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Research Article

Occurrence of Bovine Haemoprotozoan Parasites in 'Bathan' Areas of Sirajganj and Pabna Districts in Bangladesh

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ABSTRACT

Haemoprotozoan parasites, mainly transmitted by ticks, significantly impact the health of domestic animals in Bangladesh. A study was conducted to investigate the prevalence of haemoprotozoan parasites in cattle in the 'Bathan' areas of Bangladesh from September 2019 to August 2020. Blood samples were randomly collected from five distinct areas during three different seasons. These samples were examined by Giemsa-stained thin smear method. In this study, a total of 120 cattle were examined, and 34 (28.33%) were infected with different haemoprotozoan parasites: *Babesia* sp. (8.33%), *Anaplasma marginale* (12.50%), and *Anaplasma centrale* (3.33%). Additionally, among the examined samples, 4.16% of cattle had co-infections with *A. marginale* and *A. centrale*. The higher prevalence of *Anaplasma* spp. (25.0%) and *Babesia* sp. (10.0%) was observed in older animals (more than 3 years old). Furthermore, females showed a higher prevalence of both the *Anaplasma* sp. (23.44%) and *Babesia* sp. (10.94%) compared to males, with prevalence rates of 11.12% and 2.78%, respectively. Moreover, the prevalence of haemoprotozoan parasites was higher in the rainy season compared to the summer and winter seasons. The findings will provide crucial insights for developing effective control measures against haemoprotozoan parasites in the region.

Keywords: Haemoprotozoan Parasites, *Anaplasma* sp., *Babesia* sp., Bathan, Sirajganj, Pabna.



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INTRODUCTION

Livestock is a vital sub-sector of agriculture in Bangladesh, playing a crucial role in maintaining human health and contributing significantly to the national economy. Eighty percent of rural households in Bangladesh rear indigenous cattle, with many relying on traditional dairy farming practices (Siddiki et al., 2010). However, bovine hemoprotozoan parasites, including babesiosis, anaplasmosis, and theileriosis, threaten cattle health and productivity worldwide. These diseases are transmitted by arthropod vectors, particularly ticks, which cause substantial losses in the livestock industry worldwide (Ananda et al., 2009). The geo-climatic conditions of Bangladesh, characterized by moderately hot and humid weather, short winters, and a prolonged rainy season, are highly conducive to the development and survival of ticks (Kohli et al., 2014; Mohanta and Mondal, 2011). Ticks act as biological vectors for several economically important protozoan diseases, including babesiosis, theileriosis, and anaplasmosis, all of which adversely affect livestock (Mohanta and Mondal, 2011).

Haemoprotozoan parasites significantly impair cattle health and productivity (Malayar and Farid, 2018) throughout the world, causing major illness and economic losses (Rajput et al., 2005). Ticks and tick-borne diseases impact 80% of the world's cattle population (Ghosh et al., 2007). Ticks fever is one of the most economically significant arthropod-borne diseases of cattle worldwide. In Bangladesh, haemoprotozoan parasites species such as *Babesia bigemina*, *B. bovis*, *Theileria mutans*, *T. annulata*, *Anaplasma marginale*, *A. centrale* have been reported in cattle (Hosen et al., 2020). Clinically, theileriosis manifests as enlargement of superficial lymph nodes, congested mucous membranes, fever, corneal opacity, and emaciation. Babesiosis is characterized by fever, pale mucous membranes, brown coffee-colored urine, hemolytic anemia, and respiratory and neurological disorders leading to death. On the other hand, anaplasmosis is manifested by fever, anorexia, lethargy, weight loss, cough, abortion, and decreased milk production. Moreover, these diseases cause tangible losses to the livestock industry worldwide (Ananda et al., 2009) due to death, decreased productivity, reduced working efficiency (Uilenberg, 1995), increased control costs (Makala et al., 2003), and limited genetic improvement of cattle in the area (Radostits et al., 2000). Generally, haemoprotozoan parasitic infections are thought to result from complex interactions among pathogens, vectors, ages, sexes, breeds, geographical locations, seasons, and feeding systems. Aged animals are likely to be infected with haemoprotozoan parasites than young animals. Moreover, female cattles are more prone to haemoprotozoan parasites for keeping longer time in the farm (Alim et al., 2011). Additionally, rainy season is the most important factor in the prevalence of anaplasmosis and babesiosis due to availability of green grasses in the rainy season (Mohanta and Mondal, 2011).

In Bangladesh, nearly half of the total milk production is obtained from the northern areas, particularly the 'Bathan' regions, located in the Pabna and Sirajganj districts (Hemme et al., 2004). In these areas, the prevalence of anaplasmosis, babesiosis, and theileriosis was recorded at 25.82%, 2.27%, and 5.82%, respectively (Al Mahmud et al., 2015; Belal et al., 2014). However, more recent studies reported the prevalence of *A. marginale* (10.51%), *A. bovis* (63.77%), *A. phagocytophilum* (0.72%), *Babesia bigemina* (49.28%), *B. naoakii* (1.09%), *B. bovis* (0.72%), *B. ovis* (32.26%), *Theileria annulata* (6.52%), and *T. orientalis* (46.01%), respectively in Bangladesh (Mohanta et al., 2024; Mohanta et al., 2023; Mohanta et al., 2023b). Therefore, this study was undertaken to estimate the prevalence of haemoprotozoan parasites in cattle in various areas of Pabna and Sirajganj district

MATERIALS AND METHODS

Study area and period

This study aimed to determine the prevalence and identification of haemoprotozoan parasites in cattle across different seasons in the Bathan areas of Bangladesh. The Sirajganj and Pabna districts were selected for its unique livestock practices called Bathan. Bathan areas are expanses of fallow grassland where animals are housed and maintained during the dry season, from December to June (Mohanta et al., 2023b). In Bathan areas, the highest temperature is 36.7°C in April and 12.9°C in December with an average relative humidity (RH) of 77%. Therefore, ticks and tick-borne diseases are prevalent in the area. Data were collected from five locations within the Sirajganj (24° 27' 27.76" N to 89° 42' 28.87" E) and Pabna (24° 0' 46.2816" N to 89° 15' 32.6052"E) districts, encompassing three Upazilas in Sirajganj (Royganj, Chowhali, and Shahjadpur) and two Upazilas in Pabna (Bera and Santhia). The samples were collected over one year, from September 2019 to August 2020, to cover all three prominent seasons in Bangladesh, namely, winter (November to February), summer (March to May), and the rainy season (June to October). Since the Bathan areas undergo submerged due to flooding in the rainy season, the cattle are shifted to the farmer's house. Therefore, the samples in the rainy season were collected from the household instead of the 'Bathan'.

Blood sampling

During the study periods, 120 blood samples were collected from cattle of different ages groups such as 6 months to 2 years (n=40), 2 to 3 years (n=40), and more than 3 years (n=40). Approximately 2 ml of blood samples were aseptically drawn from the jugular vein and placed in EDTA-containing vials (purple color) for subsequent smear preparation and staining. The collected samples were stored in a cool box in the field and refrigerator (4°C) in the laboratory.

Preparation of blood smears and staining

A drop of blood was placed near on a clean, dry glass slide to prepare thin blood smears. A spreader slide, inclined at about 45°, was pushed horizontally to create a thin smear with a clear tail. The smear was air-dried and fixed with methanol for 2 minutes. For staining, a 10% Giemsa working solution (1:9) was prepared and stored in a coplin jar. This solution was replaced daily. The air-dried, methanol-fixed blood smear slides were immersed in the Giemsa solution for

30 minutes, rinsed with running tap water and air-dried. Finally, the stained slides were examined under high power magnification (100X). The identification was done according to the keys and descriptions of Soulsby (1982).

Statistical analysis

The collected data were organized in MS Office Excel, and statistical analysis was performed using the Chi-square (χ^2) test. Results were expressed as percentages along with the p -value. A result was considered significant when it was $p < 0.05$.

RESULTS

Overall prevalence

In the present study, out of 120 cattle examined, 34 were positive for haemoprotozoan parasites. The overall prevalence of haemoprotozoan parasites was 28.33%. Microscopic identification revealed three species of haemoprotozoan parasites, namely, *Babesia* sp., *A. marginale*, and *A. centrale* (Table 1).

Species-wise prevalence

Among 120 cattle examined, 8.33% were found positive for *Babesia* sp., while 12.5% and 3.33% of samples were positive for *A. marginale* and *A. centrale*, respectively. Additionally, 4.16% of the tested cattle had a co-infection with *A. centrale* and *A. marginale*. Moreover, single infections were almost six times more common than mixed infections (Table 1).

Table 1. Species-wise prevalence of haemoprotozoa in cattle.

Blood protozoa		Prevalence (%)
<i>Anaplasma</i> spp.	<i>A. marginale</i>	15 (12.5%)
	<i>A. centrale</i>	4 (3.33%)
	Co-infections (<i>A. marginale</i> and <i>A. centrale</i>)	5 (4.16%)
<i>Babesia</i> sp.		10 (8.33%)
Total		34 (28.33%)

Age-wise prevalence

Samples were categorized into three age groups, namely, six months to 2 years ($n=40$), 2 to 3 years ($n=40$), and more than three years ($n=40$). The prevalence of *Anaplasma* sp. was 15.0% in the six months to 2 years age group, 20.0% in the 2 to 3 years age group, and 25.0% in the more than three years age group. For *Babesia* sp., the prevalence was 5.0%, 10.0%, and 10.0% in these respective age groups (Table 2).

Table 2. Age-wise prevalence of haemoprotozoa in cattle.

Age groups	No. of cattle examined	No. of positive cases	
		<i>Anaplasma</i> spp. (%)	<i>Babesia</i> sp. (%)
6 months-2 years	40	6 (15.0%)	2 (5.0%)
2-3 years	40	8 (20.0%)	4 (10.0%)
More than 3 years	40	10 (25.0%)	4 (10.0%)

Sex-wise prevalence

The study revealed that the prevalence of both *Anaplasma* sp. (23.44%) and *Babesia* sp. (10.94%) was higher in females than that of *Anaplasma* sp. (11.12%) and *Babesia* sp. (2.78%) in males. Overall, *Anaplasma* sp. had a higher prevalence compared to *Babesia* sp. in both males and females (Table 3).

Samples were collected during three seasons namely, winter ($n=40$), summer ($n=40$), and the rainy season ($n=40$). The highest prevalence of *Anaplasma* sp. was recorded during the rainy season at 32.5%, followed by 17.5% in summer and 10.0% in winter, respectively. Similarly, *Babesia* sp. showed a prevalence of 12.5% in the rainy season, followed by 7.5% in summer, and 5.0% in winter (Table 4).

DISCUSSION

The combined prevalence of haemoprotozoan parasites in Pabna and Sirajganj districts was 28.33%, similar to Kamani et al. (2010) in Nigeria, who found 25.9% of infection in cattle. On the other hand, this study showed a lower prevalence than Hosen et al. (2020) in Sylhet, Bangladesh, and Ananda et al. (2009) in Bangalore, India. Again, this study showed a higher prevalence than Alim et al. (2011), who reported 12.02%-16.18% in cattle in Chittagong, Bangladesh. Moreover, Al-Shammari et al. (2024) recorded lower prevalence 19.82% in Babylon, Iraq. Moreover,

the prevalence of *Anaplasma* sp. and *Babesia* sp. were found to be 19.99% and 8.33%, respectively, which aligns with previous studies (Arunkumar and Nagarajan, 2013; Hosen et al., 2020; Ibrahim et al., 2012). However, this study shows comparatively lower prevalence in the case of *Anaplasma* sp. than the previous reports conducted by Belal et al. (2014) in Sirajganj district (25.82%), Chowdhury et al. (2006) in Sirajganj Sadar (70%), and Hosen et al. (2020) in Sylhet district (28%).

Table 3: Sex-wise prevalence of haemoprotozoa in cattle.

Sex	No. of cattle examined	No. of positive cases	
		<i>Anaplasma</i> spp. (%)	<i>Babesia</i> sp. (%)
Male	36	15 (11.12%)	1 (2.78%)
Female	64	4 (23.44%)	7 (10.94%)

Table 4. Seasonal dynamics of haemoprotozoa in cattle.

Season	No. of cattle examined	No. of positive cases	
		<i>Anaplasma</i> spp. (%)	<i>Babesia</i> sp. (%)
Summer	40	7 (17.5%)	3 (7.5%)
Rainy	40	13 (32.5%)	5 (12.5%)
Winter	40	4 (10.0%)	2 (5.0%)

Furthermore, in the case of *A. marginale*, this study is quietly similar to Mohanta et al. (2023), who recorded a 10.51% prevalence of *A. marginale* from Sirajganj, Jhenaidah, Bogura, and Bandarban districts, and Naikhongchari in Bangladesh. Similarly, the study is contradicted by Bhatnagar et al. (2015), who documented a 15.65% prevalence of babesiosis in the Rajasthan state of India, and Banerjee et al. (1983), who recorded a 14.53% prevalence of *B. bigemina* in Bangladesh. These dissimilarities might be due to the variation in geographic location, high number of cattle populations, availability of vectors, high number of lowland/floodplain land, and study period. However, Chowdhury et al. (2006) provided additional support to this study by reporting no cases of *Theileria* sp. prevalence in Sirajganj.

This study shows that *Anaplasma* sp. had prevalence rates of 15.0%, 20.0%, and 25.0% among cattle aged six months to 2 years, 2 to 3 years, and over three years, respectively. *Anaplasma* sp. infects bovines of all ages, where the severity and mortality rate increase in aged animals (Richey, 1984). Similar findings worldwide indicate higher susceptibility to bovine anaplasmosis in the adult population (Atif et al., 2013; Chakraborti, 2002; Kamani et al., 2010; Khan et al., 2004). *Babesia* sp. had a prevalence of 10.0% in older cattle and 5.0% in younger ones. This age-related resistance pattern is consistent with reports showing higher prevalence in adults compared to younger cattle (Kamani et al., 2010; Zintl et al., 2005). The study attributes frequent infections in adult cattle to endemic stability, whereas newborn calves are protected by colostral immunity and maternal antibodies, reducing clinical outbreaks in young animals (Cynthia et al., 2011).

In this study, *Babesia* sp. was more prevalent in female cattle (10.94%) than in male cattle (2.78%), contrasting with the findings of Hosen et al. (2020), where males showed a higher prevalence (13.04%) than females (5.54%). Conversely, anaplasmosis had a higher prevalence in females (2.59%) compared to males (1.60%), consistent with Belal et al. (2014). The higher prevalence of babesiosis in female cattle might be due to the more extended periods of breeding and milk production for females, insufficient feed supply against high demand (Kamani et al., 2010), or variation in sample size, geographical locations, and study period. On the other hand, the trend showed an opposite direction in anaplasmosis.

The prevalence of *Anaplasma* sp. and *Babesia* sp. peaked in the rainy season, followed by summer and winter, respectively. Observation of the higher prevalence in the rainy season aligns with the report of Belal et al. (2014), Rony et al. (2010), Ananda et al. (2009), and Radostits et al. (2000). On the other hand, these findings are quite dissimilar with Hosen et al. (2020) who documented higher prevalence in the summer season. The severity of hemoprotozoan parasites tends to increase shortly after the peak of tick populations, influenced by temperature, humidity, and rainfall, potentially explaining the higher prevalence during the rainy season (Salih et al., 2008; Zahid et al., 2005). However, *Babesia* sp. also showed notable prevalence in summer (21.58%), closely resembling the rainy season. This rise in summer infestation may be linked to increasing temperatures following late winter, which facilitates gradual tick population growth and, subsequently, higher infection rates (Roy et al., 2000).

CONCLUSION

The study investigated the occurrences of *Babesia* sp. and *Anaplasma* sp. pathogens in cattle of Bathan regions of Bangladesh. The higher prevalence was diagnosed in female and older animals, and during rainy season. The research emphasizing the need for measures associated to sex, age and seasonal variations for the effective control of the protozoa infections in the local areas.

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AUTHOR CONTRIBUTIONS

Amit Kumar Bosu: Writing original draft, Methodology, Investigation, Conceptualization. Al Wasef: Writing original draft, Review, Methodology, Investigation, Data analysis. S.M. Abdullah: Methodology, Investigation. Akash Samadder: Methodology, Investigation. Shamarendra Narayan: Methodology, Investigation. Uday Kumar Mohanta: Review and editing, Methodology, Investigation, Resources, Funding acquisition.

COMPETING OF INTEREST

The authors have stated that they do not have any conflicts of interest related to this research.

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