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Temporal Trends of Tick-borne Haemoparasitic Infections in Cattle of Assam, India: A Five-year Retrospective Study

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ABSTRACT

Background: Haemoparasitic diseases in cattle are infectious conditions primarily transmitted by arthropods, particularly ticks. In India, especially in the northeastern region (NER), these diseases lead to significant economic losses for farmers, impacting both production and treatment costs. This study aims to investigate the prevalence of haemoparasitic diseases in cattle around Guwahati, as there is a lack of comprehensive data on their prevalence over an extended period in this area.

Methods: The study was conducted for a period of five years from 2017 to 2021. To study the prevalence of haemoparasite Giemsa-stained blood samples from cattle presented to OPD of Veterinary Clinical Complex, CVSc, AAU, Khanapara were examined. The study was carried out concerning breed, age and sex of the animal and also regarding the prevailing seasonal changes.

Result: A total of 2100 cattle were screened, out of which 1767 (84.14%) were found to be infected with haemoparasites. In the present study, infection with *Theileria* was found to be the highest (72.94%) followed by *Anaplasma marginale* (12.00%) and *Babesia bigemina* (11.09%). A mixed infection of haemoparasite was also recorded as 3.96%. The parasites were identified based on morphology in Giemsa-stained blood smear. The highest infection was recorded in the age group of >5 years (89.23%) followed by 1-5 year group (85.99%). Similarly female animals are found to be more infected (84.92%) than that of the male (77.14%). Seasonally, the prevalence of haemoprotozoa was found highest in monsoon (97.38%) and lowest in winter (46.13%). The present study provides systematic data on haemoparasite infection in cattle.

Key words: Cattle, Haemoparasite, Prevalence.

INTRODUCTION

The North eastern region of India is bestowed with several livestock population among which cattle is of prime importance. According to the 19th livestock census there is a considerable number of cattle (1.98 million) in Assam. That plays a pivotal role in livelihood and nutritional security of farming community. Cattle also play an important role in providing dung and other animal wastes which serve as very good farm yard manure and the value of it is worth several crores of rupees. In addition, it is also used as fuel (bio gas, dung cakes) and for construction as poor man's cement (dung).

Various parasitic diseases are serious constraints for the improvement of livestock production out of which diseases caused by haemoparasites plays a crucial role causing severe economic loss to the livestock owner. Losses due to these haemoprotozoan diseases include mortality, reduction in milk yield, loss of body weight, abortion, infertility, decrease draught power and the cost of treatment of affected animals. In India the economic losses due to tick- and tick-borne diseases in animals have been estimated to the tune of more than US$ 498.7 million per annum (Ghosh and Nagar, 2014; Minjauw and McLeod, 2003). The most important haemoparasites prevalent in Assam are *Theileria spp.*, *Babesia spp.*, *Anaplasma spp.* and *Trypanosoma spp.* (Kakati *et al*., 2015; Mushahary *et al*., 2020). These haemoparasites are transmitted through tick and except Trypanosome all are intracellular parasites species.

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These vectors borne haemoparasitic diseases have been found to exist in our country since long back history. But, with the import of exotic breed of cattle for upgrading the indigenous stock to improve their milk yield, these diseases have gained greater significance in our country (Kakati *et al*., 2015). Among various tick-borne diseases bovine babesiosis is one of the important haemoparasitic

diseases that cause significant morbidity and mortality in cattle. Bovine babesiosis is a haemolytic disease due to intra-erythrocytic protozoan of the genus *Babesia*. The disease is characterized by high rise of temperature, haemoglobinuria, pale visible mucous membrane, diarrhoea, sudden drop in milk yield (Barman *et al*., 2018; Behera *et al*., 2023). *Theileria orientalis* causes 'Oriental theileriosis, in a wide range of tropical and sub-tropical zone of Asia and Europe including India and it is transmitted by *Hyalomma* and *Boophilus* ticks (Kakati *et al*., 2015). Bovine anaplasmosis is caused by *Anaplasma marginale*, a rickettsial intraerythrocytic pathogen, infects only ruminants and primarily cattle (Kocan *et al*., 2010). This parasite is transmitted by biting flies and most tick species. Trypanosomasis commonly known as 'surra' is a chronic wasting disease of cattle caused by the flagellated hemoprotozoan parasite, *Trypanosoma evansi*. It is the most prevalent and pathogenic trypanosome throughout the tropical and subtropical areas of the world owing to its ability of mechanical transmission by hematophagous tabanid flies as well as wide host range (Aregawi *et al*., 2019; Luckins, 1988).

Prevalence of *Babesia* and *Anaplasma* in cattle was reported earlier from North Eastern region of India (Ghosh *et al*., 2020; Laha *et al*., 2015; Singh *et al*., 1978), the first report on the occurrence of *T. orientalis* and involvement of pathogenic strain responsible for the clinical disease of emerging nature in Assam, was done by Kakati *et al*. (2015) Despite wide spread prevalence reports of *T. evansi* in cattle from Northern India (Juyal *et al*., 2005; Pathak and Chhabra, 2011) this parasite was not reported from the North Eastern part of India until 2015 (Sarmah *et al*., 2015). Chandu *et al*. (2021) reported seroprevalence of *Trypanosoma evansi* in cattle of Mizoram, North-east India which indicates its transmission potentiality among suitable vectors and hosts. Devi *et al*. (2022) reported the prevalence of Trypanosomasis in buffalo population of Assam.

Perusal of literature confirms that, there are very few and sporadic work has been done to know the prevalence of tick-borne hemoparasite infection in cattle in Assam in general and Guwahati in particular. The duration of those prevalence studies whatsoever done, are found to be only for one year or so, which cannot draw a conclusive decision about the actual trend of the infection prevailing in a particular area. Therefore, a systematic study was felt necessary on the prevalence of tick borne haemoparasite of cattle, in a particular period, to form a baseline dataset that will help to formulate a reliable and appropriate control and management strategy.

Considering the above facts, the present work was undertaken with the objectives to know the trends in tick-borne haemoparasitic infection in cattle in and around Guwahati, the capital city of Assam.

The present communication summarizes the investigation conducted in the cattle produced in the outpatient department of Veterinary Clinical Complex of College of Veterinary Science, Assam Agricultural University, Khanapara for a period of five years from 2017-2021 to elucidate the prevalence of haemoprotozoan diseases of cattle from this part of India.

MATERIALS AND METHODS

Study area

The present study was conducted on the cattle presented to the outpatient Department (OPD) of the Teaching Veterinary Clinical Complex of the college of Veterinary Science under Assam Agricultural University, Khanapara, Assam, India (Fig 1). The animals are brought to the clinics from different organized as well as unorganized cattle farm in and around the greater Guwahati.

Selection of animal and sampling

Blood samples were collected from all cattle irrespective of sex, breed and age that were presented to the OPD for clinical examination. A total of 2100 cattle were examined

Source: Sarmah and Das (2018).

Fig 1: Location of the study area (Guwahati) in Assam with respect to India.

out of which 210 were male and 1890 were female. Out of 2100 cattle examined 521 were below 1 year of age, 687 were between 1 to 5 years old and 892 were above 5 years of age.

The prevalence study was recorded based on animal sex (Male and Female), age (< 1 year, 1–5 years and > 5 years) and breed (cross-bred Jersey, cross-bred Holstein-Friesian and zebu) and on the basis seasonal changes, *i.e.* pre monsoon (March-May), monsoon (Jun–September), post monsoon (October-November) and winter (December-February), respectively.

Collection of blood sample

2 ml blood samples were collected from each of the animals by jugular vein puncture and kept in properly labeled EDTA vacutainer and brought to the laboratory for examination. Parasitological study was done on the day of blood collection.

Examination of blood smear

Thin blood smear was prepared from each blood sample, in clean and grease free micro slide. The blood smear was allowed to dry and the fixed with methanol for 5 minutes. The smears were stained with Giemsa stain in 1:10 dilutions following standard protocol. After 45 minutes of staining the smears were washed with running tap water. Then the smears were air dried and observed under oil immersion (× 100 magnification) for presence of haemo-protozoa (Soulsby 1982). The parasites were identified based on characteristics morphology under microscope (Soulsby 1982; Taylor *et al*., 2016).

Prevalence study

The prevalence of haemoprotozoa was recorded according to age, sex, breed and season. The prevalence (P) was estimated according to standard method (Thrusfield, 2018) and by the formula as given below:

$$P = \frac{\text{No of infected cattle during specified period}}{\text{Total cattle surveyed}} \times 100$$

Statistical analysis

The data regarding the tick borne hemoparasite was compiled according to season; breed, age and sex of cattle. The data generated were statistically analyzed by Chi square test for significance.

RESULTS AND DISCUSSION

A total of four different haemoparasites (both hemoprotozoan and hemorickettsia) were found to be prevalent in the study area. They are *Theileria sp.*, *Babesia bigemina* and *Anaplasma marginale*. Sporadic incidence of *Trypanosoma evensi* was also recorded. Out of 2100 cattle examined 1767 were found to be infected with haemo-parasites. The overall prevalence of haemoparasites (Hemoprotozoa and Hemorickettsia) was found to be 84.14%. Peripheral blood examination revealed presence of *Theileria* (Fig 1a-b), *Anaplasma marginale* (Fig 2a-b) and *Babesia bigemina* (Fig 3a-b). Infection with *Theileria* was found to be the highest (76.91%) followed by *Babesia bigemina* (11.09%) and *Anaplasma marginale* (12.0%). Moreover, the prevalence of *Trypanosoma evensi* was sporadic in nature (Fig 4a-b). A 3.96% mixed infection of hemo- parasite was also recorded in the present study (Table 1).

It has been noticed that the overall percent positivity of different hemoparasite was highest in 2019 and lowest during 2018.The prevalence of *Theileria* was highest in 2021 and lowest in 2018. Similarly, *Anaplasma marginale* infection was found to be highest in 2020 and lowest in 2021 and mixed infection was also recorded to be highest in 2021 and lowest in 2017. While infection of *Babesia bigemina* was found highest during 2017 and lowest during 2021 (Table 1). Statistical analysis of the data of year-wise prevalence of hemoparasites by Chi-square test revealed that year has a significant influence on the prevalence of hemoparasites (P value: 0.023289).

It was noticed in this study that the prevalence of hemoparasite follows a particular season-wise pattern. Irrespective of the type of parasite maximum prevalence was recorded during monsoon followed by pre-monsoon and post-monsoon and lowest in winter season during the five years study period (Table 2).

Highest infection was recorded in the age group of 1-5 years (89.23%) followed by >5-year group (85.99%) and least infection was recorded in the age group of <1

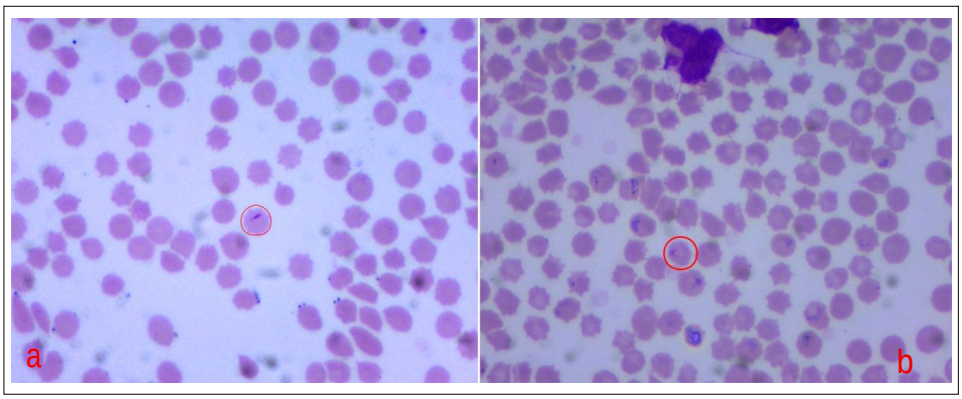

Fig 1(a-b): Peripheral blood smear showing rod shaped and annular form of *Theileria* (Red encircled) inside RBC (100x).

year (74.28%). Similarly female animals are found to be more infected (84.92%) than that of the male (77.14%) (Table 3). Statistical analysis of the data of age-wise prevalence of hemoparasites by Chi-square test revealed that age of the animal has no influence on the prevalence of hemoparasites (P value: 4.73599).

It has been noticed that female cattle are infected more with hemoparasite than their male counterparts (Table 4). Statistical analysis of the data of sex-wise prevalence of hemoparasites by Chi-square test revealed that sex of the animal has a significant influence on the prevalence of hemoparasites (P value: 0.001567196).

Highest prevalence of haemoparasite was recorded in cross bred animal of Holstein-Frisian (90.65%) followed by Jersey cross (81.91%) and lowest was recorded in Shahiwal breed of cattle with a prevalence rate of 60%. It has also been noticed that Zebu cattle shows a greater degree of hemoparasitic infection (62.5%) than other cross-bred cattle (Table 5). Statistical analysis of the data of age-wise prevalence of hemoparasites by Chi-square test revealed that breed of the animal has a significant influence on the prevalence of hemoparasites (P value: 0.001175).

Guwahati, the capital city of Assam, situated in the northeastern region of India, experiences moderately hot and very humid climate with high annual rainfall which favors the propagation of vectors and many vector-borne diseases of animals. The area is also known to have problems of tick infestation predominantly with *Rhipicephalus* (*Boophilus*) *microplus* which is proved to be a vector of several haemoparasites in cattle (Kakati *et al*., 2015; Lahkar, 1991). The area is also known to be endemic for *Babesia* and *Anaplasma* of cattle (Barman *et al*., 2018; Singh *et al*., 1978) and *Theileria* (Kakati *et al*., 2015) . In the recent past, veterinary practitioners from the region have also been experiencing *Trypanosome evansi* sporadically in cattle (Sarmah *et al*., 2015) in buffalo (Devi *et al*., 2022) and even in tiger (Deka *et al*., 2021).

In the present study, the overall prevalence of haemoparasites (Hemoprotozoa and Haemorickettsia) was found to be 84.14% out of which *Theileria* was found to be the highest (72.94%) followed by *Anaplasma marginale* (12%) and *Babesia bigemina* (11.09%). Previously, Sarmah and Kakati (2014) recorded prevalence of *Theileria* (35.95%) to be the highest followed by *Babesia bigemina* (20.11%) and *Anaplasma marginale* (3.83%) which is corroborated

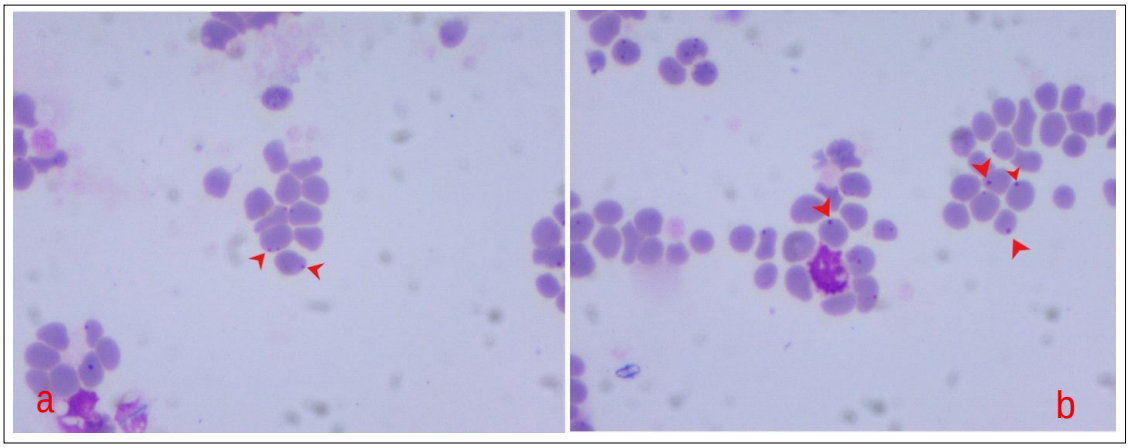

Fig 2(a-b): Peripheral blood smear showing *Anaplasma marginale* (arrow head) inside RBC(100x).

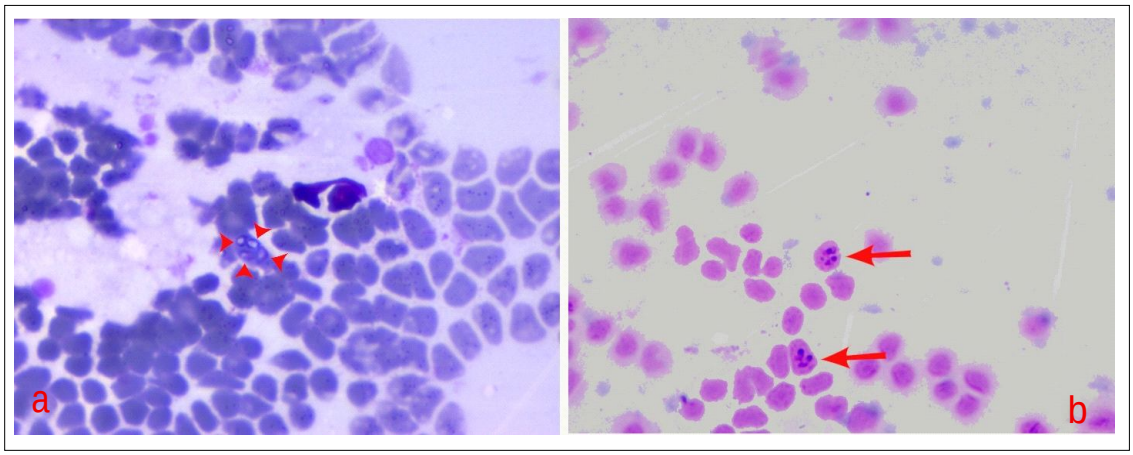

Fig 3(a-b): Peripheral blood smear showing *Babesia bigemina* (arrow head and arrow) inside RBC(100x).

with the present finding. On the contrary, Velusamy *et al*. (2014) recorded the highest prevalence of Theileriosis (13%), followed by Anaplasmosis (2.64%) and babesiasis (1.0%) in Tamil Nadu. Whereas Chaudhri *et al*. (2013) found theileriosis being the most common (32.6%), followed by anaplasmosis (2.4%) and babesiosis (2.0%) among the bovines of Harayana. These differences observed in the prevalence may be due to the difference in geographical locations of the study areas, time periods and various methods of sample analysis.

Present study recorded highest infection in the age group of 1-5 years (89.23%) followed by more than 5 year group (85.99%) and least infection was recorded in the age group of less than 1 year (24.81%). Same trend was recorded Ghosh *et al*. (2020) in Mizoram. They recorded higher infection rate in 1-5 years age group followed by > 5 years and <1 years. The higher prevalence in the age group of 1-5 years might be due to stresses, increased number of lactations, weakening of body immune system along with presence of some diseases which depress the immunity (Velusamy *et al*., 2014), as minimizing of stress and maintenance of optimum immunity is essentially required to prevent emergence and re-emergence of haemoparasitic diseases. Sarmah, 2014 and Kakati *et al*., 2015 recorded the prevalence of haemoparasitic infection to be more in the adult cattle as 58.76% and lower (3.33%) in calves from Assam. Ghosh *et al*. (2020) also recorded age-wise highest prevalence of haemoprotozoa in 1–5 years (44.33%) age group followed by >5 years age group (25.06%) and lowest in <1 year (22.92%) age group. The lower prevalence of haemoparasitic infection in calves is due to lesser grazing activity in calves since they are mostly kept confined in the sheds. As a result, they get lesser chance of contact with the vector ticks. In the present data and the previous findings of Taylor *et al*. (2015) substantiated the “inverse age resistance” phenomenon in case of blood protozoan diseases in animals.

Sex wise prevalence revealed that female animals are more infected (84.92%) than that of the male (77.14%). Ghosh *et al*. (2020) also recorded similar trend of haemoparasites infection of cattle in Mizoram. In the current study the lower level of haemoparasites in male animal may be attributed to small sample size. This sex difference might be due to the fact that female cattle were under high level of stress than males during pregnancy and lactation which made them more prone to infection (Ghosh *et al*., 2020).

In this present study, screening of breed wise prevalence data revealed that cross-bred HF (90.65%) and Jersey (81.91%) cattle were mostly infected with haemoparasites compared to zebu cattle (62.00%). Although very less number of cases presented, Sahiwal cattle also showed 60.00% prevalence of hemoparasite. Similar findings were recorded by several workers previously (Brahma *et al*., 2018; Ghosh *et al*., 2020; Sarmah and Kakati, 2014). The higher prevalence in Holstein-Friesian may be due to high milk yielding of the breed which acts as a stress factor.

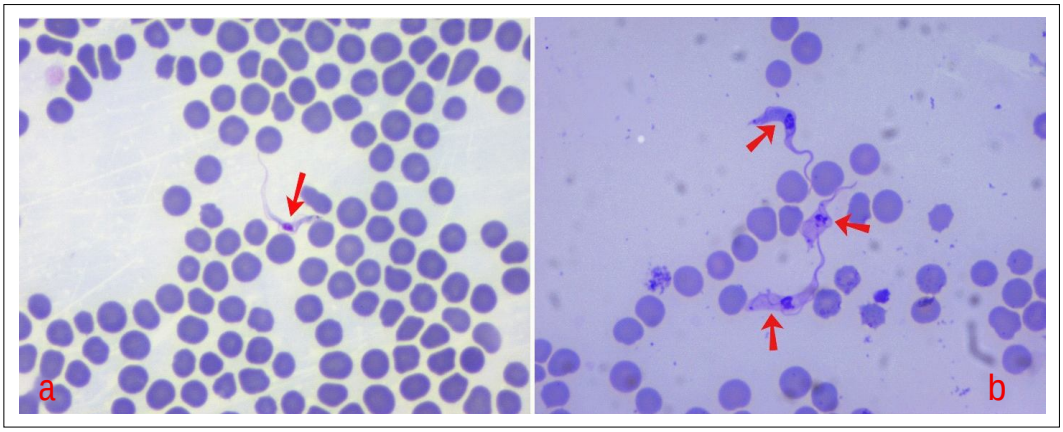

Fig 4(a-b): Peripheral blood smear showing *Trypanosoma evansi* (Arrow) (100x).

Table 1: Year wise overall prevalence of haemoparasites in peripheral blood smears examination.

Year	Total number examined	Total number positive (%)	Theileria (%)	Babesia bigemina (%)	Anaplasma marginale (%)	Mixed infection (%)
2017	495	441 (89.09)	324 (73.46)	60 (12.12)	47 (9.49)	10 (2.26)
2018	454	314 (69.16)	220 (70.06)	36 (7.93)	46 (10.13)	12 (3.82)
2019	530	512 (96.60)	381 (74.41)	57 (10.75)	57 (10.75)	17 (3.32)
2020	359	299 (83.29)	211 (70.56)	30 (8.36)	42 (11.70)	16 (5.35)
2021	262	201 (76.72)	153 (76.11)	13 (4.96)	20 (7.63)	15 (7.46)
Total	2100	1767 (84.14)	1289 (72.94)	196 (11.09)	212 (12.0)	70 (3.96)

P<0.05.

These findings also indicate the facts that the cross bred cattle are at a high risk and a preferred host of hemoparasites as compared to zebu cattle.

An effort was made to know the influence of seasonal variation on the prevalence of haemoprotozoan diseases. Theileriosis was found to be significantly high during monsoon (75.87%) and least in winter seasons (55.70%). The present investigation is in conformity with the report from Brahma *et al*. (2018), who also recorded a highest (53.85%) prevalence of Theileria in monsoon season followed by pre-monsoon season (48.84%). Ghosh *et al*. (2020) also recorded highest prevalence of haemoprotozoa infection in monsoon. However, there was no well-defined pattern of seasonal influence on the prevalence of babesiasis and anaplasmasis. The high prevalence of theileriasis observed in the monsoon season may be due to high abundance of tick vector, because high temperature and humidity is ideal for survival and breeding of ticks (Velusamy *et al*., 2014). The tick vectors are abundant during monsoon season but are found throughout the year, this may be the reason of prevalence of *Babesia bigemina* and *Anaplsma marginale* throughout the year.

Table 2: Season wise prevalence of Haemoparasites in peripheral blood smear.

Year	Season	Total number examined	Total number positive	Theileria	A. marginale	B. bigemina	Mixed infection
2017	Pre-monsoon	120	117	87	15	13	2
	Monsoon	240	231	177	28	22	4
	Post monsoon	83	69	50	10	6	3
	Winter	52	24	10	7	6	1
2018	Pre-monsoon	127	105	81	10	12	4
	Monsoon	207	126	93	12	17	4
	Post monsoon	71	53	31	7	11	2
	Winter	49	30	15	7	6	2
2019	Pre-monsoon	147	141	101	19	16	5
	Monsoon	227	226	172	27	20	7
	Post monsoon	106	104	83	6	12	3
	Winter	50	41	25	5	9	2
2020	Pre-monsoon	95	93	68	7	13	5
	Monsoon	138	130	93	15	16	6
	Post monsoon	81	45	32	2	7	4
	Winter	45	31	18	6	6	1
2021	Pre-monsoon	56	61	50	3	4	4
	Monsoon	74	66	56	2	5	5
	Post monsoon	83	51	32	6	4	4
	Winter	49	23	15	2	2	2
		2100	1767 (84.14%)	1289 (72.94%)	196 (11.09%)	212 (12.0%)	70 (3.96%)

Table 3: Age wise prevalence of Haemoparasites in peripheral blood smear.

Age group	Total number examined	Total number found +ve (%)	Theileria (%)	Babesia bigemina (%)	Anaplasma-marginale (%)	Mixed infection (%)
<1 year	521	387 (74.28)	231 (59.69)	98 (25.32)	53 (13.70)	5 (1.29)
1-5 year	687	613 (89.23)	339 (55.30)	141 (23.00)	104 (16.97)	29 (4.73)
>5 year	892	767 (85.99)	508 (66.23)	189 (24.64)	34 (4.43)	36 (4.69)

P<0.05.

Table 4: Sex wise prevalence of Haemoparasites in peripheral blood smear.

Sex	Total number cattle examined	Total number found +ve (%)	Theileria (%)	Babesia bigemina (%)	Anaplasma marginale (%)	Mixed infection (%)
Male	210	162 (77.14)	81 (50.00)	49 (23.33)	17 (8.10)	15 (9.26)
Female	1890	1605 (84.92)	932 (58.07)	426 (22.54)	192 (10.16)	55 (3.43)

P<0.05.

Table 5: Breed wise prevalence of Haemoparasites in peripheral blood smear.

Breed	Total number examined	Total number found +ve	Theileria (%)	Babesia bigemina (%)	Anaplasma-marginale (%)	Mixed infection (%)
Jersey cross	978	801(81.91)	641(80.02)	72(7.36)	52(5.31)	36(4.49)
HF cross	942	854(90.65)	673(78.81)	96(10.19)	61(6.48)	24(2.81)
Sahiwal	20	12(60.00)	8(66.67)	1(8.33)	2(10.00)	1(8.33)
Zebu	160	100(62.00)	67(67.00)	8(5.00)	16(10.00)	9(9.00)

P<0.05.

The comparison of seasonal prevalence study of *Theileria* over five years reveals that, from 2017 to 2020 monsoon season shows highest prevalence, followed by pre-monsoon ,post monsoon and lowest in winter. Similar trend was also observed in case of *Babesia bigemina* and *Anaplasma marginale*.

Although negligible in number, prevalence of intercellular haemo-flagellate *Trypanosoma evensi* was also could be detected from some of the blood samples which indicate that this haemo-flagelates can now be considered as an emerging parasitic risk for the livestock farmers of this region.

CONCLUSION

The present study suggests that the area in and around Guwahati, the capital city of Assam is highly endemic for hemoparasitic diseases in cattle which includes theileriosis, babesiasis and anaplasmasis. Along with sporadic occurrence of trypanosomiasis is also recorded. The study suggests that *Trypanosoma evensi* is an emerging parasitic risk in this area. Occurrence of the haemoparasitic disease is highest during monsoon season (June-September) followed by pre-monsoon (March-May) and post-monsoon (October-November). Least incidence is recorded during winter season (December-February). Cross-bred Holstein-Friesian animals, aged 1-5 years are highly susceptible to these haemoprotozoan diseases than indigenous animals. Female animals are more prone to haemoparasitic infection than male animals.

The outcome of the present study would help to forecast disease outbreak not only in this region but also applicable to other parts of country. There is a need for further investigations using molecular techniques to study the carrier status of haemoparasites and their vectors in this region of the country.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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