Chapter-1: Introduction

Bangladesh is a developing country where duck plays a vital role for the fulfilment of nutrient demand. The increasing demand for animal protein and the economic benefit obtained through rising poultry in both scavenging and semi-scavenging condition have created a great deal of interest among the farmers in this country (Das *et al*., 2008). The duck population in Bangladesh is 52.24 millions (DLS, 2015-2016). It plays an important role in rural economy. Local ducks are ubiquitous in the country and smallholder farmers keep them under a subsistent level of management (Islam *et al.,* 2003). But duck rearing is hindered by various problems, of which parasitic infections is one of a major problem. Many recent studies have focused on avian blood parasites as a model system for host-parasite interactions is an evolutionary and ecological context (Bensch *et al.,* 2004; Hellgren *et al.,* 2004 and Ricklefs *et al.,* 2005). *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* are known genera as avian haematozoa (Valkiunas *et al*., 2005). Avian haemoprotozoa are intracellular blood parasites that are transmitted by blood sucking insects including s**i**mulidae (black flies), mosquitoes, biting midges (*Culicoides)* etc. (Laurance *et al*., 2013). The Prevalence of *Leucocytozoon*is 16% in domestic poultry in Iran (Dezfoulian*et al.,* 2013). In one survey, 13.6% of backyard chickens in South Carolina, USA were infected with *Leucocytozoon caulleryi (*Noblet *et al*., 1976). *Haemoproteus* (4.8%), *Plasmodium* (0.6%) and *Leucocytozoon* (0.3%) were also reported in north western Costa Rica (Valkiunas *et al*., 2005).The Prevalence of *Haemoproteus columbae* was 21% in pigeon. The highest infection rate was observed in autumn (44%) while the lowest in spring in Iran (Senlik *et al*., 2005). *Leucocytozoon* (5.5%), *Haemoproteus* (3.6%) and *Plasmodium* (20.0%) are also prevalent in wild birds in Tsushima Island of Japan (Tanigawa *et al*., 2013). 13.2% of birds were infected with *Haemoproteus* spp., 15.1% with *Plasmodium* spp. and 0.6% with *Leucocytozoon* spp. in wetlands birds in Bangladesh (Elahi*et al*., 2014). Infections with multiple species and genera of haematosporidia are common (Adlard*et al*., 2004). Although, *Leucocytozoon*, *Haemoproteus*and *Plasmodium*species have been implicated in disease outbreaks (Bennett et *al*., 1993).Malaria parasites are supposed to have strong negative effects on host fitness because this intra-cellular parasite causes dramatic reductions in the efficiency of metabolism (Chen, *et al.,* 2001). Ultimately, bird can lead to progressive weakness, declines in food consumption and activity levels, loss of up to 30% body

weight (Atkinson, *et al*., 2000) and eventually, death. The role of blood parasites as a potential source of physiological stress for avian hosts in the wild was Studied. Previously, blood parasites were considered low pathogenic organisms (Bennett*et al*., 1993) in spite of them causing disease and death in captive birds. Only a few published reports are available on haemoprotozoan infection in Bangladesh (*Islam et al*., 2013). Information is not available on haematozoan parasites in resident ducks of haor areas in Bangladesh but recently *Leucocytozoon* spp. in domestic ducks and *Haemoproteus* spp. in domestic pigeons has been reported (Dey *et al*., 2008).Two kinds of blood smears that are used for microscopic examination of malaria parasites which are thick bloods smears and thin blood smears (Shetty *et al*., 2009). Large number of ducks is circulating in haors areas where they are infected by different parasitic diseases. I did my research to assess the status of blood protozoal infection in ducks at haor areas in sylhet division, Bangladesh.

Objective:

- The objective of the study was to assess the prevalence of blood protozoa in ducks at Hakaluki and Tanguar Haor areas of sylhet division in Bangladesh.

Chapter-2: Review of Literature

2.1: History of blood protozoa

Scientist Bennet first discovered the blood protozoa in birds, described three types of protozoans, considered to be closely related to Haemogregarina previously described by Danilewsky, (1885). A second type recognized as belonging to the genus *Trypanosoma*» A third referred to as a haemocytozoon which, after "Exkapsulation," also became free-swimming in the blood. Within erythrocytes were shown clear, uncolored, transparent "Vakuolen" of various shapes and sizes containing strongly light-refractile, glossy-black particles. "Pseudovakuolen" were very common in certain species of birds. Ring-like structures were lying alongside the nucleus of the erythrocytes. The more developed forms took on a spherical shape, altering the outline of the red blood corpuscles, which at the same time became more and more distorted. From this last description, it is evident that dealing was with a species of bird malaria (Bennet *et al*., 1890).

Identification of the similarity between two types of infection as evidenced by the subsequent work of (Grassi and Feletti, 1890). These investigators placed the intracorpuscular parasites of birds in the same genus as those described from man and established the genus Laverania.

The generic name of *Haemoproteus* was established by (Kruse, 1890), however, which also included the avian forms described by Danilewsky, has priority, since it appeared shortly before the work of Grassi and Peletti (1890). Confirmation of Danilewsky's descriptions of blood parasites from avian hosts was done by (Laveran, 1890) and envisioned birds as convenient laboratory hosts through which the mysteries of human malaria might be studied. The *Haemoproteus* parasitizing different species of birds varied in size and appearance and concluded that there were many species of the genus. Subsequent investigations proved him correct **(**Minchin, 1912).

The genus *Haemoproteus* was published by (Coatney, 1936) by checklist and host-index that included 45 species of *Haemoproteus*, most of which were described from birds. A recent checklist was published on host-index of the blood protozoa from birds of North America by (Herman, 1944). Few reports with *Haemoproteus saoharovi* were concerned solely with its prevalence in nature. The initial description

of the parasite was by (MacNeal *et al.,* 1904) who obtained specimens from the blood of the mourning dove, *Zenaiduramacroura*.

Identification of *H. saoharovi* from the mourning dove Zenaidura macroura was done by **(**Herms *et al*., 1939**).** Only one other time, namely, who described the infection as occurring in the blood of one of four doves examined in California. 14- Most of the literature concerning *H. saoharovi,* however, deals with its prevalence in the eastern mourning dove, *Zenaidura macroura* Carolinensis. Studying the incidence of blood parasites in Nebraska birds found *H. saoharovi* in two mourning doves **(**Herms *et al*., 1939**).** .

Similar organisms were described by (Coatney *et al*., 1938) who found, in examining the blood of 13 doves over periods of from one to 66 days, that all 13 were infected.

Primarily the *Leucocytozoon*species observed and described *H. saoharovi* from two mourning doves. She noted that gametocytes of this parasite disappeared from the blood for days at a time (Wetmore, 1941). In Illinois, reported 103 of 206 mourning doves to be infected with H. saoharovi (Levine *et al*., 1952).

Reported *H. saoharovi* in 11 of 27 of these doves taken in Arizona and California. They also observed similar organisms in the blood of the Western white winged dove **(**Wood and Herman, 1943).A species of *Leucocytozoon* was described from the blood of the European turtle dove, *Streptopelia turtur*. His descriptions and figures of this organism, however, resemble *H. saoharovi* rather than a *Leucocytozoon*. Another important host reported for this organism is the common pigeon, *Columba livia* (Franchini, 1929).

2.2 Morphology of Blood Protozoa

Haemoproteus nasimii sp

The blood of *C. livia* revealed gamogonic stages of *Haemoproteus*. The male (microgametocyte) is distinguishable from the female (macrogametocyte) by its larger and more diffuse nucleus. Usually the concentration of the parasite was sparse (1–6 pars/100 RBC) but occasionally a high degree of erythrocytes parasitization was visible (10–20 pars/100 RBC). Occasionally, the parasite infected two adjacent cells, at times there was close approximation of cells parasitized with micro and

macrogametocyte. Immature and mature gametocytes were visible in blood films (Zajac *et al*., 2012).

Immature gametocyte: The young and immature forms (8.4 × 3.7 μm) develop lateral to the host cell nucleus and have no contact with the host cell membrane or the host cell nucleus (Zajac *et al*., 2012).

Mature form: Mature forms could be differentiated into macrogametocytes (randomly scattered granules, nucleus with clear margins) and microgametocytes (granules polar, nucleus diffused with cytoplasm (Zajac *et al*., 2012).

Macrogametocyte: Macrogametocytes are broadly sausage shaped, slightly halteridial and usually laterally situated to the erythrocytic nucleus. The fully grown parasite reached the poles of the infected erythrocyte but never encircled its nucleus. The margins of the gametocyte were mostly smooth and rarely amoeboid. Variations in the shape of the macrogametocyte were quite evident. Sometimes, a large space between the gametocyte and the host cell membrane could be seen in the central zone and in such cases, the gametocyte was thin in the central zone and broad at the ends. On the other hand, some gametocytes adhered to the host cell membrane in the central area thereby broadening it. Some gametocytes were broad at one end and narrow at the othe. Occasionally, the ends of the parasite curved around the erythrocytic nucleus. Almost mature forms displaced the host nucleus towards one pole and sometimes, the parasite twists the host cell nucleus. A nearly mature form with host cell nearing enucleation could be seen in typical polar position (Zajac *et al*., 2012).

Cytoplasm of the parasite was moderately coarse and stained pale blue with Giemsa's stain. The granules were median or small sized and dispersed randomly in all parts of the cytoplasm averaging 20 per parasite. When the granules were small, their numbers was higher and were black to yellow- brown in colour. The parasite nucleus was median and stained pink with Giemsa's stain, averaging 1.8 μm in length and 1.6 μm in width. The measurements of mature form varied from 13.0 to 16.0 μm in length and 4.0–6.9 μm in width (average length 13.9 μm and width 4.7 μm in). The parasite occupied approximately three-quarters of the host cell and sometimes completely filled the host cell cytoplasm (Zajac *et al*., 2012).

Microgametocyte: Microgametocyte was slightly smaller than the macrogametocyte, slightly halteridial and usually laterals to the host cell nucleus similar to the

macrogametocyte. The ends of the parasites are usually rounded and the margin entire. The gametocytes almost adhere to the host cell membrane at the polar zone but sometimes, in the central zone as well. A fully-grown microgametocyte fills the poles of the affected erythrocyte and may displace its nucleus towards the pole. An enucleated erythrocyte also contained microgametocyte (Zajac *et al*., 2012).

Microgametocytes are also capable of twisting the host cell nucleus by approximately 90° similar to the macrogametocyt. Cytoplasm of the mature form was fairly granular and stained only lightly with Giemsa's stain or occasionally it was colourless. The granules were localized only at the poles of the parasite averaging 11 per parasite and are yellow–brown or black in color. Parasite nucleus was fused and not easily distinguishable from the cytoplasm of the parasite. Microgametocytes varied in size from 13.0 to 15.0 μm in length and 4.0 to 6.0 μm in width (average 14.0 μm in length and 4.3 μm in width). Mature form occupied the major part of the infected erythrocytes (Zajac *et al*., 2012).

Host nucleus: Erythrocytic *Haemoproteus* displaced the host cell nucleus and NDR was 0.2 in the parasitized erythrocytes. In some cases the nucleus shifted to one corner of the cell (Zajac *et al*., 2012).

Extra corpuscular form: Macrogametocytes could be seen escaping from the red blood cells or lying free in the plasma. The extra corpuscular forms lying in the plasma were halteridial), elongated or spindle-shaped in shape. Cytoplasm was fairly granular, granules being dispersed throughout the parasite. The extra corpuscular forms varied in size from 15.0 to 17.8 μm in length and 3.9 to 7.3 μm in width while the average measurement was 16.7 μm in length and 5.8 μm in width. The nucleus took a pink stain with Giemsa's stain and is situated at the center of the parasite averaging 2.0 μm in length and 1.8 μm in width (Zajac *et al*., 2012).

Double gametocyte infection (DGI): DGI and trigametocyte infection (TGI) are rarely reported in vertebrate erythrocytes. This phenomenon has been suggested to enhance apicomplexon transmission (Jovani et al. 2004). During the present investigations, infrequently cases of DGI) were encountered. No cases of TGI or multiple gametocyte infection (MGI) were recorded (Zajac *et al*., 2012).

***Haemoproteus* (Para*Haemoproteus*) pastoris, Mello, 1935**

Morphology: The gametocytes are amoeboid, adhering to the erythrocyte nucleus and envelope filling the erythrocyte up to their poles. They displaced the erythrocyte nucleus slightly. The measurement of the macrogametocytes is 14.575 ± 0.4 μm × 4.12 ± 0.3 μm. The nucleus of the macrogametocyte is compact and in subcentral position measuring 3.15 ± 0.02 μm × 2.575 ± 0.1 μm. 9 to 17 small roundish pigment granules were seen randomly scattered in the cytoplasm. The NDR was 0.5 ± 0.2. Invasion intensity was found to be 17 parasites per 100 microscopic fields (Zajac *et al*., 2012).The genus *Plasmodium* belongs to the family Plasmodiidae given their own order Haemosporidia **(**Corradetti *et al.*, 1963**).** There are currently 450 recognized species in this order. The genus *Plasmodium* includes 13 subgenera in which 5 subgenera *Giovannola, Haemamoeba, Huffia, Novyella* and *Bennettinia* were created for the known avian malaria species.

According to Corradetti et al, (1963) and Garnham, (1966), the subgenera can be identified as follows:

Features for the identification of the species in the subgenus *Bennettinia*

- Schizonts contain scant cytoplasm and are often rounded.
- Schizonts do not exceed the size of the host nucleus and stick to it.
- Gametocytes while varying in shape tend to be round or oval, do not exceed the size of the nucleus and stick to it

Features for the identification of the species in the subgenus *Giovannola*

- Schizonts contain plentiful cytoplasm, are larger than the host cell nucleus and frequently displace it.
- They are found only in mature erythrocytes.
- Gametocytes are elongated.
- Exoerythrocytic schizogony occurs in the mononuclear phagocyte system.

Features for the identification of the species in the subgenus *Haemamoeba*

- Mature schizonts are larger than the host cell nucleus and commonly displace it.

- Gametocytes are larger, round, oval or irregular in shape.
- Gametocytes are substantially larger than the host cell nucleus.

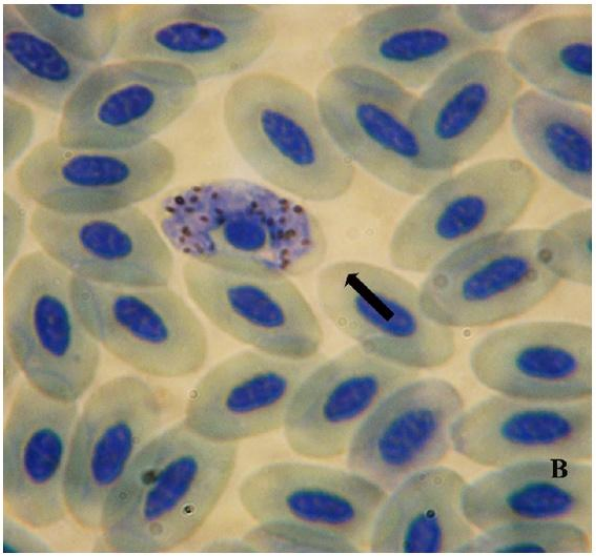
Features for the identification of the species in the subgenus *Huffia*

- Mature schizonts, while varying in shape and size, contain plentiful cytoplasm.
- Schizonts are commonly found in immature erythrocytes.
- Gametocytes are elongated.

Features for the identification of the species in the subgenus *Novyella*

- Mature schizonts are either smaller than or only slightly larger than host nucleus.
- Schizonts contain scanty cytoplasm.
- Gametocytes are elongated.
- Sexual stages in this subgenus resemble those of *Haemoproteus.*

Exoerythrocytic schizogony occurs in the mononuclear phagocyte systems



Figusre-1: Macrogametocyte of *H. nettioni*

Plasmodium sp. produces an insoluble golden brown or black deposit of haemozoin pigments in the parasite cells were confirmed by (Friend and Franson, 1999**)**.

The morphological characteristics of *Plasmodium* species discovered from *C. livia* clearly place it in the subgenus *Haemameoba* with round or oval gametocytes, schizonts in mature erythrocytes and with erythrocytic gametocytes lacking noticeable cytoplasm and being larger than the host cell nucleus (Zajac *et al*., 2012).

Plasmodium (*Haemamoeba*) *guptii* sp.

General morphology**:** Erythrocytic and exoerythrocytic forms of this parasite were observed in the blood smears.

Description of erythrocytic stages

Trophozoites: The smallest parasites (1.5 × 1.5 μm) have no visible cytoplasm, vacuole or pigments. A thin gray cytoplasm visible in 2.7 × 2.7 μm sized trophozoites which lacked pigment. Uninucleate parasites usually elongate, but as they approach the first nuclear division, often become rounded or oval. As they grow, some of them appear to migrate to the polar end of the host cell where they often assume characteristic U-shape, bending about the end of the erythrocyte nucleus. No nuclear displacement of the host cell due to trophozoites was evident (Zajac *et al*., 2012).

Schizonts: Schizonts usually lateral to the host cell nucleus, always marginal and visible in various stages of development: early schizont. They change the shape of the infected erythrocyte and displace the host cell nucleus towards one side. Pigments usually found in clumps and are more conspicuous at the extremities of the parasite. Schizonts were 5.9 × 4.1 μm in size and their nuclei usually distributed in the form of a rosette (Zajac *et al*., 2012).

Gametocytes: Stained mature gametocytes showed characteristic sexual differences, macrogametocytes staining blue and microgametocytes appearing pink or white in colour. Gametocytes usually appeared oval or round when occurring in a polar position in the cell and sometimes the host cell nucleus was oblique in position. Mature gametocytes can fill the entire host cell cytoplasm.Pigment ranules small, dispersed and vary greatly in number in macrogametocyte whereas in microgametocytes, they cluster at one end of the parasite. Macrogametocytes averaged 7.8 × 7.7 μm and microgametocytes 7.8 × 7.6 μm in size. All gametocytes seen were pigmented (Zajac *et al*., 2012).

Host nucleus: The gametocyte displaced the host cell nucleus. Sometime nucleus shifted to the one pole of the cell. NDR was 0.3 with a range of 0.1–0.5 in the host cell (Zajac *et al*., 2012).

Exo-erythrocytic stages: The prevalence of exoerythrocytic forms in the blood is highly variable, sometimes being frequent or usually quite sparse. They are usually round in shape and may be seen escaping from the RBC or lying free in the plasma (Zajac *et al*., 2012).

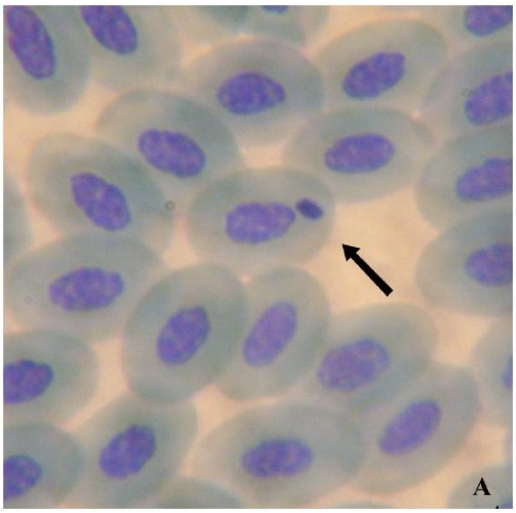


Figure-2: Trophozoite of *P. relictum*

Genus *Leucocytozoon* sp.

The genus is divided into two subgenera: Akiba and *Leucocytozoon* — based on the vector species.The only known member of the subgenus Akiba is *Leucocytozoon* (Akiba) caulleryi which uses members of the genus Culicoides as its vectors.The remaining species in the genus use members of the genus Simulium as their vectors.In 1977, Greiner and Kocan in an extensive examination of species in the order Falconiformes declared that the only valid species infecting this order was *L. toddi* (Zajac *et al*., 2012).

Morphology: They are macrogametocytes and microgametocytes. They are very few in number. They measured 10.8 ± 1.34 µM × 8.7 ± 1.43 µM. The dark blue cytoplasm with many small vacuoles appeared to be coarsely granulated. Small round organelles resembling the pigment granules are seen (Zajac *et al*., 2012).

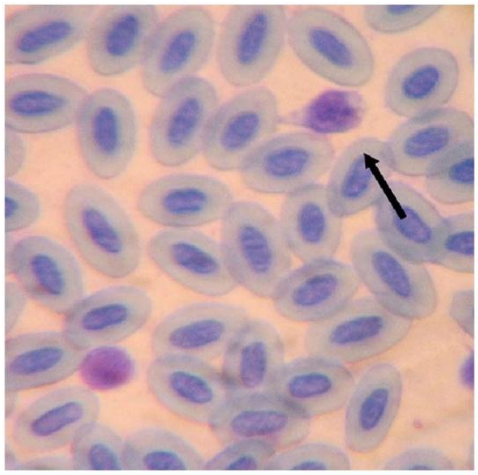


Figure-3: Macrogametocyte
of *Leucocytozoon sp.*

2.3 Life Cycle of Blood Protozoa

Life cycles of avian malaria parasites are similar in their basic features to those of human and other mammal *Plasmodium* species (Marzal, 2012**)**.

Malaria parasites are obligate heteroxenous protists, with merogony in cells of fixed tissues and also blood cells. Gametogony occurs in red blood cells, and sexual process and sporogony are completed in Culicidae mosquitoes. However, the life cycles of avian *Plasmodium* species differ from those of the parasites of mammals, particularly due to their relatively low host specificity and marked variation in patterns of development in avian hosts and vectors. For example, *Plasmodium (Haemamoeba) relictum* infects and completes its life cycle in birds belonging to over 300 species and 11 orders, and *Plasmodium (Huffia) elongatum*, *Plasmodium (Novyella) vaughani* and many other species also have a broad range of avian hosts**(**Zehtindjiev *et al.*, 2012).

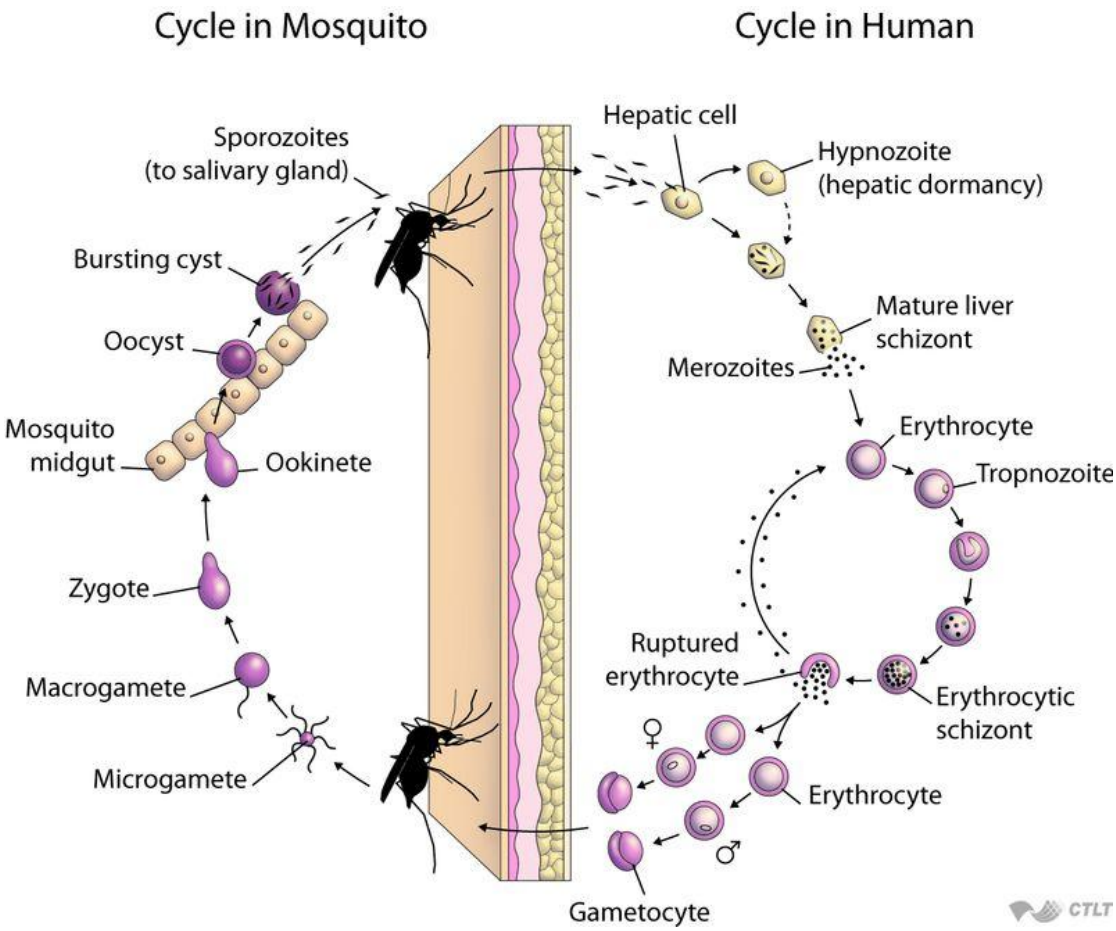


Figure-4: Life cycle of *Plasmodium* Sp. Adapted from Soulsby, (1982)

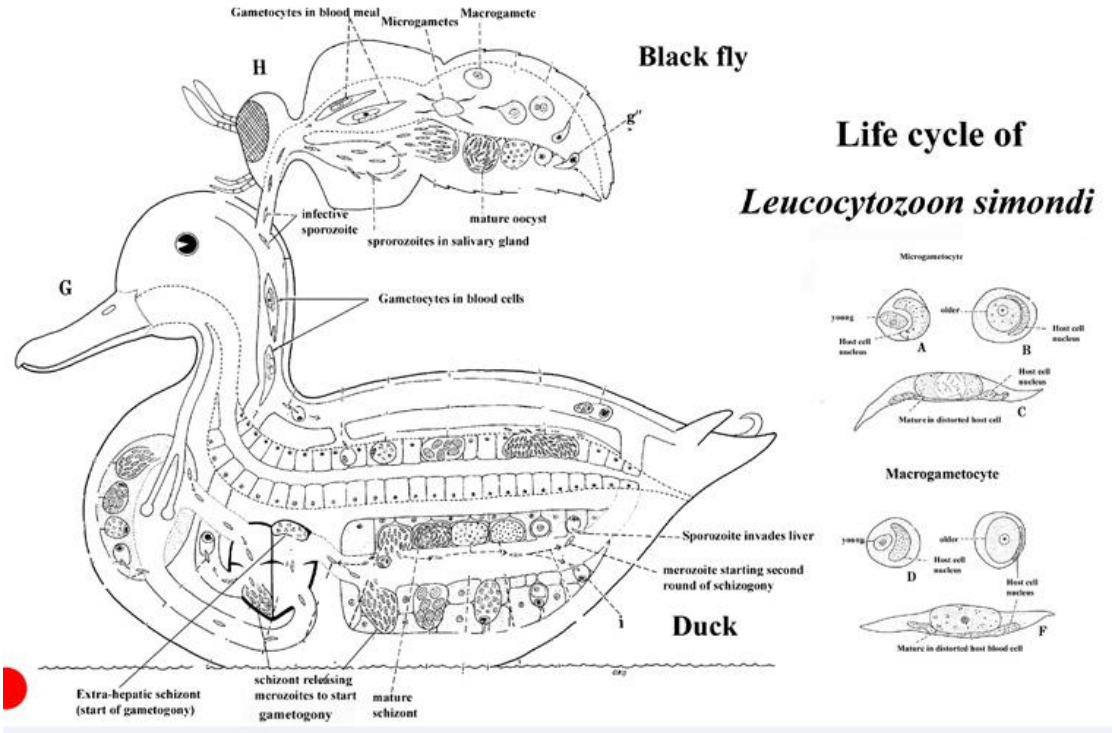


Figure-5: Life Cycle of *Leucocytozoon simondi*. Adapted from Soulsby, (1982)

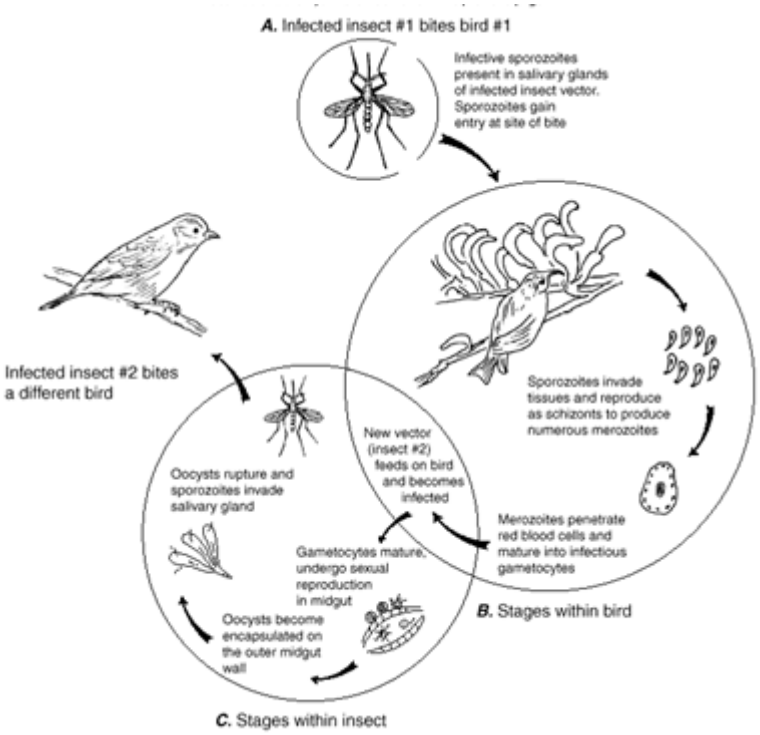


Figure-6: Life cycle of *Haemoproteus sp* Adapted from (Soulsby, 1982)

The exflagellation of microgametocytes and the union of gametes in blood drawn from a crow infected with 8 Haemoproteiis. In 1898, he reported an analogous process in what is now known as *Plasmodium* falciparum Welch. Aspects of the sexual phase of both human and avian malaria were thus demonstrated. Furthermore, encouraged by these observations of MacCallum, (1897 and 1898).Ookinetes Form in in the gut of mosquitoes and in a mite **(**Aragao, 1916). On the other hand, failed to observe ookinete formation in the gut of *Culex quinquefasciatus* and *Aedes albopictus*. More recently, species of Culicoides (Ceratopogonidae) have been suggested as suitable intermediate hosts for *Haemoproteus* nettionis (Johnson and Cleland) of ducks **(**Kartman, 1949).

Regardless of the development time of the schizonts, the mature schizonts will eventually rupture and releases thousands of *merozoites* into the bloodstream and infect the erythrocytes (red blood cells).This marks the start of erythrocytic schizogeny stage. Each merozoite will infect each erythrocyte. The merozoite that entering an erythrocyte will starts to reproduce asexually and transforming into

trophozoite. The trophozoite will then divides gradually and mature to schizont. Eventually the infected erythrocyte ruptures and releases new merozoites, which will continue to infect more erythrocytes (Coatney and West, 1940).

During erythrocytic schizogeny phase, instead of forming trophozoites, some of the merozoites will develop into immature gametocytes .The gametocytes are stimulated and mature to *microgamete* (female) in the guts of female anopheles mosquito after the mosquito ingested the human blood containing gametocytes. The microgamete and macrogamete will then reproduce sexually and this starts the third stage of *Plasmodium* life cycle. Sporozoites will be the product of this stage and another cycle will begins when the Anopheles mosquito ingests blood of a new human host (Fishman and Fishman, 2006). Erythrocytic merozoites of many avian malaria parasites can induce secondary tissue merogony in birds (Silva-Iturriza *et al.*, 2012).The exo-erythrocytic merogony occurs in cells of the reticuloendothelial and haemopoietic systems, but has not been reported in hepatocytes(Palinauskas *et al.*, 2016). Pedunculated oocysts were discovered in *Plasmodium (Bennettinia) juxtanucleare*; these oocysts possess leg-like outgrowths which attach the oocysts to the mosquito midgut wall (Bennett, *et al.,* 1966). Described some of other features which are not characteristics of malaria parasites of mammals, and this is reflected in genetic differences between these groups of parasites and their different position in molecular phylogenies(Pacheco *et al.,* 2018**)**. Malaria, the disease caused by parasites of the genus *Plasmodium*, has traditionally been viewed as a disease of the blood and blood forming tissues of vertebrate hosts, with exo-erythrocytic stages of development causing little or no pathology (Adams *et al.*, 2014).

There is recent experimental evidence of unexpected pathology associated with obstructive development of secondary exo-erythrocytic stages of *Plasmodium* in brain capillaries that can lead to ischaemia and rapid death in birds that have very low intensity parasitaemias during chronic stage of infection **(**Palinauskas *et al*., 2015).

2.4 Transmission of Blood protozoa

The possibilities of a natural vector being responsible for transmission were discussed and an extensive survey of the ectoparasites of these birds was undertaken. The vector was not discovered, however. Other vertebrate hosts recorded as being infected with H. saoharovi include the Western mourning dove, Zenaidura 16 macroura marginella

(Hanson *et al.*, 1957). The first successful transmission of avian malarial parasites was from bird to bird by blood inoculations, who worked with *Plasmodium*. Attempts by earlier workers to do this had been unsuccessful probably because they were dealing with *Haemoproteus* rather than *Plasmodium*. They believed that the malarial parasites of man and of birds, although similar, were not identical (Farmer, *et al*. 1960).The nature of malarial transmission was demonstrated, utilizing Gulex mosquitoes in transferring *Plasmodium* to sparrows. Without these facts, stemming for the most part from studies of avian malaria, it is probable that our present knowledge of human malaria would not have advanced as rapidly as it (Hayward *et al.*, 1898).

. A checklist of blood parasites was published of birds of the order Columbiformes in which eight species of *Haemoproteus* are recorded. It is apparent that members of this genus are among the most common malarial parasites of birds. Information concerning their host-parasite relationships, however, is sparse. This lack of information is undoubtedly due to the difficulties involved in maintaining laboratory strains. Bird-to-bird transfer of the parasite demands a suitable invertebrate host. Investigations concerning host-parasite relationships become complicated when the vector is unknown. Life histories are known for very few avian species of *Haemoproteus* (Levine and Kantor, 1959). *Haemoproteus* columbae Kruse is normally transmitted from pigeon to pigeon by the bite of the hippoboscid (Sergent and Sergent, 1906). Hippoboscids incriminated in the transmission of *H. columbae* are *Lynchia lividеolor* Aragao, *L. brunea* Aragao, and Microlynchia pusilla Lutz (Aragao, 1916). Extensive monograph concerning the Hippoboscidae, states: "The name *Lynchia maura, L. lividcolor* and *L. capensis*, sometimes cited also among the vectors of pigeon malaria, are all synonyms of Pseudolynchia canariensis **(**Bequaert, 1953).At one time *Microlynchia pusilla* (Speiser) was described among the bird-flies transmitting *H. columbae* in Brazil; unfortunately he never described his experiments with this fly **(**Aragao, 1916). In accordance with this apparent invertebrate host specificity, Kartman, (1949), studying *Haemoproteus* infections of Hawaiian pigeons, reported finding oocysts of 11 *H. columbae* on the midgut of P. canariensis.

In England a species of *Omithornyia latreille*was indicated to be a vector of *H. columbae* in wood pigeons, *Columba palumbus*. California quail, *Lophortyx californica*, may contract a severe malaria-like disease caused by *Haemoproteus*

lophortyx **(**Baker, 1967**)**. A young quail was injected with the macerated salivary glands and with part of the gut of an infected hippoboscid, *Lynchia hirsuta*, taken from a wild quail infected with *H. lophortyx*. After a period of 27 days, gametocytes of *H. lophortyx* were observed in the blood of the young bird. He also described sporogonic stages (ookinetes, oocysts and sporozoites) in some wild *Lynchia hirsuta* collected from infected quail (O'Roke, 1930).

Sporozoites in the salivary glands were described and body cavity of *Stilbometopa impressa* (Bigot). This material, including the salivary glands, was inoculated into a young quail. Twenty-one days after injection, parasites were observed in the blood **(**Herman and Bischoff, 1949). *H. lophortyx* was demonstrated which may be transmitted to quail by the bite of infected S. impressa (Tarshis, 1955).

Laboratory-reared Pseudolynchia maura Bequaert were used to transmit *Haemoproteus saoharov*i and *Haemoproteus maccallumi* Novy and MacNeal from the mourning dove to domestic pigeons. He doubted, however, that 12 this pigeon louse fly was responsible for the transmission of these parasites in nature. The possibility that invertebrate hosts other than hippoboscids are involved in the transmission and life history of H. columbae has also been investigated **(**Huff, 1965). Investigating *H. nettionis* infections in domestic ducks in Algonquin Park, Ontario, Canada, observed an abundance of black flies, biting midges and mosquitoes. These blood-sucking insects were collected from caged ducks and from their immediate surroundings. Clean ducks were inoculated with suspensions of these insects after comminuting of the latter in blood. *H. nettionis* infections developed in ducks injected with the specimens of Culicoides. However, the insects employed in these experiments were not specifically identified. Further 13 investigations may show that *H. nettionis* is transmissible by the bite of Culicoides (Fallis and Wood, 1957).

The successful transmission of *H. saoharovi* was reported from this host to domestic pigeons, using laboratory-reared Pseudolynchia maura Bequaert. Huff's source of *H. saoharovi* in these studies was from four naturallyinfected doves. Since only one of these, however, had a single infection, it alone was used in the transmission experiments. After the flies had been allowed to remain on this dove for two to eight days, they were placed upon laboratory-reared pigeons. Thirteen days after the first flies had been transferred; gametocytes resembling those of *H. saoharovi* appeared in the blood of one of the pigeons. This particular infection persisted up to the time the

bird was sacrificed, a period of three months **(**Huff, 1965). The first to transmit *H. saoharovi* to pigeons experimentallywas (Huff, 1965).*Haemoproteus* Kruse (*Haima*—blood and *Proteus*—sea god having the power of assuming different shapes) is a genus of Apicomplexa that are parasitic in birds, reptiles and amphibians. Three other genera like *Halteridium*, *Haemocystidium* and *Simondia* are now considered to be synonyms of *Haemoproteus.* Within the genus, there are 133 species, 5 varieties and 1 subspecies, maximum occurring in birds (114). They are transmitted by blood sucking insects including mosquitoes; louse flies (*Hippoboscidae*) and biting midges (*Culicoides*). Infection with this genus is sometimes known as pseudomalaria because of the parasites' similarities with *Plasmodium* species (Bennet *et al*., (1890).

2.5 Public Health Significance:

Malaria parasites of the genus *Plasmodium* (Haemosporida, Plasmodiidae) inhabit all major groups of terrestrial vertebrates. Avian malaria parasites is a peculiar group among them, particularly due to the ability of numerous species to develop and complete life cycles in numerous bird species belonging to different families and even orders(Clark *et al.,* 2015). The same is true for invertebrate hosts (vectors) of these parasites (Santiago-Alarcon *et al*., 2012). Many species of avian *Plasmodium* use Culicidae mosquitoes belonging to different genera (*Culex, Coquillettidia, Aedes, Mansonia, Culisetta, Anopheles, Psorophora*) for completing sporogony and transmission (Njabo *et al*., 2009). This is not the case in mammalian malaria parasites whose are transmitted mostly by *Anopheles* species (Ejiri et *al.,* 2011**)**. Furthermore, sporogony of many avian *Plasmodium* parasites is completed relatively fast in susceptible vectors at relatively low temperatures **(**Valkiunas *et al.,* 2015). These features likely contributed to the global distribution of some avian malaria infections, which are actively transmitted in countries with warm and cold climates, including regions close to the Polar Circles (Howe*et al.,* 2012).

Based on current taxonomy, four families of haemosporidians can be recognized. These are Plasmodiidae, Haemoproteidae, Leucocytozoidae and Garniidae (RK *et al.,* 2015). Blood stages of species of *Plasmodium* are particularly similar to those of relatively rare haemosporidian parasites of the genera *Fallisia* and *Garnia* of the family Garniidae **(**Gabaldon *et al.,* (1985).

While available evidence still supports this view for the primate and rodent malarial parasites, there is increasing evidence that the pathogenicity of tissue stages of avian species of *Plasmodium* has been significantly underestimated. Even more, avian malaria is often a more severe disease than human malaria **(**Valkiunas *et al*., 2017).

The severity of disease caused by a given lineage of *Plasmodium* often varies markedly in different species of avian hosts, from absence of any clinical symptoms to high mortality because of broad vertebrate host specificity, and the same *Plasmodium* species can infect distantly related birds (Vanstreels *et al*., 2015).

2.6 Diagnosis of Blood Protozoa

The blood of six from 86 mourning doves that were examined on Cape Cod, Massachusetts was positive for *H. saoharovi* (Herman, 1938**)**. 188 doves blood smears were examined, 15 trapped for banding in various regions of the United States. Of these birds, he found 51 to be infected with *H. saoharovi* and 34 others to have both *H. saoharovi* and *H. maccallumi.* In Nebraska, Coatney and West (1940) reported *H. saoharovi* from 11 of 20 nestling doves. They offered this as evidence that this parasite was acquired in the North and not necessarily after migration. The natural vector was not found, however (Huff, 1939).The first natural infections were found in the common pigeon. These investigators initially observed natural infection of *H. saoharovi* in an adult pigeon and two squabs. Further study uncovered six infections in 17 adult pigeons and five infections in 33 squabs that were examined **(**Coatney and West, 1938 and 1940). Its presence in pigeon squabs reared in a colony at Gilbert, Iowa l. Reference was made to abnormally enlarged spleens and to granular gizzards observed in a number of sacrificed birds. Some of the blood smears made from these 17 particular birds were diagnosed as positive for *H. saoharovi.* This pigeon colony was the source for another report by (Becker et al., 1957), who examined 114 stained blood films made from pigeons ranging in age from two to eight weeks. Blood samples were taken from the birds at various times during the summer of 1956, and it was shown that two squabs harbored patent *H. saoharovi* infections. This summary of investigations concerning *H. saoharovi* indicates that this parasite enjoys a relatively high natural incidence among columbiform birds and a fairly wide geographical distribution. On the other hand, it emphasizes the lack of information concerning the biology of the organism.

According to World Health Organization (2012), Malaysia was listed as pre-elimination country with 5306 malaria cases in Malaysia in 2011. Even though the situation of Malaria infection in Peninsular Malaysia is under controlled, but Sarawak and Sabah are still being found as the highest endemic area in Malaysia. Hence, early diagnosis and effective treatment is the key factor to reduce the fatality rate of malaria infection. The diagnosis of malaria disease is done based on presumptive analysis on infection symptoms, followed by clinical diagnosis supported by the detection of parasites in the blood (parasitological or laboratory diagnosis) and additional haemotological examinations.The laboratory diagnosis by microscopic examination of malaria based on Giemsa-stained blood smears remain as the common reference standard or the chosen procedure for the detection and identification of *Plasmodium* parasites (World Health Organization,(2012).

Two kinds of blood smears are used for microscopic examination of malaria parasites, which are thick bloods smears and thin blood smears **(**Shetty *et al.*, (2009). Thick blood smears are best for establishing the presence of *Plasmodium*parasites while thin smears aid in species identification both thick and thin blood smears should be prepared and used for morphological identification of malaria parasites. Principally, blood smears are prepared by placing a drop of blood on a clean glass slide and spread the drop of blood to approximately 4 times its original surface.The slides can then be stained using staining solution after extensive drying. Staining is a colourisation process of blood cells in blood samples that is used for microscopy visual detection. There are several staining methods available for malaria blood smear staining, such as Giemsa stain, Leishman stain and Wright stain. However, Giemsa stain is the widely used staining method for malaria blood smears due to its stability in tropical conditions. Giemsa staining solution stains up nucleic acid, thus red blood cells (RBCs), white blood cells (WBCs), platelets and *Plasmodium sp.* parasites will be colourised differently throughout the process and thus aid in identification of blood cells and the presence of *Plasmodium sp.* Parasites (Houwen, 2002).

Malaria parasites were described in a well stained thick smear show deep red chromatin and pale blue cytoplasm while schizonts and gametocytes are also easily recognizable if present in thin blood smear, the size of the infected red blood cells and the presence of characteristic dots such as Schuffner's dots will be observed Laboratory diagnosis by microscopic examination of stained blood smears is a skill-

based manual diagnostic procedure which requires well-trained and competent microscopist to execute the task. The whole process is to determine parasitemia and distiguish between parasitic cells and non-parasitic stained cells such as erythrocytes, white blood cells, platelets and artifacts. The identification of different species in thin blood smears based on the morphological characteristics such as erythrocyte size, shape, crenation, characteristic dots, pigment structure and color at different life cycle stage is also necessary. A well-trained microscopist should be able to detect the *Plasmodium* species correctly in thick blood smears which is relatively low in parasite density **(**Cuomo, 2009).

Misidentification or error in estimation of the species in microscopic image can still be quite frequent, even in routine microscopy **(**Breman *et a.l*, 2007).

World Health Organization, (2009) said this may be due to degradation of quality of blood smears with time , poor staining of blood smears, lack of experience in observing the parasitic cell (especially in non endemic area) and other human errors.

Diagnosis of *Haemoproteus* infection is generally accomplished by microscopic examination of a Giemsa-stained peripheral blood smear. Gametocytes are only present within erythrocytes. Organisms may appear similar to *Plasmodium*, but the pigment within the intra-erythrocytic gametocytes is more dispersed and schizonts are not seen in the peripheral blood smears (Bennet *et al*., 1890). These pigment granules (haemozoin) are derived from the digestion of haemoglobin found within the host's erythrocytes and appear as refractile, yellow to brown granules within the host's erythrocyte. The gametocytes partially encircle the erythrocyte nucleus forming a halter-shaped appearance with little displacement of the host cell nucleus. *Haemoproteus* gametocytes often occupy over one half of the erythrocyte cytoplasm. Parasite may cause slight enlargement of infected host cells and displacement of the red blood cell nucleus to one side. Based on the above generic characters, the genus is identified as *Haemoproteus* Kruse, 1890(Friend and Franson, 1999).

Definitive diagnosis of a *Plasmodium* was described dependent on detecting the presence of asexually reproducing stages of its life cycle (schizonts) in the red blood cells of the infected host. The U-shaped forms, resembling the elongate ones except in position, are unlike the usual type of *Haemoproteus*. Mature schizonts are larger than

the host cell nucleus and commonly displace it. Microgametocytes and macrogametocytes are also formed within erythrocytes in *Plasmodium* infections but are observed infrequently. Gametocytes are larger, round, oval or irregular in shape and substantially larger than the host cell nucleus (Corradetti et al., 1963).

Vertebrate host identity cannot be used as a taxonomic feature during identification of avian malaria parasites .This raises questions about parasite species identification if the same pathogen is found in unusual avian hosts (Valkiunas *et al.,* 2002**)**.

Molecular characterization is helpful in diagnosis of malaria infections, and has been developed for detection of some avian *Plasmodium* species **(**Dimitrov *et al.,* 2015). Molecular markers are essential in diagnosis and identification of exo-erythrocytic and vector stages, which cannot be identified using morphological features (Dinhopl *et al.,* 2015). Molecular diagnostics using general primers the main diagnostic tool currently used in wildlife malariology) is often insensitive in distinguishing of avian *Plasmodium*spp. Co-infections, which are common and even, predominate in many bird populations (Bernotiene *et al.,* 2016). Specific molecular markers for the majority of avian *Plasmodium* species have not been developed, and currently are difficult to develop due to significant genetic diversity of malaria parasites, which remain undescribed in wildlife. Morphological identification using microscopic examination of blood films remains important in malaria diagnostics in the wild, and is particularly valuable if it is applied in parallel with polymerase chain reaction (PCR)-based diagnostic tools (Mantilla *et al.,* 2013).

During the past 15 years, numerous avian *Plasmodium* parasites were named and described using morphological features of their blood stages **(**Mantilla *et al.,* 2013).

Molecular markers for parasite detection were developed in a handful of these descriptions. The keys that are available for identification of avian *Plasmodium* species.There should be reworked in the light of the newly available information **(**Valkiunas *et al*., 2005).

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Chapter-3: Materials and Methods

3.1 Study area and duration of study

Blood samples were collected from different villages at two haor sites in Bangladesh: Hakaluki haor (Figure: 08) (N 21°33″698, E 091°51″682) in Sylhet and Moulvibazar districts (200 birds) and Tanguar haor (figure: 09) (N 25°08.794′, E 091°04.088′) in Sunamganj district (200 birds) (figure: 7) during the period of summer (April-May) and rainy season (june-July), 2018.

3.2. **Sampling strategy**:

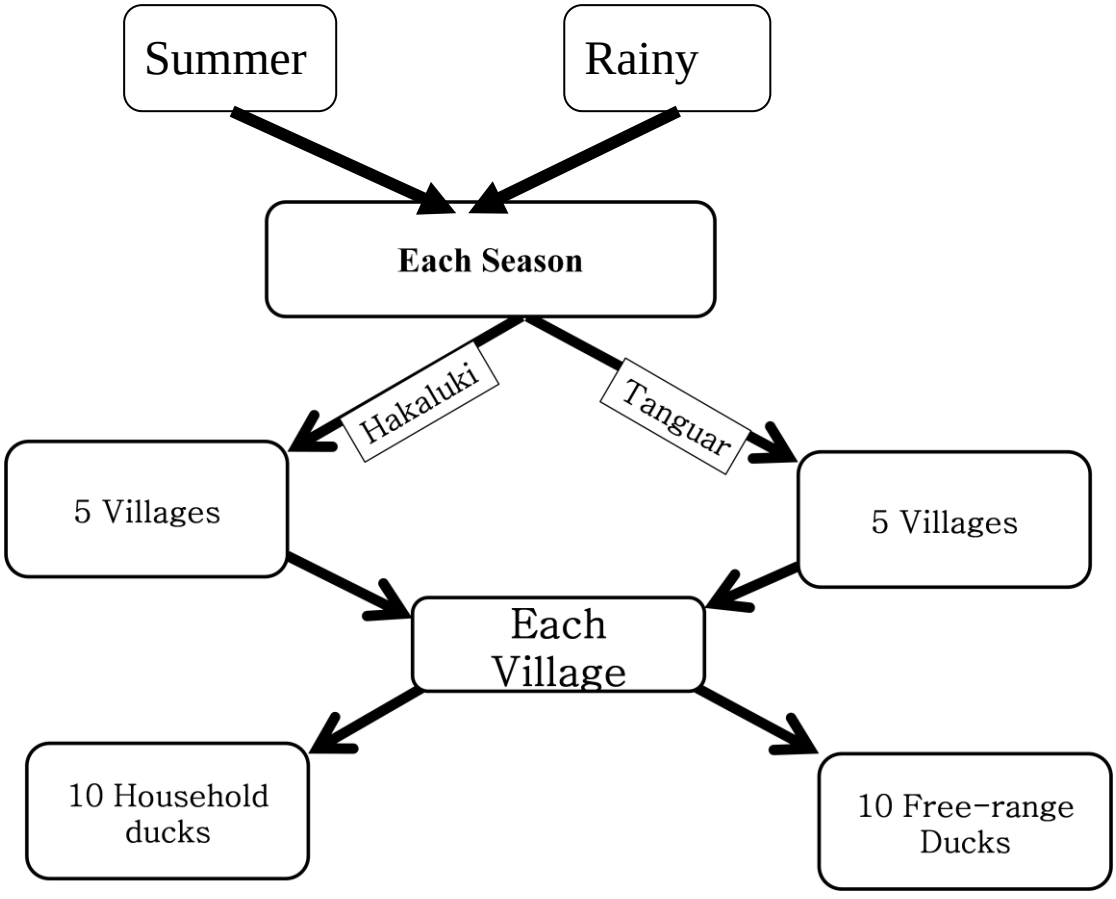

Figure 7: Sample selection statragy from village of sampling area

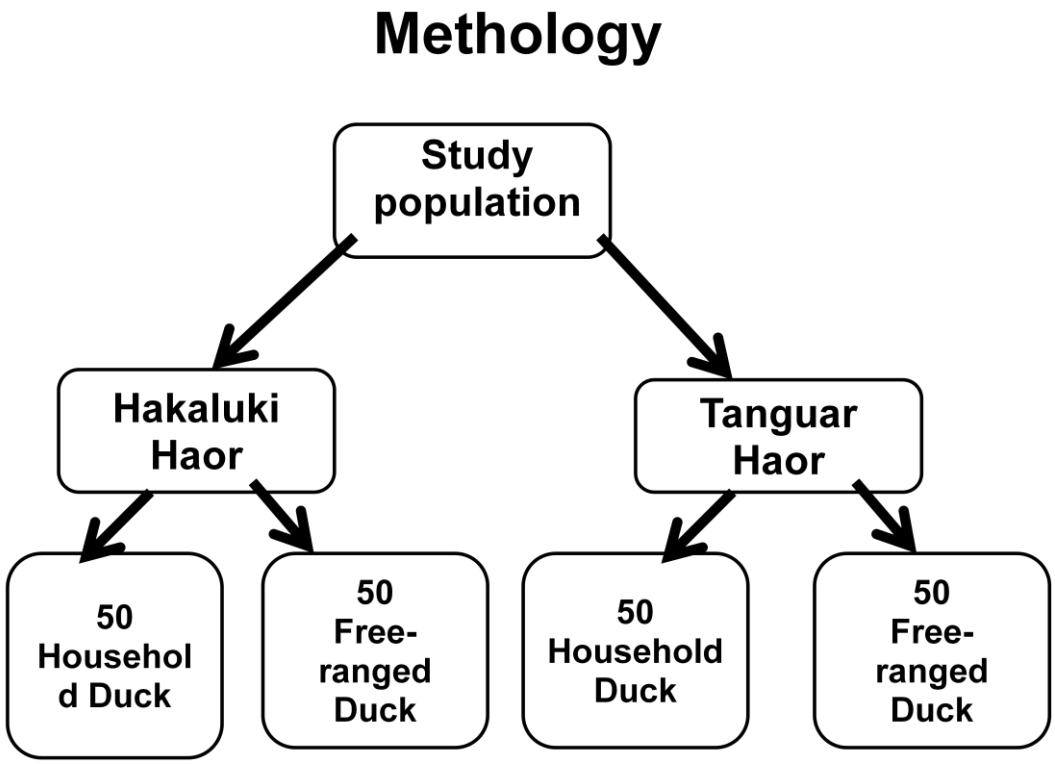

Figure 8: Sampling strategy of duck from sampling area

3.3 Sample collection

A total 400 (200 during summer and 200 rainy season)domestic ducks were sampled belonging to three breeds randomly irrespective of breed, age, sex, Deworming, Type of Scavanging, Type of Housing directly from farmer's hosehold and free-range. Hakaluki haor and Tanguar haor are seasonal water bodies located in northern Bangladesh which dry up during winter when they provide habitat for resident and migratory wild birds.Ducks were gently handled by parting of feathers against their natural direction by the owner of the farms.Clipping and using of antiseptic to the blood collection area of duck.Blood was drawn from tarsal vein of the ducks (Figure: 13). Peripheral blood samples were collected with the help of syringe and needle and taken in a vial with sufficient Ethylene Diamine Tetra Acetic acid (EDTA) and kept in ice box.

3.4 Ante-mortem examination

After collection of birds age and sex were recorded in accordance with the history from the owners. According to sex, birds were divided into male and female. Birds were further divided into two groups (6 months ≤) young and adult (> 6 months) in accordance with age.

3.5 Preparation of blood smears and identification of protozoa

A thin smear was made immediately after the collection of blood in field condition on clean, grease-free slides (Figure: 14). All slides were fixed in absolute methanol for one minute in the field, then the smears were sent to the parasitology laboratory under the department of Pathology and Parasitology of Chittagong Veterinary and Animal Sciences University, stained with 20% Giemsa (Zajac *et al.,* 2012) (Figure: 15)and air dried. The slides were examined under microscope in higher magnification (40X and 100X) for the detection of blood protozoa (Figure: 16). Identification was based on the morphology as described by (Springeret*et al.,* 1997; Levine, 1985 and Soulsby, 1982). If any parasite was found within 100 fields of microscopic observation, the slide was considered as positive; otherwise it was considered as negative. All parasites in 100 microscope fields at 1000x magnificationwere counted to calculate the intensity of invasion of the parasites.

3.6 Statistical analysis

To compare the prevalence of blood parasites in relation to sex, age, breed, deworming, type of scavenzing and type of housing, the obtained data was imported, stored and coded accordingly using Microsoft Excel-2007. Then this data was transferred from MS Excel-2007 to STATA/IC-13.0 (Stata Corporation College Station, Texas) for analysis. Descriptive analysis was performed by means of frequency (N, %) of positive and negative sample test results overall and stratified by different explanatory variables.

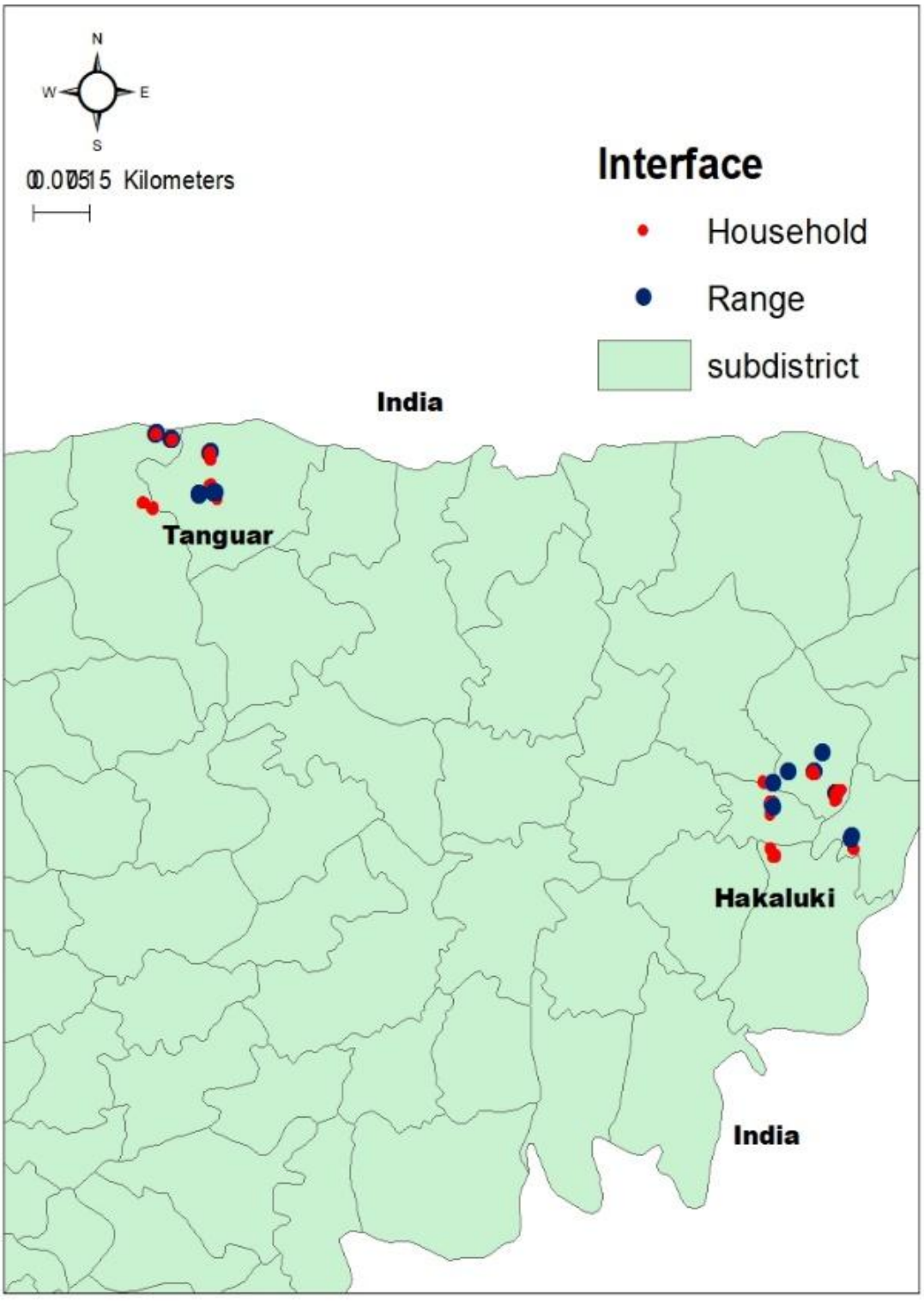

Figure-9: Study area of Hakaluki and Tanguar Haor at a glance

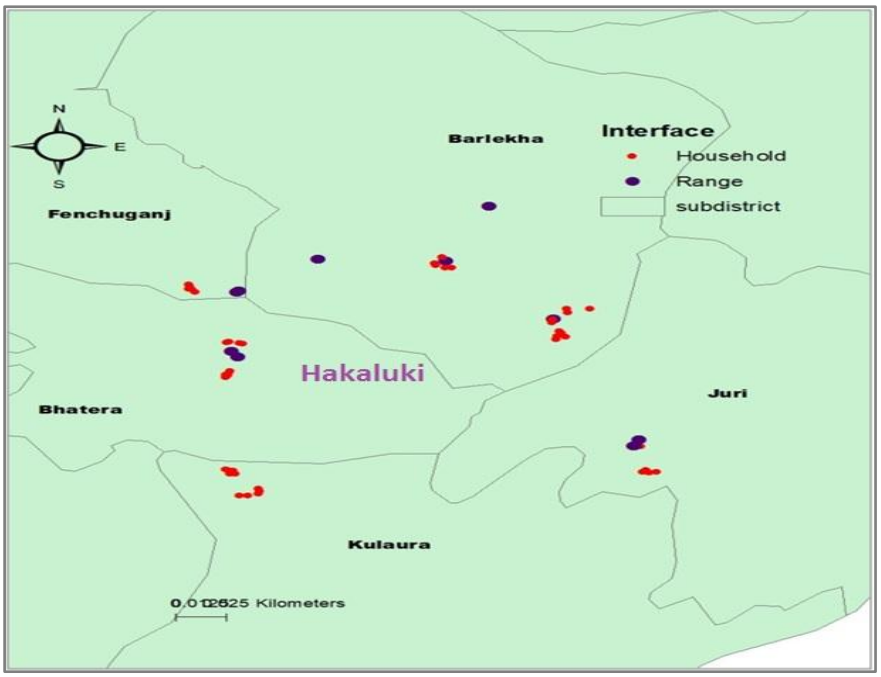

Figure 10: Sampling sites of Hakaluki Haor

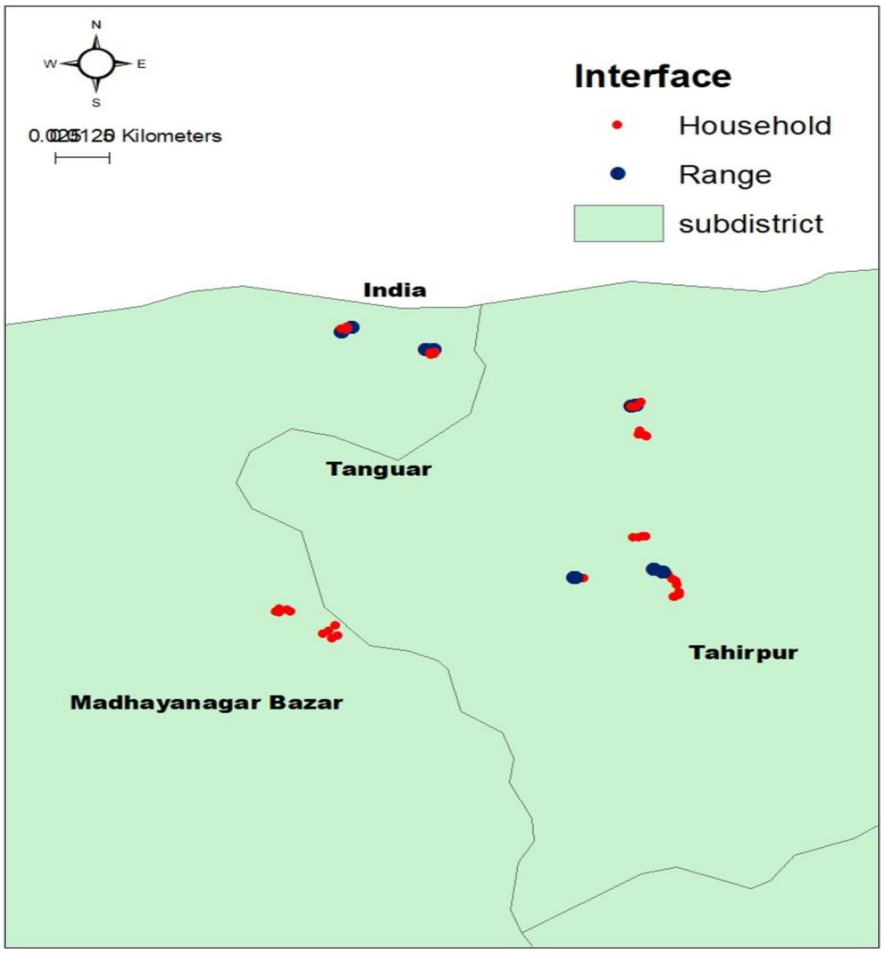

Figure 11: Sampling sites of Tanguar Haor

Figure 12: Duck of Haor Areas, Sylhet Division

Figure 13: Collection of blood from tarsal vein

Figure 14: Preparation of blood smear andfixing the smear

Figure 15: Staining of smear and air drying

Figure 16: Observation under microscope with 100X by using emersion oil

Chapter-4: Results

4.1. Prevalence of blood protozoa and Intensity of Infection

The overall prevalence of infection of the birds studied was 21.5% (44/200) birds during summer season and 15% (30/200) during rainy season. Among, 14% (28/200) prevalence of infection was recorded for *Haemoproteus* spp., 7% (14/200) for *Plasmodium* spp., and 3.5% (7/200) for Leucocytozoon spp. during summer season and 8.50% (17/200) prevalence of infection was recorded for *Haemoproteus* spp., 11% (22/200) for *Plasmodium* spp., and 3% (6/200) *Leucocytozoon* spp.during rainy season. Two birds were positive for parasites of both *Haemoproteus* and *Plasmodium* genera.

The prevalence of infection for the three genera varied considerably among the three breeds of ducks. Among the three breeds with sufficient sample size, the highest prevalence was found in the DPD breed: 13.64% (15/110 birds) for genus *Haemoproteus* during summer season and highest prevalence in rainy season was found in DPD breed: 10.83% (13/120) for genus *Plasmodium*.The intensity of invasion varied across different parasite genera. The lowest intensity of invasion for parasites of *Leucocytozoon* genus was 6, while the highest was 28 parasites per 200 microscopic fields for *Haemoproteus*.

4.2 Prevalence according to breeds

Among these ducks samples, in case of ***Haemoproteus* spp**., 13.79% (12/87) of ducks (KK) were positive during summer season and 8.97% (7/78) during rainy season.In case of DPD, 13.64% (15/110) and 8.33% (10/120) wrer positive subsequently during summer and rainy season for.But in Muscovy duck, 33.33% (1/3) waspositive during summer season and no positive was found in rainy season.

Again, in case of ***Plasmodium* sp**., 4.60% (4/87) of ducks (KK) were positive during summer season and 10.26% (8/80) during rainy season.In case of DPD, 8.18% (9/120) and 10.83% (13/120) are positive subsequently during summer and rainy season. But in case of Muscovy duck, 33.33% (1/3) was positive during summer season and 50% (1/2) was positive in rainy season.Again, in case of ***Leucocytozoon* sp.,** 5.75% (5/87) of ducks (KK) were positive during summer season and 6.41% (5/78) during rainy

season.In case of DPD 1.82% (2/120) and 0.83% (1/120) are positive subsequently during summer and rainy season.In case of Muscovy, no positive ducks were found.

4.3 Prevalence according to sex

Infection rate was higher in female than male.Highest infection rate in female was 15.24% (25/164) in case of *Haemoproteus* during summer season and lowest infection rate was 3.09% (5/158) during rainy season in case of *Leucocytozoon*.On the other hand, highest infection in male was13.16% (5/38) in case of *Plasmodium* during rainy season and lowest infection in male was 0.00% (0/36) during summer season in case *Leucocytozoon*.

4.4 Prevalence according to age

Highest infection was 40.0% (6/15) in the birds whose age was less than six month in case of *Haemoproteus* and lowest infection was found 3.24% (6/185) in these birds whose age was more than six month in case of *Leucocytozoon* during summer but highest infection was 10.64% (20/188)) in the birds whose age was more six month in case of *Plasmodium* and lowest infection was found 0.00% (0/12) in these birds whose age was less than one six month in case of *Leucocytozoon* during rainy season.

4.5 Prevalence according to vaccination status

Non vaccinated duck became more infected than vaccinated.In summer season, highest infection was 14.29% (17/119) in case of *Haemoproteus* and lowesr infection was 2.47% (2/81) in case of *Leucocytozoon* On the other hand, in rainy season; highest infection was 12.40% (15/121) in case of *Plasmodium* and lowest infection was 1.27 % (1/79) in case of *Leucocytozoon*.

4.6 Prevalence according to deworming status

Non-dewormed duck became less infected than dewormed duck for both during rainy and summer season. Highest infection was 15.25% (9/59) for *Haemoproteus* and lowest was 3.39% (2/59) for *Leucocytozoon*during summer seasonbut in rainy season highest infection was 14.04% (8/57) for *Haemoproteus* and lowest was 1.75% (1/57) for *Leucocytozoon*.

4.7 Prevalence according to health status

Sick ducks became more infected than healthy duck.Highest infection was 26.32% (5/19) during summer season in case of *Haemoproteus* and lowest infection was

3.31% (6/181%) in case of *Leucocytozoon*.During rainy season, highest infection was 13.33% (2/15) in case of *Plasmodium* and lowest infection was 0.00% (0/15) in case of *Leucocytozoon*.

4.8 Prevalence according to scavenging system

Highest infection was 33.33% (4/12) during summer season in case of household scavenging for *Haemoproteus* and lowest infection was 0.00% (0/4%) in case of Ponds cum wetland for *Haemoproteus*, *Leucocytozoon* and *Plasmodium*.During rainy season, highest infection was 16.33% (8/49) in case of wetland for*Plasmodium* and lowest infection was 0.00% (0/4) in case of Ponds cum wetland for *Haemoproteus*, *Leucocytozoon* and *Plasmodium*.

4.9 Prevalence according to Housing system

The highest infection was found to be 14.29% (10/70) during summer season in case of wetland for *Haemoproteus* and the lowest infection was 0% (0/1) in case of yard cum house for *Haemoproteus*, *Leucocytozoon* and *Plasmodium*.During rainy season,highest infection was 18.97% (11/58) in case of within house for*Plasmodium* and lowest infection was 0.00% (0/1)in case of yard cum house for *Haemoproteus*, *Leucocytozoon* and *Plasmodium*.There was no statistical significant in the infection rates among the study sites.

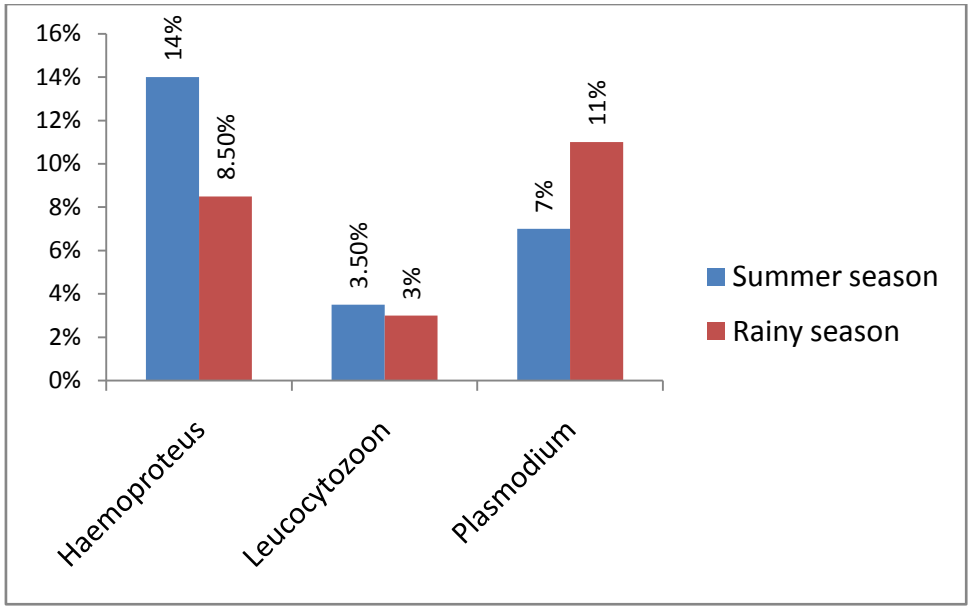

Figure 17: Overall prevalence of blood protozoa in between summer and rainy season

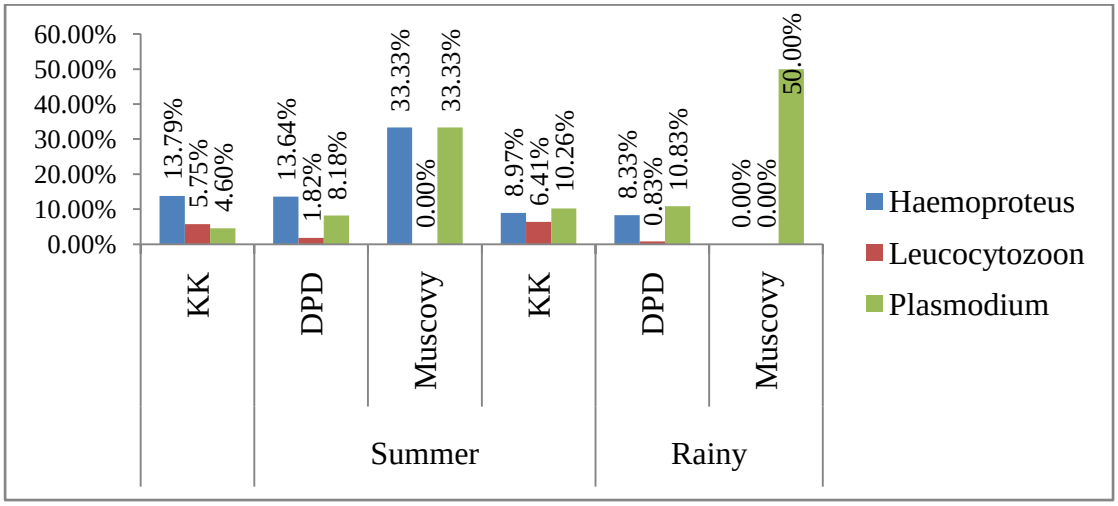

Figure -18: Prevalence of blood protozoa among breeds

Table-1: Prevalence of blood protozoa according to age

Season	Age	*Haemoproteus*	*Leucocytozoon*	*Plasmodium*
Summer	≤ 6 month	40.00% (6/15)	6.67% (1/15)	20% (3/15)
	> 6 month	11.89% (22/185)	3.24% (6/185)	5.95% (11/185)
Rainy	≤ 6 month	16.67% (2/12)	0.00% (0/12)	16.67% (2/12)
	>6 month	7.98% (15/188)	3.39% (6/188)	10.64% (20/188)

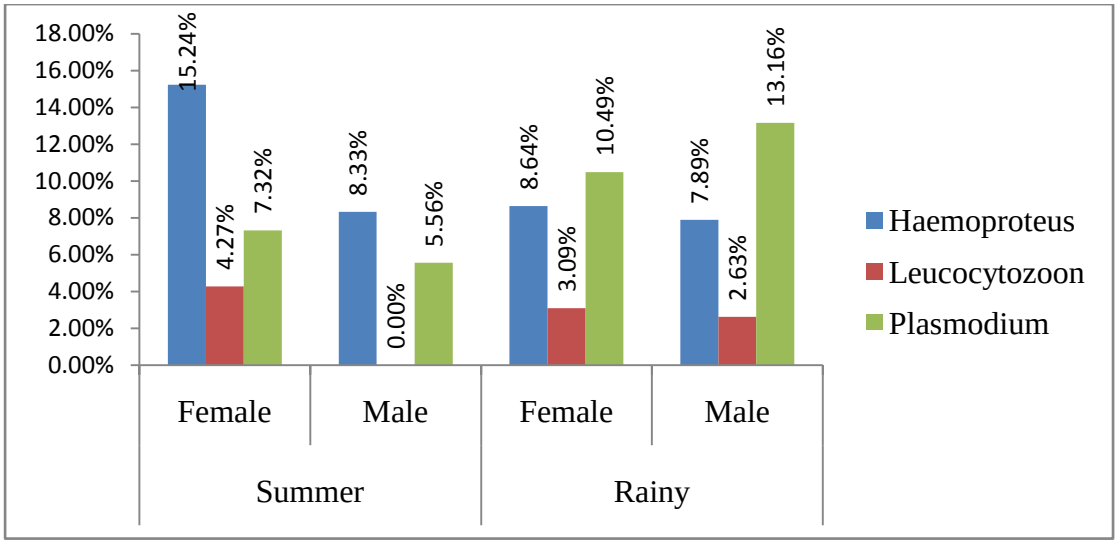

Figure 19: Prevalence of blood protozoa according to sex

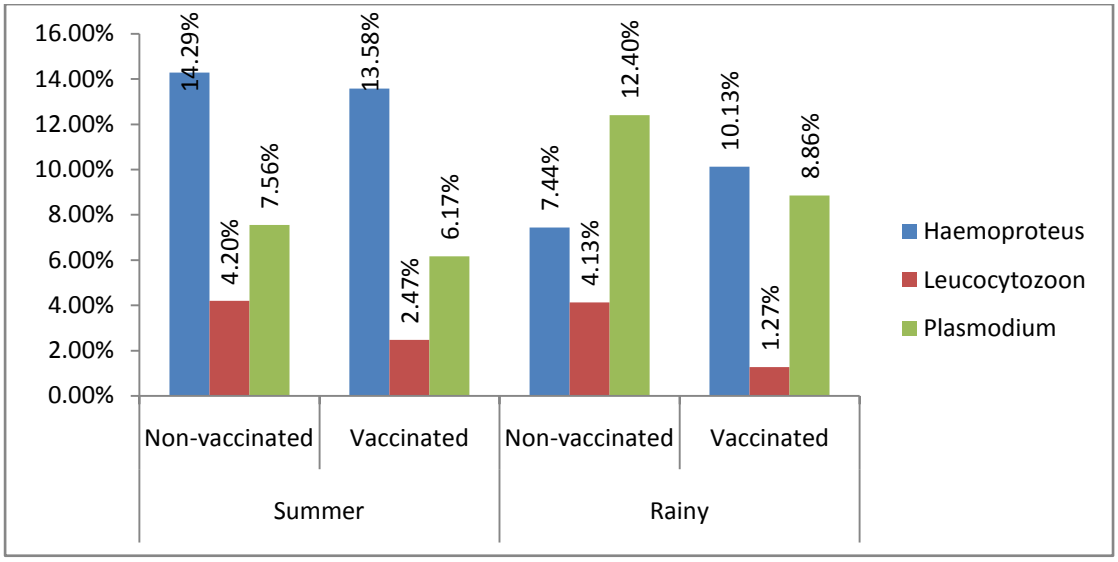

Figure 20: Prevalence of blood protozoa according to vaccination status

Table-2: Prevalence of blood protozoa according to deworming status

Season	Deworming status	*Haemoproteus*	*Leucocytozoon*	*Plasmodium*
Summer	Non-dewormed	19 (13.48%)	5 (3.55%)	10 (7.09%)
	Dewormed	9 (15.25%)	2 (3.39%)	4 (6.78%)
Rainy	Non-dewormed	9 (6.29%)	5 (3.50%)	18 (12.59%)
	Dewormed	8 (14.04%)	1 (1.75%)	4 (7.02%)

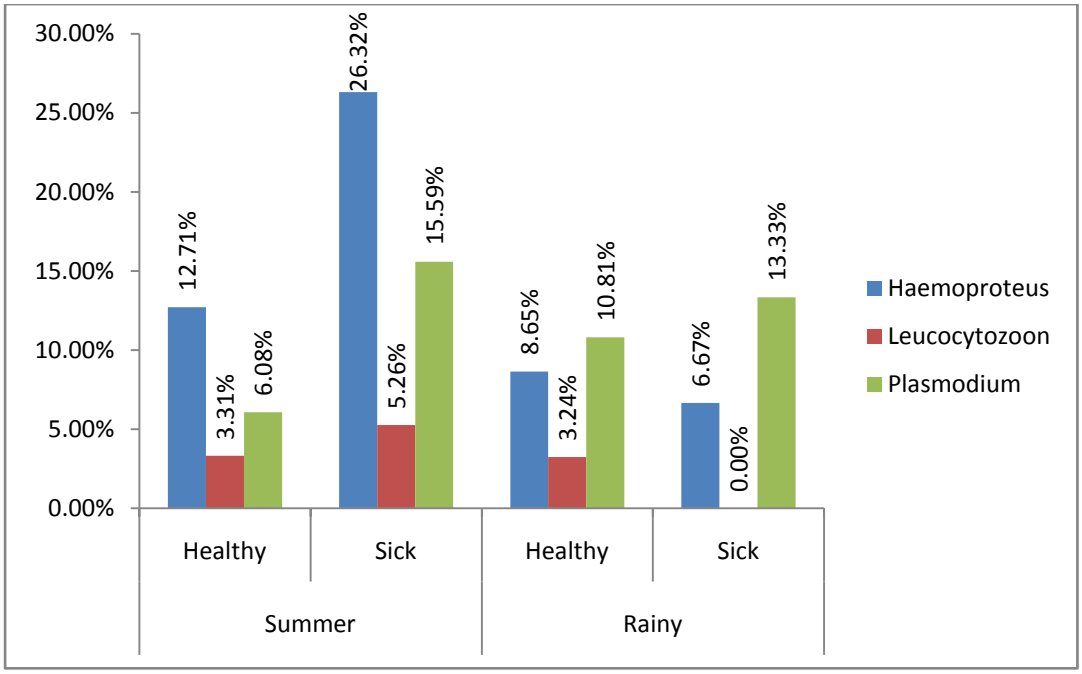

Figure -21: Prevalence of blood protozoa according to health status

Table-3: Prevalence of blood protozoa according to scavenging system

Season	**Place of scavenging**	***Haemoproteus***	***Leucocytozoon***	***Plasmodium***
Summer	Wetland	2 (7.14%)	1 (3.57%)	3 (10.71%)
	Rice paddy field	6 (10.17%)	1 (1.69%)	1 (1.69%)
	Ponds	14 (15.56%)	4 (4.44%)	9 (10.00%)
	Household	4 (33.33%)	1 (8.33%)	0 (0.00%)
	Ponds + Wetland	0 (0.00%)	0 (0.00%)	0 (0.00%)
	River	2 (28.57%)	0 (0.00%)	1 (14.29%)
Rainy	Wetland	2 (4.08%)	3 (6.12%)	8 (16.33%)
	Rice paddy field	8 (14.29%)	1 (1.79%)	5 (8.93%)
	Ponds	5 (6.67%)	2 (2.67%)	7 (9.33%)

	Household	1 (9.09%)	0 (0.00%)	1 (9.09%)
	Ponds + Wetland	0 (0.00%)	0 (0.00%)	0 (0.00%)
	River	1 (14.29%)	0 (0.00%)	1 (14.29%)

Table-4: Prevalence of blood protozoa according to housing system

Season	**Housing system**	***Haemoproteus***	***Leucocytozoon***	***Plasmodium***
Summer	Wetland	10 (14.29%)	1 (1.43%)	5 (7.14%)
	Within house	8 (17.78%)	2 (4.44%)	5 (11.11%)
	Yards	10 (11.90%)	4 (4.76%)	4 (4.76%)
	Yard + House	0 (0.00%)	0 (0.00%)	0 (0.00%)
Rainy	Wetland	8 (11.59%)	2 (2.90%)	4 (5.80%)
	Within house	4 (6.90%)	2 (3.45%)	11 (18.97%)
	Yards	5 (6.94%)	2 (2.78%)	7 (9.72%)
	Yard + House	0 (0.00%)	0 (0.00%)	0 (0.00%)

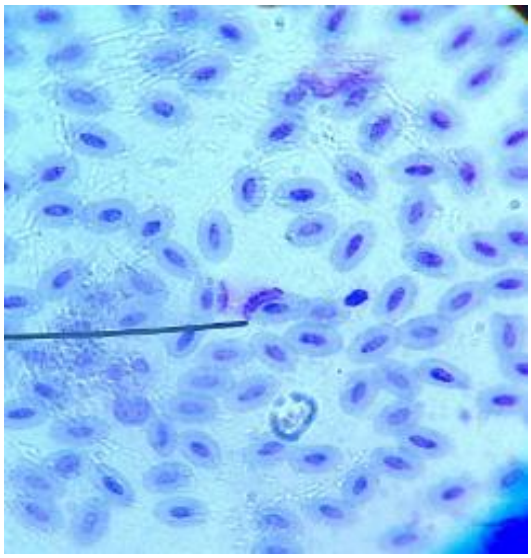

Figure 22: Gametocyte of *Haemoproteus* sp

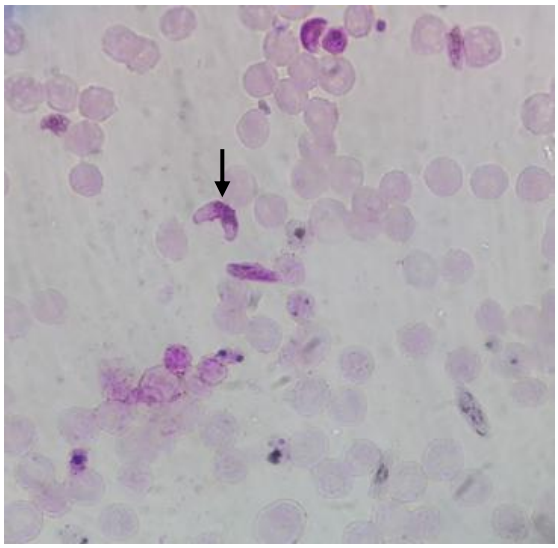

Figure 23: Gametocyte of *Plasmodium* sp

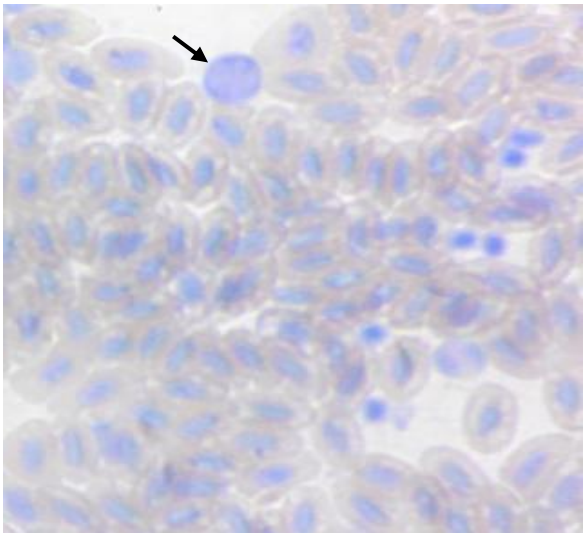

Figure 24: Gametocyte of *Leucocytozoan* sp

Chapter-5: Discussion

In this study, *Haemoproteus* spp., *Plasmodium* spp. and of *Leucocytozoon* spp. were identified in domestic ducks from two haor areas of Bangladesh although there are reports of *Plasmodium* sp, *Leucocytozoon* sp. and *Haemoproteus* sp. in migratory and non-migratory birds in wetlands of Bangladesh (Elahi*et al.,* 2014). There are also reports of *Leucocytozoon* sp. and *Haemoproteus* sp in domestic pigeons (Dey *et al.*, 2010) and ducks (Khan *et al*., 2008) in Bangladesh, but a comprehensive report on avian haematozoans is lacking. The findings of this study provide a comprehensive report.A prevalence of 14% (28 /200) of birds were infected with *Haemoproteus* spp., 7% (14/200) with *Plasmodium*s pp. and 10.50% (21/200) with *Leucocytozoon* spp. during summer season and 8.50% (17/200) of birds were infected with *Haemoproteus* spp., 11% (22/200) with *Plasmodium*s pp. and 3%(6/200) with *Leucocytozoon* spp. during rainy season was observed where *Plasmodium* nearly similar to wetlands areas in Bangladesh but *Haemoproteus* and *Leucocytozoon* slightly higher (Elahi*et al.,*2014).

The Prevalence of these studies was lower than in India and Myanmar, which are neighboring countries of Bangladesh. In India 18% Prevalence was reported for *Haemoproteus* and 28% for *Plasmodium* in wild birds and in Myanmar 40% for *Haemoproteus* and 60% for *Plasmodium* in wild birds (Gering *et al*., 2007).Their reported Prevalence was higher, likely because they used molecular methods to detect parasites, which are known to be more sensitive.

The intensity of invasion per 100 microscopic fields was higher in *Haemoproteus* than in *Plasmodium* and *Leucocytozoon*. *Haemoproteus* gametocytes persist in the peripheral blood for a long time (Paperna *et al*., 2008) while some species of *Leucocytozoon* prefer visceral circulation (Gill *et al.,* 2005); therefore, *Leucocytozoon* may have escaped our attention. In contrast to *Haemoproteus* and *Leucocytozoon*, for parasites of *Plasmodium* genus, though they prefer peripheral blood circulation, the schizogonic cycle in the erythrocytes lasts for only few days (Valkiunas *et al.,* 2005). As a result, they may also have escaped our attention.

The Prevalence in female was greater than male which was similar to them who reported Prevalence of blood protozoa in poultry in Tangail, Bangladesh (Momin *et al*.,2014) but percentage was different,it may be due to geographical location, availability of vector etc.

Highest prevalence in case of age was 40.00% (6/15) in young and 11.89% (22/185) in adult which was contradictory with the other researchers in the world where adults (59.2%) and young birds (17.4%) in Taingail, Bangladesh (Momin *et al*., 2014), in Tanzania (63% and 11%) recorded by (Msoffe *et al*., 2010), in Egypt (60.7% and 20%) in adult and young pigeons respectively (El-Magd *et al*., 1988) which may be due to age difference.In this study, the age of the 92.75% (29/400) ducks were more than six month.

Non vaccinated duck (14.29%) became more infected than vaccinated (13.58%) which is supported by (Callow *et al.,* 1977).

Non-dewormed duck became less infected than dewormed duck for both during rainy and summer season which is contradictory with other researchs (Stoltzfus *et al.*, 1998). It may be due to small number of sample size (only 116 birds dewormed/400).

Sick ducks (26.32%) became more infected than healthy ducks (12.715%) which was supported by the other Scientists (Roper et al., 1996) within the population of a Sudanese village.

In this study, highest Prevalence was 33.33% (4/12) during summer season in case of Household scavenging for *Haemoproteus* and was 16.33% (8/49) in case of wetland for *Plasmodium* during rainy season which is supported by reports (Hadipour *et al*., 2011).

Variations in the prevalence of infection in different duck breeds have been reported in this study, a finding supported by different studies around the globe (Lapointe. 2012; Shurulinkov et al., 2002). These variations among the present and previous studies may be due to the differences in geographic niches, climatic conditions, and breed of birds, management factors, availability of vectors and the method of study. In this study, only resident ducks were included and their management was relatively poor. They are frequently infested by various arthropods. Pseudolynchia canariensis (Dey et al., 2010), Simulium sp. and Culicoides sp. are abundant in Bangladesh. They

act as potential vector of blood protozoa of duck. Probably these factors play a vital role in the prevalence of blood protozoa in haor ducks in Bangladesh.

Chapter-6: Conclusion

The research was first time in resident duck of haor area in Bangladesh. It will help to raise awareness among the the farmers about blood protozoa in duck.The variation in the prevalence of parasites in relation to their age, sex, breed, health status; dewormed status was investigated.Only two seasons was compared in this study. The seasonal dynamics on prevalence of these parasites were not studied which would be more helpful in the planning of a control measures against blood protozoa in ducks at haor areas in Bangladesh. Therefore, more epidemiological studies are necessary to know the exact situation of haemosporidian parasites in poultry of Bangladesh.

Chapter-7: References

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