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Research Article
Parasitological and Molecular Based Detection of Cerebral Babesiosis in Kankrej Bullock and its Successful Therapeutic Management

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Abstract

Background and Objective: Bovine babesiosis is an economically important tick borne disease of tropical and subtropical parts of the world including India. The disease accounts for significant morbidity and mortality in cattle and buffaloes. The current study investigates the rare occurrence of cerebral form babesiosis in a Kankrej bullock by microscopy and polymerase chain reaction (PCR) based assay and its successful therapeutic management. **Materials and Methods:** A 4-years-old Kankrej bullock was brought to Teaching Veterinary Clinical Complex (TVCC), College of Veterinary Science and A.H., Junagadh, Gujarat with symptoms of high temperature (108°F), dullness, dyspnoea, pale mucous membrane, icterus, anaemia, hyperexcitability and convulsions. Blood sample was collected and subjected to various haematological parameters estimation, microscopic examination and PCR for detection of haemoparasites. **Results:** Optical microscopy based thin blood smear revealed the presence of *Babesia* piroplasm in the circulating erythrocytes. It was further confirmed by PCR that amplified an approximately 278 bp specific for *Babesia bigemina* (*B. bigemina*). The haemogram revealed suppressed haematological indices. The clinical and haematological findings coupled with detection of piroplasm by conventional and molecular techniques in circulating erythrocytes made us infer that the bullock was suffering from cerebral form of babesiosis. The bullock was treated with Diminazene aceturate along with the supportive therapy. The bullock promptly responded to therapy within 72 h and effectively restored the normal haematological indices and erythrocytes were found to be free from the piroplasms. **Conclusion:** The current study highlights the importance of microscopy complemented with PCR for accurate detection and could be used for diagnosis of latent infection in carrier animals. This seems to be the first report of cerebral form of babesiosis in a Kankrej bullock and placed on the record.

Key words: *Babesia bigemina*, bullock, cerebral babesiosis, diminazene aceturate, haemogram, therapeutic management

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The bullock plays an important role in the economic upliftment through various form in the agricultural operational works for poor and marginal farmers in the under developed and developing countries. Amongst the haemoprotozoan diseases of animals, babesiosis is an intraerythrocytic tick-transmitted disease caused by protozoans of the genus *Babesia* and it is characterized by haemolytic anemia and fever, with occasional hemoglobinuria and death[1-3]. It is a disease with a world-wide distribution affecting many species of mammals with a major impact on cattle and affecting growth and productivity of the animal[3,4]. Among the 6 species causing bovine babesiosis, babesiosis associated with *B. bigemina* and *B. bovis* is the most important disease of tropical and subtropical regions including India[5]. The one host tick *Boophilus microplus* is the vector of the disease in India, transmitting the disease both trans-stadially and trans-ovarianly[6,7]. During acute phase of infection, the parasite can be easily diagnosed by optical compound microscopy based detection method. But it is quite difficult to detect in carrier cattle owing to low numbers of parasites in peripheral blood. With the advent of molecular techniques, PCR has proven to be very sensitive particularly in detecting *Babesia* spp. in carrier cattle[8-15]. The cerebral form of disease has been rarely reported from this part of the country. This paper highlighted the diagnosis of an infrequent and interesting case of cerebral form of babesiosis in a Kankrej bullock by traditional as well as molecular based technique and its successful therapeutic management.

MATERIALS AND METHODS

History and clinical observations: A 4-years-old bullock having approximately 400 kg weight was presented at the Teaching Veterinary Clinical Complex, College of Veterinary Science and A.H., Junagadh, Gujarat for the treatment. The owner reported fever, subsequent anorexia since last few days and circling movement. Clinical symptoms revealed high temperature (108°F), dullness, dyspnoea, pale mucus membrane, anorexia, ruminal hypotonicity (1/3 min), aggressiveness, grinding of teeth, cessation of defecation, circling movement, icterus, anaemia, hyper excitability and convulsions.

Sample collection: Blood samples were collected from the ear tip by using a sterile sharp needle in clean and dry vial containing di-sodium EDTA as an anticoagulant to check for any haemoparasite, isolation of genomic DNA and for evaluation of various haematological parameters. Additionally, prescapular lymph node was aspirated to check for presence of haemoparasite, if any. Coprological samples were also collected per rectally. Ticks from body coat were collected in 70% alcohol and processed for identification.

Conventional parasitological method: Thin blood films were prepared, air dried and fixed in absolute methyl alcohol for 1-2 min. Subsequently, stained with freshly prepared giemsa stain for 45 min and then washed with distilled water to remove excess of stain. The slides were left for drying and examined under oil immersion lens.

Haematological analysis: The hematology of the whole blood was done with fully automated analyzed haematology system (Mindray, China) as per instructions of the manufacturer.

Molecular diagnostic method

Preparation of DNA template: Genomic DNA was extracted from whole blood collected in EDTA coated vacationer using GENEJET whole blood genomic DNA purification mini kit as per the given protocol (Thermo Scientific, Lithuania). The genomic DNA of infection free leucocytes separated from the blood of a 3-days-old neonatal bovine calf was included as negative control while genomic DNA isolated from *Babesia bigemina* infected erythrocytes of clinically infected bullock was used as positive control.

PCR protocol: The PCR assay was initially optimized for genus level identification with primers specific for *Babesia* spp. targeting a portion of ssu-r DNA[16]. The PCR positive samples were further analyzed for species level identification with the primers specific for *B. bigemina*[10]. The details of the primers used in the current study are depicted in Table 1 along with the expected amplicon size. The PCR assay in a final volume of 25 µL was carried out in a PCR thermal cycler (Applied Biosystem, USA). The master mix consisted of 2.5 µL of Dream *Taq* buffer (Thermo Scientific, USA), 0.5 µL of 10 mM dNTP mix (Thermo Scientific, USA), 1 µL each (20 pmol) of the primers, 0.2 µL of recombinant *Taq* DNA Polymerase (Thermo Scientific, USA) and 1 µL of template DNA isolated from infected bovine blood. The volume was made up to 25 µL with nuclease-free water. The PCR cycling conditions for were set in automated thermal cycler with the following programme: Initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 1 min, extension at 72°C for 1 min and the final extension at 72°C for

Table 1: Primer sequences along with expected amplicon size

Target organism	Nucleotide sequence	Product size (bp)	References
Babesia spp.	Ba F: 5'AATACCCAATCCTGACACAGGG3'	410	Olmeda *et al.*[16]
	Ba R: 5'TTAAATACGAATGCCCCCAAC 3'		
Babesia bigemina	BabF:5'CATCTAATTTCTCTCCATACCCCT CC 3'	278	Figueroa *et al.*[10]
	BabR:5'CCTCGGCTTCAACTCTGATGCCAAAG 3'		

10 min. The PCR cycling conditions for *B. bigemina* was similar except the annealing temperature at 57°C for 1 min.

Treatment: The affected bullock was treated with diminazene aceturate (Injection Berenil® 7%, Intervet, India) at 3.5 mg kg^{-1} b.wt., deep intramuscularly single time along with supportive therapy i.e., antibiotic like long acting oxytetracycline (Injection Intamycin LA, Intas Pharmaceuticals, India) at 20 mg kg^{-1} b.wt., at 48 h interval on 2 occasions, analgesics like ketoprofen (Injection Neoprofen, Zoetis, India) at 2.2 mg kg^{-1} b.wt., intramuscularly at 12 h interval for 3 days, hematinic (Injection Feritas, Intas Pharmaceuticals, India) at 10 mL intramuscularly thrice weekly for 1 week, liver stimulant drug (Livoferol, Vetcare, India) at 50 mL orally twice a day for 10 days along with intravenous infusions in prescribed doses. Butox (Hoechst) at 2 mL L^{-1} of water was recommended for external application on the body as well as on premises for tick control and the animal owner was advised to take special care of the bullock in shaded area with complete rest for 3 days.

RESULT

Clinical examination of the bullock revealed high temperature (108°F), ruminal hypotonocity (1/3 min), anorexia, aggressiveness, grinding of teeth and cessation of defaecation, circling movement, icterus, anaemia, paleness of conjunctival mucous membrane, hyper excitability and convulsions. Haematological analysis revealed suppressed haematological indices (Hemoglobin-10.0 g dL^{-1}, PCV-36%, TLC-4.0×10^3 μL^{-1}) and altered differential cell counts (Neutrophils 22%, Lymphocytes 71%, Monocytes 3% and Eosinophils 4%). In the present study, examination of giemsa stained blood smears revealed intra-erythrocytic pyriform shape of *Babesia* spp. (Fig. 1). Examination of coprological sample did not reveal any parasitic ova, egg or cyst. Based on the morphological features, the ticks were identified as *Boophilus microplus*. Specific primer directed amplification of PCR assay revealed the amplicon at 410 and 278 bp in 1.2% agarose gel corresponding to *Babesia* genus and *Babesia bigemina*, respectively (Fig. 2 and 3). The animal was managed with therapeutics and showed prompt recovery.

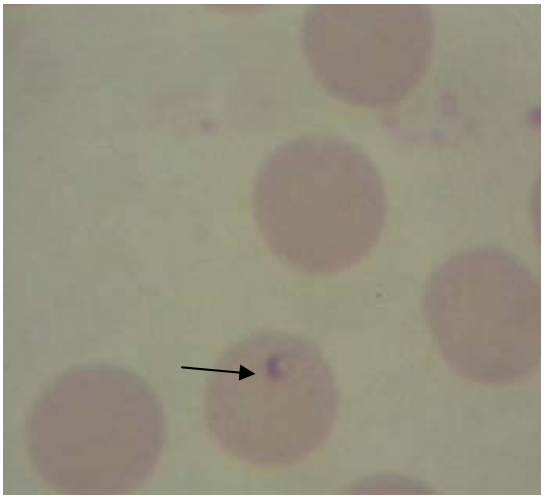

Fig. 1: Intra erythrocytic *Babesia bigemina* organism under oil immersion lens (x1000)

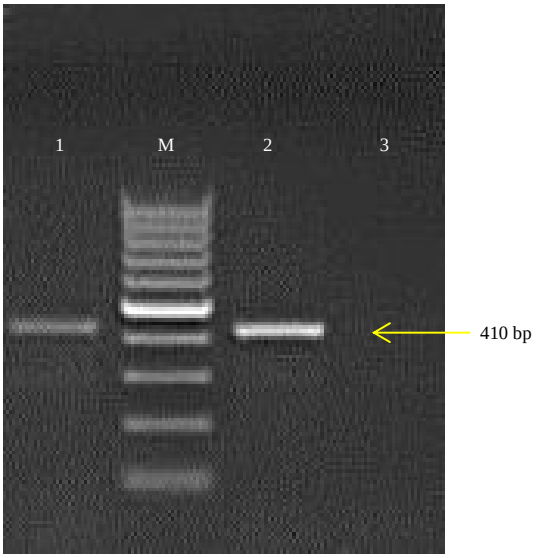

Fig. 2: Agarose gel 1.2% electrophoresis showing amplified DNA from *Babesia* spp. (410bp), Lane M: Molecular marker 100 bp, Lane 1: Amplification of *Babesia* genomic DNA from the blood of bullock infected with *B. bigemina*, 2: Amplification of *Babesia* genomic DNA from the blood of animal positive for infection (Positive control), 3: Negative control (Genomic DNA from host leucocytes)

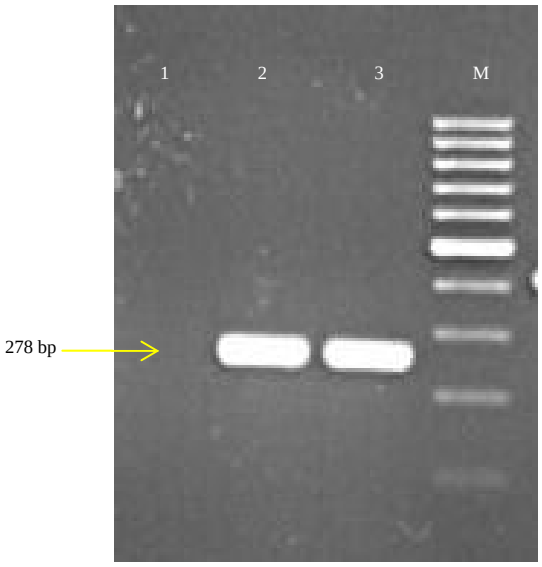

Fig. 3: Agarose gel 1.2% electrophoresis showing amplified DNA from *Babesia bigemina* (278 bp), Lane M: Molecular marker 100 bp, Lane 1: Negative control (Genomic DNA from host leucocytes), 2: Amplification of *B. bigemina* genomic DNA from the blood of animal positive for infection (Positive control), 3: Amplification of *Babesia bigemina* genomic DNA from the blood of infected bullock

DISCUSSION

Clinical examination of the presented case is suggestive of cerebral babesiosis. Extremely high temperature and heat stress during summer months are recognized as major predisposing factors[17]. The sudden onset of high fever (40-41°C) as response to effect of unspecific toxic substances produced during the metabolism of *Babesia*. Subsequently, the heart rate was increased, marked dyspnea was developed and visible mucous membranes were first congested but very soon became pale and icteric. More or less similar findings were also reported by several workers in cattle suffering from cerebral babesiosis[17,18]. Hematological findings were suggestive of the animal suffering from a milder form of anaemia, severe leucopenia, lymphocytosis and moderate eosinophilia. The present findings were in conformity with various workers[19,20]. Anaemia was possibly due to the destruction of RBC by emerging parasites, increased phagocytic activity of non-infected erythrocytes by reticulo-endothelial cells and suppression of erythropoiesis. The nervous signs appeared in the present case might be attributed to blood stasis incidental to clogging of brain capillaries by agglutination of parasitized erythrocytes[9]. Optical microscopy revealed pear shaped piroplasm in RBC suggestive of *Babesia* species. The observations are in accordance with the previous reports[8]. Morphological variability may make precise species identification difficult on the basis of smear examination. With the advent of molecular techniques, PCR has proven to be very sensitive particular in detecting *Babesia* spp. in carrier cattle[21,22]. In the present investigation, PCR assay further confirmed the diagnosis by using genus and species specific primers targeting *B. bigemina* genomic DNA revealing a fragment of size approximately 410 and 278 bp, respectively, in 1.2% agarose gel. The successful treatment depends on early diagnosis and the prompt administration of effective drugs[23]. Treatment with injection of berenil deep intra-muscularly along with injection of intamycin LA found to be very effective in early elimination of *Babesia* organism from blood circulation. The supportive therapy with injection of feritas revamped anaemia whereas liquid livoferol and intravenous infusions helped to restore rumen motility and appetite. Blood report after 3 days showed magic improvement in blood parameters with absence of piroplasms. Similar line of treatments for babesiosis have also been reported using diminazene aceturate (3-5 mg kg^{-1})[24]. Diminazene aceturate is the most commonly used anti-trypanosomal agent in the treatment of bovine babesiosis[25]. Diminazene binds irreversibly to double-stranded DNA, in the groove between complementary strands, via specific interaction with sites rich in adenine-thymine base pairs[26]. Diminazene aceturate is effective against *B. bigemina* but less effective against *B. bovis* and *B. divergens*[27]. Additionally, supportive therapy such as blood transfusions, anti-inflammatory drugs, tick removal, iron preparations, dextrose, vitamins (B-complex), purgatives and fluid replacements, may be necessary in severe cases of babesiosis.

CONCLUSION

This paper highlighted the diagnosis of an infrequent and interesting case of cerebral form of babesiosis in a Kankrej bullock by traditional as well as molecular based technique and its successful therapeutic management. The molecular technique used in the present study seems to be helpful for the diagnosis of babesiosis in animals in the initial phase of infection and in carrier animals by DNA amplification and it may be used as a tool for epidemiological investigation and successive disease eradication.

SIGNIFICANCE STATEMENTS

This study reports the rare occurrence of cerebral form of babesiosis in Kankrej bullock by traditional as well as molecular based technique that can be beneficial for target selective treatment of affected animal. This study will help the researchers for the diagnosis of babesiosis in animals in the initial phase of infection and in carrier animals by DNA amplification and it may be used as a tool for epidemiological investigation and successive disease eradication.

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