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Research Article

SEASONAL INFLUENCE ON PREVALENCE OF HAEMOPROTOZOAN PARASITIC DISEASES IN CROSSBRED CATTLE UNDER TERAI-DOOARS REGION OF WEST BENGAL, INDIA

Nonigopal Shit[1], Dilip Kumar Hajra[*2], Mrityunjay Mandal[3], Rahul Deb Mukherjee[4]

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ABSTRACT: : Diseases caused by hemoprotozoans in cross-bred cattle result in a large financial loss. It is thought that persistent climate change raises the risk of hemoprotozoan infection. Records regarding the frequency of these illnesses in West Bengal's Terai Region are scarce. To implement control measures, such data is required. Because of this, a study was conducted in the Terai Region of West Bengal to find out how common hemoprotozoan infection is in cross-bred cattle. By finding parasites in blood smears taken from animals exhibiting certain clinical symptoms typical of hemoprotozoan infection, the illness was identified. Over five years, 1308 blood samples from cattle that were suspected of being cross-bred were taken from various locations in the Terai-Dooars region. The blood smear was stained by Giemsa's stain and scrutinized under oil immersion microscopy. Blood biochemical parameters were analyzed to compare the impact in affected and healthy animals. *Theileria* sp., *Anaplasma* sp., and *Babesia* sp. were found in the screened samples. Seasonality has a major impact on the frequency of hemoparasitic infections. The wet season accounted for the majority of the occurrences (63.94%), with summer coming in second (52.52%) and winter third (31.76%). All told, hemoparasites were found in 51.98% of the suspected cases. Compared to *Babesia* sp. (13.68%) and *Anaplasma* sp. (31.03%), the prevalence of Theileria sp. (46.02%) was higher. The hematological observation showed statistically a significant ($p<0.05$) reduction of Hb (g/dl), PCV (%), and TEC (million/cm) in positive groups of crossbred cattle compared to the healthy animals. The result indicated that half of the suspected cases in the Terai zone of West Bengal would carry active haemo-parasites. There is a seasonal and species variation in the occurrence of haemo-parotozoan infection which can be capitalized for strategic control measures.

Keywords: Haemoprotozoa, Season, Haematology, Crossbred cattle, Terai Region.

INTRODUCTION

India has over 51 million exotic and crossbred dairy cattle, or roughly 26.5% of the country's total bovine population, according to the 20th Livestock Census. These animals produce a lot, but they are quite vulnerable to hemoprotozoan infection. According to reports, Tropical Theileriosis alone caused a loss of over US $800 million [1, 2], while Babesiosis and Anaplasmosis in India caused a loss of roughly, US $57 million [3]. Clinical signs in animals might range from fever, anorexia, anemia, emaciation, respiratory distress, coughing, jowl or brisket edema, enlarged superficial lymph nodes, hemoglobinuria, abrupt decrease in milk production, and severe cases, death from infections [4, 5, 6]. The identification of any disease is crucial to its management. For the identification of hemoprotozoan illnesses in animals, several molecular and serological diagnostic procedures are recommended [7], while the traditional method is the microscopical demonstration of infectious stages in blood or tissue [5, 8]. Since the development of resistance by the parasites is a persistent issue, research is being done to create effective techniques for controlling the carrier and intermediate hosts through

[1]RRS-Terai Zone, [2]Department of Agronomy, [4]SMS, KVK Cooch Behar, Uttarbanga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India.
[3]Asst Director, ARD Department, Govt. of West Bengal, India.
** Corresponding author. e-mail: dhajra@gmail.com*

innovative approaches [9, 10, 11]. To reduce hemoprotozoan infections, vaccinations against their carrier hosts are now being developed [12].

Because environmental cues that encourage tick multiplication are altered by climate change, the incidence of tick-borne disease has continuously increased [13]. Considering that ticks are a constant source of infection for susceptible animals, India's hot, humid atmosphere is perfect for tick development and survival [14]. These diseases limit the production and survival of cross-bred cattle in India and result in large economic losses because of mortality, reduced milk yield, reduced animal draught power, and increased management expenses [15, 16, 17]. West Bengal's Terai-Dooars region raises 13.40% of the state's total cattle population, contributing more than 9.15% of the breedable cross-bred population, which supports a potential dairy business for better living in this area [21, 22, 23]. The geo-climatic characteristics of this area, however, are conducive to tick survival and population growth, which raises the incidence of hemoparasitic illnesses transmitted by ticks. In this region, two additional risk factors for hemoprotozoan infection are malnutrition and traditional management practices. There are scant reports on the prevalence of these disorders in the Terai (Dooars) region of West Bengal. Many times, to strategize control measures of a disease it demands reports on the prevalence. Given this, a study was carried out in West Bengal's Terai-Dooars region to determine the prevalence of hemoprotozoan parasite infections in cross-bred cattle.

MATERIALS AND METHODS

Study area

The study was conducted in the Terai-Dooars region (foothills of Eastern Himalayas) of West Bengal constituted the districts of northern part of Cooch Behar, Alipurduar, Jalpaiguri and the plains of Kalimpong district lies between 22°38' and 23°38' North Latitude and between 86°36' and 87°46' East Longitude (Fig. 2). The area was witnessed with an average temperature 29.22-30.95ºC, humidity 82.54-90.70% and rainfall 236.44-326.42 mm throughout the study period during 2016-20 (Fig. 3).

Study period and season

This study was carried out over five years, from January 2016 to December 2020, using random sampling throughout the location, based on the population of cross-bred cattle. Three of the most notable seasons in the area were included in the prevalence study: summer (February-May), rainy (June-September), and winter (October-January).

Sample size

From January 2016 to December 2020, a total of 1308 cross-bred cattle exhibiting clinical signs that could be linked to hemoprotozoan disease were screened for the investigation of hemoprotozoan parasitic disease (Fig. 6). Blood samples were taken directly from livestock owners' doorsteps as well as from veterinary polyclinics and hospitals run by the West Bengal government's Animal Resource Development Department.

Preparation and examination of blood smear

Blood samples were collected only from clinically suspected cross-bred cattle either from the ear or jugular vein in an EDTA-coated vacutainer. A thin blood smear was prepared on a grease-free glass slide [24] following a label indicating the site and date of collection. The smears were stained using Giemsa's stain for 25-30 min after methanol fixation for a minute and examined under an oil immersion lens (100X magnifications) for the presence of haemoparasites (*Theileria* sp., *Babesia* sp. and *Anaplasma* sp.) individually [25] as depicted in Fig. 1. The percent prevalence of the different haemoparasites was determined [26].

Haemato-biochemical study

Samples from the healthy animals (control) and clinically suspected cases were collected and screened for hematological parameters.

1. Using Sahli's method [27], the hemoglobin concentration (Hb) was calculated and represented in grams per deciliter (gm/dl).
2. The Wintrobe hematocrit method was used to estimate packed cell volume (PCV), and the result was given as a percentage (%) of the total volume [27].
3. The hemocytometer was used to estimate the total erythrocyte count (TEC) and total leucocyte count (TLC), which were expressed as millions per cubic millimeter ($\times 10^6$/cm) and thousands per cubic millimeter ($\times 10^3$/cm), respectively.
4. Using a standard methodology, mean corpuscular hemoglobin (MCH) concentration and mean corpuscular volume (MCV) were assessed. The results were compared to look for any notable changes.

Data management and analysis

Data was organized and cleaned using Microsoft Excel 2007 (Microsoft Corporation, USA). The occurrence of positive cases for different haemoprotozoan diseases was expressed in percent. Variation in seasonal prevalence was analyzed by the Chi-square (χ^2) test using Statistical Package for Social Science [29]. The level of significance was shown by the p-value. Hematological parameters were analyzed by taking blood from 10% of the affected animals and were compared against values found in unaffected animals (n=25), considered as the control population using a t-test.

RESULTS AND DISCUSSION

The overall prevalence of haemoprotozoan infection

This study screened a total of 1308 blood samples from suspected crossbred cattle in the study location during five years (Jan 2016-Dec 2020) and 680 samples were found positive *i.e.*, the overall prevalence was 51.98% (Fig. 6). Out of these, 313 (46.03%) animals were positive for *Theileria* sp., 93 (13.68%) for *Babesia* sp. and 211 (31.03%) for *Anaplasma* sp. (Fig. 5).

The current study's findings supported reports from several other Indian states that hemoprotozoan infection is common in the Terai-Dooars region of West Bengal. The current study's infection incidence was higher than that of earlier findings [19], which may be related to the area's current agroclimatic conditions, which are ideal for vector survival and development. Notably, the majority of the animals who were suspected had symptoms for longer than a week. Higher incidence of Anaplasmosis and Theileriosis may be related to the involvement of the same ticks (*Hyalomma* sp.) carrying the two hemoprotozoan parasites.

Seasonal effect on prevalence

The occurrence of haemoprotozoan infection varied significantly ($p<0.05$) due to season.

More than 50 percent of the suspected cases were found positive in summer (52.52%) and rainy seasons (63.94%), while in winter it was 31.76 percent. The present result is in congruence with previous reports which showed a higher prevalence of Theileriosis during the rainy season [20, 30]. On the contrary, a few reports indicated a higher incidence of haemoprotozoan infections during summer [15, 31, 32]. Dissimilar demographic and agro-climatic conditions of the study locations might be responsible factors for such differences. The current findings may be explained by the fact that the rainy season promoted the growth of the vector population, or tick population, which could account for the increased incidence of hemoprotozoan infections. Wintertime lows in temperature and humidity, however, are not as beneficial for vectors. The study population experiences a lower prevalence of some diseases as a result of them hiding in cracks and crevices throughout the season as engorged females, nymphs, larvae, and unfed adults [33, 34]. Changes in the macroclimate, which is necessary for tick reproduction, could be the cause of a significant seasonal variation in the occurrence of hemoprotozoan infections.

Year-wise prevalence

Year-wise prevalence of haemoprotozoa infection is given in Fig. 4 and Fig. 5. The highest number of positive cases was recorded in 2018 (62.43%) compared to other years. Throughout the study period, the prevalence of *Theileria* sp. was dominant over other infections followed by *Anaplasma* sp. and *Babesia* sp. which could be due to the population density of the respective vectors in this geographical location.

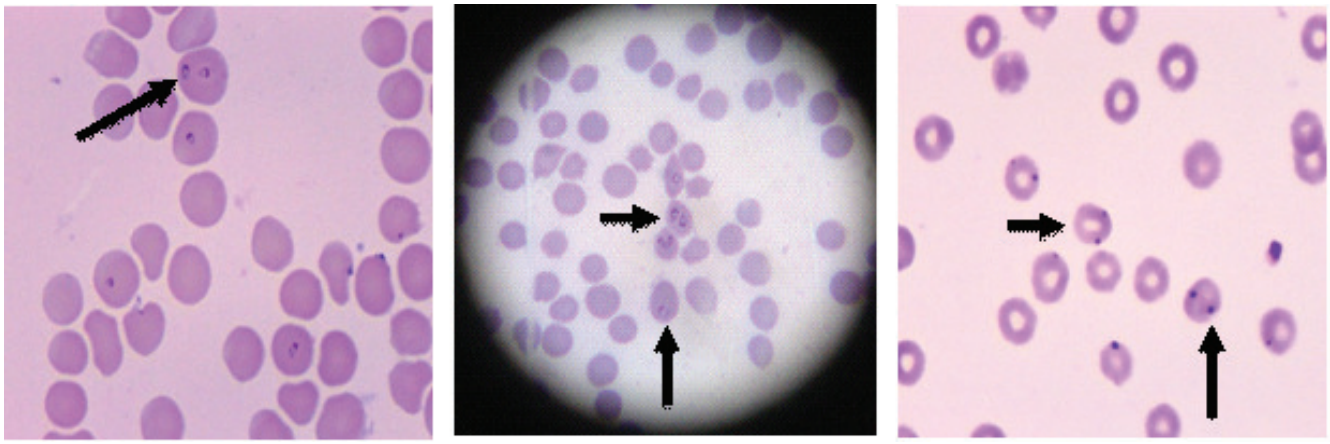
Changes in hematological parameters

There was a significant ($p<0.05$) reduction in PCV (29.01% vs 34.78%), Hb (8.12g/dl vs 11.26g/dl), and TEC (3.94 million/cm vs 8.09 million/cm) concentration in affected animals compared to the healthy ones (Fig. 7). On the contrary, considerably ($p<0.05$) increased values of MCHC (33.52 vs 31.73) and MCV (41.73 vs 39.57) were noted in infected individuals over the healthy crossbred cattle.

Bovine Theileriosis, Babesiosis, and Anaplasmosis infections resulted in macrocytic hypochromic anemia in animals with aberrant erythrocyte shape, including anisocytosis, poikilocytosis, basophilic stippling, hypochromasia, incinerated red blood cells, and polychromasia. When comparing clinically suspected cases to animals that appeared to be in good condition, the considerable decrease in PCV, Hb, and TEC concentration demonstrated a detrimental effect of hemoprotozoan infection on hematological value (Fig. 7). The changes in hematological parameters observed in this study are in agreement to some of the previous findings [35, 36]. The current findings may be linked to erythro-phagocytosis [38], destruction of erythrocytes by piroplasms that infect and multiply in them, and chronic blood loss by blood-sucking ticks that cause anemia [37, 38]. The organisms inside the

Table 1. Season-wise prevalence of tick-borne infection on blood smear examination of cross bred cattle under Terai-Dooars region of West Bengal.

Particulars	Summer (Feb - May)	Rainy (June - Sept)	Winter (Oct - Jan)	p value
No of animals screened (N)	535	477	296	-
No of positive cases (n)	281	305	94	-
Prevalence percentage (n/N)	52.52	63.94	31.76	p<0.05



A) *Theileria* sp. **B) *Babesia* sp.** **C) *Anaplasma* sp.**

Fig. 1. Slides of blood smear showing the presence of *Theileria* Spp. (Panel-A), *Babesia* Spp. (Panel-B) and *Anaplasma* Spp. (Panel-C) under oil immersion (100 X) magnification.

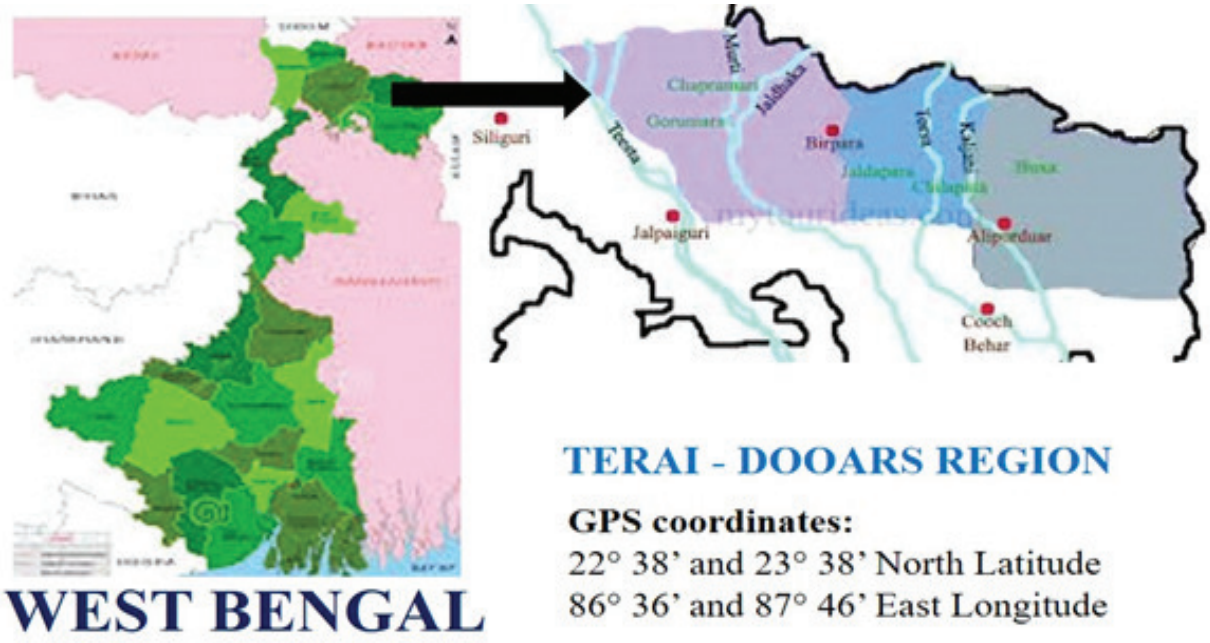


Fig. 2. Map showing the geographical study location of Terai-Dooars region of West Bengal, India.

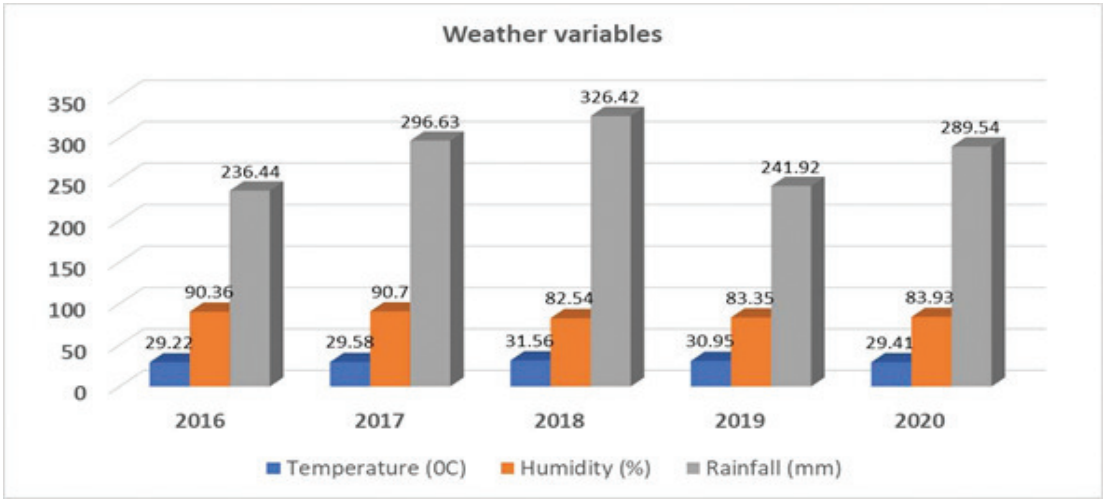


Fig. 3. Report of weather variables (Temperature, Humidity and Rainfall) during the study period in the Terai-Dooars region of West Bengal, India.

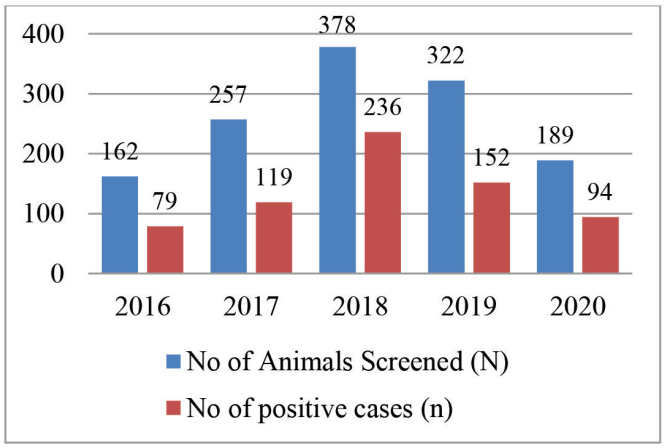


Fig. 4. Number of positive cases against the total number of samples screened for haemoprotozoan disease in cross bred cattle during the study period under Terai-Dooars region of West Bengal, India.

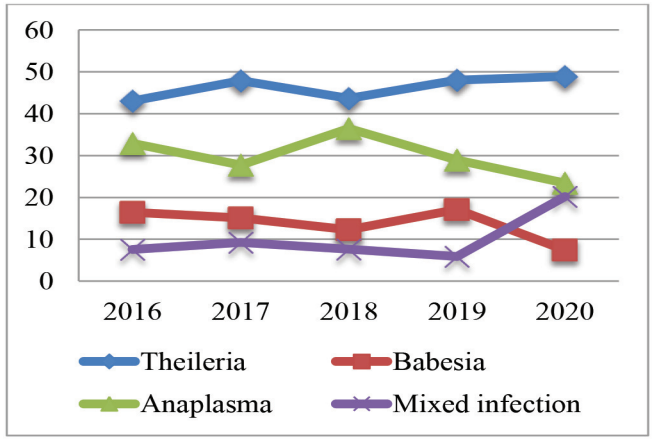


Fig. 5. Year wise prevalence (per cent) of different haemoprotozoan diseases in cross-bred cattle under Terai-Dooars region of West Bengal, India.

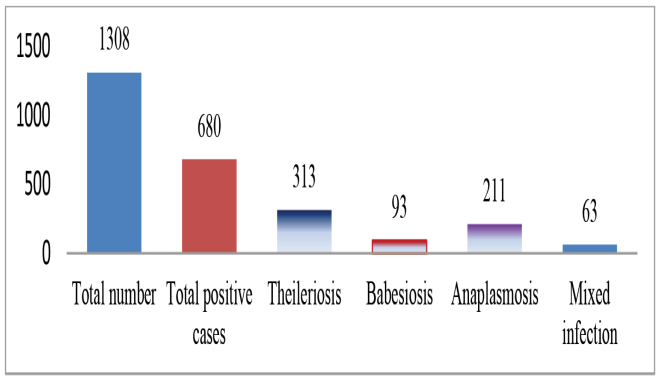


Fig. 6. Overall incidence (number) of tick-borne haemoprotozoan disease on blood smears examination in cross bred cattle recorded under Terai-Dooars region of West Bengal, India.

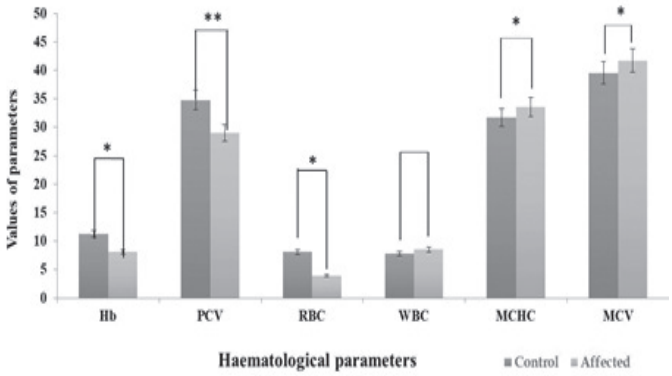


Fig. 7. Changes of haematological parameters in tick-borne haemoprotozoan disease and non-infected groups of cross bred cattle under Terai-Dooars region of West Bengal, India. [*p<0.05, **p<0.01; Control - N=25; Affected - 10% of sample; Hb (g/dl), PCV (%), RBC (10^6/mm^3), WBC ($\times 10^3$/µl), MCHC (g/dl) and MCV (fl); Hb- Haemoglobin; PCV- Packed cell volume; RBC- Red blood cells; WBC -White blood cells; MCH- Mean corpuscular haemoglobinand MCV- Mean corpuscular volum]

red blood cells during their growth may have caused damage, which could explain the change in the hemogram [15, 18]. All of these hemogram abnormalities were brought on by anemia brought on by the toxic metabolites of tick-borne hemoprotozoa, which damage bone marrow by interfering with erythropoiesis [39]. Escalation of leucocyte count was also reported in a similar study [40]. *Theileria annulata* has been reported [41] to cause lower hemoglobin and total erythrocyte counts. Alteration in the antigenicity of erythrocytes due to the entry of parasites evokes an autoimmune reaction in the body which triggers the removal of infected erythrocytes from circulation by macrophages (erythro-phagocytosis) causing anemia [42].

CONCLUSION

It can be concluded that the Terai-Dooars region of West Bengal, India, is highly endemic for haemoprotozoan parasites. Theileriosis (46.03%), Babesiosis (13.68%), and Anaplasmosis (31.03%) are the predominant haemo-protozoan diseases. Farmers and clinicians of the region need to be cautious during summer and rainy seasons as the haemoprotozoan infection is likely to occur in 50 percent of the suspected cases in cross-bred cattle. The changes in haematological parameters indicated physiological deviation which would subsequently affect production efficiency including current production loss. With the available diagnostic facility for the animal resource of the state, strategic screening of blood smears under a microscope may be adopted to control the haemoparasite. However, policy-level intervention is needed to adopt robust control measures to resist losses of dairy farmers from haemoprotozoan diseases.

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