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Study of prevalence and diagnosis of babesiosis in cattle

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Abstract
The present investigation was conducted to study the prevalence and diagnosis of in and around Guwahati, the headquarter of Kamrup (Metropolitan) district and the capital city of Assam. The study was conducted from October 2015 to September 2016 for a period of one year. In the present study, the overall prevalence of babesiosis in cattle was 8.78 percent. Age wise distribution of the positive cases showed the highest prevalence in the age group of 2.5-7.5 years. The highest seasonal prevalence of babesiosis in cattle was recorded in the monsoon season and lowest in the winter season. Although it affects animals of both the sexes, yet, the prevalence rate was more in the female. Clinical signs of anorexia, the presence of ticks on body coat, pale mucous membrane, high rise of body temperature, coffee colored urine and drop in milk yield were recorded. In 33.34% of cases, the reddish faecal matter was observed.

Keywords: Prevalence, babesiosis, cattle, north east region, agro-climatic condition

1. Introduction
Livestock sector plays a significant role throughout the world in terms of food, energy, raw material and manure [1]. It also plays an important role in the upliftment of rural economy in India, whereas 68 percent of the people are dependent on agriculture and animal husbandry. Profitability and viability of a dairy farm depends mainly on the production. However, disease stands as a major constraint in the production, besides adoption of good managemental practices. Among various tick-borne diseases bovine babesiosis is one of the important haemoparasitic diseases that cause significant morbidity and mortality in cattle [2]. Annual economic losses to the livestock industry due to babesiosis in India are estimated to be about 57.2 million US dollars [3]. The tick responsible for the spread of the organism is *Boophilus microplus*. The susceptible host (cattle) acquires the infection by the introduction of sporozoite stage of the Babesia species into the blood circulation from the salivary gland of a tick during a blood meal. On reaching the circulation, it penetrates the cell membrane of Red Blood Cell and undergoes asexual mode of reproduction i.e. binary fission [4]. The disease is characterized by high rise of temperature, haemoglobinuria, pale visible mucous membrane, diarrhoea, sudden drop in milk yield [5]. The disease causes significant economic loss in terms of milk production and death of the animal. Sometime it occurs without showing any typical clinical sign. The recovered animals remain as carriers with subclinical infection lasting for several months or years. Looking into the problems and losses incurred by the dairy farmers by the disease, it would be judicial to carry out a systemic study on the disease for the well-being of the livestock as well as improvement in their production.

2. Materials and Methods
The present study was conducted in and around Guwahati, the headquarter of Kamrup (Metropolitan) district as well as the capital city of Assam. The southeast periurban area of Guwahati, bordering the Ri-Bhoi district of Meghalaya is important as a recognized cattle trade center of the Northeast having the highest population of Jersey and Holstein-Friesian crossbred cattle under unorganized livestock farming private sector to supply a sizeable amount of milk to the Guwahati city as well as adjoining areas. A total of 239 animals both crossbred and indigenous were included in the present study conducted in one year period from December' 2015 to November' 2016. All the crossbred animals were purely stall-fed whereas the indigenous animals were of open grazed type.

Prevalence of babesiosis in cattle of organized and unorganized sheds were ascertained on the basis of herd history, clinical case study and parasitological examination of blood by microscopy. Some of the samples, found negative in microscopy were subjected to a molecular assay for diagnosis. Information was also obtained from veterinary practitioners in regards to their experience on coming across such clinical cases/previous laboratory diagnosis and treatment of animals against haemoparasitic infections in the locality.

About 5 ml of blood samples from each of 239 animals were obtained by venipuncture and collected partly in properly labeled vacutainer containing Ethylene Diamine Tetra Acetic Acid (EDTA) and in sterilized vials without anticoagulant for separation of serum. The samples were brought to the laboratory for parasitological, hematological and molecular studies. Parasitological and hematological studies were done on the same day of blood collection. For molecular and serological studies, the serum was separated using centrifugation and was preserved in deep freeze at -20ºC until use.

A thin blood smear was prepared from each of the anticoagulated blood samples by spreading a small drop of well-mixed blood over a clean glass slide and fixed with methanol after drying. Fixed smears were then stained with commercial Giemsa stain diluted in buffered water (Soren Sen Buffer; pH = 7.4) and were kept for 45 minutes. The stained smears were then washed thoroughly with tap water and air dried. Stained smears were later examined under oil immersion objective (100X) of a compound microscope for detection of *Babesia* organism within and outside the red blood cells. The parasites when present were identified on the basis of their characteristic morphology [4]. Failure to detect parasite in a smear even after examination of at least 500 oil immersion fields was recorded as a negative blood sample.

Twenty-one blood samples that were negative for *Babesia* in microscopy were randomly selected and screened for the detection of parasite DNA using Polymerase Chain Reaction (PCR) as per standard method. The test was conducted for identification of *B. bigemina* and *B. bovis*, using published standard primers.

DNA Extraction was carried out using the DNeasy Blood and Tissue kit (Qiagen® Kit, Catalogue No. 69504) as per manufacturer's protocol. About 100 µl of anti-coagulated blood was taken individually from each of the samples in a 2 ml microfuge tube and then it was lysed in 20 µl proteinase K and the volume was adjusted to 220 µl by adding Phosphate Buffered Saline (PBS). The tubes were then incubated at 56°C in water bath for 10 minutes. Final elution was done with 100 µl elution buffer (Buffer AE) and the templates were preserved at -20°C, until further use.

The standard primers for molecular identification of *B. bigemina* and *B. bovis*, the published primer sequence pairs (forward and reverse), amplification targets and product size in base pairs (bp) has been presented (Table.1).

PCR assays to detect *B. bigemina* and *B. bovis* were carried out using the protocols mentioned (Table. 2) in a Techne-500 thermal cycler (Bibby Scientific). The PCR products were subjected to electrophoresis in an agarose gel prestained with Ethidium Bromide (0.5 µg/ml) and subsequent visualization done in gel documentation system (DNR Mini Lumi, Applied Bioimaging).

All the animals under the study were subjected to physical examination individually for record of body temperature, the colour of mucous membrane, appetite status, depression, respiratory rate, heart rate, coloration of dung /urine and mortality if any.

3. Results and Discussions

The overall prevalence of babesiosis in cattle was 8.78 percent through microscopy (Fig. 1) and P.C.R. (Fig. 2). A similar finding was reported by Jyotishree *et al.* [5] from Andhra Pradesh, who recorded an overall prevalence of 8.02 percent. The prevalence was recorded lower through microscopy (5.85%) than PCR (33.34%). It might be due to the efficacy of PCR to detect even very low grade of parasitemia that is not possible through microscopy. Moreover, in the present study, the number of samples screened through PCR was quite lower than that of microscopy.

Age wise distribution of the positive cases revealed the highest prevalence (10.48%) in the age group of 2.5-7.5 years which might be attributed to the production associated stress experienced by this group of animals. This finding is in accordance with Wadha *et al.* [6] and Ananda *et al.* [7], who in their studies, found that the age group of 4-6 years was mostly affected. No prevalence was recorded in the age group below 6 months.

In the present study, the highest prevalence of babesiosis in cattle was recorded in the monsoon season (13.54%) and lowest in the winter season (2.56%). This might be due to the abundant growth and multiplication of tick vector of the Babesia organism in the hot and humid weather of monsoon season. This finding is in agreement with other workers [5, 8].

Although it affects animals of both the sexes, yet, the prevalence rate was more in the female which was due to maximum numbers of sample collected from the females under study.

The present study on babesiosis in cattle recorded clinical signs like anorexia, the presence of ticks on body coat, pale mucous membrane, high rise of body temperature, coffee colored urine and drop in milk yield. Various workers from different parts of India and abroad recorded similar findings in their studies [6, 9, 10]. In 33.34% of cases, the reddish faecal matter was observed which is specific finding for *Babesia bigemina* infection as documented by [4]. Some of the positive cases showed no typical clinical abnormalities indicating a latent or subclinical infection. Kakati *et al.* [8] also recorded the presence of subclinical babesiosis in cattle.

Table 1: Standard primers used for identification of *B. Bigemina* and *B. Bovis* along with their amplification targets and product size

Parasite	Primer sequence pair	Amplification Target	Product Size	Reference
Babesia bigemina	Bbi:F1:5'-TGG CGG CGT TTA TTA GTT CG-3' Bbi:R1:5'-CCA CGC TTG AAG CAC AGG A-3'	A portion of the *Babesia bigemina* mitochondrial DNA	1124 bp	Laha *et al.*, 2012
Babesia bovis	Bbo:F1:5'-GGG TTT ATA TAG TCG GTT TTG T-3' Bbo:R1:5'-ACC ATT CTG GTA CTA TAT GC-3'	A portion of the *Babesia bovis* mitochondrial DNA	711 bp	Nutcha *et al.*, 2004

Table 2: Thermocycling conditions of Pcr for detection of *B. Bovis* and *B. Bigemina*

Parasite	No. of Cycles	Initial Template Denatu-ration	Denatura-tion	Primer Annealing	Primer Extension	Final Extension	Hold
Babesia bigemina	40	94 ˚C, 2 min	94 ˚C, 30 sec	55 ˚C, 30 sec	72 ˚C, 1 min	72 ˚C, 5 min	4 ˚C
Babesia bovis	30	94 ˚C, 6 min	94 ˚C, 1 min	55 ˚C, 2 min	72 ˚C, 3 min	72 ˚C, 7 min	4 ˚C

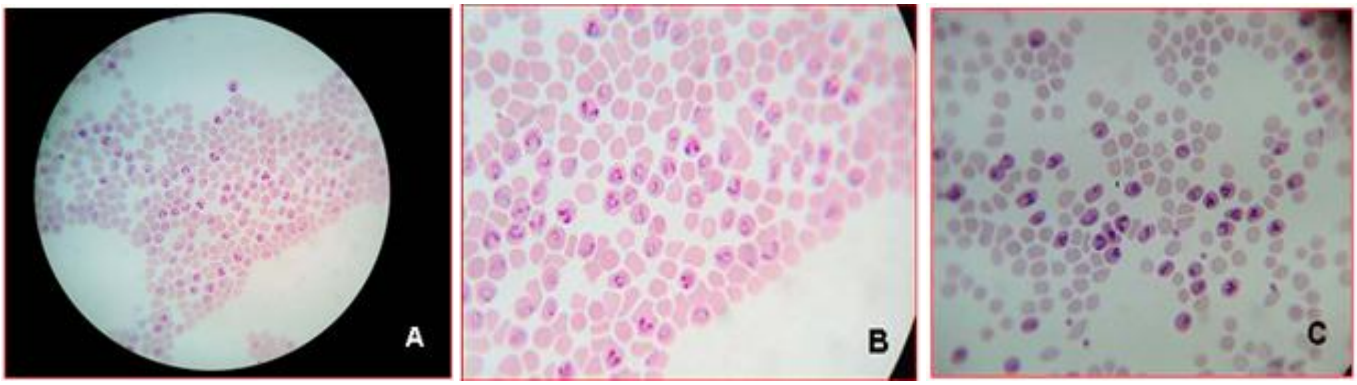

Figure 1(A, B, C): Photomicrograph showing pear shaped *Babesia bigemina* organism inside red blood cells.

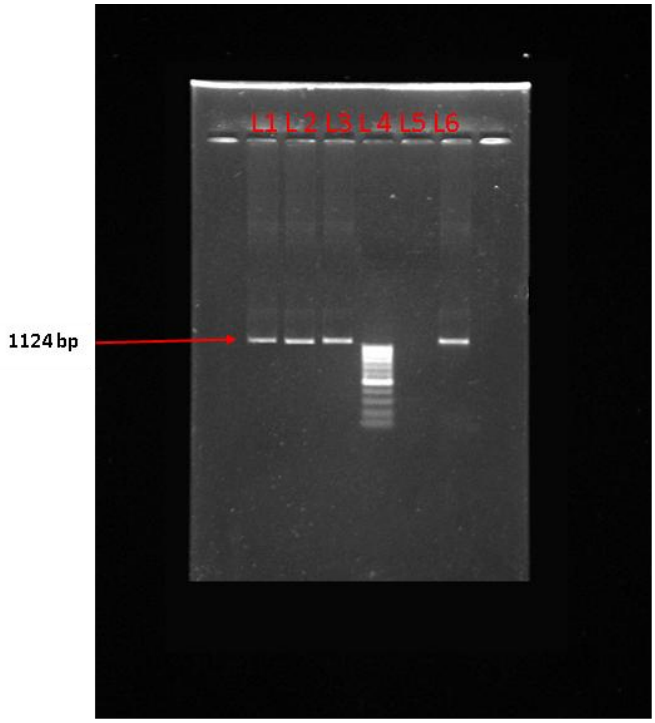

Fig 2: Photograph showing 1124 bp fragment of *Babesia bigemina* dna in 1.5 % agarose gel. L1 positive sample (1124 bp) L2 positive sample L3 positive sample L4 DNA ladder (100bp plus) L5 negative control L6 positive control

4. Conclusion

In the present study, the overall prevalence of babesiosis in cattle was recorded 8.78 percent. The prevalence recorded through microscopy (5.85%) was lower than PCR (33.34%). Age wise distribution of the positive cases revealed the highest prevalence in the age group of 2.5-7.5 years (10.48%) and lowest in 6 month to 2.5 years (6.94%). The highest seasonal prevalence of babesiosis in cattle was recorded in the monsoon season (13.54%) and lowest in winter season (2.56%). Although the disease can occur in both the sexes, yet, the prevalence rate was more in female in comparison with male. The present study recoded the presence of an atypical clinical symptom of reddish color faecal matter in 33.34% of the total positive cases besides the clinical symptoms like anorexia, the presence of ticks on body coat, pale mucous membrane, high rise of body temperature, coffee colored urine and drop in milk yield.

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