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-protoza in blood smears of fruit and small bats. Giemsa was used to stain the pre-prepared slides and emulsion oil was used to visualize the protozoa under microscope. A total of 350 blood smears were included in the study and the prevalence of *Babesia* and *Plasmodium* were 2.65%, 2.32%, and 6.25%, 0%, respectively in fruit (*Pteropus giganteus*) and small bats (*Megaderma lyra*) The highest prevelence of Babesia was found in Khagrachari (6.90%) and lowest in Chakaria, Dinajpur and Ramnagar but the highest prevelence of *Plasmodium* was found in Faridpur (4.76%) and lowest in Khagrachari, Rangpur, Chakaria and Dinajpur. The prevalence in both fruit and small bat were found no significant ($p>0.05$) variation. It is necessary to conduct more research on heamoparasties of bats due to public health concern.

Key words: Fruit bats, Small bats, Haemoprotozoa, Prevelence, *Babesia, Plasmodium*

CHAPTER I

INTRODUCTION

Bangladesh is a moderately hot and humid country with short winter and prolonged rainy season. The geo-climatic condition of Bangladesh is suitable for the development and survival of various parasites within the wild mammal. Bats in tropical lowlands are excellent indicators of ecosystem health and ecosystem change due to their species richness, differential reactions to fragmentation and crucial ecological functions. Bats also provide value to ecosystems as primary, secondary, and tertiary consumers that support and sustain both natural and human dominated ecosystems ranging from the simple to the complex. Bats have long been postulated to play important roles in arthropod suppression, seed dispersal and pollination; as well as maintain the ecosystem. The rich diversity of dietary habits of bats, ranges from species that feed on insects and other arthropods to those that feed on fruit, nectar, and flowers, thus provide valuable ecosystem services (Khan, 1982). Bangladesh as a deltaic flood plain of recent origin has a variety of habitats suitable for diverse fauna. As many as 125 species of mammals has been reported from the country (IUCN Bangladesh 2000). This is inclusive of 31 species of bats (Khan, 1982). Sarker, (1988) reported only 17 species while IUCN Bangladesh, (2000) largely based on checklist of Khan, (1982) listed 29 species of bats from Bangladesh. However, they are also associated with diseases deadly to humans. Several highly fatal diseases have been linked to bats. Rabies is perhaps the most well known disease associated with bats. Along with animals such as dogs, foxes, raccoons, and skunks, bats are one of the primary animals that transmit rabies. In addition, Histoplasmosis is caused by a fungus that grows in soil and material contaminated with droppings from animals, including bats. It is widespread in certain areas of the U.S.A (CDC, 2013).

Fruit bats, also known as ‘flying foxes’ of the genus *Pteropus* are natural reservoir hosts of the Nipah and Hendra viruses. The virus is present in bat urine and potentially, bat feces, saliva, and birthing fluids. Nipah and related viruses are also associated with the same group of bats in Southeast Asia and parts of Africa, although outbreaks of disease in humans have so far been limited to Malaysia, Singapore, India, and Bangladesh (OIE, 2008). Ever since bats have been identified as possible reservoirs of *Trypanosoma cruzi*, *Babesia* & *Plasmodium* they have been

included in medical epidemiological surveys. This interest has been fuelled by the presence of bat-specific blood protozoa. In previous decades large efforts given upon regarding the bats could be reservoirs for human pathogenic haemoparasites have been undertaken.So the knowledge is essentialy need for adequate risk assessments about chances of disease transmission, either it is transmittable between bats and humans. The incidence of infectious diseases, especially general vector-borne parasitism, often increases as species diversity decreases. Haematozoa are protozoan parasites living within blood cells or free in the blood of their hosts. These blood stages are usually part of complex life cycles, which may include both sexual and asexual reproduction in a wide range of cells and tissues of the same host or in a second, vector, host required for the parasite's transmission. The haematozoa of bats were first reported at the end of the nineteenth century in Italy Dionisi, (1899) who examined these mammals as possible hosts of human malaria and, in doing so, discovered. Previous records of heamoparastie of bats have been limited to localized studies involving a small number of bats, representing only four of the 15 British species. Several species of bat protozoa was identified in bat, among them *Babesia, Trypanosome, Plasmodium* of several species etc are pronounced Coles, (1914).In the genus of *Babesia* the *B. vesperuginis* is found in bats (Baker *et al.,* 1963). The results demonstrate that *B. vesperuginis* can be pathogenic in its natural host because splenomegaly, depressed blood hemoglobin, debilitation and possibly haemoglobinuria were observed. Congestion of the spleen resulting from *B. vesperuginis* infection could interfere with vascular changes. During these periods the spleen acts as an erythrocyte reservoir, permitting alterations to the blood composition, and probably easing the workload of the heart; release of erythrocytes from the spleen reverses this condition as soon as arousal begins (Worth, 1932). The genus *Polychromophilus* (one of the genus of the *Hemosporidia*) was first described by Dionisi, (1899) who named two species *P. murinus* and *P. melanipherus. Polychromophilus* a malaria parasite of Chiropteran is reported at first in England (Gardner *et al.,* 1987). As far known no study was conducted to evaluate the prevalence of heamo protozoal harbouring bats in Bangladesh. So the present study was undertaken with regarding the following objectives**:**

Objectives:

1) To know the prevalence of hemo-parasite in fruits and small bats
2) To know the prevalence of bat hemo-parasite at different location in Bangladesh

CHAPTER II

REVIEW OF LITERATURE

Bats are mammals of the order Chiroptera whose forelimbs form webbed wings, making them the only mammals naturally capable of true and sustained in fly. Bats do not flap their entire forelimbs, as birds do, but instead flap their spread-out digits (Hunter, 2007) which are very long and covered with a thin membrane or patagium. Bats represent about 20% of all classified mammal species worldwide, with about 1,240 bat species divided into two suborders: the less specialized and largely fruit-eating megabats, or flying foxes, and the highly specialized and echo- locating micro bats (Hunter, 2007). About 70% of bat species are insectivores. Most of the rest are frugivores, or fruit eaters. A few species, such as the fish-eating bat, feed from animals other than insects, with the vampire bats being hematophagous most microbats are nocturnal and are active at twilight. A large portion of bats migrate hundreds of kilometers to winter hibernation dens while some pass into torpor in cold weather, rousing and feeding when warm weather allows for insects to be active . Others retreat to caves for winter and hibernate for six months. Bats rarely fly in rain, as the rain interferes with their echolocation, and they are unable to locate their food Fenton & Brock (2001).

The social structure of bats varies, with some bats leading solitary lives and others living in caves colonized by more than a million bats. The fission-fusion social structure is seen among several species of bats. The term "fusion" refers to a large numbers of bats that congregate in one roosting area, and "fission" refers to breaking up and the mixing of subgroups, with individual bats switching roosts with others and often ending up in different trees and with different roost mates. Studies also show bats make all kinds of sounds to communicate with others. Scientists in the field have listened to bats and have been able to identify some sounds with some behavior bats will make after the sounds are made. Insectivores make up 70% of bat species and locate their prey by means of echolocation. Of the remainder, most feed on fruits. Only three species sustain themselves with blood. Some species even prey on vertebrates. The leaf-nosed bats (*Phyllostomidae*) of Central America and South America, and the two bulldog bat (*Noctilionidae*) species feed on fish. At least two species of bat are known to feed on other bats:

the spectral bat, also known as the American false vampire bat, and the ghost bat of Australia. One species, the greater noctule bat, catches and eats small birds in the air. Bats are present throughout most of the world, performing vital ecological roles of pollinating flowers and

Fig-1: Fruit bat *(Pteropus giganteus*)

Fig-2: Small bat (*Megaderma lyra*)

dispersing fruit seeds. Many tropical plant species depend entirely on bats for the distribution of their seeds. Bats are important, as they consume insect pests, reducing the need for pesticides Fenton & Brock, (2001),

2.1Hunting behavior

Most bats are nocturnal creatures. Their daylight hours are spent grooming and sleeping; they hunt during the night. This means by which bats navigate while finding and catching their prey in the dark. These bats were placed in a room in total darkness, with silk threads strung across the room. Even then, the bats were able to navigate their way through the room. When bats fly, they produce a constant stream of high-pitched sounds only bats are able to hear. When the sound waves produced by these sounds hit an insect or other animal, the echoes bounce back to the bat, and guide them to the source (Lauber, 1968).

2.2 Feeding and diet

The majority of food consumed by bats includes insects, fruits and flower nectar, vertebrates and blood (Johnson & Sylvia, 1985). Almost three-fourths of the world's bats are insect eaters. Bats consume both aerial and ground-dwelling insects. Each bat is typically able to consume one-third of its body weight in insects each night, and several hundred insects in a few hours. This means a group of one thousand bats could eat four tons of insects each year. If bats were to become extinct, it has been calculated that the insect population would reach an alarmingly high number (Shebar & Sharon, 1990).

2.2.1 Insectivores

Occasionally, a bat will catch an insect in mid-air with its mouth, and eat it in the air. However, more often than not, a bat will use its tail membrane or wings to scoop up the insect and trap it in a sort of "bug net". Then, the bat will take the insect back to its roost. There, the bat will proceed to eat said insect, often using its tail membrane as a kind of napkin, to prevent its meal from falling to the ground. One common insect prey is *Helicoverpa zea*, a moth that causes major agricultural damage (Fitt, 1989).

2.2.2 Frugivores

Fruit eating or frugivores, is a specific habit found in two families of bats. Mega chiropterans and micro chiropterans both include species of bat that feed on fruits. These bats feed on the juices of sweet fruits, and fulfill the needs of some seeds to be dispersed. The fruits preferred by most fruit-eating bats are fleshy and sweet, but not particularly strong smelling or colorful. To get the juice of these fruits, bats pull the fruit off the trees with their teeth, and fly back to their roosts with the fruit in their mouths. There, the bats will consume the fruit in a specific way. To do this, the bats crush opens the fruit and eat the parts that satisfy their hunger. The remainder of the fruit, the seeds and pulp, are spat onto the ground. These seeds take root and begin to grow into new fruit trees (Shebar & Sharon, 1990). Over 150 types of plants depend on bats in order to reproduce. Some bats prefer the nectar of flowers to insects or other animals. These bats have evolved specifically for this purpose. For example, these bats possess long muzzles and long, extensible tongues covered in fine bristles that aid them in feeding on particular flowers and plants. When they sip the nectar from these flowers, pollen gets stuck to their fur, and is dusted off when the bats take flight, thus pollinating the plants below them (Shebar & Sharon, 1990). The rainforest is said to be the most benefitted of all the biomes where bats live, because of the large variety of appealing plants. Because of their specific eating habits, nectar-feeding bats are more prone to extinction than any other type of bat. However, bats benefit from eating fruits and nectar just as much as from eating insects (Hodgkison *et al.,* 2003).

2.2.3 Blood

A few species of bats exclusively consume blood as their diet. This type of diet is referred to as hematophagy, and three species of bats exhibit this behavior. These species are the common, the white-winged, and the hairy-legged vampire bats. The common vampire bat typically consumes the blood of mammals, while the hairy-legged and white-winged vampires feed on the blood of birds Greenhall & Arthur (1961).

2.3 Drinking

In Fenton & Brock, (2001), discovered that it skimmed again to get a second drop of water, and so on, until it has had its fill. A bat's precision and control during flight is very fine, and it almost never misses. Other bats, such as the flying fox or fruit bat, gently skim the water's surface, then land nearby to lick water from chest fur (Jones, 2000).

2.4 Pathogens and role in the transmission of zoonoses

Bats are natural reservoirs for a large number of zoonotic pathogens including rabies, severe acute respiratory syndrome (SARS), Henipavirus (i.e. Nipah virus and Hendra virus) and possibly ebola virus. Their high mobility, broad distribution, and social behaviour (communal roosting and fission-fusion social structure) make bats favourable hosts and vectors of disease. Many species also appear to have a high tolerance for harbouring pathogens and often do not develop disease while infected. However, this is not true of rabies, which is as fatal to bats as it is to all other species. However, a bat may be ill with rabies for a longer time than other mammals (Halpin *et al.,* 2000). In regions where rabies is endemic, only 0.5% of bats carry the disease. In the United States, bats typically constitute around a quarter of reported cases of rabies in wild animals. However, their bites account for the vast majority of cases of rabies in humans. Of the 36 cases of domestically-acquired rabies recorded in the country in 1995–2010, two were caused by dog bites and four patients were infected by receiving transplants from an organ donor who had previously died of rabies. All other cases were caused by bat bites (CDC, 2013) Rabies is considered fully preventable if the patient is administered a vaccine prior to the onset of symptoms. There is evidence that it is possible for the bat rabies virus to infect victims purely through airborne transmission ("cryptic rabies"), without direct physical contact of the victim with the bat itself (Messenger *et al.,* 2002).

2.5 Haemoprotozoa in bat

The haemoprotozoa are protozoan parasites living within blood cells or free in the blood of their hosts. These blood stages are usually part of complex life cycles, which may include both sexual and asexual reproduction in a wide range of cells and tissues of the same host or in a second, vector, host required for the parasite's transmission. The haematozoa of bats were first reported

at the end of the nineteenth century in Italy by Dionisi, (1898 and 1899) that examined these mammals as possible hosts of human malaria and, in doing so, discovered. Previous records of haematozoa of bats have been limited to localized studies involving a small number of bats, representing only four of the 15 British species.

2.5.1 *Babesia* spp.

Members of the genus *Babesia*, are primarily intra-erythrocytic parasites of mammals and birds. Levine, (1971) listed 70 species of *Babesia* from a wide range of wild and domestic mammals, and Peirce, (1975) listed 10 avian species, about which little is known. Dionisi, (1898) was the first to describe a piroplasm of bats, from *Nyctalus noctula* in Italy, which he later named Achromaticus vesperuginis Dionisi, (1899). He suspected that infected bats were anaemic, but could not confirm it. Schingareff, (1906), also found *Babesia vesperuginis* in *N. noctula* in Italy; he noticed that the blood of 3 heavily infected bats was very pale, and inferred that it had a low haemoglobin content. In addition to these observations Goedbloed, (1964) in Holland reported a 'sick' *Pipistrellus pipistrellu*s harbouring a heavy infection of *B. vesperuginis*. They also found 5 other *P. pipistrellus*, 1 *Myotis nattereri*, 2 *M. daubentoni*, 3 *M. mystacinus*, 2 *N. noctula* and 3 *Plecotus auratus* infected but did not associate the infection with any pathogenicity. *B. vesperuginis* has been previously reported from *P. pipistrellus* in Britain by a number of authors Coles, (1914) previously reported *B. vesperuginis* in the blood of 19 of 206 *P. pipistrellus* and 1 of 11 *M. mystacinus* examined.

It can be demonstrate that *B. vesperuginis* can be pathogenic in its natural host because splenomegaly, depressed blood haemoglobin, debilitation and possibly haemoglobinuria were observed. A infected wild bat showed that the course of infection observed in experimentally infected bats may occur in nature; splenomegaly also occurs in infected wild bats. There was a significant elevation of the reticulocyte counts in naturally infected bats in captivity compared with uninfected captive bats. Baker *et al.*, (1963) and Krampitz, (1979) found evidence of splenomegaly in *Microtus agrestis* infected with *B. microti*, although no evidence of pathogenicity in *M. agrestis* in the wild was obtained. The spleens of laboratory-bred *M. agrestis* infected with *B. microti* enlarged to up to 6 % of body weight within 3 weeks of infection and then decreased gradually in size, although they remained elevated indefinitely Krampitz, (1979).

This pattern appears to be similar to that encountered with *B. vesperuginis* in *P. pipistrellus.* Krampitz, (1979) reported haemoglobinuria and urine retention in *M. agrestis* experimentally infected with newly isolated *B. microti*, but he did not find differences in haematological parameters between infected and uninfected *M. agrestis* in the field. The significantly lowered haemoglobin levels in the experimentally infected bats indicate that they were not fully compensating for the parasite-induced red blood cell destruction. It is likely that haemoglobin levels were depressed early in the parasitaemia as seen in experimental *Babesia* infections of rodents Nowell, (1969). If the debilitation observed in infected bats was experienced in the wild, as seems to occur, infected bats would be unable to forage effectively. Congestion of the spleen resulting from *B. vesperuginis* infection could interfere with vascular changes associated with hibernation. During these periods the spleen acts as an erythrocyte reservoir, permitting alterations to the blood composition, and probably easing the workload of the heart; release of erythrocytes from the spleen reverses this condition as soon as arousal begins (Worth, 1932). The soft tick *Argas vespertilionis* was frequently found on infected *P. pipistrellus*. In Holland, Goedbloed *et al.*, (1964) found *B. vesperuginis* in 6 species of bat and reported finding only argasid ticks. Before Gunders & Hadani, (1974) demonstrated that the natural vector of *B. meri* in *Psammomys obesus* was an argasid tick. *Ornithodorus erraticus*, it had been considered that ixodid ticks were the only vectors of *Babesia* spp. The only ixodies tick found on bats during the survey on the prevalence of haematozoa of British bats (Gardner *et al.*, 1987) was *Ixodes pipistrelli* from *Rhinolophus hipposideros*. The absence of *Ixodes* from *P. pipistrellus* (Gardner *et al.*, 1987) and the presence of *Argas vespertilionis* on *P. pipistrellus* infected with *B. vesperuginis* indicate that this argasid tick is the vector.

2.5.2 *Plasmodium* spp.

Plasmodium falciparum (Apicomplexa, Haemosporidia), the most dangerous of human malaria parasites, is responsible for at least one million deaths a year (Peterson *et al.*, 1995). It has been suggested that its exceptional virulence, compared to the three other species of human *Plasmodium*, is due to its relatively recent host-shift from birds to humans and the short period for the latter to adapt to the parasite. Given the heavy burden of *P. falciparum* on human populations around the tropics, there is a critical need to better understand the origin and

evolution of this parasite and related organisms. *Plasmodium falciparum* belongs to a group that also infects a considerable range of birds, squamates, crocodilians, chelonians and non-human mammals. These parasites are known to be virulent, invasive pathogens in a variety of wild animals and contribute to the parasite burden of natural populations, including several threatened species (Peterson *et al.,* 1995). Host switching by these parasites could be the trigger for emerging virulent diseases Madagascar and Cambodia are two biodiversity "hotspots" (Siemers *et al.,* 2004). The faunas of these areas, which are, in evolutionary terms, distant from one another, provide an attractive system for characterizing haemosporidian parasite species and for evaluating host-parasite coevolution and exchange. After rodents, bats are the largest order of mammals (at least 1,100 species, more than 20% of extant mammal species). The Chiroptera are very diverse and they are distributed almost worldwide and have extremely diverse life history traits and morphology. Based on recent molecular work, they are classified into four super-families that apparently diversified in different areas during the early. In developing echolocation and different flight strategies, the ancestors of modern bats colonized various ecological niches (Siemers *et al.,* 2004) , where birds and their associated blood parasites are thought to have been present, thus favoring host switching from birds to bats. Furthermore, *Myotis goudoti* and *Miniopterus manavi* often share common day roost sites in tree hollows, caves and rock shelters (Peterson *et al.,* 1995), which expose considerable numbers of densely packed individuals to the same potential blood parasite vectors in bats.

2.5.3 *Polychromophilus murinus*

Polychromophilus murinus, a malaria parasite of Chiroptera is reported from *Myotis daubentoni* in England. The vector was suspected to be the ectoparasitic Nycteribiid fly, *Nycteribia kolenatii*. *N. kolenatii* collected from wild-caught *M. daubentoni* were found to have oocysts on the midgut and sporozoites in the salivary glands. Wild-caught *N. kolenatii* were maintained on two wild-caught *M. daubentoni* harbouring heavy (patent) infections of *P. murinus*; both oocysts and sporozoites were found in these flies. Sporozoites were straight or slightly crescentic and had a mean length of 7-4 µm. Electron microscopy of immature and mature oocysts revealed morphology similar to that of malaria parasites. Sporozoites were also similar in structure to Plasmodium sporozoites and were found in the epithelial cells of the salivary gland and within

the lumen; a cytostome was present and transverse sections revealed 21 microtubules arranged evenly around the periphery. Sporozoites were observed within the basement membrane of the salivary gland of *N. kolenatii;* such sporozoites appeared to be penetrating the gland, a process hitherto not described in malaria parasites. Rickettsia-like bodies were found within the cytoplasm of the epithelial cells of the salivary gland. Exflagellation of microgametocytes was achieved. An ultra-structural study of the gametocytes revealed a structure similar to that described in other Haemoproteidae. A common feature of infected erythrocytes was a projecting erythrocyte membrane. Attempts to find schizogony in impression smears and sections of tissues of two infected *M. daubentoni* were not successful Coles, (1914).

The genus *Polychromophilus* was first described by Dionisi, (1899) who named *P. murinus* and *P. melanipherus* from *Vespertilio murinus* and *Miniopterus schreibersi* respectively; they were distinguished by small differences in gametocyte size and pigmentation (erythrocyte enlargement in *P. murinus* and coarser pigment in *P. melanipherus*) although the identification was probably complicated by mixed infections in the bats. Schingareff, (1906) found gametocytes of Polychromophilus in the blood of *Myotis daubentoni* and *Miniopterus schreibersi* and identified them as *P. murinus* and *P. melanipherus* respectively. He also described small schizonts with 20-22 merozoites seen within macrophages in impression smears of the liver and spleen and, infrequently, in macrophages in the peripheral circulation. Schingareff, (1906) suspected that nycteribiid flies were the vectors of *Polychromophilus* but, he dissected and examined 6 flies, was unsuccessful in his search for oocysts. Coles, (1914) found short, stumpy, slightly crescentic sporozoites which were very probably *Polychromophilus* in 2 nycteribiids in Italy, whilst Mer & Goldblum, (1947) briefly described sporozoites from a nycteribiid taken from a bat infected with *Polychromophilus* in Israel. Mer & Goldblum, (1947) also found schizonts in the bone-marrow, lung, kidney and liver of *Myotis myotis* and kidney and liver of *M. nattereri*. They later saw oocysts and stumpy sporozoites in two species of flies, *Penicillidia dufouri* and *Pen. cospicua* Garnham, (1966).

Garnham, (1966) attempted to clarify the problem of differentiation of species of *Polychromophilus* and redescribed the two parasites, confining *P. melanipherus* to records from *Miniopterus schreibersi* and *P. murinus* to other genera of vespertilionid bats. Contrary to Dionisi's, (1899) observations on gametocyte sizes, Garnham, (1966) stated that *P. murinus*

tended to be smaller than *P. melanipherus*, but that this was not a reliable feature for identification. As a result of recent work on *Polychromophilus*, Beveridge & Baccam, (1980) have erected two sub-genera *P. (Polychromophilus)*, type species *P. (P.) melanipherus*, and *P. (Bioccala)*, type species *P. (B.) murinus*, laying emphasis on differences of the gametocytes. They attribute small schizonts (under 30 µm in diameter), those described by Schingareff, (1906) from *M. daubentoni* and Mer & Goldblum (1947) from *M. myotis* and *M. nattereri*, to *P. (Bioccala)* spp. and large schizonts to *P. (Polychromophilus)* spp. They considered all records of sporogonic stages to date to belong to *P. (Bioccala)* spp. Records of sporogenic stages in nycteribiid flies associated with *Polychromophilus* infections were listed by Garnham (1973). These have involved a very small number of flies, and the identity of the parasite observed was not always certain. In a recent study a high proportion of *M. daubentoni* (Daubenton's bat) (25%) has been found infected with *P. murinus* (Gardner *et al.,* 1987). The apparent absence of *P. melanipherus* from British bats means that sporogonic stages found in *Nycteribia kolenatii* collected from *M. daubentoni* could be confidently attributed to *P. murinus*.

2.5.4 *Schizotrypanum* & *Megatrypanum* (sub genus of *Trypanosoma* spp*)*

Trypanosomes of the subgenus *Megatrypanum* are a heterogenous group of large mammalian trypanosomes whose kinetoplast is typically situated near the nucleus and far from the posterior extremity of the body Coles, (1914). A number of species have been described from bats but, until recently, only from African and American microchiroptera. Coles, (1914) listed 9 species of *Megatrypanum* from bats and there have been a number of unnamed *T. (M.) heybergi* like trypanosomes reported from African bats (Heisch & Garnham, 1953). Four further species have been described since Coles, (1914) listed: *T. lizae* (Miltgen & Landau, 1979) from *Hipposideros cyclops* in the Gabon; *T. megachiropterum* from *Pteropus tonganus* in Tonga (Marinkelle, 1979), the only record of *Megatrypanum* from the Pacific area and from a megachiropteran host; *T. rhinopoma* in India (Bandyopadhyay *et al.,* 1982) and *T. scotophila* in China. Coles, (1914) recorded of *Megatrypanum* from Asian bats in the later. The first record of *Megatrypanum* from a European bat was by Coles, (1914) who found a *Trypanosoma* he identified as *T. incertum.* Pittaluga, (1905) in 3 of 21 *Pipistrellus pi pistrellus* from East Anglia Coles, (1914). This parasite was found in 33 of 206 *P. pipistrellus* examined in a recent survey (Gardner *et al.*,

1987). The original description of this parasite was unclear. Coles, (1914) found a large trypanosome in 2 *Pipistrellus kuhli* in Algeria. Coles, (1914) drawing of the parasite was devoid of a free flagellum nucleus and kinetoplast. *T. heybergi,* however, if the trypanosome is compared with the erythrocytes in the illustration. Coles, (1914) named their *T. vespertilionis*, an unavailable junior homonym, as this name had earlier been given to a *Schizotrypanum* of bats in Italy by Battaglia, (1904), and was renamed *T. incertum* by Coles, (1914). Hoare, (1972) did not refer to Coles, (1914) work and hence no reference to this named species appears in his monograph. Nicolle & Comte, (1906) found a large trypanosome in the blood of 9 of 112 *P. kuhli* examined from the same region as those examined by the Coles, (1914) and considered it to be the same species. However, Nicolle & Comte, (1906) believed the larger trypanosomes to be a multiplicative stage of the smaller *Schizotrypanum* also found in the same bats.

Nicolle & Comte, (1906) found a large trypanosome in the blood of 9 of 112 *P. kuhli* examined from the same region as those examined by the Coles, (1914) and considered it to be the same species. However, Nicolle & Comte, (1906) believed the larger trypanosomes to be a multiplicative stage of the smaller *Schizotrypanum* also found in the same bats. Nicolle & Comte, (1906) description and measurements of the parasite correspond with those of Coles, (1914). The overall knowledge of the subgenus *Megatrypanum* is meager. Most of the detailed work has been undertaken of the trypanosomes on domestic cattle and sheep Wells, (1976). The biology of *Megatrypanum* species in bats is even less well understood. There is no information on the methods of multiplication and only a few species have been grown in culture. Mites, cimicid bugs, and sandflies (Zeledon & Rosabal, 1969) are suspected vectors. The only experimental evidence of a vector of a bat *Megatrypanum* is that of Coles, (1914) incriminating the human bed bug, *Cimex lectularius.*

Trypanosomes of the subgenus *Schizotrypanum* are restricted to bats of the Old and New World, except for *Trypanosoma cruzi cruzi* which infects many other mammals in Latin America Coles, (1914). Two species of *Schizotrypanum, T. (S.) vespertilionis* and *T. (S.) d. dionisii* have been identified from the British bats *Pipistrellus pipistrellus* and *Nyctalus noctula* (Baker, *et al.*, 1972). These trypanosomes cannot be differentiated by their blood forms but are distinguishable by their morphology in vitro (*T. d. dionissi* producing short broad and long thin metatrypanosomes. The bat bug, *Cimex pipistrelli*, was suspected of being the vector of *T.*

vespertilionis by Pringault, (1914). However, from his descriptions of the blood forms of the trypanosomes it is apparent that the bats he studied were probably infected with both *Schizotrypanum* and *Megatrypanum*; furthermore, the 'uninfected' bats to which the trypanosomes were allegedly transmitted were recently captured bats with possible sub-patent infections. Rodhain, (1939) showed that metatrypanosomes developed in the gut of the bed bug *C. lectularius* fed on cultures of *T. vespertilionis*, and concluded that transmission was contaminative. Williams, (1976) in Canada, demonstrated experimentally that *C. brevis* is the vector of *T. hedricki*.

CHAPTER III

MATERIALS AND METHODS

3.1 Study area

The study was carried to measure the prevalence of blood parasitic diseases in fruit and small bats of Khagrachari, Faridpur, Rangpur, Dinajpur districts and Chakaria, and Ramnagar upazila of Bangladesh.

3.2 Study period

The field study was undertaken between 2010 to 2013 by joint research team of Ecohealth Alliances and ICDDRB 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 350 blood smears were collected randomly from fruit bats and small bats from Khagrachari, Faridpur, Chakaria, Rangpur, Dinajpur and Ramnagar during the study period.

3.4 Processing of bats

1. At first live bats were placed in a cotton bag and kept in a cool dry place until sampling time. Then bat weight was taken in bags using a Pesola hanging scale. After that the bat was removed from the bag for sampling and bag weight taken and subtracted from previous total
2. Then manually restrain the bats during collection of blood. Three peoples were used for these manipulations, one person used for safely restrain the bat, one person for to take samples and third one to manage the tubes and record samples
3. Ketamine (5mg/kg) was used for anesthesia of bat. At that time the person was restraining the bat were monitored respiration. At that time of the handler also monitor any type of severe stress

4. Bat that had not any disease or abnormality in health was selected for sample collection. The animal's health and safety is always more important than collecting samples. It can be mentioned that bats can die from stress at any time
5. In live bats caution was taken to maintain a ratio not greater than 10µl of blood to 1 gm of bat body weight which is equivalent to 1% of bodyweight
6. During the time of blood collection pressure was applied to the site of bleeding using a cotton ball until bleeding ceased(approximately 1 minute).Then the collected blood was placed into conical eppendorf tubes
7. Blood smears from each bat were collected from wing vein by puncturing with sterile needle. The slides were touched to the coming out blood and then spread by another slide. The slides were air dried and fixed by 100% methyl alcohol for 2 min.

3.5 Staining and examination of blood smears

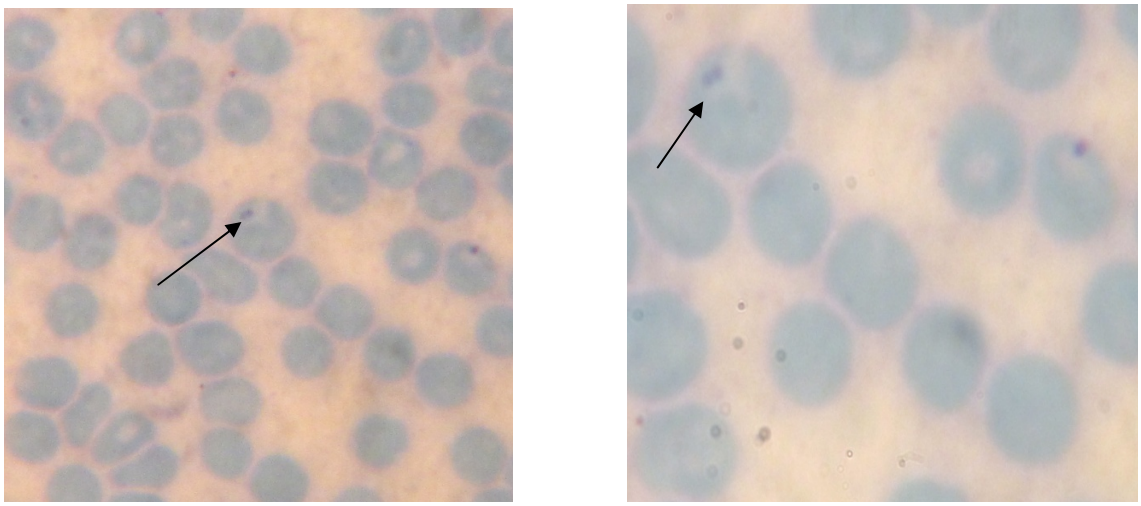
The prepared blood smears were stained with the Giemsa stain (working solution) for 25 to 30 minutes. After rinsing with water of the stained blood smears, they were air dried and examined under microscope (10X100x) with immersion oil for the identification of blood parasites as described by Soulsby, (1986).

3.6 Identification of parasites

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

3.6.1 *Babesia* spp.

The parasites in the erythrocytes were ring shaped, oval bizarre amoeboid shapes, frequently appearing vacuolated (Fig-1). Ring or oval-shaped parasites usually had a single nucleus on their periphery, and larger parasites had 2 or 3 nuclei (Fig-3).



(1a) (1b)

Fig-3: *Babesia* spp

3.6.2 *Plasmodium* spp: Numerous inclusion bodies were seen. They were circular or oval in section (Fig-4), oval forms, bounded by discrete walls and containing fibrous material. The bodies

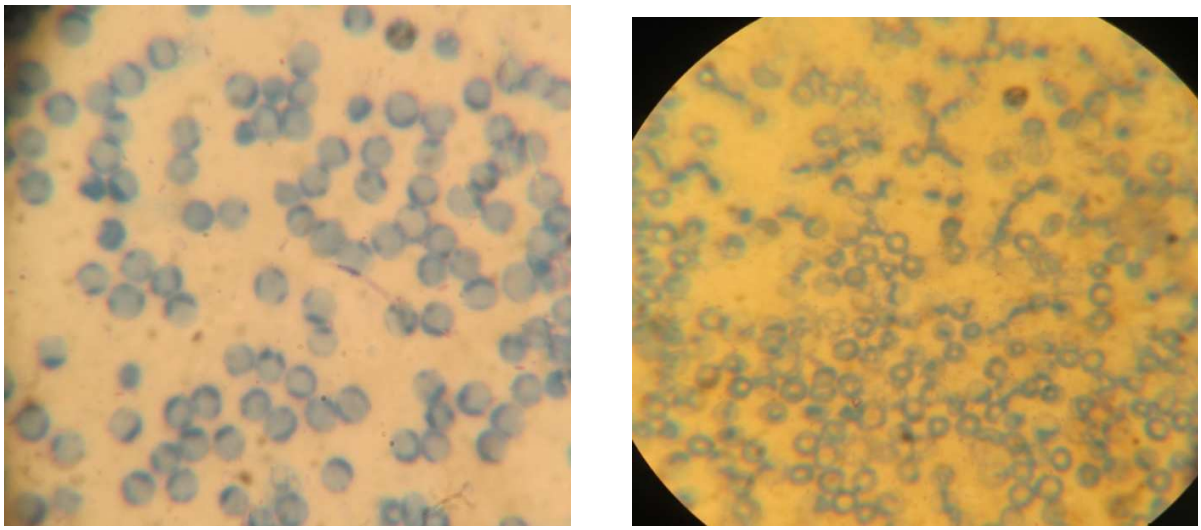


Fig-4: *Plasmodium* spp

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.

3.7 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 Chi-square 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

Table-1: Descriptive analysis of species, location and haemo-parasites of Bats (n=350)

Variable	Category	Percentage (%)	SEM	95% CI
Species	Fruit Bats	86	1.84	82.66-89.90
	Small Bats	14	1.84	10.09-17.33
Location	Khagrachari	8.28	1.47	5.38-11.18
	Rangpur	5.42	1.21	3.04-7.8
	Chakaria	10.28	1.62	7.08-13.48
	Dinajpur	17.14	2.01	13.17-21.11
	Faridpur	36	2.56	30.94-41.05
	Ramnagar	22.85	2.24	18.43-27.27
Parasities	Negative	94.85	1.18	92.53-97.18
	Babesia spp.	3.14	0.93	1.30-4.97
	Plasmodium spp.	2.0	0.74	0.52-3.47

CI=confidence interval;

Table-1 shows analysis among the positive case where the prevalence of haemo-parasites in the fruit bat (86%) is comparatively greater than the small bats (14%). In terms of locations highest prevalence was found in Faridpur district (36%) followed by Ramnagar (22.85%), Dinajpur (17.14%), Chakaria (10.28%), Khagrachari (8.28%) and Rangpur (5.42%) respectively. From the total blood smears 94.85% (N=350) of the sample is found as a negative and rest of them are positives in relation to the presence of haemo-protozoa.

Table-2: Comparative scenario of parasitic infection on different species and locations (n=350)

Variable	**Category**	**Percentage (Positive)**		**P-value**
		Babesia spp.	*Plasmodium* spp.	
Species	Fruit Bats	2.65(8)	2.32(7)	0.24
	Small Bats	6.25(3)	0	
Location	Khagrachari	6.90(2)	0	0.02
	Rangpur	5.26(1)	0	
	Chakaria	0	0	
	Dinajpur	0	0	
	Faridpur	6.35(8)	4.76(6)	
	Ramnagar	0	1.25(1)	

The overall prevalence of *Babesia* within the positive samples were bit higher in small bats (6.25%) than fruit bats (2.65%) & there were significant variation in prevalence of *Babesia*, on the other hand prevalence of *Plasmodium* was higher in fruit bats (2.32%) than small bats but the differences were not significant ($p > 0.05$). On the basis of location, the highest prevelence of *Babesia* was found in Khagrachari (6.90%) and lowest in Chakaria, Dinajpur and Ramnagar but the highest prevelence of *Plasmodium* was found in Faridpur (4.76%) and lowest in Khagrachari, Rangpur, Chakaria and Dinajpur. The overall prevalence of *Babesia* and *Plasmodium* within location varies significantly ($p < 0.05$) (Table-2).

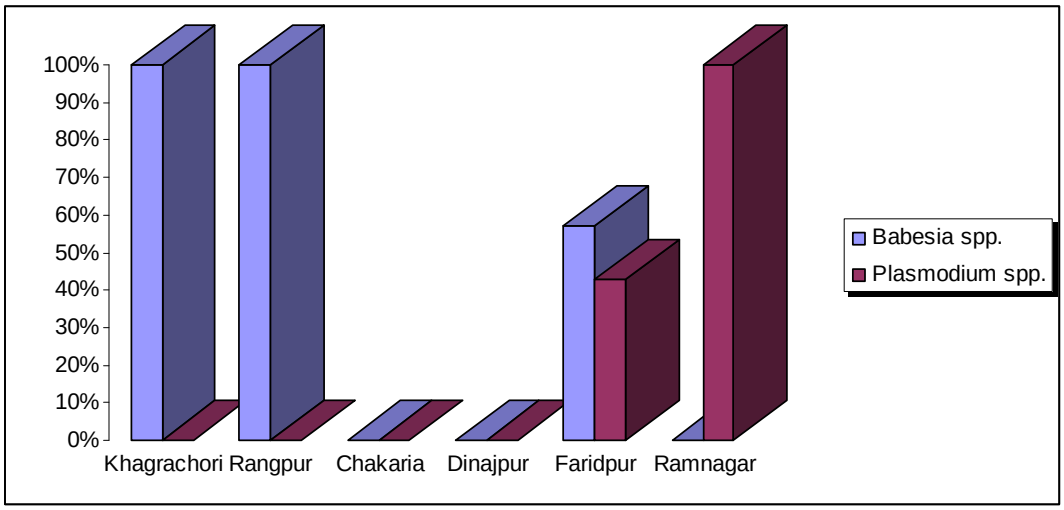


Fig-5: Distribution of haemo-parasitic infection in bats of different areas.

In case of graphical distribution of haemo-parasite *Babesia* was found much higher in the Khagrachori and Ranpgur district, comparatively to other locations of Bangladesh. But in case of *Plasmodium* Ramnagar represents the highest percentages of positive samples (Fig-5). The figure represents the percentages of haemoprotozoa only within the positive samples.

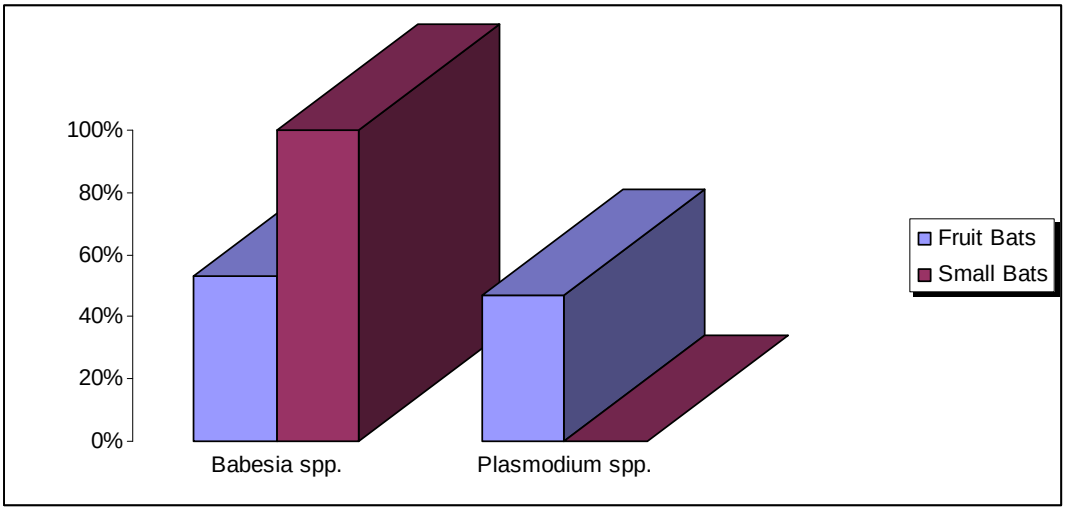


Fig-6: Haemo-parasitic infection in different bat species.

Fig-6 represents the percentage of haemo-protozoa fruit and small bat species. Small bats is found as a highest percentages of prevalence of *Babesia* was higher in small bats in comparison to fruit bats. Other side both bats are infected with *Plasmodium.*

CHAPTER IV

DISCUSSION

Prevalence of *Babesia* was found in fruits and small bats of current study. Gardener, (1988) also reported *Babesia* and selected ten bats randomly from where found prevalence of *Babesia* 50% within the bats in Cambridgeshire. Monaska *et al*., (2011) reported that the proportional prevalence of *Babesia* infection of bats was 14.94% in Canada which is more than the present study result.

Worth, (1932) reported that 63.73% bats were infected with *Babesia* spp in Mexico which is more than the study result in Bangladesh. This variation may be varying due to area, duration of study and resistance of the bats.

In case of *Plasmodium* Mer & Goldblum, (1947) found that the prevalence of fruit bats were 47% which is greater than our present study. Gardener, (1987) found a high proportion (25%) of *P. murinus* in fruit bat. Siemers *et al.,* (2004) reported that after rodents, bats are more susceptible to *Plasmodium* which is nearest about 58%.

In current report the sample is much higher (n=350) including fruit bats and small bats. The overall prevalence of *Babesia* within the positive samples were 6.25% in small bats than fruit bats 2.65% and the infected rate of fruit bats within the positive sample is 86% and in case of small bats it is 14%.

The prevalence of *Plasmodium* in current study is more or less similar with the finding of Garnham, (1966) who found (3-18%), (6-16%) and (3%) in August, September & October respectively. In regards it is not possible to ensure the presence of heamoparasties within the bat without any molecular characterization. This molecular characterization would help to give a significant result.

CHAPTER IV

CONCLUSION

In conclusion, these results appear to be compatible with assumption that the and small bats (*Megaderma lyra*) which are the most part, younger than the fruit bats (*Pteropus giganteus*), harbor with parasites of the *Plasmodium* spp, which I consider to be the most archaic, while small bats harbor parasites of the *Babesia* is comparatively more. From this study, it might be concluded that among the infected bat with haemo-parasites the highest prevelence of *Babesia* was found in Khagrachari and lowest in Chakaria, Dinajpur and Ramnagar but the highest prevelence of *Plasmodium* was found in Faridpur and lowest in Khagrachari, Rangpur, Chakaria and Dinajpur. Besides this the fruit bats is more susceptible to *Babesia* then the *Plasmodium* and this rate of susceptible is vary from one place to another place.

CHAPTER V

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