Chapter-I

INTRODUCTION

Livestock has made important contributions to human well-being in social and economic terms since the time of civilization and domestication of wild animals. Livestock is an important part of the agricultural system that makes a better contribution to the development of Bangladesh's economy. According to DLS, Bangladesh's total ruminant livestock is 55.139 million. There are 24.09 million cattle, 1.48 million buffalos, 26.1 million goats and 3.47 million sheep in the total livestock population (DLS, 2018). The constant price contribution of the animal farming sector to GDP is 1.53 percent and the contribution to GDP of this sector in the agricultural sector was 13.46 percent in FY 2017-18 (Economic review, 2019). This has led to a growing trend in the share of the contribution of the livestock sector to agricultural development, suggesting its strong potential to contribute positively, at an increasing pace, to national income. Small ruminants, especially sheep and goats, play an important role in Bangladesh's rural economy and has significant contribution to foreign currency earnings through the export of skins and other by-products (Kamaruddin, 2003). The average number of goats and sheep per household is 0.90, mostly raised by poor, small, and medium-sized farmers (Banglapedia, National Encyclopedia of Bangladesh). Unleashed parasitic infestation is the most considerable of all the limitations in livestock production. It is an omnipresent phenomenon that affects all livestock groups, particularly sheep and goats (Jithendran et al., 1998). In most parts of the world, persistent productivity losses due to common parasitic infection are a major and common problem for the development of small ruminants (Skyes, 1994). Economic losses due to worm infestation are very high, either internal or external worms such as tape worms, round worms, flukes, ticks, and lice, although there is no exact figure available (Ayaz et al., 2018).

Gastrointestinal parasite infection (Helminthiasis) can cause a major problem in small ruminant production because of the parasite’s increasing resistance to several anthelmintics (Silvestre et al., 2001 and Eke et al., 2019), especially in tropics and subtropics. Moreover, GI parasite infection can cause severe economic losses through a reduction in food intake, weight gain, fertility rate, increased treatment costs, and mortality in heavily parasitized animals (Zajac and Gipson, 2000; Kaplan, 2004; Waller, 2006). Economic losses of these

parasites are decreased animal output and weight gain, carcass condemnation and/or slaughter, care costs and mortality in extreme cases (Over et al., 1992; Nari et al., 1997; Gatongi et al., 1997; Perry and Randolph, 1999; Perry et al., 2002). The main nematodes causing severe losses to the livestock industry are *Trichostrongylus*, *Haemonchus contortus*, *Oesophagostomum* sp., *Cooperia* sp. and *Trichostrongylus* sp. (Waller, 2006). *Haemonchus contortus* is a blood-sucking nematode of small ruminants, it is responsible for huge losses and massive animal welfare problems worldwide which causes clinical and pathological manifestations that incorporate with hemorrhagic anemia, hypoproteinemia, and parasitic gastroenteritis (Ahmed and Ansari, 1989; Albers et al., 1990). Infections by *Fasciola* sp. also result in major economic losses in sheep and goats (Anonymous, 1994; Hansen and Perry, 1994; Urquhart et al., 1996). The construction of dams and the installation of irrigation systems increased their distribution by providing favorable conditions for their intermediate hosts on the snail (Anonymous, 1994). Less relevant parasites are the smaller flukes of liver (*Dicrocoelium* sp.) and rumen (*Paramphistom* sp.). However, small ruminants can experience intermittent losses (Anonymous, 1994; Urquhart et al., 1996). Continual losses in productivity due to widely prevalent parasitic infection is crucial and frequent problem for small ruminant production in nearly all parts of the world (Skyes, 1994).

In addition to GI parasitic infection, small ruminants are also highly prone to haemoprotozoan parasites (Maske et al., 1990). Tick-borne haemoparasitic diseases (TBHDs) caused by protozoans (*Theileria, Babesia*) and bacteria (*Anaplasma/Ehrlichia*) enforce a serious constraint upon livestock production in tropical and sub-tropical regions where the distributions of host, pathogen, and vector intersect (Jongejan and Milenberg, 1994; Muraleedharan, 2005). These infestations occur on all continents, whereas the mode of distribution of these infections changes constantly due to the immigration and passage of vectors and animals and an increased globalization of both animals and their products. These changes will have an enormous effect on the distribution and establishment of both pathogens and vectors (Shope, 1991; Geiger et al., 2015). Many of these illnesses threaten animal production and some even affect human health (Bilgic et al., 2017). TBHD losses include deaths, loss of manufacturing, diagnosis/treatment expenses for veterinarians and

tick control (Jonsson et al., 2008). A survivor of acute disease create long-term carrier state , with substantial development and economic losses. In addition, transportation of pathogens to tick larvae, nymphs and/or adults is critical for the transmission of pathogens.

Ectoparasites including lice, sheep ked, ticks and mange mites have reportedly caused a broad range of medical problems, such as mechanical tissue injury, pain, inflammation, hypersensitivity, abscesses, weight loss, lameness and anaemia (Nyangiwe and Horak, 2007; Ofukwu et al., 2008; DeMatos et al., 2009; Beyecha et al., 2014; Hurtado et al., 2018) as well as may result loss of productivity, mortality, and skin diseases, in considerable financial loss to farmers. They often cause large skin pre-slaughter defects and cause small ruminant skin to weaken and reject (Yacob, 2014). In addition, ectoparasites have zoonotic value and are capable of passing on different disease pathogens from animals to animals and from animals to humans because of their blood sucking habits (Ofukwu et al., 2008 and Yacob, 2014).

Despite of many advancement in diagnostic technological aspect in the world, we are still lagging behind to identify the vector biology for disease transmission, actual disease occurring causal agents, pathogenicity of known organisms, associated factors for changing the mode of infection. In our country we mostly depend upon light microscopy for the diagnosis of parasitic infection. Along with investigation of epidemiological pattern of the parasitic diseases in the different agro-climatic zones of the country and using basic identification technique, molecular identification provides a basis for developing strategic and tactical control systems against them. In Bangladesh, several epidemiological studies have been conducted on haemoprotozoan diseases and gastrointestinal parasites of goats and sheep (Hassan et al., 2011; Rahman et al., 2017; Islam et al., 2017; Islam et al., 2019; Bhowmik et al., 2020; Dey et al., 2020; Jewel et al., 2020) but very limited investigations have been done on hilly areas (Mohanta et al., 2011). Among all investigations most of them are on the basis of light microscopic examination, only one study has been conducted by Rahman et al. (2017), performed molecular investigation to detect blood protozoa of goats and sheep. To the best of our knowledge, there is no molecular investigation report

of detection of blood protozoa in their suspected vectors in Bangladesh. Considering the above circumstances, the present study was conducted to fulfill the following objectives

- To investigate the prevalence of gastrointestinal and haemoprotozoan parasitic diseases in goats and sheep in Panchori, Golabari and Sadar union of Khagrachari district.
- To determine the effect of different factors such as, breed, age, sex, seasons etc. in the occurrence of such diseases.
- Molecular detection of haemoprotozoan in blood and tick samples to investigate the clinical, sub-clinical infection and responsible vectors for their transmission.
- Analysis of genetic diversities of haemoparasites of goat and sheep and assisting in the understanding of molecular epidemiology of *Anaplasma* sp., *Babesia* sp. and *Theileria* sp. using data generated from this study.

Chapter II

REVIEW OF LITERATURE

Admissible literature on gastrointestinal and haemoprotozoan disease occurrences along with their associated risk factors in sheep and goats are reviewed in this chapter. This chapter will represent the current and more relevant information concerning the research work.

- Gastrointestinal parasites: Epidemiology, Factor affecting the size of gastrointestinal parasitic infections
- Diagnosis of Gastrointestinal parasitism.
- Prevalence of Gastrointestinal parasites in Bangladesh
- Prevalence of Gastrointestinal parasites in other countries of the world
- Haemoprotozoan diseases: Consecutive discussion on Etiology, Epidemiology, and Risk factors of Babesiosis, Anaplasmosis and Theileriosis
- Diagnosis of Haemoprotozoan diseases
- Prevalence of Haemoprotozoan diseases in Bangladesh
- Prevalence of Haemoprotozoan diseases in other countries of the world

2.1. Helminthoses

Helminth infections, or helminthiases, refer to a complex condition caused by the Nematode, Cestode, and Trematode parasites. Although, the above-mentioned parasites can infect any sheep and goat, low worm burdens typically have slight effect on the health of animals. However, as the quantity of worm increases, weight loss and appetite are decreased. Medical signs such as weight loss, diarrhea, and anemia (bottle jaw) may develop with heavier worm burdens (Lughano and Dominic, 2013). The phyla Platyhelminthes (flatworms) and Nemathelminths (roundworms) are the parasite found in small ruminants (Soulsby, 1986; Urquhart et al., 1996). The first phylum consists of two classes Cestode and Trematode (flukes). The Taeniidae family is responsible for the most important parasites of ruminants for both public and veterinary health. *Moniezia, Avitellina*, *Thysanosoma* and *Stilesia* are the adult cestode parasites usually found in small ruminants in African countries (Urquhart et al., 1996). The Digenea sub-class comprises all trematode species that are parasitic in small ruminants. Liver flukes, *Fasciola gigantica*,

Fasciola hepatica, *Dicrocoelium* sp. and rumen flukes (Paramphistomes), *Paramphistomum* sp. are the most important of these species in Asia (Soulsby, 1986; Hansen and Perry, 1994; Anonymous, 1994; Urquhart et al., 1996).

The causes of helminth parasitism in ruminant livestock are multiple and often interactive, the vast majority of cases are due to any of the following basic reasons.

(1) An increase in the number of infective stages on grazing land

(2) Variation in host susceptibility

(3) The insertion of susceptible stock into an infected environment

(4) The introduction of infections into an environment

(5) Ineffective parasite elimination from the host animals due to the use of sub-standard anthelmintic doses and/or the development of anthelmintic resistance (Urquhart et al., 1996).

2.1.1. Nematode

There are many superfamilies of veterinary significance in the Nemathelminth (nematodes). These included Trichostrongyloidea, Filarioidea, Oxyuroidea, Ascaridoidea, Metastrongyloidea, Ancylostomatoidea, Rhabditoidea, Trichuroidea and Spiruroidea. The most important Gastrointestinal nematodes in small ruminants are members of the order Strongylida, which includes all of the four superfamilies, Trichostrongyloidea, Strongyloidea, Metostrongyloidea, Ancylostomatoidea but most belong to the Trichostrongyloidea superfamily. An entire population of these Strongylid nematodes has infected both pastured sheep and goats, whose clinical effect is known as parasite Gastroenteritis (Zajac, 2000*). Haemonchus contortus, Oesophagostomum columbium, Trichostrongylus colubriformis, Trichostrongylus axei, Bunostomum trigonocephalum, Cooperia curticei, Trichuris ovis, Trichuris globulosus, Strongyloides papillosus, Gaigeria pachyscelis* and *Chabertia ovina* are most common nematodes in small ruminants in most of the Sub-Saharan countries. Parasitic bronchitis, particularly in young animals, is caused by lungworms, as *Dictyocaulus filaria, Mullerius capillaris, and Protostrongylus rufescens Haemonchus contortus, Teladorsagia circumcincta* and *Trichostrongylus* sp. (The most significant of these is *T. axei, T. colubriformis* and *T. vitrinus)* (Hansen and Perry,1994; Lughano and Dominic, 2013).

2.1.1.1. Life cycle

The life cycles are direct and require no intermediate hosts for all Strongylid parasites of small ruminants, which are economically relevant (Hansen and Perry, 1994; Urquhart et al., 1996). In these cycles, the adult female parasites in the GI tract produce eggs that occur within the fecal mass and that embryo of the eggs into larvae (L1) which develop into larvae in the second stage (L2), removing the protective cuticle of the animal. The larvae feed on bacteria during this period. The L2 moult in larvae (L3) in the third stage but hold the cuticle out of the last moult. The L3 is the infective stage that migrates to the surrounding vegetation where the sheep and goats are available to feed. The L3 larvae migrate to the abomasum or intestine where they are ex-sheathed after ingestion. The L3 of the Trichostrongylus glands penetrate or join the stomach glands into the epithelial layer of the mucous membrane (Teladorsagia). In normal growth, the L3 moult becomes a fourth stage larval (L4) within 2–3 days, which persists for another 10–14 days in the mucous membrane or gastric glands. Lastly, the L4 emerges and turns into young adult parasites. The duration between consumption of L3 and the adult parasite (referred to as the prepatent period) varies among parasites but is typically from 3 to 5 weeks. Species of *Nematodirus, Trichuris, Bunostomum* and *Strongyloids* are exceptions to the cycle of life.

The larval stages of *Nematodirus* species develop in the hull, which is extremely tough, drought and freezing resistant. It takes very long for larvae to develop and early summer eggs to fall will have earlier entered the 3rd stage of infectious larvae. The adult *Trichuris* lives in the broad stomach. Females lay eggs in the eggs and grow into infectious larvae within a couple of weeks. It takes a couple of days for *Bunostomum* egg to pass into feces prior to the development of infectious larvae. Through the skin and mouth, larvae reach the body. Embryonic eggs are lay with *Strongyloides*. The eggs spring up rapidly, the larvae fleeing into the intestine lumen. Those with their faces move through the body, others rapidly grow and enter the body through the lower bowel and migrate through the tissue until they reach the small intestines, in which case they become adults and reproduce. This multiplies *Strongyloides* in the body (Soulsby, 1986; Urquhart et al., 1996).

2.1.1.2. Factors affecting nematode abundance

Parasite epidemiology is focused on factors such as the stresses on infection in the environment and host species susceptibility (or individual). The pressure for the infection, in turn, depends on factors that influence the free and medium-term phases such as temperature, precipitation and humidity. In addition, the existence of a large number of responsive permanent and intermediate hosts would enhance the reproductiveness of the parasites and result in a high number of parasites.

2.1.1.2.1. Climatic factors

The growth, survival, and transmission of the phases of free life of nematode parasites are affected by microclimate factors in the pellets and herbal products. These include sunshine, temperature, precipitation, moisture, and soil humidity (Lughano & Dominic, 1996). Development takes 7 to 10 days under optimum conditions (high moisture and warm temperature) (Soulsby, 1986; Hansen and Perry, 1994; Urquhart et al., 1996). Temperatures are usually conducive to larval growth in the climate in most African countries. Trichostrongylid larvae are produced at temperatures about 10 – 36°C, trichostrongylid larvae do not grow below 5°C, whereas temperature above 40°C is lethal (Lughano and Dominic, 2013). For the production of most organisms, optimal humidity is needed at 85%. Although desiccation may be lethal during the life stages, the significant nematode parasites can either be present in embryonic or infective larvae. Depending on species and particularly the weather conditions, the L3 of Trichostrongylid nematodes could remain for varying periods. In the L3 desiccated state they may survive a few months, but when they hydrate, they become active and exhaust their reserves quickly.

Greater worm burdens and parasitic gastroenteritis outbreaks are observed in goats and sheep in sub-Saharan regions in and after rainy seasons (Lughano and Dominic, 2013). There is a graded environmental influence, ranging from deserts and vast pastures to browsing plants to the extreme pasture areas of permanent watercourses (lakes and rivers) and irrigated land, in arid tropical climates of Ethiopia's lowlands, Somalia's, and Sudan. A change in the environments thus occurs that varies from being hostile to those most suitable for the production and survival of the parasites' free life stages.

Some trichostrongylid larvae, such as *Trichostrongylus colubriformis* and *Oesophagostomum columbianum,* have been known to be resistant to drought and thus lived at extreme temperatures, low or high (Lughano and Dominic, 1996).

During hypobiosis or the arrested growth of larvaes (usually L3 or early L4) inside the hosts, gastrointestinal nematodes may withstand harsh conditions. Hosts live in hot and seasonal dry conditions as adults in the absence of hypobiosis nematodes. In general, the humid tropical climate helps gastrointestinal nematodes for survival, growth, and transmission (Hansen and Perry, 1994; Urquhart et al., 1996). However, the combined effects on the pasture of L3 and the subsequent prevalence of worm burdens in hosts can lead to seasonal fluctuations in the availability of L3. Animal control schemes have a major effect on gastrointestinal nematodes' epidemiology. This seasonal variation of parasite population dynamics has been described in several studies in many African countries (Vercruysse, 1983; Pandey et al., 1994; Tembely et al., 1997; Nginyi et al., 2001; Debela, 2002).

2.1.1.2.2. Management systems

The high stocking density makes the ecosystem polluted with nematode eggs or larvae more available to susceptible animals. In the conventional husbandry systems, the accumulation of high worm burdens is not associated with high levels of storage and intensive management at limited to minimal rotational grazing, as well as with high pasture contamination and clinical helminthosis outbreaks. Animals may also be infected massively with ego- or larvae in pastures at watering points during the dry season, resulting in the outbreak of gastroenteritis parasite. This forces animals to gristle close to faecal material, which contributes to more infective larvae being taken in addition to leading to pasture degradation and soil erosion (Lughano and Dominic, 2013).

Anthelmintic therapy decreases the incidence, severity, and epidemiological effect of gastrointestinal nematode infections (Lughano and Dominic 2013), however established resistance to one or more accessible anthelmintic groups has been a challenge to control efforts on a worldwide basis (Jackson and Coop, 2000; Miller and Waller, 2004; Kaplan, 2004; Waller, 2006).

2.1.1.2.3. Host factors

Host factors such as age, breed, diet, physiological condition, and presence and/or lack of intercurrent infections may also affect the occurrence and severity of infection with gastrointestinal nematodes, for example, children and lambs are known to be more susceptible than adults, and the weight of worm in goats and sheep is likely to decrease at increased age. Some races of goats and sheep are known to be genetically resistant to GI infections (Lughano & Dominic, 1996). For example, adult female nematodes in sheep and goats will increase their egg yield during the period of partition.

This phenomenon is known as the peri-parturient rise (PPR) and has been reported in various African countries for the epidemiology of nematodes of small ruminants (Tembely 1995; Ng'ang'a et al., 2006). Every second week prior to lambing and kidding, a PPR was found in nematode egg excretion, and lasting up to eight weeks after lambing and kidding occurred during wet seasons. Thus, pregnant, or lactating ewes became the main source of infection for newborn lambs and children. (Tembely 1998; Sissay 2007).

2.1.1.2.4. Parasite factors

The intrinsic multiplication rate of the nematode species determines the rate of establishment and size of the nematode burden in the host. The multiplication rate is determined by the fecundity of the adult worms, the prepatent period, and the survival and development rate of the parasite in the environment. For example, *Haemonchus contortus* is one of the most prolific nematodes; a female *Haemonchus contortus* may produce thousands of eggs each day, and larval numbers on pasture can rapidly increase during the wet seasons (Soulsby, 1986; Hansen & Perry, 1994; Urquhart et al., 1996).

2.1.2. Trematodes:

Life cycles of essential trematode species of small ruminants include intermediate hosts, such as various aquatic or terrestrial snails, ants for Dicrocoelium species (Fasciola hepatica, *Fasciola gigantica*, *Schistosoma* Sp., *Paramphistomum* sp. and *Dicrocoelium* sp.) (Urquhart, et al., 1996).

The availability of suitable intermedial hosts and favorable climatical and ecological conditions rely on several variables for fluke infections in small ruminants. Temperature,

precipitation, humidity, and moisture are such environmental influences. The factors affecting both the larval phases of the Flukes and their intermediate hosts are close to those of the nematode parasites (Anonymous, 1994; Hansen and Perry, 1994; Urquhart et al., 1996; Torgerson and Claxton, 1999). Temperatures and humidity of fluke ivory hatching, growth, and survival of fluke larvae in the intermediate host and snail host breeding are favorable in most parts of Africa. Cercariae shedding starts at the beginning of the dry season, when the water level tends to drop and is still high. During the dry season, animals then ingest the metacercariae and clinical complications, depending on the infection rate, arise at the end and the beginning of this wet season (Urquhart et al., 1996). In the transmission of *F. hepatica,* the most significant intermediate host is the widespread aquatic snail *Lymnaea trucatula*.

The most important intermediate of *Fasciola gigantica* is *Lymnaea auricularia*. Also found to be the intermediate hosts of *Fasciola gigantica* was *Lymnaea natalensis* in Africa. The intermediate hosts for *Paramphistomum* sp. are snails such as *Planorbis* sp. and *Bulinus* sp. (Soulsby, 1986).

2.1.2.1. Life cycle

The eggs are placed in the adult *Fasciola* sp. and transferred by bile into the gall bladder. The eggs are then removed from the host with face after the gall-bladder contracts. The eggs have miracidia under ideal temperature and humidity conditions. The latter pierces snail hosts actively and grows through the phases of the sporocyst, redial and cercaria. The cercariae leave the snacks and become metacercariae, the infection stage, and encyst on the weeds below the water level. Metacercariae are taken with polluted grass or water by grassing animals. They excyst into the duodenum, penetrate the intestinal wall and pass through the abdominal cavity into the liver. In the liver parenchyma the immature flukes migrate and reach the bile ducts where they mature, then eggs are produced (Soulsby, 1986).

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Paramphistomum sp. transmission is similar to *Fasciola* sp. transmission. However, the immature Paramphistomes migrate up the food tract after excystation and attachment of the duodenum to the epithelial of the rumen and the reticulum (Soulsby, 1986). Duodenum and Ileum are immature Paramphistomes that provide plug feed and lead to hemorrhage, which can lead to bleeding, diarrhea, and bleeding for a long period of time (Soulsby, 1986).

Cercariae, which are free to live in freshwater can, penetrate healthy human skin in the complex life cycle. The head of the cercaria transforms into an endoparasitic larva, the schistosomula. The schistosomules then go into the vein and gradually move to the lung for several days in the skin. Then they pass toward the hepatoportal circulation via the circulatory system, to mature in adult worms and cotton. The schistosomes migrate to their last region of infection on the bladder or intestine where the females start the development of eggs, depending on the species. The eggs are fastened to the lumen wall. The eggs reach the lumen wall, which is supported by lytic elements isolated from the eggs and by heavy tissue perforation at the point of the egg.

The fecals or urine is then expelled. The miracidium, free from the shell, is looking for snail hosts to enter an eventually free-living process that looks for hosts (Semuguruka, 1992; Fritz et al., 2001). In Sudan, two species of *Bulinus* (*Schistosoma intermediate*) are found in Kosti and in the Jabal Aulia Dam along the White Nile (William and Hunter, 1968). Sulaiman and Ibrahim (1985) reported *Bulinus truncatus* and *Biomphalaria pfeifferi* snails in the same area.

2.1.3 Cestodes

The cestodes have indirect life cycles and the intermediate host may be the soil-inhabiting oribatid mites such as *Oribatula* sp., *Galumna* sp. in the case of *Moniezia* (Soulsby, 1986).

2.1.3.1 Life cycle

The adult worms are found in the small intestine of goats and sheep. Proglottids and eggs are passed out in the faeces of the infected animal. In the environment, the eggs may be ingested by oribatid mites where they develop into cysticercoids. The cysticercoids which

are the infective forms are produced in 4 months. Ruminants are infected by the ingestion of the infected mites with herbage. The prepatent period is 37-40 days (Soulsby, 1986)

2.1.4 Diagnosis of parasites in small ruminants

The diagnosis of parasites of small ruminants is based on demonstrating the presence of eggs or oocyst and larvae, in faecal samples, or the presence of parasites recovered from the digestive tracts or other viscera of the animals. Although a great variety of methods and modifications have been described for such diagnosis, standardization of techniques, such as egg or larval counts, worm counts, pasture larval counts, etc., does not exist. Therefore, in practice, most diagnostic laboratories as well as teaching and research establishments apply their own set and protocols of test procedures (Kassai, 1999).

However diagnostic procedures as described by (Soulsby, 1986; Thienpont et al., 1986 and Margaret et al., 1989) are usually adopted.

2.1.5. Prevalence of gastrointestinal parasites in Bangladesh

In Tangail, 63.4 percent were found to have a complete prevalence of GI infection in small ruminants. In general, there was a prevalence of 52.11%, 2.11%, 36.62% and 10.33% respectively of nematodes, cestodes and protozoa. The prevalence of parasites was variable with *Fasciola* sp. (8.45%), *Paramphistomum* sp. (28.17%), *Moniezia* sp. (2.11%), *Haemonchus* sp. (31.22%), *Trichuris* sp. (1.17%), *Oesophagostomum* sp. (10.80%), *Strongyloides* sp. (4.93%), *Trichostrongylus* sp. (2.35%), *Bunostomum* sp. (1.64%), *Eimeria* sp. (24%) and *Balantidium* sp. (6.34%). Stoll's ova dilution technique has processed and tested fecal samples under a microscope. In infection rates between goats (64.09%) and sheep, no substantial ($p>0.05$) differences were observed (60.67%). Seasonal variability between winter (56.72%) and summer (61.82%) was found to be significant in the rainy (72.44%) season (Rahman et al., 2017).

In Mymensingh, 74.8% of GI parasites in small ruminants were found. In Mymensingh, an investigation Prevalence from a species point of view suggested a parasite infection that occurred at 77.0% for goat and 65.9% for sheep. Nine species of GI parasites were identified in the study area namely *Strongyloides* sp., *Haemonchus* sp., *Oesophagostomum*

sp., *Trichostrongylus* sp., *Trichuris* sp., *Paramphistomum* sp., *Fasciola* sp., *Eimeria* sp., *Balantidium* sp., *Oesophagostomum* sp., *Trichostrongylus* sp., *Trichuris* sp., *Balantidium* sp., *Paramphistomum* sp., *Fasciola* sp. And *Eimeria* sp. have been described in the study area. Young small ruminants (78.4%) showed significantly higher prevalence as compared to adult (68.8%). In female (83.6%) higher prevalence than male was found among sexes (64.7%). In poorly conditioned, small ruminants (82.1%) were substantially infected by the infection, compared to moderate ruminants (72.2%) and good body conditions (53.8%). The highest prevalence in the rainy season was observed in the season (83.6%), followed by the summer (78.6%) and the winter (59.4%) (Islam et al., 2018).

There was another research to examine the prevalence of goats in all topographical Bangladeshi gastrointestinal nematodes. The research adopted standard flotation techniques and updated McMaster. The identified nematodes were strongyles (51.9%), *Strongyloides* sp. (19.0%) and *Trichuris* sp. (2.9%). Young age, other than Black Bengal, bad livestock, rearing of backyard, muddy houses, analphabet farmers and rainy seasons have been reported significantly in relation to GIN infections (Dey et al., 2020).

In Chattogram, the gross prevalence of GI parasitic infections was 68.64% in sheep and 61.82% in goats and was epidemiologically examined in gastrointestinal parasites. The eggs / oocytes of helminths and protozoan parasites were found in all samples subject to routine coproscopy (e.g., direct smear, flotation, and sedimentation). In the sheep and goats of all three areas of study, the occurrence of the nematodes and trematodes was higher compared to cestodes and protozoa. In comparison with the young population, adult goats reported substantially higher parasite GI infections. Women were more likely to be infected by GI parasites, whilst goats found the opposite pattern (Bhowmik et al., 2020).

The prevalence of ecto and endoparasites in semi-scarves Black-Bengal goat (*Capra hircus*) in Pahartali Thana was investigated in another study in Chattogram. Gastrointestinal and ectoparasite prevalence respectively stood at 63% and 57.72%. Of all the endoparasites *Strongyloides* sp. was 51.74%, *Haemonchus* sp. was 41.79%, *Paramphistomum* sp. was 39.30%, *Trichostrongylus* sp. was 36.32%, *Oesophagostomum* sp., *Bunostomum* sp., *Fasciola* sp. and *Cooperia* sp. remained between 10.95% and 12.94%. The rest of the species (*Capillaria* sp., *Moniezia* sp. *and Chabertia* sp.) were just in between 1.5 and 2.0% (Hasan et al., 2011).

In Sylhet, the overall prevalence of gastrointestinal nematodiasis was 82.66% in goat. 86.51% young (12) and 77.04% adult (>2 years) were found to have GIN infection with respect to age. Prevalence in females is observed at 80.41% while male prevalence was observed at 86.79%. The EPG samples for nematodal species collected were counted with a simple McMaster method using saturated salt and sugar solution (Islam et al., 2019).

2.1.6. Prevalence of gastrointestinal parasites in other countries

In India, one study found 83.08% overall prevalence of endoparasitic infection, consisting of 85.16% and 79.24% in sheep and goats, respectively. The associated risk factors with the prevalence of gastrointestinal tract (GIT) parasites showed that females (85.97%) were significantly more susceptible than males (69.23%). Age wise the adults (>6 months) were significantly more prone to parasitic infection as compared to young ones (<6 months). Seasonal variation was recorded throughout the year and was significantly highest during monsoon (90.10%), followed by winter (83.84%) and summer (78.35%) (Singh et al., 2017).

Another study in Jabalpur of India investigated on prevalence of gastrointestinal parasite and found 97% overall prevalence. Maximum infection recorded was of strongyles (79.08%) followed by coccidia (45.38%). Considerable infection of Trichuris (18.19%) and amphistome (17.53%) was also recorded. Strongyloides infection was recorded in 3.91% animals, whereas 3.57% Fasciola infection was observed. A meager infection of *Moniezia* (2.63%) and a negligible infection of *Toxocara* (0.39%) were also recorded (Gupta et al., 2013).

In Pakistan, to determine the prevalence of various endoparasites in and around twin cities of Rawalpindi and Islamabad one study was carried out. Overall prevalence was 65.7% for endoparasites. The prevalence of gastrointestinal parasites tended to be higher in sheep (72%) than in goats (63.7%). The endoparasites identified in sheep included *Haemonchus* (80.64%), Coccidia (51.61%), *Trichuris* (32.25%), *Nematodirus* (29.03%) and *Fasciola* (4.38%) while only *Haemonchus* (75%), *Trichuris* (62.5), and Coccidia (57.5%) were recovered from the fecal samples of goats. Identification of endoparasitic infection was

done using direct microscopic examination, centrifugation floatation and sedimentation technique (Gadahi et al., 2009).

In Nigeria, prevalence of gastrointestinal parasitic infection in sheep was investigated and found 64% overall prevalence. The results presented eleven species of GI parasites identified in the study area namely, *Eimeria* sp. (40.6%), *Strongyloides* sp. (8.9%), *Oesophagostomum* sp. (19.4%), *Trichostrongylus* sp. (13.9%), *Fasciola* sp. (6.1%), *Bunostomum* sp. (2.5%), *Haemonchus* sp. (5%), *Paramphistomum* sp. (0.5%), *Neoascaris* (0.5%), *Dicrocoelium* (1.7%), *Avitellina* sp. (0.5%). Adult sheep (66.7%) showed no significant difference in prevalence when compared to young (55.3%). higher prevalence was found in females, (64.6%) than males, (63.2%) (Maimadu et al., 2020).

2.2 Haemoprotozoan diseases

2.2.1 Babesiosis

Babesiosis is a combined term for diseases caused by parasites of the genus Babesia. The disease affects both domestic and wild mammals. Under the genus, Babesia over 100 species have been described (Homer et al., 2000) in which a large number occur in domesticated animals (Nyindo, 1992). Clinical cases are described as babesiosis whereas sub-clinical infections found in young animals and those recovered from clinical attack are termed babesiasis (Nyindo, 1992).

2.2.1.1 Etiology

Babesia's ovine and caprine is caused by three antigenic species: *B. motasi* is a large and more virulent form that occurs in erythrocytes individually or pairs; *B. ovis* is a small form of *Babesia crassa* (Sahinduran, 2012). (Hashemi-Fesharki and Uilenberg 1981; Levine 1985). Of the three babesia, *B. crassa* is non-pathogenic (Bashiri Bod, 1993 and Motavalli Haghi et al., 2013).

2.2.1.2. Epidemiology

2.2.1.2.1. Geographical occurrence

Babesia is widely spread all over the world. In 2009, babesiosis in various African, Asian, European, Oceania, North and South American countries is reporting according to OIE. Found in Southern Europe, in the former Soviet Union in Middle East, Asia and Africa, and in Tropics in Southern Europe, Middle East Africa, Asia and the former Soviet Union, *Babesia Motasi* is present.

2.2.1.2.2. Transmission

While *Babesia* infections in the ticks continue through moults (trans-stadian maintenance), they are transmitted transovarial (Lewis & Young, 1980). (Herwaldt et al., 2011). In the absence of infected mammalians, ticks can also sustain their population over many generations (Donnely & Peirce, 1975). In tick gut, haemolymph, ovary, salivary glands, the stage of development is recognized (Nyindo, 1992).

Four Genera, *Rhipicephalus, Ixodes, Haemophysalis* and *Hyalomma* within the Ixodidae have been reported as vectors of *Babesia* sp. *B. ovis, B. ovis, B. motasi* and *B. crassa.* are primarily transmitted via *Haemaphysalis qinghaiensis* and *H. longicornis* (Niu et al., 2016). Moltman et al., (1982) described the fine structure of *B. ovis* in *Rhipicephalus bursa.*

2.2.1.2.3 Risk factors

2.2.1.2.3.1. Susceptible Host

Mammal species usually remaining to the same or related genera are receptive to the same *Babesia* species. Thus *B. bigemina* and *B. bovis* can infect African and Asian buffalo taurines and zebu; *B. motasi* and *B. ovis* infect sheep and goats, and all wild species or subspecies of the subfamily Ovinae; *B. caballi* and *B. equi* infect horses, donkeys, and zebras; whereas dogs, various wolves and jackals are receptive to *B. canis* and *B. gibsoni* (Morel, 1989; Smith et al., 1972). Sheep and goats are equally receptive to *Babesia*, but, depending on the parasite species or strain of a given species, goats are usually low pathogenic and may only have a latent infection.

2.2.1.2.3.2. Age

As far as age is concerned, younger is less vulnerable to babesiosis between young and older animals (Urquhart et al., 1996). Inverse age tolerance is unique in contrast to other infectious diseases that more significantly impact young animals. This effect is noticeable for cattle and horses only and lasts longer than antibodies that are passively transferred (Goff et al., 2003; Smith et al., 2000; L'Hostis, 1995). Innate resistance and independent of mother's immune status is thought to be the cause of this phenomenon (Christensson, 1987).

2.2.1.2.3.3. Environmental factors

The distribution of babesiosis primarily depends on its distribution of the vector. The distribution of babesiosis vector depends upon some factors such as latitude, altitude, and its impacts (sunlight, temperature, rainfall, wind) (Morel, 1989). Seasonal changes in a bioclimatic environment may further benefit or impede the production or behavior of a tick for certain periods (Morel, 1989; Rabo et al., 1995). The predominant factor in tropical conditions is precipitation (Morel, 1989). In Southwest Nigeria, tick infestation is greater in the rainy season than in the dry season (Dipeolu, 1975). In the northern hemisphere, temperate climates disease distribution typically has a bimodal seasonal distribution with a spring peak between April and June and an autumn peak from August to October.

2.2.1.2.3.4. Other factors

The existence of the spleen seems to be another factor which provides non-specific protection against *Babesia*. Splenectomised calves become completely contagious (Edelhofer et al., 1998) and unnaturally resistant hosts may develop highly infected diseases if the spleen is removed. The most remarkable example of this is zoonotic *B. divergens* infections in splenectomized humans (Gorenflot et al., 1998). The normal or learned defense mechanism often affects the physiological state. Any deterioration of condition due to fatigue, nutritional issues (lactation, mistletoe, gestation) or anabolism increases the susceptibility of the animal to an infection or recurrence (Urquhart et al., 1996; Morel, 1989). The style of animal breeding also affects babesiosis. In the endemic areas where many infected ticks are observed, host immunity is preserved at a high level

by persistent challenge and overt disease is uncommon. In some areas, the occurrence of swine babesiosis is assessed by the interaction of domestic pigs with wild suidaes (in some freely paced herds). By comparison, where there are few ticks or small areas, some young livestock are not infected, protected against and susceptible to maternal antibodies or other innate mechanisms. Animals managed under intensive management systems in tick-vector-free areas do not become infected, but these cattle are highly vulnerable if they are introduced to endemic areas where the infection is high (Losos, 1986).

2.2.2. Anaplasmosis

The most important intraerythrocytic disease in livestock transmission of tick is anaplasmosis (Ristic & Kreier, 1984). The causative agents in bovine and wild ruminants and in the ovate of the sheep and goats are *Anaplasma marginale* and *Anaplasma centrale*. *Anaplasma mesaeterum* is closely linked to *A. ovis* and can also lead to a disease in sheep and goats (Nakamura et al., 1993; Uilenberg et al., 1979).

2.2.2.1. Etiology

Anaplasma ovis is spread across the world and is regarded to be the most common cause of small ruminant anaplasmosis but appears less pathogensome compared to other species of *Anaplasma* (Kuttler, 1984). The causative agent for tick-transported fever (TBF) in domestic ruminants such as sheep was found to be *Anaplasma phagocytophilum* first (Gordon et al.,1932). Molecular analyzes have shown that sheep are often infected with *A. phagocytophilum* (Stuen et al., 2002b). Subclinical infections by *A. marginale* have been recorded both in sheep and in goats (Stoltsz, 2004)

2.2.2.2. Epidemiology

2.2.2.2.1. Geographical occurrence

Anaplasmosis occurs worldwide (approximately 40° N to 32° S) in tropical and subtropical areas, including Central and South America, USA, Southern Europe, Africa, Asia, or Australia. It is transmitted by a diverse community of biological and mechanical vectors, is endemic and sporadically distributed in temperate climatically in tropical and subtropical regions that sustain these vectors.

2.2.2.2.2. Transmission

The bulk of transmissions occur by ticks, and more than 20 species worldwide are accused of biological vectors (Kocan, 1995). A. Ticks of the genus of *Rhipicephalus bursa, Rhipicephalus bursa turanicus, Dermacentor silvarum*, *Dermacentor marginatus*, *Dermacentor andersoni,* and *Haemaphysalis sulcata* (Alessandra et al., 2012). *A. phagocytophilum* is transmitted by Ixodidae ticks. In Europe *A. Phagocytophilum* transmits transstadial in these ticks, but the transovarian transmission is not evidence (Ogden et al., 2002b). Biting dipterans transmission is considered essential in parts of the United States (Kuttler, 1979). Transplacental transmission takes place and is possibly underestimated (Zaugg, 1987; Barry & Niekerk, 1990; Potgieter and Stoltsz, 1994). Infection of the dam is commonly associated with in the second or third gestation trimester. Anaplasmosis can also spread over infected needles, dehorns, castrations, or other surgical tools (iatrogenic transmission) via blood transfer, unless care is taken to clean instruments on each animal between use (Ristic, 1968; Hungerford and Smith, 1997; Abdala et al., 1998).

2.2.2.2.3. Susceptible Host

Wild ruminants reported to have anaplasmosis include white tailed deer, blacks, mules, elks, pancakes, bighorn sheep and several exotic specimens. Home ruminants including bovine animals, sheep and goats are also resistant to this disease. The contaminated non-rumen wildlife includes mice, coyotes, fishes and mountain lions, People, dogs, and horses may be infected as well.

2.2.2.2.4. Risk factors

2.2.2.2.4.1. Age

Immune mother calves are covered partially against colostral antibodies (Corrier & Guzman, 1977). This protection lasts for around 3 months and is typically accompanied by age period of about 9 to 12 months for animals (Jones et al., 1968; Paul et al., 1980). Calves exposed to anaplasmosis rarely show clinical symptoms but develop a solid, long-lasting immunity, when the maternal or age resistance is high. It is therefore possible to have both *Anaplasma* sp and vectors present on an area without animal losses or clinical diseases.

This situation is known as endemic stability. Unlike in the case of bovine anaplasmosis, age susceptibility to ovine and goat infection does not appear to be important. Young and adult animals typically develop only a mild disease, although different stressors in individual cases can worsen this (Stoltsz, 1994).

2.2.2.2.5. Other factors

Immune-compromised animals have shown that they are vulnerable to heterologous threats, either through splenectomy or by treating immunosuppressants such as cyclophosphamide and corticosteroids (Kuttler et al., 1984). It was also proposed that cattle's immunity could be weakened under environmental stress or other stressors (Kuttler et al., 1984), but Wilson (Wilson, 1979) demonstrated that cattle had more serious primary *Anaplasma* reactions in a good nutrient plane than in a hunger ration.

2.2.3. Theileriosis

2.2.3.1. Etiology

T. lestoquardi, *T. seprata,* and *T. ovis* are primarily involved in various *Theileria* species that cause sheep theileriosis. *T. Lestoquardi* causes malignancy in sheep and goat while *T. sepratum* causes low or nonpathogenic malignant theileriosis (Sayin *et al.,* 2009). *Theileria ovis* is an important etiological agent of ovine theileriosis that causes significant economic losses in tropical and subtropical areas of sheep and goats (Durrani et al., 2011; Iqbal et al., 2011; Esmaeilnejad et al., 2012 and Naz et al., 2012)

2.2.3.2 Epidemiology

2.2.3.2.1. Geographical occurrence

In thirteen countries of sub-Saharan Africa, *Theileria parva* is occurring. In any region with annual rainfall exceeding 20 pounds, the tick vectors can be found from sea level to more than 8000 feet. In southern European countries, North Africa, Middle East, and Asia, *Theileria annulata* occurs., *Theileria lestoquardi* (*T. hirci*) occurs in infecting small ruminants in the Middle East, East and North Africa, China, East, Central Asia and Eastern and Southern Europe. This infects small ruminants. Their virulence differs greatly, from serious disease development to full and benign forms, resulting in high mortality.

2.2.3.2.2. Transmission

Ticks disperse *Theileria* bacteria. The largest vector for *T. parva* is *Rhipicephalus appendiculatus* and in southern Africa it is *R. zambeziensis. Theileria orientalis* is transmitted by *Haemaphysalis* sp., or other genera of ixodid ticks. *H. longicornis* is the vector in Australia, New Zealand, and Japan. Mechanical transmission occurs through routine husbandry practices in case of *T. orientalis* transmission. *Theileria lestoquardi* is transmitted by ticks of the genus *Hyalomma. Theileria luwenshuni* and *T. uilenbergi* are transmitted by ticks of the genus *Haemaphysalis.*

2.2.3.2.3. Susceptible Host

This agent includes both wild and domestic *Bovidae* all over the world and also contains small ruminants by certain species. Several *Theileria* sp. have been described that Infect cattle; the most significant pathogenic and economic is *T. Parva*, causing triggering East Coast fever (ECF), *T. annulata,* which causes Tropical theileriosis (TT) or Mediterranean theileriosis and *T. orientalis* (*T. orientalis/buffeli* group), which causes Oriental theileriosis (OT) or *Theileria*-associated bovine anaemia (TABA). *Theileria lestoquardi* (*T. hirci*), which causes Malignant ovine theileriosis (MOT), *Theileria uilenbergi* and *Theileria luwenshuni* are the most pathogenic species of economic significance infecting small ruminants

2.2.4. Diagnoses of Haemoprotozoan diseases

Diagnosis of hemoparasite infection is based primarily on clinical symptoms and on parasitological methods to detect the causative agent. Although the traditional Giemsa-stained blood smears has many drawbacks, it is still known as the world-wide gold standard for the diagnosis of haemoparasite infections (Marcondes, 2017). However, all hemoprotozoan, particularly subclinical cases, are not detected through a microscopic examination. In addition to the direct microscopic technique, antigens, or antibodies against TBPs may also be identified with indirect diagnostic techniques such as multiple serological assays (e.g., ELISA, indirect fluorescence assays and CFT) (Snodgrass et al., 1971; Lew-Tabor, 2016; Lempereur et al., 2017; Shabana et al., 2018; Ghaffar et al., 2020).

For diagnosing parasites such as *Babesia* sp., indirect fluorescent antibody technique has been used for long (Morzaria et al., 1977; Anderson et al., 1980). ELISA is used as a basis for the assessment of the vaccine program (Guglielmone et al., 1997) and the epidemiological surveys (Passos et al., 1998). Microscopic and serological methods are of limited importance due to many limitations such as lower sensitivity and precision, cross reactivity, incapacity to detect carriers and the need for time and expertise (Igarashi et al., 2014; Mans et al., 2015; Lew-Tabor, 2016). These limitations have been overcome by use of highly sensible molecular approaches, including conventional PCR (cPCR), quantitative PCR (qPCR), nested PCR (nPCR), reverse line blotting (RLB), Loop Medium Isothermic Amplification (LAMP), high-resolution melting (HRM) assays, high-throughput microfluidics-based real-time PCR and the next-generation sequencing (NGS) (Schnittger et al., 2004; Criado-Fornelio et al., 2009; Alessandra et al., 2012; Johnsen et al., 2013; Michelet et al., 2014;Wang et al., 2019).

Microscopy is the most commonly used tool of scientific studies in Bangladesh for the recognition of TBPs. Field diagnosis is generally made based on clinical evidence and tick exposure history, mainly because well-equipped veterinary diagnostics laboratories are not available in the region (Afzal, 2009; Jabbar et al., 2015; Ghafar et al., 2020). In more research, changes in the haematological profiles of TBP animals have also been investigated and a reduction in haemoglobin and the volume of packaged cells associated with anaplasmosis, babesiosis and theileriosis has also been recorded (Ali et al., 2014; Ullah et al., 2018; Ghaffar et al., 2020).

2.2.5. Prevalence of haemoprotozoan diseases in Bangladesh

Epidemiologic research on common blood parasites have been limited and molecular detection of hemoprotozoic diseases has been carried out in Bangladesh, both clinically and subclinically suspected sheep and goats. All experiments have been done by traditional microscopy on the basis of blood smear. In Sylhet, however, 5% (Jewel et al., 2020) and 16.63 percent (Mohanta et al., 2011) in hilly areas of Bangladesh were prevalence of *Babesia* sp. infection in goat areas.

In the area of Sylhet, however, there was a 12% (Jewel et al., 2020) prevalence of *Anaplasma* Sp., and 14.94% (Mohanta et al., 2011) in Hilly regions. Two previous studios

were performed using a molecular technique using microscopic, while Hasán et al., used 2019 PCR in clinically positive cases for detecting haemoprotozoan where *Anaplasma* sp. was 43%, *Babesia* sp. 19%, *Anaplasma* sp. with *Babesia* sp. 33%, *Theileria* sp. 4% and *Anaplasma* sp. with *Babesia* and *Theileria* sp. was 1% in Dhaka, Sirajganj and Nikhangsori.

2.2.6. Prevalence of haemoprotozoan diseases in other countries

In India, the prevalence of haemoprotozoan diseases in sheep and goats were recorded 3% and 11% respectively out of 242 sheep and 312 goats' samples from the period of 2004 to 2013 in the North-Western part of Tamil Nadu. The haemoparasites recorded the highest in summer followed by winter and rainy season. In the western Bengal of India, haemoprotozoan infection was detected by Giemsa's staining technique. Higher infection was observed in does (39.67%) than bucks (10.78%). Age-wise, the highest prevalence of haemoprotozoan was found in 1–3 years (36.39%) age group followed by >3 years (27.95%) and <1 year (23.52%), respectively. Seasonal variation was also observed, the prevalence of haemoprotozoan was found highest in monsoon (42.84%) and lowest in winter (15.10%) (Patra et al., 2018).

Theileriosis is Pakistan's most commonly studied TBD in sheep and goat, with 20 vertebrate and three ticks' investigations. However, somewhat higher prevalence was reported in sheep (13.9–55.3%; 23.9–36.8%; and 28–47.2%) than goats (1.7–30.7%; 20.6–32.8%; and 25.3–34.8%) using microscopic, serological (enzyme-linked immunosorbent assay and molecular methods, respectively. The prevalence of Anaplasma sp. in both sheep and goats was the highest (25.3–55.3%) in Punjab province followed by Khyber Pakhtunkhwa (1.7–47.2%) and Sindh (13.3%). So far, only one study has projected the occurrence of *Anaplasma* sp. in ticks of small ruminants from Khyber Pakhtunkhwa province which stated a higher occurrence of the bacteria in ticks from sheep (39.1%) than those collected from goats (35.5%) (Ghafar et al., 2020). Babesiosis is the minimum studied disease among all the key TBDs of small ruminants in Pakistan (Punjab = 5; Khyber Pakhtunkhwa = 4). 7–41.7% and 23.9–55% of the studied goat and sheep population were positive, respectively, using light microscopy and molecular methodologies, based on the data on occurring babesiosis in small ruminants.

In Italy, the serological and molecular prevalence of anaplasmosis, babesiosis and theileriosis were investigated. The serological prevalence for sheep were higher (82.9%) for *A. ovis* than (11.9%) *A. phagocytophilum*; for goats *A. ovis* were 74.9% and *A. phagocytophilum* were 15.2%. Molecular prevalence for sheep were 47.3% for *A. ovis* and 5.4% for *A. phagocytophilum*, for goats they were 31.7% for *A. ovis* and 2.5% for *A. phagocytophilum.* The prevalence of piroplasmosis (babesiosis/theileriosis) was detected by RLB technique, 28.1% in sheep and 11.7% in goats (Alessandra et al., 2012).

Anaplasma ovis, Babesia ovis and *Theileria ovis* have been discovered in Nigeria. Of these haemoparasites encountered, *A. ovis* had the highest prevalence (12.62%) in sheep and (11.34%) in goats followed by *T. ovis* with a prevalence of 6.79% in sheep and 4.12% in goats. *Babesia ovis* had the lowest prevalence rate of 0.97% in sheep and 2.06% in goats (Jatau et al., 2011).

An alternative test was performed with 126 randomly chosen blood samples. PCR was used for the detection of 18S rRNA gene of *Babesia/Theileria* sp. (B/T) and the 16S rRNA gene of *Trypanosoma brucei/Ehrlichia* sp.(A/E) (*Hemoplasma*). A total of 34.1%, 23% and 51.6% of samples were positive in the cases of B/T, A/ E and *Hemoplasma,* respectively. The findings indicate the links between hematology and PCR (Happi et al., 2020).

Babesiosis has a prevalence of 12.2% in Iran, with 9.9% of infection from sheep and goats (2.3%) detected through microscopic screening. 4.2% sheep, and 0.5% goat blood samples have been infected by *Babesia* by molecular method (PCR and semi-tested PCR) (Naderi et al*.,* 2017).

In Turkey, the presence of *Theileria* sp. OT1, *T. luwenshuni, T. uilenbergi, T. separata, Babesia motasi* and *B. crass* small ruminants were verified using molecular techniques. A high prevalence of mixing infections, which showed that 52.24% and 35.42% of animals were co-infected with multiple species with PCR and RLB approaches. More than 80% of the mixed infections contained *T. ovis* and/or *A. ovis* (Bilzic et al., 2017).

Chapter-III

MATERIALS AND METHODS

3.1. Description of study areas

The study was conducted in three upazilla under the Khagrachari district namely Panchori, Sadar, and Golabari. The study was conducted in hilly areas to know how the pattern of different climates and geographical conditions affect the occurrence of different parasitic diseases.

3.2. Study period

The study was commenced for a period of ten months starting from April 2019 to January 2020 where two seasons were included Summer (April 2019 to July 2019) and Winter (October 2019 to January 2020).

3.3. Selection of animals and Survey Design

3.3.1. Target animals

Goats of different breeds (Indigenous, Black Bengal, and Jamnapari) and indigenous sheep breed were selected randomly from the selected hilly areas of Khagrachari district.

3.3.2. Selection of age groups

To determine the age and breed susceptibility of different parasites, sheep and goats were divided into three subgroups. It was divided into lamb or kid (0-6 months), young (6-12 months), and adult (>12 months).

3.3.3. Target sampling

Total 279 samples were collected from randomly selected small or medium-scale farms of which 182 samples were from sheep and 97 samples were from goats. In summer we collected 44 samples from goats and 74 samples from sheep. In winter we collected samples from 161 animals including 108 Sheep and 53 Goats. Samples were taken in each season from randomly selected animals from different houses located in Panchori, Sadar, and Golabari union of Khagrachari. Samples were collected in two consecutive seasons (Summer and winter) where new animals were considered for each season.

A questionnaire was used to track information like owner's name and address, animal identification (ID), farm size, breed, age, sex, deworming history, Vaccination history, presence or absence of tick, etc.

3.4. Sample collection and preservation

Four different types of biological samples (blood smear, blood, faeces, and ticks) were collected during this study and an individual animal was considered as a sampling unit. Smear was prepared from blood taken from a jugular vein puncturing with a sterile needle. First, a thin smear was prepared by taking a drop of blood on the glass slide then air-dried and fixed with absolute methanol for 3-5 minutes. On the other hand, another two thin blood smears were prepared by touching the coming out fresh blood and then spread by another slide.

Faeces (approximately 5-10 gm) were collected directly from the rectum and stored in plastic containers. Then, the container was filled with formalin (10%) and transferred to cool box. At the time of sample collection, labeling of the samples was strictly maintained to prevent misinterpretation.

Ectoparasites (ticks) were collected from a different region of the body and then put into a zipper bag.

3.5. Examination of samples

3.5.1. Blood Smears Examination

All the examinations were carried out at the Parasitology laboratory, Chattogram Veterinary and Animal Sciences University (CVASU). The prepared thin blood smears (Hendrix, 2006) were stained with the Giemsa stain for 25-30 minutes. After washing with water, the stained blood smears were air-dried and examined under a binocular microscope (X100) with immersion oil for the identification of blood parasites (Benbrook and Sloss 1962; Soulsby, 1986 and Urquhart et al., 1996).

3.5.2. Fecal samples Examination

After gross examination of faecal samples (color, consistency, odor, blood, or mucus, etc.) three separate types of qualitative tests, namely direct smear, floatation, and sedimentation

techniques used to examine the fecal samples. Sugar Salt solution was used as flotation fluid. At least, two smears were prepared from each sample for each test to identify the morphological characteristics of eggs, cysts, Oocysts, etc. (Hendrix, 2006; Foreyt, 2001; Urquhart et al., 1996 and Soulsby, 1986).

3.5.3. Morphological Identification of tick

Ticks were identified by light microscopy in accordance with the morphology of ticks, especially the dorsal and ventral surfaces of adult males and females. Ticks were detected using recognized morphological keys (Parola et al., 2013). The collected ticks were counted and categorized to species, and sex. Tick species were determined morphologically using taxonomic keys of Estrada-Pena et al. (2004).

3.6. Molecular characterization of blood protozoa from goats and sheep

3.6.1. DNA extraction from blood and tick sample

From blood and tick specimens, DNA was extracted by using the tissue genomic DNA extraction mini kit (AddPrep Genomic DNA Extraction Kit) according to manufacturer instructions. The samples (blood and ticks) were taken in eppendorf tubes and labeled accordingly. Ethanol was added in the eppendorf tube. The samples were air-dried to remove the ethanol. After adding binding and lysis solution properly, the mixture was vortexed. Then 20µl proteinase-k was added into the eppendorf tube. Again, the mixture was properly mixed by pulse vortex. Mixture was incubated at 60°C for 15 minutes. After incubating, 200 µl concentrate ethanol (96-100%) was added into the mixture then vortexed and spin centrifuged for 30 seconds. Mixture was transferred to a spin column and centrifuged at 8000 rpm for 1 minute again. After centrifuging, we have the discarded the lower part of the mixture (discarded the drops from the inside of the lid). Then 500 µl wash buffer-I was added into the tube and centrifuged at 8000 rpm for 1 minute. This process was repeated twice. Then empty spin column was centrifuged at maximum speed at 13000 rpm for 3 minutes to remove ethanol. Then 100 to 200 µl elution buffer was added and incubated at room temperature for 1 minute. Later, mixture was centrifuged at 13000 rpm for 2 minutes and DNA was collected into new eppendorf tube. Finally, the DNA product was preserved at -20° C for PCR.

In case of tick sample, the whole tick and eggs were washed with 70% ethanol first and then air-dried in sterile filter paper. The whole tick and eggs were transferred to an eppendorf tube and considered as a single sample.

3.6.2. Amplification of gene by polymerase chain reaction

Each individual DNA extract from blood and tick sample were homogenized and DNA extracted using the procedure as described above. For PCR, amplification was conducted using following primers (Table 1). The Reactions were made 25µl volume containing 4µl of extracted DNA, 2µl of each primer, 12.5µl master mix (2X) and 4.5µl double distilled water or nuclease free water.

Thermo profile for PCR of Theileria specific was containing at 94° C for 3 min., followed by 35 cycles, 1 min. at 94° C for denaturation, 1 min. at 60° C for annealing and 1 min. at 72° C for extension, with a final extension step of 72° C for 7 min. After completing of PCR reaction, it was store at 4° C.

For *Babesia* sp.*,* PCR was performed for 5 min at 95° C to initial denaturation, and then the reaction was repeated for 35 cycles under the following conditions: 1 min of denaturation at 95° C, 1 min of annealing at 56° C and 1 min of extension at 72° C following by a 5 min completion step at 72° C.

The conditions used for amplification of *Anaplasma* sp. were as follows: initial denaturing at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 10 min.

Table1: Primer pairs used for the detection of Theileria, Anaplasma and Babesia from blood and tick sample

Primer specificity	**Target gene**		**Product size (bp)**	**References**
Theileria sp.	18SSU rRNA	F:5′-AGTTTCTGACCTATCAG-3′ R:5′-TTGCCTTAAACTTCCTTG-3′	1098	(Allsopp et al., 1993)
Anaplasma sp.	16S rRNA	F: 5′-GGTACCYACAGAAGAAGTCC-3′ R: 5′-TAGCACTCATCGTTTACAGC-3′	345	(Yongshuai et al., 2017)
Babesia sp.	18S rRNA	F: 5′-GCGATGGCCCATTCAAGTTT-3′ R: 5′-CGCCTGCTGCCTTCCTTAGA-3′	146	(Theodoropoulos et al., 2006)

3.6.3. Gel Electrophoresis

An aliquot of 5-6 µl of each PCR product was subjected to electrophoresis on a 1.5% agarose gel. 0.75 gm agarose powder was added to 50ml 1X TAE buffer (Tris, Acetic acid and EDTA) was and mixed thoroughly. The mixture was then heated in the oven for 2 minutes. Then 5µl ethidium bromide was added into the mixture. Finally, the mixture / gel was poured on gel tray. Then 5-6µl PCR product was put into the gel tray. After putting of PCR product, run the gel electrophoresis, and waiting for minimum 25 minutes. The bands were visualized using the ethidium bromide and gel documentation system (UV-illuminator). A 100bp DNA ladder was use as a molecular-weight size marker.

3.7. Purification of PCR amplicons

The amplified PCR DNA was excised from the agarose gel using QIAquick Gel Extraction Kit (Qiagen, Germany) following the manufacturers instruction.

3.8. DNA sequencing and phylogenetic analysis

Twenty purified PCR amplicons of the samples amplified using primers targeting Ac-16S rRNA were sequenced with both the forward and reverse primers. The Sanger sequencing

was performed using an ABI Prism1 Big Dye Terminator v3.1 Cycle Sequencing Kit (PerkinElmer, Applied Biosystems Division, and Foster City, CA, USA) in South-Africa according to the manufacturer ‘s instructions. The obtained sequences were verified using chromatogram peaks, edited, and assembled using Chromas, BioEdit. The multiple alignments of the assembled sequences were performed using MUSCLE algorithm and Neighbor Joining cluster method on MEGA X (version 10.2.4 windows x64) and then consensus sequences were obtained. BLASTn for nucleotide analysis accessed through GenBank of the NCBI database and then used to compare the consensus sequences with the correct *Babesia*, *Anaplasma* and *Theileria* species identity. The confirmation of the species was established as the nearest BLASTn match with an identity of between 93% and 100% to those homologues found in the GenBank. The construction of three phylogenetic tree (*Anaplasma, Babesia* and *Theileria*) of evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model (Kimura, 1980). The tree for *Babesia*, *Anaplasma* and *Theileria* with the highest log likelihood are -8699.90, -3284.29, and 4933.33 were shown, respectively. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 20, 21, and 14 nucleotide sequences for Babesia, Anaplasma and Theileria, respectively. There was a total of 2158, 1456, and 1827 positions in the final dataset for Babesia, Anaplasma and Theileria, respectively. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018) version 10.2.4 windows x64. The statistical significance for the internal branches of the trees was estimated using the Bootstrapping with 1000 iterations.

3.9. Statistical Analysis

The obtained information was imported, stored, and coded accordingly using Excel of Microsoft 365 and STATA/SE-13.0 (Stata Corporation College Station) for analysis. Descriptive statistics were expressed as the proportion with Confidence Interval. The result

was expressed in percentage with P-value for the Chi-Square Test. Significance was determined when P<0.05. Molecular data were analyzed by bioinformatics tools using BLASTn, sequence alignments using MUSCLE algorithm and Neighbor Joining cluster method on MEGA X (version 10.2.4 windows x64), and phylogenetic analysis using Neighbor Joining method with Kimura - 2 algorithm on MEGA X (version 10.2.4 windows x64)

Chapter-IV
RESULTS

4.1. Prevalence of gastrointestinal parasites on the basis of microscopic identification

4.1.1. Overall prevalence of gastrointestinal parasites

The overall prevalence of gastrointestinal parasites was 55.67% and 63.19% in goats and sheep, respectively. Among different gastrointestinal parasites, prevalence of *Fasciola* sp. was highest and it was 27.84 % in goats and 21.43 % in sheep. Lowest parasitic infections were recorded in *Moniezia* sp., *Toxocara* sp. and *Ostertagia* sp. infections in sheep which were 0.55%. However, slightly higher prevalence was recorded in *Paramphistomum* sp., *Trichuris* sp., and *Trichostongylus* sp. infections than *Haemonchus* sp., *Nematodirus* sp. and *Fascioloides* sp. infection (Table 2).

Table 2: Overall prevalence of gastrointestinal parasites in goats and sheep

Gastrointestinal Parasites		**Goats (n=97)**		**Sheep (n=182)**	
Parasite groups	**Parasites**	**%(n)**	**95% CI**	**(%)**	**95% CI**
Trematode	*Fasciola* sp.	27.84 (27)	19.21-37.85	21.43 (39)	15.70-28.10
	Fascioloides sp.	0 (0)	0	2.20 (4)	0.60-5.53
	Paramphistomum sp.	6.19 (6)	2.30-12.97	9.89 (18)	5.96-15.17
Cestode	*Moniezia* sp.	0 (0)	0	0.55 (1)	0.01-3.02
Nematode	*Trichostongylus* sp.	5.15 (5)	1.69-11.62	14.84 (27)	10.00-20.84
	Stongyloides sp.	8.25 (8)	3.62-15.60	15.38 (28)	10.47-21.46
	Oesophagostomum sp.	3.09 (3)	0.64-8.77	6.04 (11)	3.05-10.55
	Trichuris sp.	6.19 (6)	2.30-12.97	8.24 (15)	4.68-13.22
	Toxocara sp.	1.03 (1)	0.02-5.61	0.55 (1)	0.01-3.02
	Nematodirus sp.	4.12 (4)	1.13-10.22	1.65 (3)	0.34-4.74
	Haemonchus sp.	3.09 (3)	0.64-8.77	2.20 (4)	0.60-5.53
	Ostertagia sp.	0 (0)	0	0.55 (1)	0.01-3.02
Overall		55.67 (54)	45.22-65.75	63.19 (115)	55.73-70.20

n= total no. of animals, CI= Confidence Interval

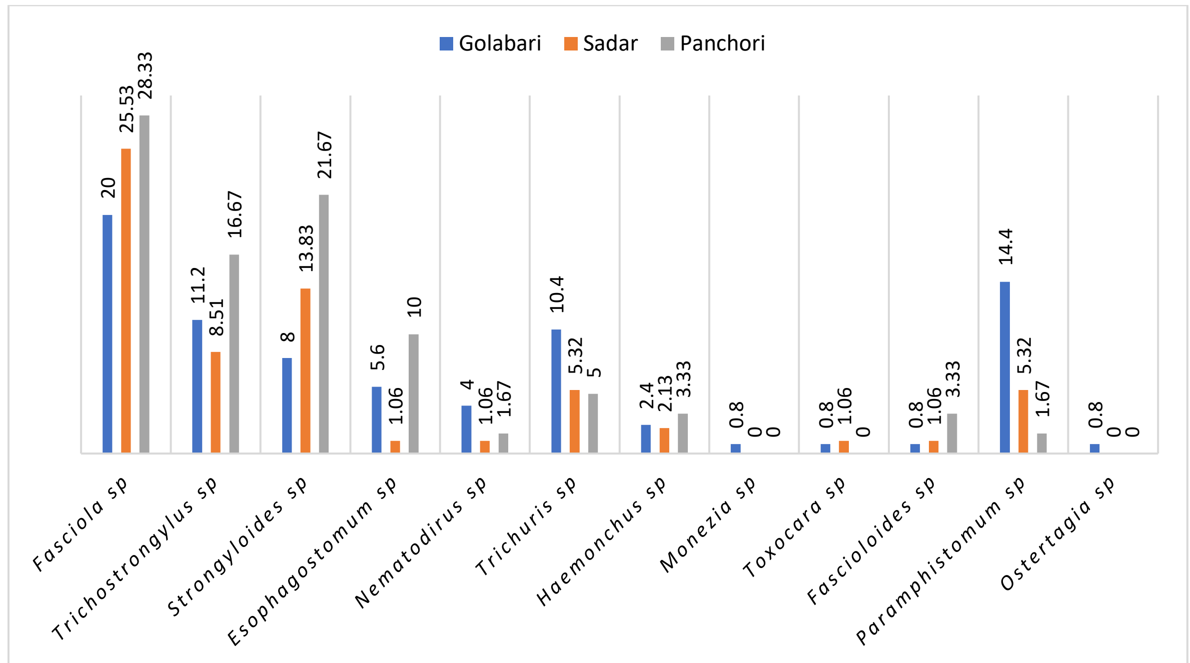


Fig. 1: Prevalence of gastrointestinal parasite in goats and sheep in different areas

In terms of location, Panchori had highest (40.11%) prevalence in relation to Golabari (38.41%) and Sadar (21.46%) besides among different parasites *Fasciola* sp. have highest prevalence followed by *Strongyloides* sp., *Trichostrongylus* sp., *Oesophagostomum* sp., *Paramphistomum* sp., *Trichuris* sp., *Haemonchus* sp. and *Nematodirus* sp.

4.1.2. Seasonal prevalence of gastrointestinal parasites

Seasonal prevalence of gastrointestinal parasites showed some variability in goats and sheep. Highest overall prevalence was recorded in winter which was 72.22% and 62.26% followed by summer (47.73% and 50%) in goats and sheep, respectively. It was also revealed that gastrointestinal parasites were more prevalent in sheep than goats. Occurrence of *Fasciola* sp. was highest in winter (33.96%) in goats whereas 25.93% was recorded in sheep. In the present study, *Trichuris* sp. (14.86%), *Paramphistomum* sp. (18.92%) and *Strongyloides* sp. (13.51%) infection were more prevalent in summer in sheep than goats. Lowest prevalence of *Toxocara* sp. (1.89%) was found in goats in winter and *Haemonchus* sp. (2.70%) infection in sheep in summer. Seasonal fluctuation was also recorded in *Nematodirus* sp. *Oesophagostomum* sp., *Strongyloides* sp. and *Ostertagia* sp. infections during this investigation.

Table 3: Seasonal prevalence of gastrointestinal parasites in goats and sheep

Gastrointestinal Parasites		Goats			Sheep		
Parasite groups	**Parasites**	**Winter (n= 53)**	**Summer (n= 44)**	**P- value**	**Winter (n= 108)**	**Summer (n= 74)**	**P-value**
		% (n)			**% (n)**		
Trematode	*Fasciola* sp.	33.96 (18)	20.45 (9)	0.139	25.93 (28)	14.86 (11)	0.074
	Fascioloides sp.	0	0	0	3.70 (4)	0	0.094
	Paramphistomum sp.	5.66 (3)	6.82 (3)	0.814	3.70 (4)	18.92 (14)	0.001*
Cestode	*Moniezia* sp.	0	0	0	0.93 (1)	0	0.407
Nematode	*Trichostongylus* sp.	5.66 (3)	4.55 (2)	0.805	22.22 (24)	4.05 (3)	0.001*
	Stongyloides sp.	5.66 (3)	11.36 (5)	0.309	16.67 (18)	13.51 (10)	0.563
	Oesophagostomum sp.	5.66 (3)	0	0.109	10.19 (11)	0	0.005
	Trichuris sp.	0	13.64 (6)	0.006*	3.70 (4)	14.86 (11)	0.007
	Toxocara sp.	1.89 (1)	0	0.360	0.93 (1)	0	0.407
	Nematodirus sp.	7.55 (4)	0	0.063	2.78 (3)	0	0.148
	Haemonchus sp.	5.66 (3)	0	0.109	1.85 (2)	2.70 (2)	0.701
	Ostertagia sp.	0 (0)	0	0	0.93 (1)	0	0.407
Mixed		9.43	9.09	0.954	15.74	13.51	0.678
Overall		62.26	47.73	0.151	72.22	50.00	0.002*

n= total no. of animals, CI= Confidence Interval

4.1.3. Age specific prevalence of gastrointestinal parasites

Occurrence of gastrointestinal parasites influenced by the different age groups of animals where significance difference ($P<0.05$) was observed only in *Strongyloides* sp. in case of goats. However, adult showed greater susceptibility to some gastrointestinal parasites especially, *Fasciola* sp., *Paramphistomum* sp., *Trichuris* sp., *Toxocara* sp. and *Trichostrongylus* sp. than young and kid. Highest prevalence of *Fasciola* sp. was 29.17% in adult in goats and 29.82% in young sheep. Occurrence of *Paramphistomum* sp. was also highest in adult goats and sheep. *Oesophagostomum* sp. and *Nematodirus* sp. infection was

highest in kid which was 9.09% and 9.62% in goats and sheep, and 4.55% and 3.85% respectively. *Trichostongylus* sp. occurred in almost all age groups of the studied goats and sheep. Infection caused by *Toxocara* sp. and *Moniezia* sp. was only recorded in kids whereas no *Strongyloides* sp. infections were recorded in lamb which was shown in Table 4.

Table 4: Age specific prevalence of gastrointestinal parasites in goats and sheep

Traits	**Goats**				**Sheep**			
	Age Group				**Age Group**			
Gastrointestinal Parasites	**Kid (n=22) %**	**Young (n=27) %**	**Adult (n=48) %**	**P Value**	**Lamb (n=52) %**	**Young (n=57) %**	**Adult (n=73) %**	**P value**
Fasciola sp.	27.27 (6)	25.93 (7)	29.17 (14)	0.954	19.23 (10)	29.82 (17)	16.44 (12)	0.164
Paramphistomum sp.	4.55 (1)	11.11 (3)	14.17 (2)	0.457	5.77 (3)	8.77 (5)	13.70 (10)	0.323
Fascioloides sp.	0	0	0	0	5.77 (3)	0 (0)	1.37 (1)	0.100
Trichuris sp.	4.55 (1)	0 (0)	10.42 (5)	0.186	3.85 (2)	10.53 (6)	9.59 (7)	0.387
Toxocara sp.	0 (0)	0	2.08 (1)	0.597	1.92 (1)	0 (0)	0 (0)	0.285
Oesophagostomum sp.	9.09 (2)	3.70 (1)	0 (0)	0.122	9.62 (5)	3.51 (2)	5.48 (4)	0.396
Haemonchus sp.	9.09 (2)	0	2.08 (1)	0.160	0	3.51(2)	2.74 (2)	0.422
Trichostrongylus sp.	4.55 (1)	3.70 (1)	6.25 (3)	0.882	11.5 (6)	14.04 (8)	17.81 (13)	0.611
Strongyloides sp.	0	0 (0)	16.67 (8)	0.012*	9.62 (5)	22.81 (13)	13.70 (10)	0.142
Nematodirus sp.	4.55 (1)	7.41(2)	2.08 (1)	0.535	3.85 (2)	1.75 (1)	0 (0)	0.249
Ostertagia sp.	0	0	0	0	0	0	1.37 (1)	0.472
Moniezia sp.	0 (0)	0 (0)	0 (0)	0	1.92 (1)	0 (0)	0 (0)	0.285
Mixed	4.55 (1)	3.70 (1)	14.58 (7)	0.203	11.54 (6)	21.05 (12)	12.33 (9)	0.278
Overall	59.09 (13)	48.19 (13)	58.33 (28)	0.650	61. 54 (32)	66.67 (38)	61.64 (45)	0.806

* Significant (P<0.05)

4.1.4. Sex specific prevalence of gastrointestinal parasites

It was revealed that female goats showed more susceptibility to different gastrointestinal parasites than male, but it was not statistically significant but in case of sheep it was opposite. However, prevalence of *Fasciola* sp. was highest in female goats (31.17%) than female sheep (21.6%). *Trichostrongylus* sp., *Oesophagostomum* sp. and *Trichuris* sp. infections were also more in male sheep. *Strongyloides* sp. and *Paramphistomum* sp. infection was more in female sheep than male. Occurrence of *Oesophagostomum* sp., *Haemonchus* sp., *Trichostrongylus* sp., *Strongyloides* sp. and *Nematodirus* sp. were highest in male goats (Table 5).

Table 5: Sex specific prevalence of gastrointestinal parasites in goats and sheep

Traits	**Goats**			**Sheep**		
	Sex			**Sex**		
Gastrointestinal Parasites	**Male (n= 20) %**	**Female (n= 77) %**	**P Value**	**Male (n= 57) %**	**Female (n= 125) %**	**P value**
Fasciola sp.	15.00 (3)	31.17 (24)	0.151	21.05 (12)	21.60 (27)	0.932
Paramphistomum sp.	5.00 (1)	6.49 (5)	0.805	7.02 (4)	11.20 (14)	0.381
Fascioloides sp.	0	0	0	5.26 (3)	0.80 (1)	0.057
Trichuris sp.	0	7.79 (6)	0.197	10.53 (6)	7.20 (9)	0.449
Toxocara sp.	0	1.30 (1)	0.608	1.75 (1)	0	0.138
Oesophagostomum sp.	5.00 (1)	2.60 (2)	0.580	10.53 (6)	4.00 (5)	0.087
Haemonchus sp.	5.00 (1)	2.60 (2)	0.580	3.51 (2)	1.60 (2)	0.415
Trichostrongylus sp.	10.00 (2)	3.90 (3)	0.271	15.79 (9)	14.40 (18)	0.807
Strongyloides sp.	5.00 (1)	9.09 (7)	0.553	14.04 (8)	16.00 (20)	0.733
Nematodirus sp.	5.00 (1)	3.90 (3)	0.825	5.26 (3)	0	**0.010***
Ostertagia sp.	0	0	0	1.75 (1)	0	0.138
Moniezia sp.	0	0	0	0	0.80 (1)	0.498
Mixed	5.00 (1)	10.39 (8)	0.459	22.81 (13)	11.20 (14)	**0.041***
Overall	45.00 (9)	58.54 (45)	0.281	68.42 (39)	60.80 (76)	0.323

* Significant (P<0.05)

4.2. Prevalence of haemoprotozoan diseases on the basis of microscopic identification

4.2.1. Overall prevalence of haemoprotozoan diseases

The overall prevalence of haemoprotozoan diseases was 42.27 % in goats and 40.11% in sheep. The highest prevalence was recorded in Anaplasmosis which was 29.90% and 32.42% for goats and sheep, respectively. Occurrence of Babesiosis was 20.62 % in goats and 13.19% in sheep. Theileriosis and mixed infection (Babesiosis, Anaplasmosis and Theileriosis) was found relatively lower during this investigation (Table 6).

Table 6: Overall prevalence of haemoprotozoan diseases in goats and sheep

Haemoprotozoan Diseases	**Goats (n=97)**		**Sheep (n=182)**	
	(%)	**95% CI**	**(%)**	**95% CI**
Babesiosis	20.62	13.07 – 30.03	13.19	8.63– 18.98
Anaplasmosis	29.90	21.02 – 40.04	32.42	25.68 – 39.73
Theileriosis	8.25	3.62 – 15.60	7.69	4.26 – 12.56
Mixed infection	12.37	6.55 – 20.61	8.79	5.10 – 13.88
Overall	42.27	32.29 – 52.72	40.11	32.92 – 47.61

n= Total no. of population, CI= Confidence Interval

4.2.2. Seasonal prevalence of haemoprotozoan diseases

The prevalence of haemoprotozoan diseases more in summer than winter season. Highest prevalence of anaplasmosis was recorded 37.04 % in sheep followed by 26.42 % in goats in winter. Occurrence of babesiosis in two seasons in goats showed statistically significant difference (P=0.013). Infection caused by Babesiosis was highest in summer which was 31.82% in goats followed by 25.68 % in sheep. On the other hand, Occurrence of theileriosis was lowest among the haemoprotozoan infections recorded in summer and winter season but in case of goats it was statistically significant. Mixed infections were also very low in the study (Table 7).

Table 7: Seasonal prevalence of haemoprotozoan diseases in goats and sheep

Haemoprotozoan Diseases	**Goats**			**Sheep**		
	Seasons			**Seasons**		
	Winter (n= 53) %	**Summer (n= 44) %**	**P-Value**	**Winter (n=108) %**	**Summer (n=74) %**	**P-value**
Babesiosis	11.32	31.82	**0.013***	9.26	18.92	0.059
Anaplasmosis	26.42	34.09	0.411	37.04	25.68	0.108
Theileriosis	1.89	15.91	**0.012***	4.63	12.16	0.061
Mixed infection	1.89	25.00	**0.001***	1.85	18.92	0.000

* Significant (P<0.05)

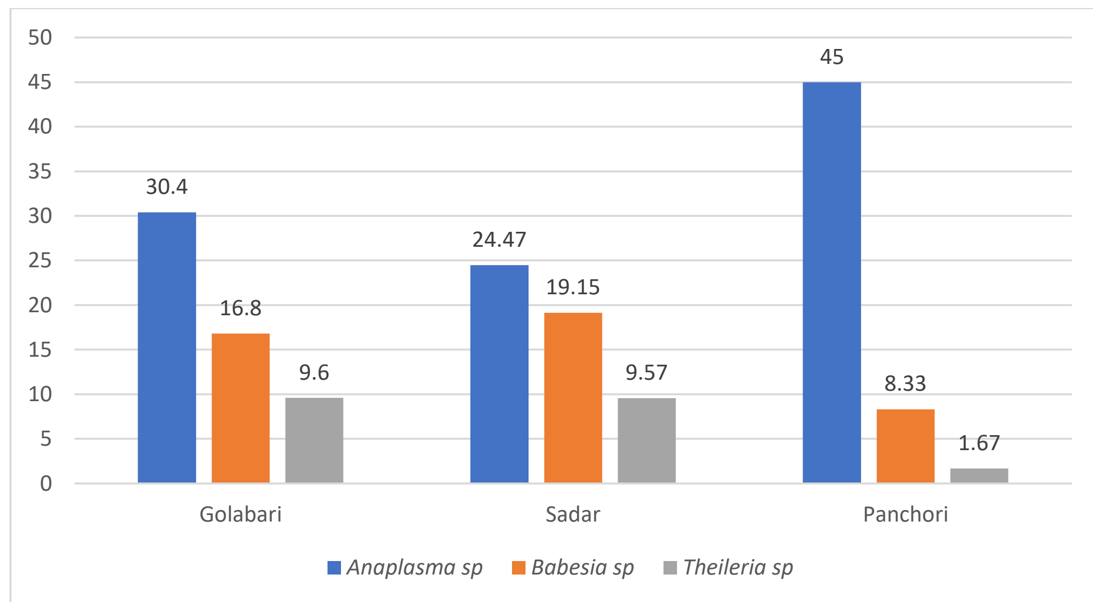


Fig. 2: Prevalence of haemoprotozoan infection in goats and sheep in different areas

In case of anaplasmosis, Panchori had highest (45%) prevalence in relation to Golabari (30.40%) and Sadar (24.47%). The prevalence of babesiosis was highest in Sadar (19.15%) followed by Golabari (16.8%) and Panchori (8.33%). The lowest prevalence of theileriosis was detected in Panchori (1.67%).

4.2.3. Age specific prevalence of haemoprotozoan diseases

Among three different age groups, young showed more susceptibility to different haemoprotozoan diseases than adult and kid and overall infection in young was 51.85% and 35.09% in goats and sheep, respectively. It was also observed that prevalence of babesiosis increased significantly ($P<0.05$) with the increase of age where highest prevalence was 33.33% and 13.46%, respectively in young goats and sheep. Occurrence of anaplasmosis was highest in kid than young and adult of the goats but in sheep, lambs had lower. Theileriosis and mixed infections were also varied according to age of animals during this study (Table 8).

Table 8: Age specific prevalence of haemoprotozoan diseases in goats and sheep

Traits	**Goats**				**Sheep**			
	Age Group				**Age Group**			
Haemoprotozoan Diseases	**Kid (n= 22) %**	**Young (n=27) %**	**Adult (n=48) %**	**P-Value**	**Lamb (n=52) %**	**Young (n=57) %**	**Adult (n=73) %**	**P-value**
Babesiosis	4.55	33.33	20.83	**0.046***	10.53	13.46	15.07	0.748
Anaplasmosis	31.82	25.93	31.25	0.868	28.07	34.62	34.25	0.698
Theileriosis	0	14.81	8.33	0.172	5.26	13.46	7.69	0.181
Mixed infection	0	14.81	16.67	0.131	9.62	7.02	9.59	0.850
Overall infection	36.36	51.85	39.58	0.479	42.31	35.09	42.47	0.647

* Significant ($P<0.05$)

4.2.4. Sex-Specific prevalence of haemoprotozoan diseases

Comparatively, higher prevalence of babesiosis was found in female which were 22.08% and 13.60% in female goats and sheep, respectively. However, male sheep showed a little more susceptibility to Anaplasmosis than female which was 38.60% but in case of goat, female (36.36%) are significantly highly susceptible than male (5.0%) which is statistically significant. Theileriosis and mixed infections also showed variations in their occurrence in male and female sheep and goat (Table 9).

Table 9: Sex-Specific prevalence of haemoprotozoan diseases in goats and sheep (Microscopic)

Haemoprotozoan Diseases	Goats			Sheep		
	Sex			Sex		
	Male (n=20) %	Female (n=77) %	P value	Male (n=57) %	Female (n=125) %	P value
Babesiosis	15.00	22.08	0.486	12.28	13.60	0.807
Anaplasmosis	5.00	36.36	**0.006***	38.60	29.60	0.229
Theileriosis	0	10.39	0.132	8.77	7.20	0.712
Mixed infection	0	15.58	0.059	8.77	8.80	0.995
Overall infection	20.00	48.05	**0.024***	47.37	36.80	0.177

* Significant (P<0.05)

4.3. Microscopic identification of ticks

Two types of ticks were found in goats and sheep in this study, namely *Boophilus* sp. and *Haemophysalis* sp. The prevalence of *Boophilus* sp. is highest in both goats (48.5%) and sheep (55.49%). Mixed infection of both *Boophilus* sp. and *Haemophysalis* sp. also found.

Table 10: Prevalence of ticks in goats and sheep

Species	Goats n=97	Sheep n=182
Boophilus sp.	48.5% (50)	55.49% (101)
Haemophysalis sp.	45.59% (47)	44.52% (81)
Mixed infection	29.1% (30)	27.47% (27.47)

4.4. Molecular identification of blood protozoa

A total 118 blood samples comprising of 44 samples from goats and 74 samples from sheep were screened by PCR for the presence of *Babesia* sp*., Anaplasma* sp. and *Theileria* sp. using primers targeting 18S rRNA, 16S rRNA, and 18SSU rRNA genes, respectively. The positive rates from amplification of 18S rRNA, 16S rRNA, and 18SSU rRNA genes in goats and sheep were 38.64% and 38.64%, 25% and 29.73%, 28.38% and 17.57%, respectively. During PCR, the amplified DNA fragment of 146 bp, 345 bp and 1098 bp for, *Babesia* sp., *Anaplasma* sp. and *Theileria* sp., respectively considered as positive (Fig. 12, 13, 14).

Tick infestation was identified in all of the samples. From total samples 12 samples were taken randomly, female ticks with eggs of both *Boophilus* sp. and *Haemophysalis* sp. were selected for DNA extraction. Extracted DNA sample was amplified for the detection of *Anaplasma* sp*., Babesia* sp. *and Theileria* sp. by amplification of specific fragment of selected genes. Four *Haemophysalis* sp. ticks were positive for the specific 146 bp fragment which indicates the presence of *Babesia* sp. (Fig. 12).

4.5. DNA sequencing:

Total seven amplicons were randomly selected for sequencing, two amplicons from three pathogen positive samples, one from goat and another from sheep for each pathogen were selected and another one was selected for one positive pathogen amplified from tick extract. All of the samples generated nucleotide sequences were suitable for further analysis. Bioinformatic analysis by BLAST method of three sequences of Babesia-18S rRNA gene were identified. Two out of three sequences from sheep and tick samples revealed homologues that were identical to *B. caballi and* another one from goat was identical to *Babesia ovis.* Next two sequences from Anaplasma- 16s rRNA revealed homologues identical to *A. bovis* and A. *phagocytophilum* one sample of caprine origin and another from ovine origin. The other two sequences of Theileria- 18ssu rRNA were from both goat and sheep yielded sequence homologues identical to *Theileria lewenshuni*. The E-values and nucleotide sequence identities of the homologous sequences are outlined in (Table 11)

Table 11: Results of *Babesia, Anaplasma* and *Theileria* species identified by BLASTn analysis using 18S rRNA, *16S rRNA and 18SSU rRNA* sequences of the isolates from goat and sheep in Khagrachari, Bangladesh

Sample	Species	Homologous sequence	E-Values	Ident.(%)
A66	Sheep	*Anaplasma phagocytophilum*	1e-107	92.58
A103	Goat	*Anaplasma bovis*	7e-154	100
B5	Sheep	*Babesia ovis*	5e-68	100
B13	Goat	*Babesia sp*	5e-68	100
BT5	Sheep; Tick	*Babesia ovis*	5e-68	100
T67	Sheep	*Theileria luwenshuni*	0.0	99.71
T104	Goat	*Theileria luwenshuni*	0.0	99.16

4.6. Results of nucleotides sequence alignment

4.6.1. Multiple sequence alignment of *Babesia* isolates with others from other regions

Multiple alignment of *Babesia* nucleotide sequences of the *B. ovis* isolate revealed that the sequences of samples from goat and sheep were conserved. The sequences of the *B. ovis* isolates from Spain, China, Iran, Turkey, and Tunisia were identical to the Khagrachari, Bangladesh isolates (Fig 3).

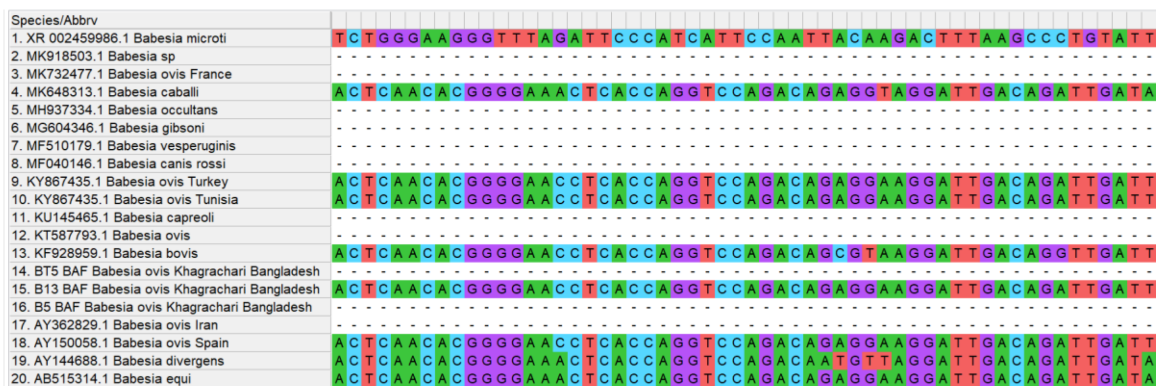


Fig. 3: A multiple sequence alignment of *18S rRNA* gene fragments for comparison of *B. sp* and *B. ovis* isolates isolated from goat and sheep. The conserved regions are represented by the dots (.) while the variable regions are indicated by the letters representing the nucleotide A, C, G and T

4.6.2. Multiple sequence alignment of *Anaplasma* isolates with others from other regions

Multiple alignment of Anaplasma nucleotide sequences of the *A. bovis* and *A. phagocytophilum* isolates revealed that the sequences of samples from goat and sheep were conserved. The sequences of the *A. bovis* isolates from China, India, Pakistan, Japan, and USA were identical to the Khagrachari, Bangladesh isolates. However, the sequences of the *A. phagocytophilum* isolates from China and India were identical to the Khagrachari, Bangladesh isolates the sequences of two isolates from South Africa and USA were different from the Kenyan Anaplasma isolates (Fig. 4).

Fig. 4: A multiple sequence alignment of *16S rRNA* gene fragments for comparison of *A. bovis* and *A. phagocytophilum* isolates isolated from goat and sheep. The conserved regions are represented by the dots (.) while the variable regions are indicated by the letters representing the nucleotide A, C, G and T

4.6.3. Multiple sequence alignment of *Theileria* isolates with others from other regions

In this study, the *Theileria* gene fragment sequences of the Khagrachari, Bangladesh isolates obtained from goat and sheep were homologous to *Theileria lewenshuni*. Multiple alignments of *Theileria lewenshuni* nucleotide sequences were done in order to compare the Khagrachari isolates with those from the other regions of the world. The sequences of

all the Khagrachari *Theileria lewenshuni* were identical (99%) to China, Turkey, and South Korea.

Sequence	Alignment
1. T104 THF Theileria luwenshuni This Study	GGGAGCCTGAGAAACGGCTACCACATCTAAG
2. T67 THF Theileria luwenshuni This Study	GGGAGCCTGAGAAACGGCTACCACATCTAAG
3. MK849886.1 Theileria orientalis	GGGAGCCTGAGAAACGGCTACCACATCTAAG
4. MH424326.1 Theileria velifera	GGGAGCCTGAGAAACGGCTACCACATCTAAG
5. L02366.1 Theileria parva	GGGAGCCTGAGAAACGGCTACCACATCTAAG
6. KY283963.1 Theileria luwenshuni Turkey	GGGAGCCTGAGAAACGGCTACCACATCTAAG
7. KU565346.1 Theileria luwenshuni South Korea	-------------------------------
8. KJ024366.1 Theileria lestoquardi	GGGAGCCTGAGAAACGGCTACCACATCTAAG
9. KC735169.1 Theileria luwenshuni China	GGGAGCCTGAGAAACGGCTACCACATCTAAG
10. DQ287944.1 Theileria annulata	GGGAGCCTGAGAAACGGCTACCACATCTAAG
11. AY533144.1 Theileria ovis	GGGAGCCTGAGAAACGGCTACCACATCTAAG
12. AF245279.1 Theileria youngi	GGGAGCCTGAGAGACGGCTACCACATCTAAG
13. AF162432.1 Theileria sp	GGGAGCCTGAGAAACGGCTACCACATCTAAG
14. AF078815.1 Theileria mutans	GGGAGCCTGAGAAACGGCTACCACATCTAAG

Fig. 5: A multiple sequence alignment of *18SSU rRNA* gene fragments for comparison of *Theileria luwenshuni* isolates isolated from goat and sheep. The conserved regions are represented by the dots (.) while the variable regions are indicated by the letters representing the nucleotide A, C, G and T

4.7. Phylogenetic analysis of the *Babesia, Anaplasma* and *Theileria* isolates

A Phylogenetic tree of *Babesia ovis* was constructed from the 18S rRNA in order to understand the genetic relationship of the *Babesia ovis* from Khagrachari, Bangladesh as compared with the isolates from others available in GenBank. The *Babesia ovis* isolates from both goat, sheep and tick from sheep has formed a well-supported clade with those from Spain, Turkey, Iran, and Tunisia (Fig. 6)

To understand the genetic evolutionary relation of the Khagrachari *Anaplasma species* and compare them with the isolates from Pakistan, India, China, Japan and USA, a phylogenetic tree was constructed from the 16s r RNA. *Anaplasma bovis* isolated from goat is very closely related with China isolate but the isolates from India (Arunachol, Mizoram) and Pakistan have the common origin. Additionally, the *A. phagocytophilum* from Khagrachari was found in the same clade of India (Fig. 7)

Another Phylogenetic tree of *Theileria lewenshuni* was constructed from the 18ssu rRNA in order to understand the genetic relationship of the *Theileria luwenshuni* from Khagrachari, Bangladesh as compared with the isolates from others available in GenBank. The *Theileria lewenshuni* isolates from both goat and sheep have formed a well-supported

clade with those from China, Turkey. The *Theileria lewenshuni* isolates from South Korea belonged to same clade but have recent common ancestor with the Khagrachari isolates. These findings indicate that some Kenyan A. ovis isolates are genetically close to isolates from Turkey, China, and South Korea (Fig. 8).

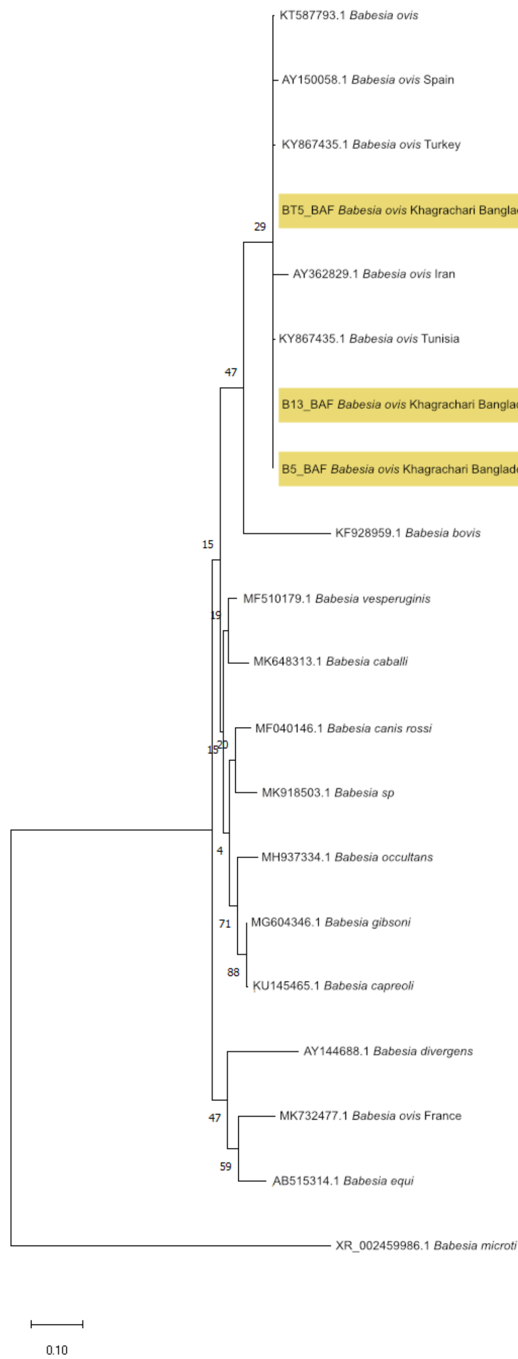


Fig. 6: Phylogenetic analysis based on partial *18S rRNA* gene sequences of the *Babesia* sp. isolated from goat and sheep in Khagrachari, Bangladesh and those from other regions of the world. The phylogenetic tree was constructed by the maximum likelihood method using MEGA X; Bootstrap analysis was performed with 1000 replicates with Kimura 2-parameter (K2) model

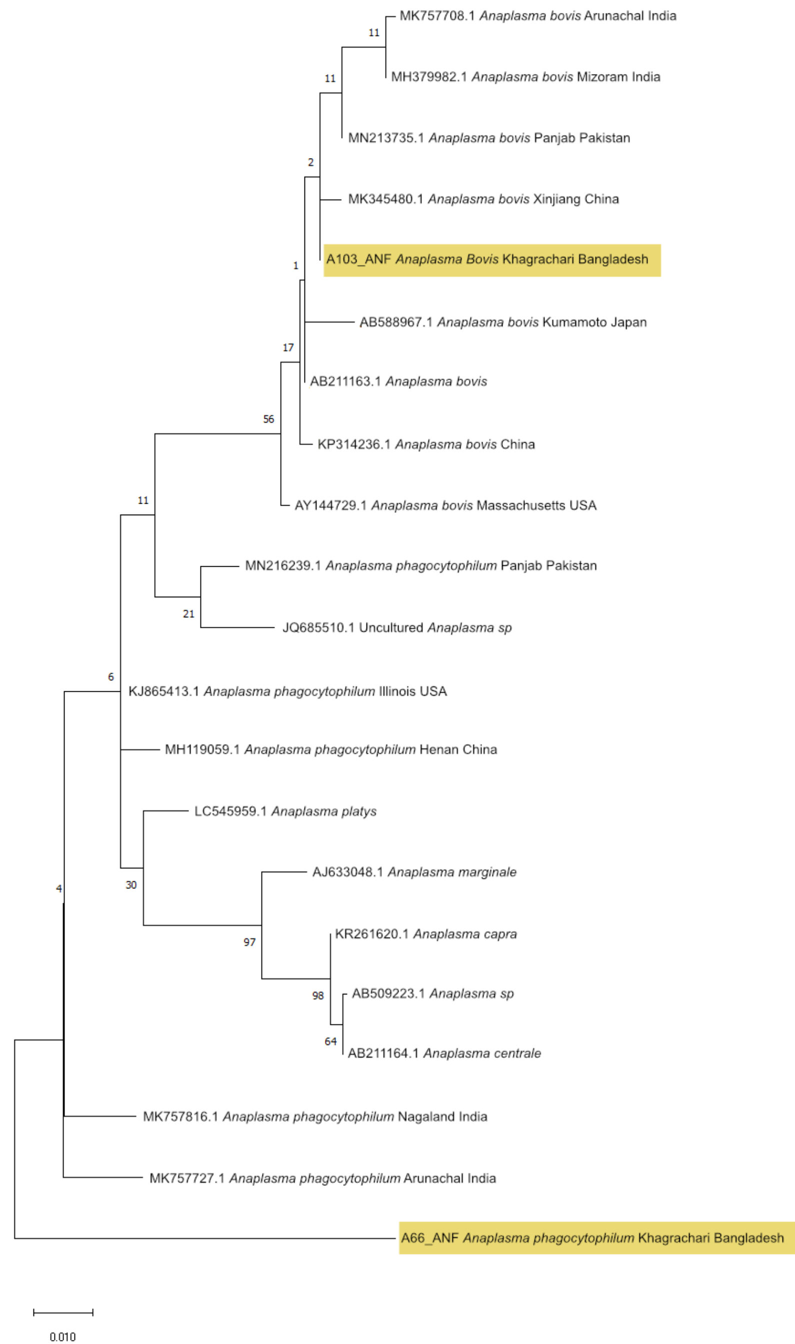


Fig. 7: Phylogenetic analysis based on partial *16S rRNA* gene sequences of the *Anaplasma* sp. isolated from goat and sheep in Khagrachari, Bangladesh and those from other regions of the world. The phylogenetic tree was constructed by the maximum likelihood method using MEGA X; Bootstrap analysis was performed with 1000 replicates with Kimura 2-parameter (K2) model

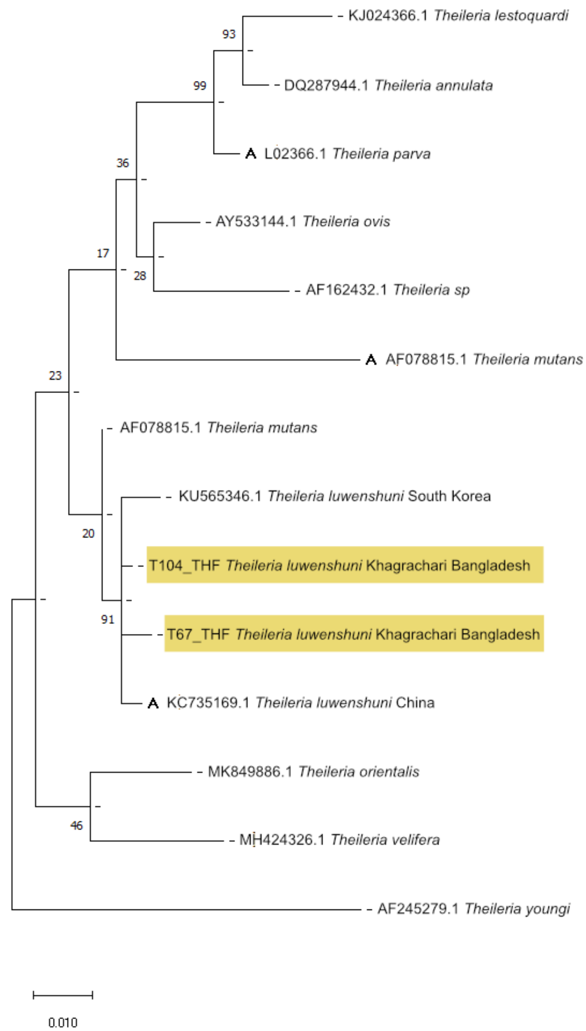
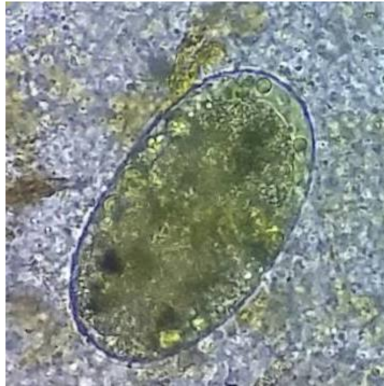
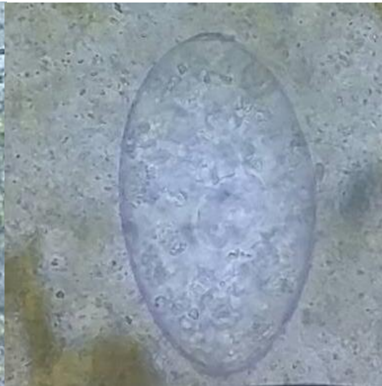
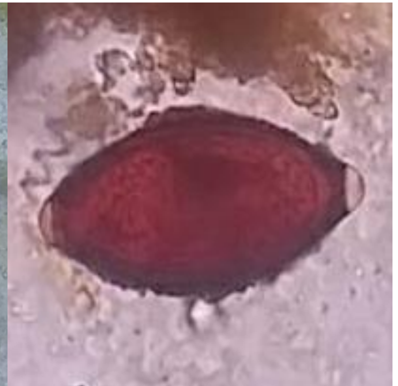
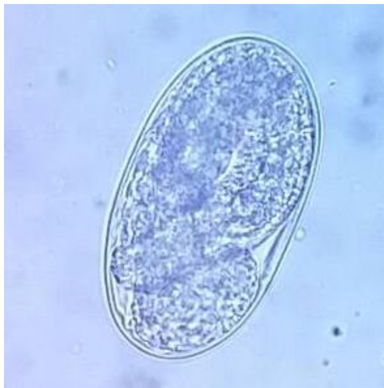


Fig. 8: Phylogenetic analysis based on the partial *18SSU rRNA* gene sequence of the *Theileria* sp. isolated from goat and sheep in Khagrachari, Bangladesh and those from other regions of the world. The phylogenetic tree was constructed by the maximum likelihood method using MEGA X; Bootstrap analysis was performed with 1000 replicates with Kimura 2-parameter (K2) model.

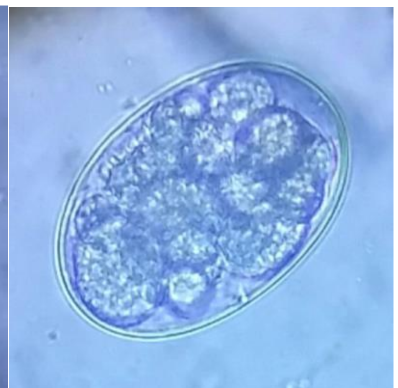

Fasciola sp.

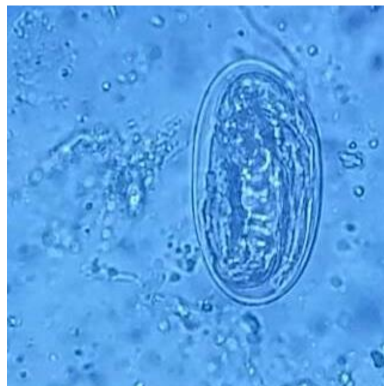

Paramphistomum sp.


Trichuris sp.


Strongyloides sp.

Trichostrongylus sp.


Haemonchus sp.


Oesophagostomum sp.

Nematodirus sp.

Fig. 9: Eggs of gastrointestinal parasites

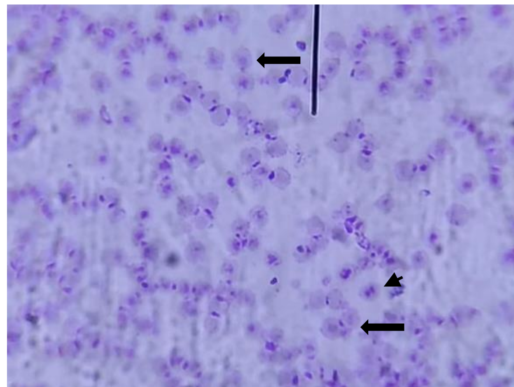


Fig. 10: Blood smear showing both *Babesia* sp. (arrowhead) and *Theileria* sp. (arrow) in a goat, Giemsa stain; 100X objective

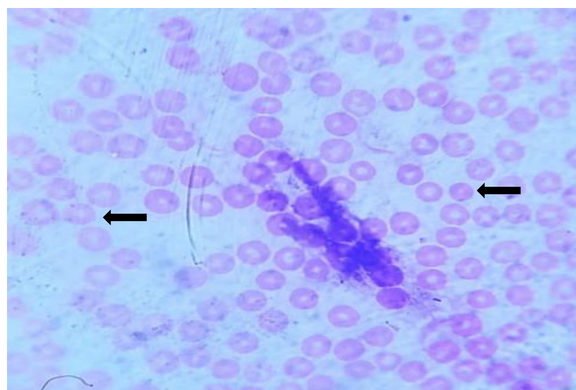


Fig. 11: Blood smear showing *Anaplasma marginale* (arrow) in a goat, Giemsa stain; 100X objective

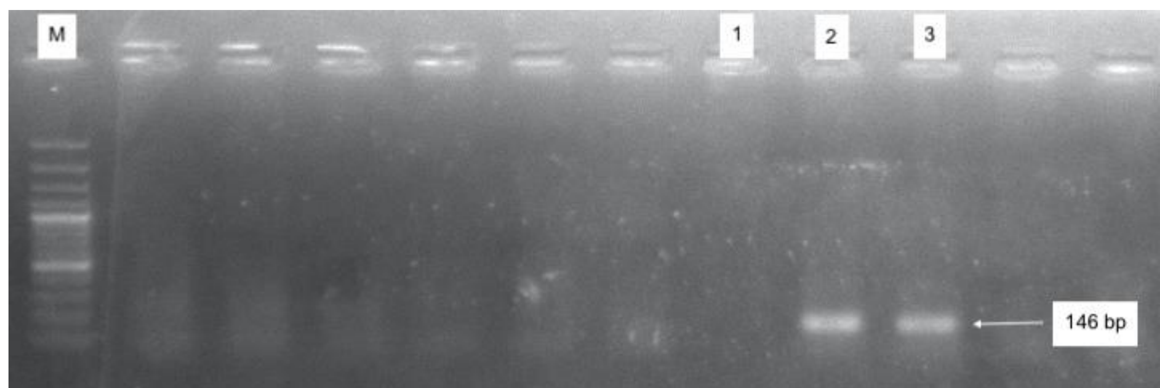


Fig. 12: Agarose gel electrophoresis of amplified PCR products obtained from *Babesia* sp. genomic DNA using *Babesia* specific primers. Lane M.: 100 bp DNA ladder; Lane 1. Negative control (Distilled water); 2.3. *Babesia* species DNA positive sample from sheep blood and tick sample, respectively

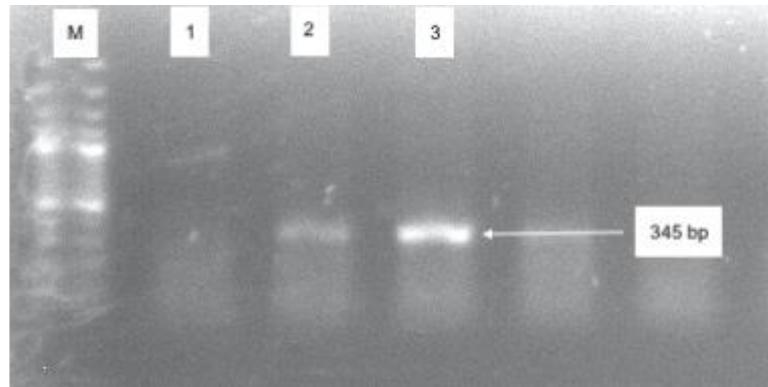


Fig. 13: Agarose gel electrophoresis of amplified PCR products obtained from *Anaplasma* sp. genomic DNA using *Anaplasma* specific primers. Lane M. 100 bp DNA ladder; Lane 1. Negative control (Distilled water); 2.3. *Anaplasma* species DNA positive sample from goat and sheep, respectively

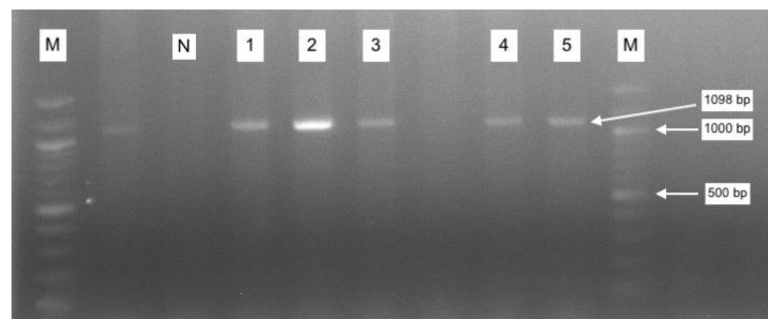


Fig. 14: Agarose gel electrophoresis of amplified PCR products obtained from *Theileria* genomic DNA using *Theileria* specific primers. Lane M. 100 bp DNA ladder; Lane N. Negative control (Distilled water); Lane 1.2.3. *Theileria* species DNA positive sample from goat; Lane 4.5. *Theileria* species DNA positive sample from sheep

Chapter-V
DISCUSSION

5.1. Prevalence of gastrointestinal parasites on the basis of microscopic identification

5.1.1. Overall prevalence of gastrointestinal parasites

The prevalence of gastrointestinal parasites in sheep is higher than goats of this study, consistence with the observation of Koinari et al., (2013), Islam et al., (2017), Chikweto et al., (2018). Similar findings also observed by Asif Raza et al., (2007); Dappawar et al., (2018); Singh et al., (2017); Win et al., (2020) who found higher prevalence of gastrointestinal infection in sheep than goats. But the previous study opposed by Islam and Taimur et al., (2008); Asif et al., (2008) and Jatau et al., (2011). However, the earlier observation partially consistence with the reports of Bhowmik et al*.,* (2020) who found 68.64% prevalence in sheep, 68.75% found in small ruminants by Singh et al., (2013). The prevalence of gastrointestinal parasites in small ruminants of the present study varied widely from the report of Jatau et al*.,* (2011) and Bhuyan (1970), who observed 95.51% in Nigeria and 71.6% in Bangladesh. Variation in the occurrence of gastrointestinal parasites might be due to geo-climatic conditions, sample size, breed, age, sex, level of nutrition, stress, accessibility of intermediate host, vegetation, grazing pattern, rearing and husbandry measures, anthelmintic therapy, genetic resistance etc. (Hansen and Perry, 1993).

Prevalence of *Fasciola* sp. in small ruminants of the current research showed similarity with the reports Islam and Taimur et al., (2008), who observed the *Fasciola* sp. is more prevalent in goats than sheep which was 14.28% in goats and 8.82% in sheep from different parts of Bangladesh. In this study fascioliosis is more prevalent in both goats and sheep which is similar with the observation reported by Bhowmik et al. (2020), Observation of this study varied from the reports of Koinari et al. (2013); Islam et al. (2018) who observed 5.5% in sheep and 18.2% in goats in Papua New Guinea and Mymensingh, Bangladesh, respectively.

Paramphistomum sp. of this study is second most prevalent trematode and this finding is consistent with the observation of Bhowmik et al., (2020). However, prevalence of *Paramphistomum* sp. showed somewhat consistency with the reports of Kakar et al. (2008) and Arman et al., (2018), who observed 7.82% in Quetta, Pakistan and 24% in

Mymensingh, Bangladesh, respectively. The earlier finding showed a discrepancy with the reports of Uddin et al., (2006); Pathak et al., (2008) and Siddiki et al., (2009), who recorded 65.28% and 56.66% in different region of Bangladesh, 80.68% in India and 38% in Chittagong district, Bangladesh, respectively. It was also reported that occurrence of Paramphistomiasis varies from 0.70% to 88.89% from place to place due to variation in geo-climatic conditions (Gupta et al., 1987 and Georgiev et al., 1980).

Occurrence of *Trichostrongylus* sp. in the present study merged with the findings of Khajuria et al., (2012); Nuruzzaman et al. (2012) and Arman et al., 2018, recorded 13.67 % in Jammu province, 16.00% in Thakurgaon district, Bangladesh and 15.56% and 5% in Chattogram and Mymensingh of Bangladesh. The earlier observation partially consistence with the report Shirale et al., (2008), who observed 4.85% in Akola district, India. Higher prevalence of *Trichostrongylus* sp. was recorded by Rajapakse et al., (2008), Kumsa et al., (2006) and Woldemariam (2003) who recorded 59%, 48.8%, 40.2%, 83-100% and 40%, respectively in different parts of the world. Lower prevalence of *Trichostrongylus* sp. observed by Lima (1998), who recorded less than 1% infection in Minas Gerais state, Brazil.

Prevalence of *Trichuris* sp. in goats and sheep was found in agreement with Iqbal et al., (2007) and Rahaman and Ahmed (1991), who reported 8.48% infection in Jhang district, Pakistan, and 7.1% in different areas of Bangladesh, respectively. But the earlier findings of this study varied widely from the reports of Radfar et al., (2011), Gadahi et al., (2008), Rajapak-se et al., (2008), Asif et al., (2008) who reported 44.75% in Iran, 35.48% in Pakistan, 59% in Sri Lanka and 62.5% in Pakistan, respectively. Conversely, lower prevalence of *Trichuris* sp. was reported by Koinari et al. (2012), and Nwosu et al. (2007) who recorded 2.9% and 3.6% respectively in Papua New Guinea, and Thailand, respectively. Variation of prevalence might be due to geo-climatic diversity, animal enterprises, husbandry measures, nutritional status, anthelmintic therapy etc. (Hansen and Perry, 1993).

Prevalence of *Oesophagostomum* sp. of this current research found inconsistence with the reports of Mohanta et al. (2007) who recorded 92%, 24.17% in different regions in Bangladesh. The observation also varied from the report of Woldemariam (2003); Waruiru et al., (2005) and Rajapakse et al., (2008), who recorded 33-83%, 88%, 13%, in different

corners of the world. In the present study, Oesophagostomum sp. infection was observed low which might be due to the relatively long-life cycle and low resistance to desiccation of the pre-infective stages of this genus (Rivera et al., 1983; Pfukeny et al., 2007).

Occurrence of *Moniezia* sp. was in accordance with the reports of Samad et al. (2004); Yadav et al. (2006) and Theodoropoulos et al. (2010) observed, who observed 1% in Mymensingh district, Bangladesh, R. S. Pura, Bishna and 0.4% in Greece, respectively. However, results of current study were not consistent with the reports of Nwosu et al., (1996) and Koinari et al., (2012) who observed 31% in Nigeriaand 9.1% in sheep and goats in Papua New Guinea.

Prevalence of *Strongyloides* sp. found in accordance with the observation of Shirale *et al.,* (2008); Arman et al. (2018) who recorded 11.14%, 6.48% and 15.56% in goats in Akola district, India, Dhaka and Chattogram, respectively. Occurrence of *Stongyloides* sp. was lower than the present study, observed by Sardar et al., (2006) and Garrels (1975), who recorded 1% infection in Mymensingh and 1.6% in Tangail, Bangladesh, respectively. Occurrence of *Strongyloides* sp. showed higher variation from the reports of Hassan et al., (2011), Lima et al., (2003), Waruiru et al. (2005), Nwosu et al. (1996) who recorded 51.74% in Chittagong district, Bangladesh, 72.8%, 51.6%, 83% in Brazil, Kenya and Nigeria, respectively.

In the present study, prevalence of *Haemonchus* sp., *Nematodirus* sp., *Ostertagia* sp., and *Fascioloides* sp. varied widely. Variation in the occurrence of helminths influenced by geo-climatic conditions (Lima, 1998 and Kakar et al., 2008), sample size, gender, age, plane of nutrition, livestock density, grazing pattern, physiological stress, concurrent infections, genetic factors as well as ecological factors of such parasites (Hansen and Perry, 1993 and Pfukeny et al., 2007). However, lower prevalence of such parasites infection in this study might be due to improved husbandry practices especially in crossbred cattle at farm level, irregular anthelmintics therapy and disease resistance capacity by the native cattle etc.

5.1.2. Seasonal prevalence of gastrointestinal parasites

The seasonal effect on the gastrointestinal parasites in small ruminants was found significant which is similar to the finding of Rahman et al., (2017). In this study, prevalence of gastrointestinal parasites was more in winter which agreed with the reports of Singh et

al., (2017) and Dappawar et al., (2018), who found higher prevalence of gastrointestinal parasites in winter than summer in western zone of Punjab, India. Findings of this study result disagree with the Yadav et al., (2006); Velusamy et al., (2015) and Islam et al., (2017), who observed highest prevalence of gastrointestinal parasites in summer than winter.

Prevalence of *Paramphistomum* sp. showed seasonal variation in both goats and sheep where highest prevalence recorded in summer which was in accordance with the earlier reports of Ollerenshaw and Rowland, (1959).

Prevalence of *Trichuris* sp. and *Strongyloides* sp. is higher in summer than winter which is supported by the observation of Khajuria and Kapoor (2003); Yadav et al., (2006) and Devina et al., (2007). Low prevalence during winter could be due to adverse climatic conditions in winter months, which help in arrested development in host and environment Occurrence of *Toxocara* sp., *Ostertagia* sp. and *Moniezia* sp. observed very low during the present study and no significant seasonal trends were noticed for these genus. It might be due to geographical diversity, mode of infection of such parasites, good hygienic measures, irregular or strategic anthelmintic therapy in cross bred cattle and genetic resistance of local cattle against such infections.

5.1.3. Age specific prevalence of gastrointestinal parasites

Though, statistically there exist no significant difference in different age groups of goats, adult goats appeared to be more susceptible to parasitic infections as compared to kid and young goats. Similar observations were reported by Sharma et al., (2013); Raza et al., (2014); Rahman et al., (2017) and Dappawar et al., (2018), who have observed that, age of the animals did not show any significant association with the prevalence of the parasites. In current study, influence of age on the occurrence of gastrointestinal parasites was observed and statistically significant variation ($P<0.05$) was found in Strongyloides sp. infection in goats. Prevalence of most of the gastrointestinal parasites of this study especially *Paramphistomum* sp., *Strongyloides* sp., *Trichuris* sp., *Trichostrongylus* sp., and *Fasciola* sp. were more in adult goats.

In case of sheep there was no significant difference among different age groups, where young are more susceptible than lambs and adults. Findings of the study showed

disagreement with Molla and Bandyopadhyay (2016), and Dappawar et al., (2018), who observed lambs are more susceptible than adults. In this study, higher prevalence in adult might be due to keeping the animal on low plane of nutrition, less availability of forage, maximum adult female animals were in lactation or in pregnancy and keeping them for a longer period of time in breeding and milk production purposes, long travelling, limited pasture fields, no extra or little feed was given to them other than grazing on nearby poor pasture fields or on roadside grasses.

5.1.4. Sex specific prevalence of gastrointestinal parasites

In the present study, infection caused by *Fasciola* sp., *Paramphistomum* sp., *Trichuris* sp., *Toxocara* sp., and *Strongyloides* sp. were found predominant in female than male goats and sheep. Findings of this study is in accordance with the reports of Rahman et al., (2017); Singh et al., (2017); Islam et al., (2017) and Dey et al., (2020), who also reported higher prevalence of helminths in female sheep and goats. On other hand, in both goats and sheep, *Trichostongylus* sp. and *Oesophagostomum* sp. infection were more in male than female which was in accordance with the reports of Dappawar et al., (2018). In this study, variation in occurrence of such helminths in male and female animals might be due to the variation in sample size or number of sample studied (Bachal et al., 2002), lowered resistance of female animals on the part of their reproductive events or temporary loss of acquired immunity near parturition (Garcia et al., 2007 and Barger, 1993), stress, genetic resistance of host and insufficient/imbalanced diet against higher needs (Raza et al., 2010 and Hansen and Perry, 1993).

5.2. Prevalence of haemoprotozoan diseases on the basis of microscopic identification

5.2.1. Overall prevalence of haemoprotozoan diseases

The prevalence of haemoprotozoan diseases in the present study were partially consistent with those of Gautam et al., (2018) who recorded 32.28% in goats in West Bengal, India. The incidence of haemoprotozoa has also been reported in different areas of the country and abroad. Mohanta et al., (2011) and Hasan et al., (2019) also found comparatively higher prevalence of haemoprotozoan diseases in livestock of hilly areas. On the contrary, Mohammed and Idoko (2013) examined the goat for haemoprotozoa and found 24.70%

goat samples were positive. According to, Zangana and Nakid (2011) the difference in the results may be due to the climatic and geographical variations of the study areas as well as may be due to the difference between husbandry practices and study design.

It was observed that haemoprotozoan diseases occurred more in sheep than goats which was supported by the earlier reports of Radwan and El Kelesh (2009); Jatau et al., (2011), Velusamy et al., (2015) and Hasan et al., (2019).

The prevalence of babesiosis of this study was higher than the observation revealed by; Jatau et al., (2011) and Jewel et al., (2020) who recorded 0.97% and 2.06% in sheep and goats, respectively and 5% in goats. However, the prevalence of Babesiosis in goats was partially consistence with those of Mohanta et al., (2011) who recorded 16.63% in hilly areas of Bangladesh. Findings of goat supported the report of Naderi et al., (2017) from Iran reported higher percentage of babesiosis in goats (17.0%) than compared to sheep (12.41%) but Razmi et al., (2003) from Iran who recorded 23.5% and 0.5% of B. ovis in sheep and goats, respectively which differs from the present study result.

Prevalence of Anaplasmosis is highest in both sheep and goat in this study which agrees with the previous reports (Okaiyeto et al., 2008; Jatau et al., 2011; Adamu and Balarabe, 2012). Similar findings also reported by Velusamy et al., (2015), Jewel et al., (2020) and Hassan et al., (2019). Moreover, anaplasmosis is ubiquitous in nature which has been reported in all the six continents (Rymaszewska and Grenda, 2008) and notably in the tropics and subtropics, due to the plenty of its tick vectors (Jongejan and Uilenberg, 2004). Prevalence of Theileriosis of this current research supported by the findings of Magzoub *et al.,* (2020), who recorded 13% *Theileria lestoquardi* infection in Sudan, and according to Irshad et al., (2010). Prevalence in sheep was 7.36%, while in goats it was 3.8% in Pakistan and partially consistent with Kamani et al*.,* (2010) and Enwezor et al*.,* (2009), who recorded 3.1% *Theileria mutans* infection in Kaduna State, Nigeria. The earlier findings of this study greatly differed from the reports of Radwan and El Kelesh (2009), who recorded Theileria infection in sheep and goats was 33.75% and 28% respectively, in Egypt.

In current research, variation in the prevalence of such haemoprotozoan diseases might be due to distinct geo-climatic conditions prevailing during the study period, sample size, breed, age, sex, seasons, vector availability, rearing pattern, genetic resistance, concurrent infections etc. However, microscopic examination alone is not a fully reliable method of its diagnosis. It may be coupled with other sero and molecular diagnostic assays.

5.2.2. Seasonal prevalence of haemoprotozoan diseases

Haemoprotozoan diseases fluctuate greatly according to seasons. The prevalence of haemoprotozoan diseases was found more in summer than winter season. This might be due to the lower temperature and humidity of winter which is less advantageous for the growth and proliferation of tick vectors which might cause to lower frequency of such diseases in the study population (Muhammad et al., 1999 and Zahid et al., 2005). The above observation was in accord with the reports of Mohanta et al., (2011), Velusamy et al., (2015), Gautam et al., (2018) and Jewel et al., (2020), who suggested that higher incidence of blood protozoa was found at summer and rainy season.

5.2.3. Age specific prevalence of haemoprotozoan diseases

Age also impacts the rate of haemoprotozoan diseases. In case of goats, prevalence of haemoprotozoan diseases in young was more than kids and adult. In case of sheep, highest prevalence was found in lambs and adults which is supported by the findings of Luo and Yin (1997) who found 78-85% and 9% in lambs and adults, respectively and Egbe-Nwiyi et al., (2017), who observed highest prevalence in adults than young.

Findings of Babesiosis of this study varied significantly ($P<0.05$) which was supported by the Soulsby, (1986); Urquhart et al., (1996) and Kage et al., (2019), who reported an inverse age resistance of the disease. According to Smith et al., (2000) and Goff et al., (2003), this phenomenon is only reported in cattle and horses. However, it is evident that puppies, kids, and lambs that are unprotected by maternal antibody can be more severely affected by Babesia than adult animals (Yeruham et al., 1998 and Bai et al., 2002) which supports the present finding of this study where the babesia infection in lambs is high.

Prevalence of Anaplasmosis was more in young followed by adult which was in line with the reports of Radostits et al., (1994) and Jewel et al., (2020), who observed higher prevalence of anaplasmosis in less than 1 year of age. Observation of this study also consistence with the findings of Mohanta et al., (2011) and Rajasokkappan et al., (2016), who recorded animal between 1-2 years of age are highly affected by Anaplasmosis. According to, Arunkumar (2014) the overall prevalence of infection was found to 9.2% and sheep above 2 years of age were highly prone for the infection. Higher prevalence in adults might be due to enhanced wandering activity for search of fodder, breeding and marketing.

Prevalence of Theileriosis in kids and lamb partially consistent with Savini et al., (1999), who observed that infection significantly increased with age, but the earlier findings differed from the report of Urquhart (1996), who observed that young stocks were more susceptible, and a degree of innate resistance usually limits mortality to a low level. In the present study, no infection was found in kids which might be due to a smaller number of sample size or acquired immunity by the kid.

5.2.4. Sex-Specific prevalence of haemoprotozoan diseases

Genders of animals have somewhat influence in the existence of haemoprotozoan diseases. The prevalence of haemoprotozoan disease in female animals showed uniformity with the opinion of Gautam et al (2018); Shah et al., (2017) and Egbe-Nwiyi et al., (2017), who recorded relatively higher prevalence in female than male goats of West Bengal, India. Females are generally believed to be more susceptible to haemo-parasitaemia due to their prolonged breeding for economic reasons (calving and milk production) as well as the stress of breeding, milking and hormonal changes associated with pregnancy, parturition, and lambing processes (Ukwueze, 2015).

5.3. Microscopic identification of ticks

In this study two types of ticks are found which are *Boophilus* sp. and *Haemophysalis* sp. and similar observation was reported by Mohanta et al., (2011) and Sarker et al., (2010). Between two species identified, *Boophilus* sp. is well distributed in all over the country.

This distribution pattern of ticks has formerly been described by Mondal et al., (1995, 1996). In this study sheep is more infested with ticks than goat which is supported by the findings of Irshad et al., (2010). *Boophilus* sp. is more prevalent in both goats and sheep than *Haemophysalis* sp. and similar finding was reported by Noor et al., (2016).

5.4. Molecular characterization of blood protozoa

The distribution of *Babesia* sp. overlaps with the distribution of transmitting *Rhipicephalus* and *Haemaphysalis* ticks. It is found that Haemaphysalis ticks are responsible for transmitting *B. motasi* (Uilenberg et al., 2006). *B. crassa* has been detected in Haemaphysalis ticks in Europe and in Turkey (Orkun et al., 2014 and Hornok et al., 2015). In this study Babesia sp. is detected by using the amplification of specific gene fragment from extracted DNA of *Haemophysalis* sp. tick which is quite similar with the findings of Mihaljica et al., (2012) in Serbia in dog, Hong et al., (2019) in Korea in Human and Orkun et al., (2019) in hedgehogs in Turkey, Azmi et al., (2016) in sheep in Palestine and Song et al., (2018) in sheep in China.

5.4.1. Detection of haemoprotozoan parasites from blood and tick sample by PCR

The prevalence of babesiosis in goats and sheep was recorded 38.64% and 29.73%, respectively. Theodoropoulos et al., (2006) found 3% and 16% of prevalence in goats and sheep. The result may differ due to the presence of tick which suggest a high risk of infection with Babesia in sheep and goats. Ticks are suitable for transmission of Babesia has been recorded in Greece (Papadopoulos et al.,1996b).

Anaplasmosis caused by *Anaplasma ovis* is the most frequent pathogen in sheep and goats, with high serological and biomolecular prevalence (Torina et al., 2010 and Liu et al., 2012). A PCR-based molecular analysis proved that Anaplasma are highly prevalent in goats in central and southern China, and the average prevalence of single infections with *A. ovis*, *A. bovis*, or *A. phagocytophilum* was 46.6%, 49.6%, or 14.5%, respectively which supports the result of present study where the prevalence is 38.64% and 28.38% in goats and sheep, respectively. whereas the prevalence of *A. phagocytophilum* was 6.7% in both sheep and goats according to Zhan et al., (2010).

Several Theileria species can cause theileriosis in sheep and goats. Of these, *T. lestoquardi* which is reflected to be the most highly pathogenic; the other species of Theileria such as *T. ovis* were less pathogenic and had lower economic significance than *T. lestoquardi* (Uilenberg, 1981). It is challenging to distinguish these two species on the basis of morphology of piroplasm and schizont stages especially in mixed infection. Also, *Theileria* species of sheep and goats can be detected routinely by conventional microscopic examination, but it lacked sensitivity and creates inconsistency of results, especially in asymptomatic or carrier animals since the parasitemia level remain very low (El-Zeedy et al.,1998). In the present study, PCR was used for the detection *Theileria* species of sheep and goats. 25% sheep and 17.57% goats were positive for *Theileria* species specific primer at 1098 bp. These results are supported by d'Oliveira et al (1995) who used *Theileria* species specific primer, determined from small subunit gene for amplification of the expected 1098 bp DNA fragment from all *Theileria* species samples.

5.5. Phylogenetic analysis of the *Babesia, Anaplasma* and *Theileria* isolates

The infection of *Babesia ovis* was found in all the surveyed areas of Khagrachari district in this study, and the high infection (38.64% and 29.73%in goat and sheep) signifies the wide- ranging distribution of this parasites in the study area. The infections were confirmed by sequencing the 18S rRNA gene, which has been proved useful for phylogenetic studies and genetic characterization of *Babesia* sp. Phylogenetic analysis of 18S rRNA sequences from Khagrachari and previously obtained 18S rRNA sequences from other countries revealed four 18S rRNA genotypes in goats and sheep from Iran, Tunisia, Turkey, and Spain, implying that it is distributed worldwide. In this study amplicon isolated and sequenced from tick has formed a highly similar clade with the isolates from goat and sheep, it indicates the presence of *Babesia ovis* and evident of its transmission through tick.

Anaplasma sp. is the most ubiquitous and highly diverse blood protozoa infecting both human and animals. Despite of having higher diversity, high resemblances of the 16S rRNA gene sequences were identified among different *Anaplasma* sp., several 16S rRNA gene variants have been identified (Katargina et al., 2012 and Kawahara et al., 2006). In this study, two 16S rRNA variants of *Anaplasma* sp. were reported in sheep and goats.

Anaplasma phagocytophilum from sheep and *Anaplasma bovis* from goat are identified. *Anaplasma phagocytophilum* under the genus *Anaplasma* shows a high degree of genetic assortment, host tropisms and variation in pathogenicity (Barakova et al., 2014). Phylogenetic analysis revealed that *Anaplasma phagocytophilum* from Khagrachari, Bangladesh, clustered with 92.58% identity, with the strains isolated from Arunachal and Nagaland from India, indicating the similarity with the neighboring country. (Fig. 7). Whereas *Anaplasma bovis* isolated from goat, clustered with 100% identity, with the strains isolated from China, Japan, India, and Pakistan, indicating that it circulates in a wide variety of vertebrate hosts in Asia.

The prevalence of Theileriosis in small ruminants in Bangladesh has been investigated in previous studies but to my understanding this is the first molecular and genetic diversity investigation of *Theileria* sp. in Bangladesh. Moreover, the phylogenetic tree revealed that all the 18SSUrRNA sequences in this study has created one main cluster with the other *T. luwenshuni* sequences from China and Turkey, despite showing little distance with South Korea. This probably suggests that there might be two distinct SSU rRNA genotypes of *T. luwenshuni*.

CHAPTER-VI

CONCLUSION

The study provides baseline information about the prevalence of different gastrointestinal parasites and haemoparasites in goats and sheep of three regions of Khagrachari district and also studied with the effect of different associated factors on the occurrences of infection. The result of the current study will give epidemiological forecasting in the occurrence of such diseases as well as more precise diagnosis of haemoprotozoan diseases and their vectors which will help to better understand the epidemiology of parasitic diseases. In future, the results of the study based on molecular investigations will help to organize effective vaccination and drugs administration schedule to control and prevent the distribution of parasitic diseases.

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