OPEN ACCESS Veterinary Sciences: Research and Reviews

Research Article

Hemoprotozoal Infections in Cattle in the Barishal Division of Coastal Region of Bangladesh: Epidemiology, Hematology, and Tick Vector Insights

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Abstract | Tick borne hemoprotozoal diseases such as babesiosis, theileriosis and anaplasmosis cause significant economic losses in livestock production. Accurate identification of both vector and pathogen residing within the vector is essential for controlling these diseases. In this study, 217 blood samples and 470 ticks were collected from cattle in selected areas of Barishal, Patuakhali, Jhalokathi and Bhola districts in the coastal region of Bangladesh. Hemoprotozoa were identified using Giemsa staining. For Giemsa-stained positive cases, total RBC count, PCV, Hb concentration, and urinalysis were performed using traditional method. The overall prevalence of tick-borne diseases was 14.74%, with cases of babesiosis, theileriosis and anaplasmosis accounting for 6.45%, 5.06% and 3.23% respectively. The prevalence of blood protozoa was analyzed concerning season, age, sex, breed, and extent of tick infestation. Babesiosis was most prevalent during the rainy season, theileriosis during summer season, and anaplasmosis during winter season. The study revealed that female and crossbred cattle were more susceptible to hemoprotozoan diseases compared to their male and indigenous counterparts. Babesiosis and theileriosis were significantly more common in older animals, whereas anaplasmosis showed no such age-related pattern. Cattle infested with ticks had a significantly higher prevalence of hemoprotozoan diseases (51.85%, 14/27) compared to cattle without tick infestation (9.47%, 18/190). Hematological findings showed that cattle affected by babesiosis, theileriosis, and anaplasmosis had significantly reduced red blood cell (RBC) counts, hemoglobin levels, and PCV percentages compared to healthy cattle. Additionally, brown-colored urine was observed in cattle infected with babesiosis, which was not seen in cases of theileriosis or anaplasmosis. The infected cattle were predominantly infested with *Rhipicephalus* (*Boophilus*) and *Haemaphysalis* ticks. The hematology and urinalysis data provide insights into treatment options for hemoprotozoal diseases, while the findings on the epidemiology and vector detection highlight the need for effective control strategies to reduce the spread of these diseases in the coastal region of Bangladesh.

Editor | Muhammad Abubakar, National Veterinary Laboratories, Park Road, Islamabad, Pakistan.
Received | December 23, 2024; **Accepted** | January 27, 2025; **Published** | February 08, 2025
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Citation | Uddin, M.R., S.S. Saha, K.J. Alam, M.S. Alam and S. Rani. 2025. Hemoprotozoal infections in cattle in the Barishal division of coastal region of Bangladesh: Epidemiology, hematology, and tick vector insights. *Veterinary Sciences: Research and Reviews*, 11(1): 26-38.
DOI | https://dx.doi.org/10.17582/journal.vsrr/2025/11.1.26.38
Keywords | Hemoprotozoa, Coastal region, Hematology, Epidemiology, Tick

Introduction

Livestock plays a vital role in the rural socio-economic system as maximum households are directly involved in livestock farming related activities. Cattle are pivotal components of the mixed farming systems in Bangladesh and are found everywhere in the country. They are mainly reared for milk and meat, although skin and manure are also important products from this animal. The population of livestock is about 560 lakh (Bangladesh Economic Review, 2021). More than 80% of rural people rear indigenous cattle. At present many crossbred cattle are available throughout the country. However, upgrading of livestock is limited by several factors, including the damage caused by various hemoprotozoan diseases transmitted by tick. Hemoprotozoan diseases, namely babesiosis, anaplasmosis and theileriosis, are the tick-borne diseases (TBDs) of cattle, distributed throughout the world, particularly in tropical and subtropical countries including Bangladesh (Ghosh *et al*., 2007). Tick-borne diseases cause substantial losses to the livestock industry throughout the world (Ananda *et al*., 2009; Rajput *et al*., 2005) as these have got a significant economic impact due to obvious reason of death, decreased productivity, increased cost for control measures (Makala *et al*., 2003) and limited introduction of genetically improved cattle in an area (Radostits *et al*., 2000).

Bovine babesiosis, caused by *Babesia bigemina* and transmitted by *Boophilus microplus*, is common in Bangladesh. The progressive development of the babesiosis is the result of hemolytic anemia caused by the parasite, and the excess production of cytokines and other components related to the immune response leads to the progression of the disease (Bock *et al*., 2004). Babesia parasites cause destruction of red blood cells resulting in anaemia, icterus, weight loss, infertility (Ellis *et al*., 2003) in a wide range of mammals including cattle. At least 7 species of Babesia parasites including *Babesia microti*, *Babesia divergens*, and *Babesia venatorum* infect humans and sometimes cause severe symptoms and occasionally death. Theileriosis, caused by *Theileria annulata* and transmitted by ticks of the genus *Hyalomma* has emerged as one of the fatal diseases of crossbred animals in last two decades. *Theileria* sp. is responsible for tropical theileriosis which has been reported from various parts of the world (Oliveira *et al*., 1995; Singh *et al*., 2017; Durrani *et al*., 2006; Tavassoli *et al*., 2011). Anaplasmosis, caused by *Anaplasma marginale* and *A. centrale*, are obligate intracellular parasite that causes anaemia by infecting erythrocytes in cattle. The reticuloendothelial cells phagocytize the infected erythrocytes causing the development of anaemia and jaundice. The pathogenicity of anaplasmosis depends on the species and age of animal. Young calves show an innate resistance to the disease while the acute form occurs in animal from 1 to 3 years. Cattle that survive anaplasmosis become carriers for life and act as a reservoir of infection for susceptible animals (Aubry and Geale, 2011).

Recent studies have shown the substantial effect of hemoprotozoal infections on livestock in South Asian countries such as India, Pakistan and Bangladesh (Bhatnagar *et al*., 2015; Atif *et al*., 2023; Hosen *et al*., 2020; Bary *et al*., 2018). These findings underscore the necessity for efficient management and preventative efforts to alleviate the effects of hemoprotozoal illnesses in South Asia. Bangladesh is usually hot and humid except in winter, and the geo-climatic condition of Bangladesh is highly favorable to a wide variety of parasites including ticks of various species such as *Rhipicephalus microplus*, *Haemaphysalis bispinosa*, *Rhipicephalus sanguineus*, *Hyalomma anatolicum*, *Hyalomma truncatum*, and *Amblyomma testudinarium* (Ghosh *et al*., 2007; Islam *et al*., 2006; Roy *et al*., 2018a). Although the presence of blood protozoa in Bangladesh has been recorded for many years (Samad *et al*., 1989; Chowdhury *et al*., 2006; Roy *et al*., 2018b), there are few available data on the prevalence and hematological analysis of hemoprotozoal disorders in the coastal region, and no comprehensive report exists on their impact on cattle in this area or in Bangladesh as a whole.

The coastal belt of Bangladesh, particularly the regions of Barisal, Patuakhali, and Bhola, is one of the country's most important dairy-producing areas, with the dairy sector expanding significantly. This region plays a vital role in the national economy, with the livestock sector contributing substantially to its growth. The topography and climatic conditions of the coastal belt are distinct from those of other regions in Bangladesh. The humid environment in this area affects the biology and spread of arthropods, including ticks, making the dynamics of disease transmission different from other parts of the country. Additionally, cattle farming systems in this region vary considerably compared to other areas, further influencing disease prevalence and management.

Precise identification of infections and vectors is essential for effectively controlling hemoprotozoal illnesses. However, there is a notable lack of statistical information on the prevalence of hemoprotozoan diseases, their impact on cattle health, and the tick vectors associated with these diseases in the coastal regions of Bangladesh. This study aims to address these knowledge gaps by focusing on the following objectives:

- To identify and assess the prevalence of haemoprotozoal diseases in cattle in the coastal areas of Bangladesh.
- To evaluate the hematological and urinalysis parameters in cattle infected with these diseases.
- To identify tick species and their role in transmitting hemoprotozoal diseases.

Materials and Methods

Sampling area

The experiment was conducted by the Department of Pathology and Parasitology, Faculty Animal science and Veterinary Medicine, Patuakhali Science and Technology University, Babuganj, Barishal, during the period from March 2021 to February 2022. Blood and tick samples were collected from 217 cattle, both tick infested and non-infested cattle, in the selected areas of Barishal, Patuakhali, Jhalokathi and Bhola districts of coastal regions of Bangladesh. Samples were collected both random basis and in-person contact with upazila livestock office of the disease outbreak area. Date, age, breed, sex and clinical signs of all studied animal were recorded in the questionnaire. Seasons were categorized into three seasons such as summer (March-June), rainy (July-October) and winter season (November-February). The research identified hemoprotozoa by Giemsa-stained blood smears, detecting *Babesia*, *Theileria*, and *Anaplasma*. Hematological characteristics (RBC count, PCV, Hb concentration) and urinalysis (brown-colored urine indicative of Babesia) were evaluated in confirmed cases. Tick vectors, mainly *Rhipicephalus* (*Boophilus*) and *Haemaphysalis*, were morphologically identified to associate infestation with disease prevalence. In order to assess tick-borne diseases in cattle, this method included clinical, hematological, and vector information. The Institutional Ethical Approval Committee of the Patuakhali Science and Technology University, Bangladesh (No. 33(1)), approved this research project on May 16, 2022. The committee found that the experimental design of the study did not violate animal ethics.

Collection of blood

Blood was collected from the ear vein of animal for making smear through needle prick. For hematological analysis, 2.5 ml of blood was collected from the jugular vein of the clinically suspected animals in EDTA containing vacutainer tubes, and transported to laboratory in ice box.

Preparation of smear

Both thick and thin blood smear was prepared for every animal. Thick smear was prepared for detection of infected animal and thin smear was prepared for the identification of parasite. Smear was air-dried, and then dipped into 100% methanol; a coupling jar with a screw top was used for doing this. After one minute, the slides were removed and placed on end to drain the alcohol. Then they are placed into a plastic slide box for complete drying. For distant places, the plastic slide box was placed into a zip-lock bag with silica gel, and they are allowed to dry overnight.

Collection and preservation of Arthropods

Tick samples were collected from the same animal where blood sample was collected. If ticks are not present in animal body, then it was collected from animal rearing area using flagging method. Tick was collected using fine tip forceps gently. Collected ticks were put into a 2 ml screw cap tube and preserved in 80% alcohol for future study.

Giemsa staining

Preparation of Giemsa stain: Giemsa stain was prepared in a traditional method. Briefly, 3.8 gram of Giemsa powder was mixed with 250 ml of pure glycerol and 250 ml of pure methanol. The mixture was kept in a well-closed bottle containing glass beads for at least 48 hours and agitated frequently during that time. The stock buffer was kept in the refrigerator for storing of months. The mixture can be kept outside at room temperature for several weeks. Stock solution was filtered through filter paper. Stain was diluted with buffered water before staining.

Preparation of staining buffer: Staining buffer was prepared by adding 10.79gm of PBS powder in 1000 ml distilled water. The suspension was heated to dissolve completely and then sterilized by autoclaving.

Steps of staining smears: A layer of stain was placed in the bottom of a coplin jar (about 3 mL), then buffer was added to a level that will just cover the slides (except for labeled end) when they are in the jar. Amount of buffer to add was adjusted after several trials. Slide was stained at room temperature for about 50 minutes. Slide was removed and rinsed by dipping a few minutes into plain buffer or streaming under tap water to remove excess stain, and then was stand on end to dry.

Examination of blood smears under compound microscope and documentation

Immersion oil was placed directly on the smear without adding coverslip for observing under compound microscope 100x. Protozoa was identified according to the identification of blood parasites as described by Soulsby (1982).

Haematological analysis

Hematological analysis was conducted using representative samples. A total of six blood samples were collected from animals that appeared to be in good health, while 28 blood samples were retrieved from animals who tested Giemsa staining positive for hemoprotozoa. The traditional approach (Rosenfeld and Dial, 2010) was used to measure the total erythrocyte count (TEC), packed cell volume (PCV), and haemoglobin.

Urinalysis

In order to do a physical examination of urine and determine its specific gravity, a recently voided midstream urine sample was collected and placed in a sterile container. The color, clarity, odor, and volume of the urine were observed and recorded. A calibrated refractometer or urinometer was employed to determine the specific gravity.

Morphological identification of tick and documentation

Collected tick was fixed on glass slides with adhesive tape. Morphological identification and documentation were performed under stereo microscope using standard taxonomic keys as described by Walker (2003) Ticks of domestic animals in Africa - a guide to identification of species.

Statistical analysis

Statistical analysis was performed using IBM SPSS statistics 25.0 for Windows. The Chi-square test was used to conduct univariate analysis in order to assess the significance of the relationships among various protozoa infections and other epidemiological variables, such as season, age, sex, intensity of tick infestations, and hematological parameters. Observed differences were considered to be statistically significant at a 0.05 threshold value ($p < 0.05$). In the table * denotes P value< 0.05, ** = $P < 0.01$, *** = $P < 0.001$.

Result and Discussion

Prevalence of hemoprotozoan diseases

Overall prevalence of hemoprotozoan diseases: A total of 217 blood samples were collected from four specific districts, namely Barishal, Bhola, Jhalokathi, and Patuakhali, located in the coastal region of Bangladesh. Of these, 32 samples (14.74%) were found to be positive for hemoprotozoan infections. The microscopic examination using Giemsa staining identified the following: specifically, 14 samples (6.45%) tested positive for babesiosis (Figure 1A), 11 samples (5.06%) tested positive for theileriosis (Figure 1B), and 7 samples (3.23%) tested positive for anaplasmosis (Figure 1C) when examined under a microscope using Giemsa stain (Table 1).

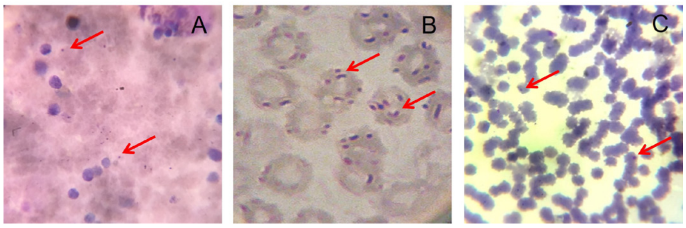

Figure 1: *Cattle RBC with Giemsa-stained hemoprotozoan parasites (100x). (A) Pear-shaped Babesia (arrowhead) was seen in thick smeared RBCs. In cattle RBCs, comma-shaped Theileria sp. (B) and dot-shaped Anaplasma sp. (C).*

Table 1: *Prevalence of hemoprotozoan diseases in cattle.*

Name of diseases	No. of cattle tested	No. of positive case	Prevalence (%)
Babesiosis	217	14	6.45
Theileriosis		11	5.06
Anaplasmosis		7	3.23
Overall prevalence		32	14.74%

Season-wise prevalence: Understanding season-wise prevalence of hemoprotozoan disease is crucial for predicting potential outbreaks and implementing effective preventative measures. The study revealed the following seasonal trends: Babesiosis was most prevalent during the rainy season (12.5%), followed by the summer (4.12%) and winter (2.08%) seasons. Theileriosis had the highest prevalence in the summer season (6.18%), with similar prevalence rates in the rainy (4.16%) and winter (4.16%) seasons. Anaplasmosis was most common during the winter season (6.25%), followed by the rainy season (2.77%) and summer season (2.06%). The prevalence of babesiosis was found the highest in rainy season (12.5%) in relation to summer (4.12%) and winter (2.08%) season. But the prevalence of theileriosis was observed the highest in summer season (6.18%), followed by rainy (4.16%) and winter (4.16%) season. The prevalence of anaplasmosis was found the highest in winter season (6.25%), followed by rainy (2.77%) and summer (2.06%) season (Table 2). These findings emphasize the need for season-specific disease management strategies to mitigate the impact of hemoprotozoan diseases.

Sex-wise prevalence: Table 3 shows the sex-wise prevalence of hemoprotozoan diseases in cattle. The prevalence of babesiosis, theileriosis, and anaplasmosis was slightly higher in females (7.38%, 6.04%, and 4.02%, respectively) than in males (4.41%, 2.94%, and 1.47%, respectively). However, these differences were not statistically significant.

Age-wise prevalence: Age-wise prevalence studies of hemoprotozoan diseases are crucial for developing targeted, effective, and age-specific prevention, management, and treatment strategies, as age plays a significant role in infection occurrence. The highest prevalence of babesiosis and theileriosis was found in cattle over 3 years of age (11.39% and 10.12%, respectively), followed by cattle aged 2 to 3 years (6.15% and 3.33%, respectively), and cattle aged 6 months to 2 years (1.36% for both diseases). On the other hand, the highest prevalence of anaplasmosis was observed in cattle aged 6 months to 2 years (4.10%), followed by cattle aged 2 to 3 years (3.07%) and cattle over 3 years of age (2.53%) (Table 4).

Table 2: *Season-wise prevalence of hemoprotozoan diseases in cattle.*

Season	Babesiosis			Theileriosis			Anaplasmosis		
	Positive cases	%	P value	Positive cases	%	P value	Positive cases	%	P value
Summer (n=97)	4	4.12	0.034* (<0.05)	6	6.18	0.45 (>0.05)	2	2.06	1.9 (>0.05)
Rainy (n=72)	9	12.5		3	4.16		2	2.77	
Winter (n=48)	1	2.08		2	4.16		3	6.25	

Table 3: *Sex-wise prevalence of hemoprotozoan diseases in cattle.*

Sex	Babesiosis			Theileriosis			Anaplasmosis		
	Positive cases	%	P value	Positive cases	%	P value	Positive cases	%	P value
Male (n=68)	3	4.41	0.40 (>0.05)	2	2.94	0.33 (>0.05)	1	1.47	0.977 (>0.05)
Female (n=149)	11	7.38		9	6.04		6	4.02	

Table 4: *Age-wise prevalence of hemoprotozoan diseases in cattle.*

Age	Babesiosis			Theileriosis			Anaplasmosis		
	Positive cases	%	P value	Positive cases	%	P value	Positive cases	%	P value
6 month - 2 years (n=73)	1	1.36	0.0373 (<0.05)	1	1.36	0.331 (<0.05)	3	4.10	0.856 (>0.05)
2-3 years (n=65)	4	6.15		2	3.33		2	3.07	
>3 years (n=79)	9	11.39		8	10.12		2	2.53	

Table 5: *Breed-wise prevalence of hemoprotozoan diseases in cattle.*

Breed	Babesiosis			Theileriosis			Anaplasmosis		
	Positive cases	%	P value	Positive cases	%	P value	Positive cases	%	P value
Local (n=90)	1	1.11	0.007** (<0.01)	1	1.11	0.025* (<0.05)	2	2.22	0.48 (>0.05)
Crossbred (n=127)	13	10.23		10	7.87		5	3.93	

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Table 6: *Hematological findings (Mean±Standard error) of hemoprotozoa affected cattle.*

Parameters	Healthy cattle (n=6)	Babesiosis (n=12)	Theileriosis (n=10)	Anaplasmosis (n=6)
TEC (10^6/cmm)	7.55±0.18	3.03±0.36***	6.18±0.29*	5.38±0.47*
Hb (g/l)	11.96±0.15	5.53±0.16***	7.56±0.33***	7.13±0.62**
PCV (%)	34.87±0.75	16.07±0.70***	25.25±1.36**	21.17±2.12**

Table 7: *Prevalence of hemoprotozoan diseases in cattle related to tick infestation.*

Tick infestation	No. of animal examined	Babesiosis			Theileriosis			Anaplasmosis		
		Positive cases	%	P value	Positive cases	%	P value	Positive cases	%	P value
Tick infested	27 (51.85%)	5	18.5	0.006** (<0.01)	5	18.5	0.00066** (<0.01)	4	14.8	0.00027** (<0.01)
Non- tick infested	190 (9.47%)	9	4.73		6	3.15		3	1.57	

Breed-wise prevalence: Studies on the frequency of hemoprotozoan diseases in different breeds are crucial for developing effective, focused, and long-lasting strategies to manage these diseases. The occurrence of babesiosis and theileriosis was markedly higher in crossbred cattle (10.23% and 7.87%, respectively) compared to local cattle (1.11% for both diseases). The prevalence of anaplasmosis was slightly higher in crossbred cattle (3.93%) than in local cattle (2.22%), although the difference was not statistically significant (Table 5).

Hematology

Hematology provides critical insights into the pathophysiology, diagnosis, management, and epidemiology of hemoprotozoan infections. Babesiosis exhibits the most prominent hematological alterations among the examined diseases, with considerably reduced Total Erythrocyte Count (TEC), Hemoglobin (Hb), and Packed Cell Volume (PCV) in comparison to healthy cattle (Table 6). Theileriosis and anaplasmosis similarly cause notable decreases in TEC, Hb and PCV, however the severity is less pronounced compared to babesiosis (Table 6).

Urinalysis

Urinalysis is a relatively simple and non-invasive diagnostic tool. Urine of affected animal examined physically; it was appeared coffee color that is the characteristics of babesiosis. No such urine color was observed in case of theileriosis and anaplasmosis affected animal. Specific gravity of urine was compared between affected and unaffected animal, but no such remarkable variation was observed.

Prevalence related with tick infestation

This investigation assessed the relationship between hemoprotozoan diseases and the presence of ticks on the surfaces of cattle bodies. The prevalence of all three hemoprotozoan diseases (babesiosis, theileriosis and anaplasmosis) was significantly higher in tick infested cattle (18.5%, 18.5% and 14.8%, respectively) compared to cattle that appeared to be tick-free (4.73%, 3.15% and 1.57%, respectively) (Table 7).

Morphological identification of tick

Tick vectors were carefully collected from the surfaces of cattle bodies to avoid any injury. The ticks were then brought to the laboratory for morphological identification. Two genera were preliminary identified namely *Rhipicephalus* (*Boophilus*) sp. (Figure 2A, B, C, D) and *Haemaphysalis* sp. (Figure 3A, B, C, D).

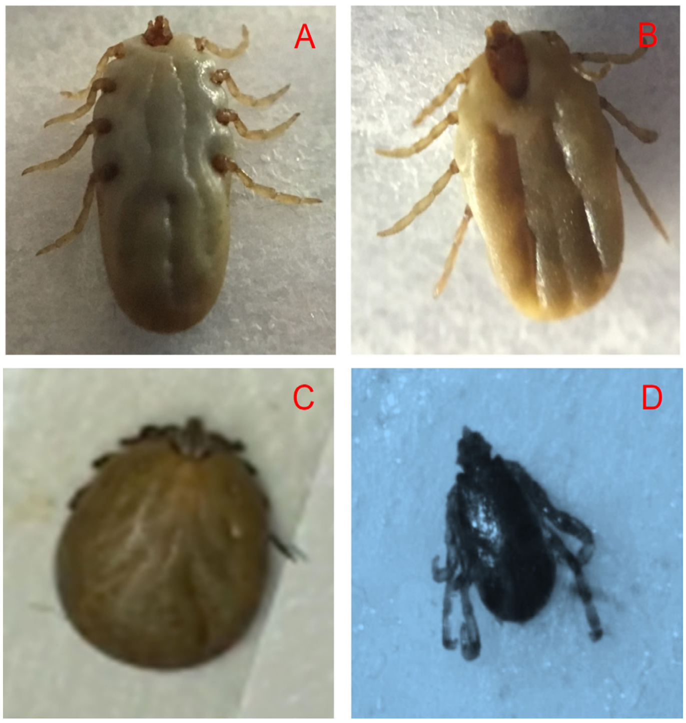

Figure 2: *Rhipicephalus sp. (A) Adult partially engorged female tick (ventral surface). (B) Adult partially engorged female tick (dorsal surface). (C) Adult engorged female tick (dorsal surface). (D) Adult unfed male tick (dorsal surface).*

Figure 3: *Haemaphysalis sp. (A) Adult unfed male tick (dorsal surface). (B) Adult unfed female tick (dorsal surface). (C) Adult unfed male tick (ventral surface). (D) Adult partially engorged female tick (Ventral surface).*

Morphological identification of Rhipicephalus (Boophilus) sp.

Major observed morphological features *R. microplus* (male) was as follows: The body was found to be broadest at the height of coxa IV, coxa IV is tringulars of moderate length with numerous setae. Coxa I posteriorly with two spurs of about even length divided by a cleft, the inner spur blunts and the external spur slender and more pointed. Anterior spurs of coxa I were dorsally conspicuous. Coxa II and III with broadly rounded internal and external spurs, less developed in coxa III. Basis capituli was subhexagonal with a straight posterior border and triangular short blunt cornua. Anterolateral margins straight to slightly convex; posterolateral margins concave; medium to long setae present transversely along the dorsal surface. Scutum covered the body antero-posteriorly, leaving a narrow band of extra-scutal region exposed on each side (Figure 2C, D). This region was finely striated longitudinally. Festoons lacking, eyes inconspicuous, setae long and absent from grooves and depressions.

Ventral surface had long white setae; genital opening on level with coxa II and anal opening at about one third to half the distance between the posterior margin of the body and the posterior edge of coxa IV. Anal groove was absent. Adanal plates were found long.

Major observed morphological features *R. microplus* (female) was as follows: The body was found to be broadest at the height of coxa IV (Figure 2B). Coxa I triangular, longer than wide with two broadly rounded spurs separated by a relatively deep cleft, the external spur is as wide as or slightly wider than internal spur. Tarsi I–IV have a terminal ventral spur. The basis capituli is subhexagonal, with a slightly convex posterior margin and indistinct cornua. A few short setae are present on the lateral margins of the basis capituli. The hypostome is short, with 4/4 dentition and one pair of very short posthypostomal setae. The scutum varies in size; in most specimens, it is slightly longer than wide with inconspicuous eyes at greatest width. The Setae on the scutum are long and sparse, usually found along the anterolateral margins to the level and around the eyes, as well as in transverse rows near the anterior margin of the scutum; a few scattered setae can be found in the middle of the scutum, but absent from cervical grooves. The ventral surface has medium to long white setae, and the genital aperture is located at the level of coxa II. The spiracular plate is broadly oval or subcircular (Figure 2A).

Morphological identification of *Haemaphysalis* sp.: Presence of spurs on the 2nd palpal segment (Figure 3) of the dorsal side are the predominant feature of *Haemphysalis* sp.

Major observed morphological features *Haemaphysalis* (male) were as follows: The posterior body margin of the body features a series of grooves, forming a pattern of festoons (Figure 3A, C). Coxae 1 had a single large spur in both sexes; while coxae 2 to 4 have spurs of varying sizes. Males do not have any sclerotized plates aligned with the anus. The anal groove is positioned posterior to the anus in both sexes.

Major observed morphological features *Haemaphysalis* (female) were as follows: Small ticks have a body and mouthparts approximately 3mm long, with a yellow-brown coloration. The mouthparts are the same length as the basis capitula (Figure 3B); and the second segment of the palps is expanded laterally, making the mouthparts wider than the basis capitula (Figure 3B). The basis capituli has straight lateral margins. The Scutum is plain, without any colored patterns and eyes are absent. The legs are free from pale rings. The posterior body margin features a series of grooves, forming a pattern of festoons (Figure 3D). The ventral surface of an adult female Haemaphysalis tick is characterized by, an elongated genital aperture, and comma-shaped spiracular plates near the fourth pair of coxae. The anal groove arches posteriorly, and sparse setae are distributed near the genital aperture (Figure 3D).

Potential vectors for the spread of hemoprotozoal illnesses such anaplasmosis, theileriosis, and babesiosis in cattle are arthropods like ticks. Arthropod biology can be impacted by variables such rising temperatures, salinities, and deforestation (Alim *et al.*, 2012; Makala *et al.*, 2003). Identification of these infections and their vectors is essential for disease prevention and control. Currently, there is a lack of comprehensive published reports on the identification of veterinary arthropods and diseases that are present in coastal regions, including Bangladesh. The study focused on host body changes, tick vector identification, and epidemiological features based on the mentioned parameters. Researchers are conducting study on vector-borne diseases in several locations across the country, as shown by Karim *et al.* (2012), and Siddiki *et al.* (2010).

In this study, three types of blood protozoa were recorded namely *Babesia* sp. (Figure 1A) *Theileria* sp. (Figure 1B) and *Anaplasma* sp. (Figure 1C). In this study, overall prevalence of hemoprotozoan diseases were observed 14.74% (Table 1). The results of the current study, 6.45% of cattle in coastal areas had babesiosis (Table 1). The results of this study were higher than those of Rahman *et al.* (2015), Al-Mahmud *et al.* (2015), Alim *et al.* (2012), and Chowdhury *et al.* (2006), who reported 1.5%, 2.27%, 1.85%, 1%, 1% and 3.3%, respectively. However, the results of this study were lower than those of Nath *et al.* (2013), Banerjee *et al.* (1983) and Mohanta *et al.* (2011) who reported 16%, 14.53%, and 16.63%, respectively. The study's total theileriosis prevalence of 5.06% (Table 1) is almost identical to the results of studies conducted by Al-Mahmud *et al.* (2015) and Alim *et al.* (2012), who found that theileriosis prevalence was 5.82% and 4.62%, respectively. The result of Khattak *et al.* (2012), which found that the overall prevalence of theileriosis on Giemsa stain was 5.20% in Khyber Pukhtoon province (Pakistan), lends more credence to the current investigation. Differences in findings may be due to climate, geography, husbandry practices, or study methods. Anaplasma infection was found in 3.23% of cattle in this study, consistent with a recent report on the disease's prevalence in Bangladesh by Rahman *et al.* (2015), which revealed a 3.50% infection rate in cattle from the Rangpur district. The current results, however, diverge from those of Chowdhury *et al.* (2006), who found that the frequency of anaplasmosis was significantly higher (70%) than in other inland reports; this difference might be due to the fact that the authors studied only clinically suspected cattle.

During this investigation, the highest prevalence of babesiosis was found in rainy season (12.5%), followed by summer (4.12%) and winter (2.08%) season (Table 2). The findings contradict the findings of Al Mahmud *et al.* (2015) where he reported that the prevalence of Babesiosis was found the highest in summer season (2.50%) in relation to rainy (2.27%) and winter (2.02%) season. The research corroborates Radostits *et al.* (2000), observing an increased incidence of haemoprotozoan diseases subsequent to peak tick populations during the rainy season. The prevalence of theileriosis was observed the highest in summer season (6.18%), followed by rainy (4.16%) and winter (4.16%) season (Table 2). These findings disagree with the findings of Al-Mahmud *et al.* (2015) who stated that the prevalence of theileriosis was recorded the highest in rainy season (6.25%), followed by summer (5.83%) and winter (5.05%) season. The present study revealed that the prevalence of anaplasmosis was found the highest in winter season (6.25%), followed by rainy (2.77%) and summer (2.06%) season. The investigation shown difference from the report of Alim *et al.* (2012) which revealed that the highest prevalence of anaplasmosis was in summer. The findings of seasonal prevalence of this study underscore the impact of climatic factors, especially in the coastal areas of Bangladesh, where elevated humidity and mild temperatures during the rainy season establish ideal circumstances for tick survival and reproduction. Vegetation and moisture-laden areas facilitate tick habitats, while communal grazing activities augment transmission. The region-specific environmental variables elucidate the heightened incidence of babesiosis in the rainy season, theileriosis in summer, and anaplasmosis in winter, as documented in this study. These observations underscore the necessity for seasonally focused tick control and management techniques adapted to the distinct climatic conditions of Bangladesh's coastal regions.

In the present study, the prevalence of babesiosis, theileriosis and anaplasmosis were found higher in female (7.38%, 6.04% and 4.02%, respectively) than male (4.41%, 2.94% and 1.47%, respectively), the data didn't show significant difference (Table 3). The result is consistent with the earlier reports of Rahman *et al.* (2015), Al-Mahmud *et al.* (2015), Singh *et al.* (2012), Khattak *et al.* (2012) and Kamani *et al.* (2010). According to Kamani *et al.* (2010), the higher prevalence in female cattle is likely due to their longer retention for breeding and milk production, coupled with inadequate feed for their high demand.

In this investigation, it was found that the highest occurrence of babesiosis and theileriosis were found in the age of above 3 years (11.39% and 10.12%, respectively), followed by 2 to 3 years of age (6.15% and 3.33% respectively) and 6 months to 2 years of age (1.36% and 1.36%, respectively) (Table 4). This finding is supported by the earlier reports of Al Mahmud *et al.* (2015), Alim *et al.* (2012) and Ananda *et al.* (2009). Zintl *et al.* (2005) estimated that the inverse age resistance might be due to rapid immune responses to primary infection by the calves through a complex immune mechanism. Cynthia *et al.* (2011) recorded that endemic instability of the study areas might be responsible for frequent infections in adult cattle where newborn calves were protected by colostral immunity. Maternal antibodies is responsible for the reduced number of clinical outbreaks in young animals. According to the present study, the prevalence of anaplasmosis was found highest in the age of 6 month to 2 years (4.10%), followed by 2 to 3 years of age (3.07%) and above 3 years of age (2.53%). The prevalence is found in line with the report of Rahman *et al.* (2015) which documented that, the prevalence of *Anaplasma* infection was 3.8% and 2.3% in young and adult cattle respectively. However, the findings are different from the reports of Nath *et al.* (2013) and Ananda *et al.* (2009) and who recorded that the occurrence of anaplasmosis found higher in cattle of more than 3 years old than that of younger cattle.

In present study, the prevalence of babesiosis, theileriosis and anaplasmosis were found higher in crossbred cattle (6.15%, 3.33% and 3.93%, respectively) than in local cattle (1.11% and 1.11% and 2.22%, respectively) (Table 5). Susceptibility of crossbred cattle to haemoprozoan diseases recorded in this study shows uniformity with the reports of Rahman *et al.* (2015), Nath *et al.* (2013) and Chowdhury *et al.* (2006). Nath *et al.* (2013) recorded that the prevalence of anaplasmosis in indigenous and crossbred cattle was 4% and 38%, respectively and the prevalence of babesiosis in indigenous and crossbred cattle was 2% and 14%, respectively. Siddiki *et al.* (2010) stated that constant exposure of infections and development of immunity against such infections might be responsible for lower prevalence in indigenous cattle. Better management of crossbred cattle reduces vector exposure but leads to lower immunity and more frequent diseases (Ananda *et al.*, 2009; Chowdhury *et al.*, 2006; Siddiki *et al.*, 2010).

This study revealed that, TEC, Hb and PCV of hemoprotozoa affected cattle was significantly lower than that of healthy one (Table 6). The present findings agree with the report of Kaur *et al.* (2021), Singh *et al.* (2017), Sharma *et al.* (2013). According to Kaur *et al.* (2021), haematological alterations, especially decrease in hemoglobin, red blood cell count and packed cell volume is evident in hemoprotozoan affected cattle. Sharma *et al.* (2013) also documented significant decrease in the RBC count, Hb and PCV levels of the *Anaplasma* infected cattle as compared to the healthy cattle. The decline in the RBC count, Hb and PCV levels could be attributed to normocytic normochromic anemia caused by intravascular hemolysis of red blood cells in babesiosis and normocytic hypochromic anemia in theileriosis. In the investigation, cattle with babesiosis had urine the color of coffee. Bock *et al.* (2004) also noted similar results. Coffee-colored urine may be the result of immune-mediated erythrocyte hemolysis (Bock *et al.*, 2004). Nevertheless, neither theileriosis nor anaplasmosis showed this kind of alteration in urine color.

According to the present study, the prevalence of all three hemoprotozoal diseases (babesiosis, theileriosis and anaplasmosis) were found significantly higher in tick infested cattle (18.5%, 18.5% and 14.8%, respectively) than tick free cattle (4.73%, 3.15% and 1.57%, respectively) (Table 7). This finding is consistent with the previous reports by Rahman *et al.* (2015) which revealed that the prevalence of *Babesia* and *Anaplasma* infections was significantly higher in tick infested cattle than the apparently tick free cattle. Tick infested cattle were about 6 times at greater risk of *Babesia* infection than the non-infested cattle. Similarly, tick-infested cattle found 7 times at greater risk of *Anaplasma* infection than tick free cattle (Table 7). Costa *et al*. (2013) and Francisco *et al*. (2013) recorded that, as blood sucking ticks are the vectors of both *Babesia* and *Anaplasma* organisms, the presence of them might influence the occurrence of infections with these organisms.

In the present study, two genera of ticks viz. *Rhipicephalus* (Figure 2) and *Haemaphysalis* (Figure 3) were recorded in cattle that supports the findings of Akter *et al*. (2019), Sen *et al*. (2012) and Roy *et al*. (2018a) who all reported some species of the two genera of tick with similar tick morphology. However, Haque *et al*. (2015) reported three genera of ticks namely *Rhipicephalus*, *Haemaphysalis* and *Hyalomma*. Islam *et al*. (2006) reported five species of tick of four genera, viz. *Rhipicephalus*, *Haemaphysalis* and *Hyalomma* and *Amblyomma* in cattle through an investigation in flood plains, hilly areas and Barind tract of Bangladesh. Mohanta *et al*. (2011) reported two ticks namely *Boophilus microplus* and *Amblyomma testudinarium* in hilly area of Bangladesh. The difference among the findings of present and previous study works with regard to genera of ticks might be attributed to geographical location, variation in sample collection technique, housing system of the cattle shed and climate of the study area.

This study successfully identified hemoprotozoa in cattle through Giemsa staining, clinico-pathological data, and tick vector identification. It provides a comprehensive understanding of the prevalence and impact of hemoprotozoal diseases in the coastal region of Bangladesh. The findings revealed that crossbred and female cattle were more susceptible to these diseases, with tick infestation identified as a major contributing factor. Hemoprotozoal infections significantly impaired cattle health and productivity, emphasizing the need for targeted prevention and control strategies.

Farmers are strongly encouraged to implement regular tick management practices, monitor livestock during high-risk seasons, and prioritize adequate nutrition and preventive healthcare. Policymakers should support integrated tick management programs, subsidize diagnostic tools and acaricides, and promote farmer education and vaccine research. Although the study provides valuable insights, it was constrained by the limitations of Giemsa staining and the absence of molecular diagnostics. Future research should focus on incorporating advanced molecular techniques such as PCR for precise pathogen identification, expanding the geographic and seasonal scope, investigating tick-pathogen relationships, and evaluating environmental and management factors influencing disease prevalence. Long-term studies and control strategies, like vaccination and tick management, are vital to reduce losses and improve livestock health.

Acknowledgements

We express our gratitude to the Research and Training Centre of Patuakhali Science and Technology University of Bangladesh for research funding.

Novelty Statement

This study provides insight into tick-borne haemoprotozoal diseases in the Barishal division of coastal region of Bangladesh, demonstrating seasonal differences in disease frequency and susceptibility among cattle populations. Tick infestation and disease occurrence have connections, and hematological and urinalysis results aid diagnosis. The identification of *Rhipicephalus* (*Boophilus*) and *Haemaphysalis* ticks as main vectors underscores the necessity for focused vector control to reduce disease transmission.

Author's Contribution

Shib Shankar Saha and Md. Shah Alam: Conceptualized and designed this study.
Md. Roies Uddin: Engaged in data curation.
Md. Roies Uddin, Shib Shankar Saha, Khondoker Jahengir Alam, and Shampa Rani: Drafted the manuscript.
All authors reviewed and approved and the final version of the manuscript.

Conflicts of interest

The authors have declared no conflict of interest.

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