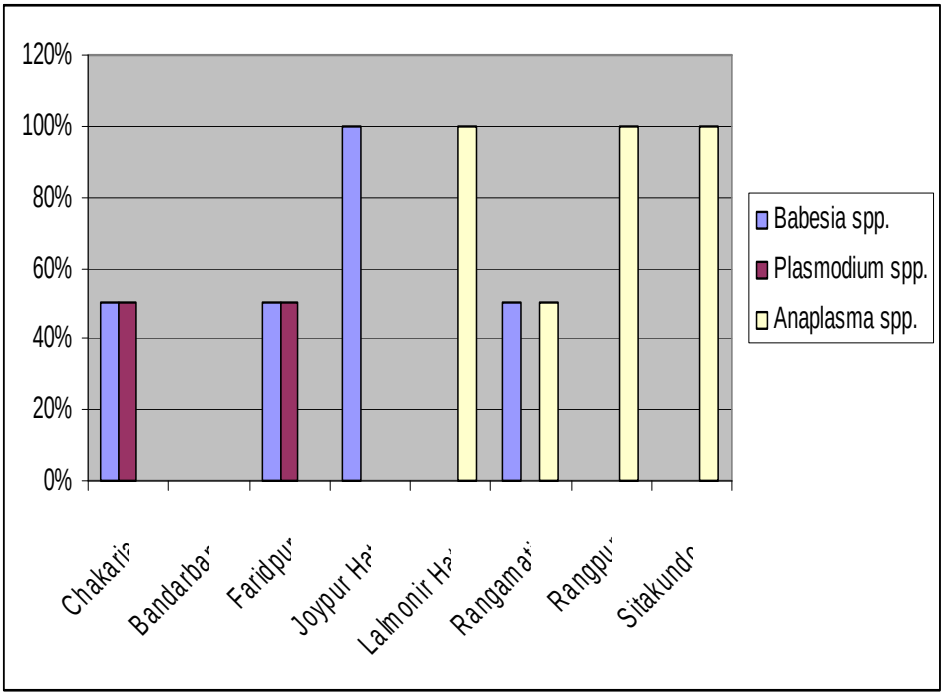

Fig-2: Distribution of haemo-parasitic infection in rodents of different areas

4.1.3. From the graphical presentation it is observed that *Babesia* was highest percentages (50%) in *Rattus rattus* and *Mus muscullus* and lowest percentages in *Vandeleuria oleracea.* On the other hand, graphical preserntation it is observed that *Plasmodium* was highest percentages (50%) in *Rattus rattus* and lowest percentages in *Vandeleuria oleracea. Anaplasma* was found almost 30% in *Rattus rattus* but there no positive cases found in the species of *Mus muscullus and Vandeleuria oleracea.* According to the location, *Babesia* predominates in the Chakaria, Faridpur, Joypurhat and Rangamati. The prevalence of *Plasmodium* was found only in Chakaria and Faridpur. On the other hand, Anaplasmosis dominated in the Lalmonirhat, Rangamati, Rangpur and Sitakunda area.

4.2. Discussion:

Babesia spp, Anaplasma spp and Plasmodium spp are arthropod transmitted haemoparasites appear to be widely distributed in many parts of the world as well as Bangladesh. Despite of large number of rodents to be susceptible to infection with haemoparasites, very few records are descried and disease due to infection is rarely mentioned. Haemoparasites are generally considered only slightly pathogenic to Rodents species. However some evidence exists that they can cause subclinical effects and depresses breeding rates and thus influence on their total population and production. In this study *Babesia spp* were observed in only 6 Rodents, *Anaplasma spp* were observes in only 4 Rodents and *Plasmodium spp* were observed in only 5 Rodents**.** Etkind *et al.,* (1980) has been suggested that haemoparasitic infection is species specific, criteria for species distribution include host range and gametocyte features such as staining characteristics, size, nature and extent of distortion of host cell and altered shape and position of the host cell nucleus. This study agrees with Etkind *et al.,* (1980) on *Anaplasma* in Europe and North America. The existence of multiple variants of the agent that cause different clinical symptoms and immunologic reactions in different host species was noted early on. Even among strains isolated from the same host species, there is a great deal of variation in virulence as measured by percentage of infected granulocytes, the degree and duration of parasitemia, and the length of the incubation period Foster and Cameron, (1970). This study was also agree in the virulency of haemoparasites in rodents as measured by percentage of infected granulocytes, degree and duration of parasitemia and the length of the

incubation period of haemoparasites. The most recent reviews of *Babesia* spp. primarily concern parasites of livestock in tropical and subtropical regions, or zoonotic species, focusing mainly on the rodent species *B. microti* (Telford *et al.*, 1993). In this study prevalance of haemoparasitic infection is high in tropical and subtropical region due to high tick prevalence in this region.

More structural and extensive study as well as molecular characterization of hemo-parasites requires finding out the actual prevalence in rats of Bangladesh.

CHAPTER-V

CONCLUSION

The field study was conducted jointly by Ecohealth Alliance and ICDDR'B but we did the laboratory works in the Department of Pharmacology to detect the prevalence of haemo-parasites in different types of rodents in relation to different location. We found *Babesia, Anaplasma* and *Plasmodium* in the blood smears. All the haemoparasites has public health importance. So, structural and extensive research will be needed to molecular characterization of hemo-parasites and find out the actual prevalence in rodents of Bangladesh

CHAPTER VI

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