Chapter -1

INTRODUCTION

In Bangladesh 45.9 percent of total households own bovine stack of livestock. The density of livestock population per acre of cultivated land is 7.37.The density has been increasing every year in the country. The country has relative density of livestock population well above the average for many other countries of the world. Cattle the largest group of ruminants in the country. Bovine Babesiosis can make significant impact on the international cattle trade for any country exporting meat and meat products (Bock et al., 2004).

Members of the genus *Babesia* are tick-transmitted intraerythrocytic protozoan parasites and can hinder the growth of livestock sector. This causes serious economic impact due to death, decreased productivity, lowered working efficiency (Uilenberg, 1995), increased cost for control measures (Makala *et al.*, 2003) and limited introduction of genetically improved cattle in an area (Radostits *et al*., 2000). Among different species affecting cattle, *B. bovis* and *B. bigemina* are the most economically important species globally.

The routine diagnosis is usually accomplished by routine microscopic examination (Giemsa or Wrights stained) of blood smears collected from the peripheral circulation. However, the classical staining techniques are suitable for detection of acute and heavy infections but unable to identify sub-clinical infections where the parasitemia is usually much lower (Almería et al., 2001; Aziz et al., 2014). Different serological techniques such as IFAT and ELISA are also commonly used for the diagnosis of subclinical infection of Babesiosis (Bock et al., 2004; Molloy et al., 1998). Epidemiological surveillance is an important aspect to control Babesiosis in the endemic areas.

In Bangladesh, the prevalence of babesiosis in cattle has been reported in different areas by some investigators. Most of the published studies were based on the classical blood smear examination (Giemsa stain) and the prevalence was recorded as 1-14% with a variable sample size (Samad et al., 1989; Siddiki et al., 2010). Those reports also investigated the risk factors associated with *Babesia* prevalence in Bangladesh based on age, sex, climate and seasons.

Chittagong District is the most important dairy belt of Bangladesh and the density of cattle population of this district is high. The climatic condition and geographical location of the areas might favor the growth and multiplication of ticks which act as natural vectors of babesiosis.. Considering the above scenario, the present study was designed with the following specific objectives:-

1. To determine the prevalence and risk factors of babesiosis in cattle in selected areas of Chittagong District, Bangladesh.
2. Develop recommendations to formulate control strategy against babesiosis.

Chapter –2

MATERIALS AND METHODS

2.1: Study areas

The study was conducted in the hilly and the plain areas of 3 different southern parts of Chittagong. These include Chittagong Metropolitan area(Plain), Anwara Upazilla (semi Hilly), Rangamati sadar (Hilly).

2.2: Target animals

Holstein Friesian (HF) crossbred (*Bos taurus* X *Bos indicus*) and indigenous cattle (*Bos indicus*) were selected for this study as target animals.

2.3: Study design

A questionnaire was used to record relevant information comprising owner's name and address, animal Identification (ID), farm size, breed, age, sex, housing history and farmer's economic status etc. Farmer's economic status were categorized into viz. i) Poor ii) Moderate and iii) ultra poor. Housing history was treated as floor type and categorized into paka (cemented) and mati (soil) floor. Selected animals were categorized into three age groups: calves (≤1 year), young (>1–2.5 years) and adult (≥2.5 years). Samples were collected randomly in two consecutive seasons; summer (March to May), rainy (June to August).

2.4: Sample collection and microscopic examination:

Approximately 3-5 ml of blood sample was collected from jugular vein using 10 ml disposable plastic syringe from each animal and carried in vials containing EDTA (7.2 mg) and two or three thin peripheral blood films by puncturing ear vein from each cattle. The vials were carried to the Parasitology laboratory of Chittagong Veterinary and Animal Sciences University (CVASU) in a cool box and kept in refrigerator at 4°C until further use.

2.5: Staining method:

The prepared thin blood smears was stained with the Giemsa stain and allowed to stay for 25-30 min. After rinsing with running tap water, the stained blood smears were air-dried and examined under binocular microscope (1000X) for the identification of blood parasites (Urquhart et al., 1996).

2.6: Data analysis:

Obtained data were analyzed by using statistical software 'STATA/IC-11.0' where descriptive statistics was expressed as proportion with 95% confidence interval (CI). For Chi-Square Test, results were expressed in percentage with P-value and significance was determined when $P<0.05$**.**

Photo Gallery

All the images related to this study were taken from sites during handling.

Fig 2.1: Clinical sign of Babesiosis. Feces of affected cows is dark, tar-like, and contain dark red to black clots of digested blood.

Fig 2.2: Thin Blood smear examination after staining with Giemsa stain.

Chapter -3

RESULTS

To investigate the prevalence of babesiosis classical microscopic technique was used for screening field samples. Total 63 whole blood samples (13 from hilly areas, 9 from semi hilly and 41 from plain areas of Chittagong Division) were collected from cattle without considering the clinical signs (Some of the cattle showing clinical sign also included). From blood smear examination (Giemsa staining technique), 12.7% animals were found positive for *Babesia* spp. Infection where the organisms in thin blood smears appeared as pear-shaped bodies usually located in periphery of the infected RBCs (Fig 3.2). Topographical study exposed that a substantial variation in prevalence of Babesiosis in different geographic areas. The highest overall prevalence was recorded in plain area Anwara (33.3%) but it was not significant.

To identify the association of different categorical variables such as season, breed, age, sex; cattle shed floor category and economic capacity of the animal owners, obtained data were analyzed by using statistical software 'STATA' (Table 3.1). The Season is one of the most influencing factors on the prevalence of blood parasitic diseases. During the present study, the prevalence of Babesiosis was higher in summer seasons (15.8%) followed by rainy and winter seasons (8%) (Figure 3.2).The overall prevalence of babesiosis was 13.3% in crossbred and 11.1% in indigenous cattle. Age specific prevalence of infections revealed that adult cattle showed more susceptibility to babesiosis than calves. The highest prevalence (15.9%) was observed in adult crossbred cattle. However, female cattle were more prone to babesiosis than male. Higher prevalence of babesiosis was recorded in female (14.3%) where male disease prevalence is low (7.1%). Economic status of the farmer also acts as an influencing factor where poor status of farmer cause more prevalence of disease than moderate status. When farm housing conditions were assessed, the prevalence of babesiosis was recorded higher in animals that are kept in uncemented (mati) floor (13.5%) in compared to animals that were kept in cemented (paka) floor (9.1%).

Variables	Categories (N)	Prevalence (%)	P value
Breed	Cross breed (45)	13.3	
	Local breed (18)	11.1	0.588
Age	Upto 1 year (14)	0	
	>1 year- 2.5 year (45)	15.9	0.315
	>2.5 year (4)	16.7	
Sex	Female (42)	14.3	0.42
	Male (13)	7.1	
Floor	Mati (52)	13.5	0.574
	Cemented (11)	9.1	
Economic status	Moderate (56)	8.9	0.21
	Poor (6)	33.3	
	Ultra poor (1)	100	

Table 3.1: Association of different categorical variables with the prevalence of Babesiosis (by using software STATA)

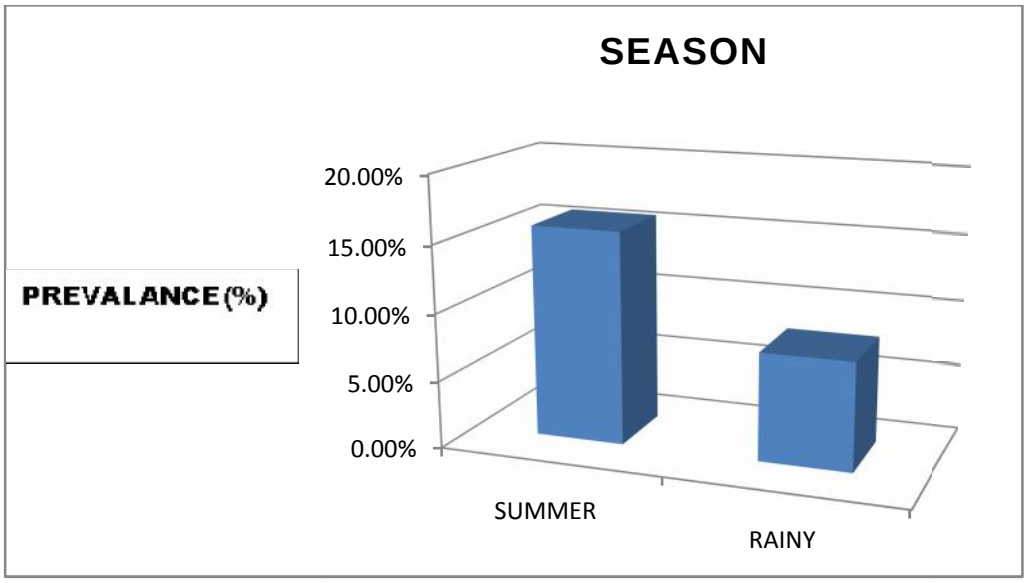

Fig 3.1: Prevalence of *Babesia* according to season
(Here X axis indicates the season and Y axis Indicate the prevalence. Higher prevalence was recorded in summer season in compared to rainy season)

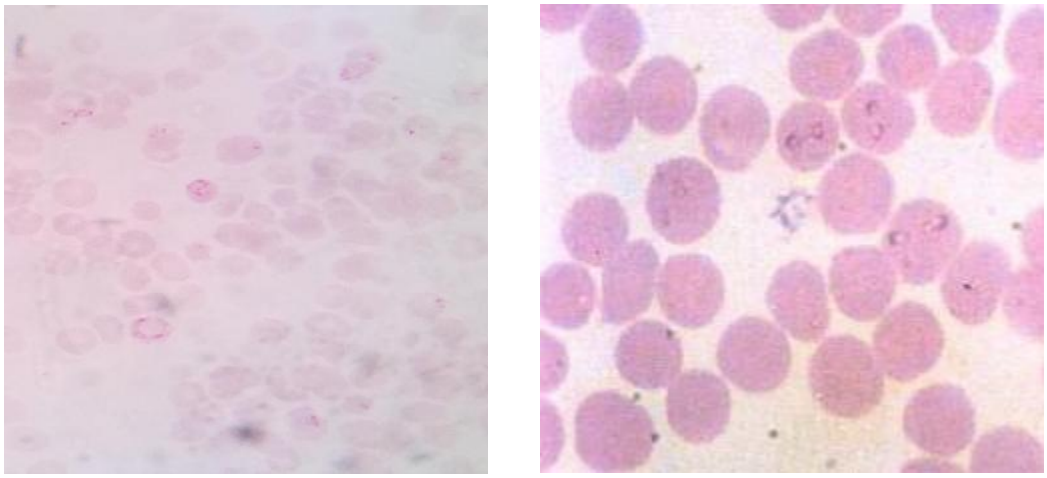

Fig 3.2: *Babesia spp.* located inside the RBCs. Identified by Giemsa stained thin blood smear.

Chapter - 4

DISCUSSION

The overall prevalence of Babesiosis recorded during this study was 12.7% (6.3-23.8%) higher than the earlier report of (Mannan *et al.* 2017) who recorded prevalence 1.43% in Chittagong. Higher prevalence in the current study might be due to small sampling no of my study and may be due to the climate-associated factors and the distribution of relevant vector ticks that might be active and available in different season as the study period of my sampling based on 2 seasons. Further higher prevalence of up to 16% was also reported by other reports where samples were collected from northern districts of Bangladesh (such as Mymensingh and Sylhet) (Banerjee et al., 1983; Nath and Bhuyian., 2013).

According to topographical location maximum prevalence of babesiosis was recorded in semi-hilly areas followed by plain areas and hilly areas. These were partially consistent with other previous reports who recorded prevalence higher in coastal followed by plain then hilly areas in Chittagong (Mannan *et al.* 2017).

Higher prevalence was recorded in case of female animals (14.3%) than male (7.1%).The prevalence of babesiosis in female cattle of this investigation showed uniformity with the report of (Alim *et al.* 2012). Higher prevalence in female cattle is possibly due the fact that they were kept longer for breeding and milk production purpose, supplied insufficient feed against their high demand (Kamani *et al.*, 2010). The similar observation was reported earlier by other investigators (Mannan et al., 2017; Kamani et al., 2010).

Age also influences the occurrence of the infections. The highest prevalence of Babesiosis was found in the aged >1-2.5 year. Similar observation was reported by other researchers (Chakraborti, 2002; Chowdhury et al., 2006; Rahman et al., 2015) and however severity of the disease increased with ageing, but the infection rate were

detected higher in young animals. It may be due to declining of immunity with the increasing of age (Wright, 1990). This observation were supported by the observation of (Annetta et al. 2005) who reported an inverse age resistance of the disease where adult showed more susceptibility than calves. This might be due to rapid immune responses to primary infection by the calves through a complex immune mechanism (Annetta et al., 2005). Endemic instability of the study areas might responsible for frequent infections in adult cattle where newborn calves were protected by colostral immunity (Cynthia et al., 2011). Age resistance, perhaps in combination in some cases with maternal antibodies, is reflected in the reduced number of clinical outbreaks in young animals.

The present study recorded a higher prevalence of Babesiosis in summer season which was observed by other researcher (Mannan et al. 2017,Al Mahmud et al., 2015; Alim et al.,2012, ,Rony et al.,2010). It may be due to prevalence of ectoparasites/ tick infestation was higher in summer season. The rise of infestation in summer may be due to rise of temperature in late winter leading to gradual increase in the load as well as percentage of infestation in May and June (Roy et al. 2001).

The prevalence of babesiosis in this study was comparatively higher in crossbred cattle than local cattle although the observations were statistically insignificant. The findings were similar to the observation by previous investigators (Al Mahmud et al., 2015; Alim et al., 2012; Chowdhury et al., 2006:). The zebu cattle, *B. indicus* reportedly show strong innate resistance to *B. bovis* and *B. bigemina* infections when compared to *B. taurus* and their crosses (Bock et al., 1997). The lower prevalence in local cattle might be linked to constant minimum exposure to infections and development of passive immunity along with the genetic makeup of each different cattle breed (Siddiki et al., 2010).

The prevalence of babesiosis was recorded higher in animals that are kept in uncemented (mati) floor in compared to animals that were kept in cemented (paka) floor. Similar finding was observed by others also. It may be due to higher vector load in muddy floor (Nath and Bhuyian, 2013).

LIMITATIONS

Small number of samples was considered for investigation which may results higher prevalence rate. Epidemiological investigation didn’t cover the all factors. Besides these molecular identification and phylogenetic investigation was not done in this study.

Chapter - 4

CONCLUSION

From the above discussion it is concluded that the prevalence of babesiosis is the most common and frequent in Chittagong district of Bangladesh which may be due to its geoclimatic conditions, high density of cattle population, low land/flood plain based area and high density of tick population. Here a number of risk factors including age, sex, season, housing condition etc., were assessed with variable results. The study indicates that regular strategic prophylactic treatment and use of acaricides should be ensured especially in summer season in order to the control of babesiosis. Further analyses with more sensitive and reliable diagnostic tools like PCR assay along with subsequent sequencing and identification of tick vectors can be useful to formulate effective control strategies of babesiosis.

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BIOGRAPHY

I am Arjuman Lima, daughter of Md. Mahabubur Rahaman and Mst. Zarna Tara Begum. We are two siblings. I was born in Chittagong. I passed my Secondary School Certificate (SSC) examination in 2008 from Kapashgola Girls High School, Chittagong and Higher Secondary Certificate (HSC) in 2010 from Govt. Women's college, Chittagong. Now I am an Intern student under the Faculty of Veterinary Medicine in Chittagong Veterinary and Animal Sciences University. I firmly believe that two factors determine one's success. Determination is the main while getting support from others is another factor. In future, I would like to work devotedly to uphold the dignity of veterinary profession in our society.

Appendix-1:

Questionnaire used for collection of data (Blood parasite- *Babesia*)

Farm level data

1. Name of the farm:……Mobile No…….
2. Address:……..Ward……Thana…….District…
3. Economic condition of owner:
   1=ultra poor, 2=poor, 3=Moderate
4. Receive training on Farming**:** 1=Yes; 2=No
5. Record of farm data? 1= Yes; 2= No
6. Current population of cattle at farm ……
7. Farm composition:
   Total adult female……
   Total calf: Male ……Female ……
   Total male animal……
8. Last year farm dynamics/changes: animals bought……..animal sold…….
9. Floor type, 1=Paka (Cemented/semi-semented), 2= Mati (uncemented)
10. Drainage system of the farm: 1= Good 2= Moderate 3= Poor 4= Nothing
11. Position of drain:
    1= Back of the stall/house 2=between animal rows (middle) 3= Other
12. Frequency of the cleaning of the floor: 1= Once daily 2=every alternate day
    3=more than once daily 4=No schedule
13. Do you use disinfectant for cleaning? 1= Yes (Frequent) 2= No
14. Do you practice routine vaccination? 1= Yes; 2= No
15. Do you practice routine deworming? 1= Yes; 2= No

Individual animal data

- Sl. no.
- Age
- Sex
- Breed
- Current status
- Clinical sign