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A CLINICAL CASE OF BABESIOSIS IN A CROSS BRED COW OF MEGHALAYA

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ABSTRACT

A cross-bred cow of aged four years, maintained in the dairy farm of ICAR research complex for NEH Region, Umiam, Meghalaya, in its 3 months of lactation was found suffering from high rise of temperature (106.4^{0}F), haemoglobinurea, anorexia, decrease milk production, anaemia and diarrhoea. Blood samples of this cow were collected in sterile condition on the day of acute stage of infection, 48 hours post treatment (PT) and then at an interval of 3 days up to a period of 21 days post treatment for diagnosis of the infection as well as to monitor the infection. Each and every time the collected blood samples were processed for diagnosis of Babesiosis using conventional parasitological and molecular techniques using polymerase chain reaction (PCR). The body of the cow was thoroughly searched for presence of ticks, if any. The disease was diagnosed as *B. bigemina* infection after examination of Giemsa stained blood smears and PCR using *B. bigemina* specific primers. The tick *Boophilus microplus* was found to present in the body of the cow. The animal was treated successfully with a single injection of 4,4' Diamidine diazoamine benzene diaceturate (Berenil) with recommended doses and no parasite was detected either by examination of Giemsa stained blood smears or PCR after a period of 48 hours PT onwards.

Key words: *Babesia bigemina*, Cross bred cow, Hilly region, Treatment.

Haemoprotozoan diseases in cross-bred cattle are the major constraint for livestock development. After adoption of cross breeding programme in cattle in India to increase the production, the incidence of haemoprotozoan diseases also increased and Babesiosis played a major role with other haemoprotozoan infections. Bovine babesiosis caused by *Babesia bigemina*, is an important tick borne disease especially in the tropical and subtropical countries of the world. Several reports on occurrence of Babesiosis in cattle in India are available (Reddy *et al.,* 1997; Jithendran, 1997; Garg *et al.,* 2004; Ravindran *et al.,* 2002, 2007; Singh *et al.,* 2007, 2009). But there are scanty reports of Babesiosis in cattle from hilly region of India. A clinical case of Babesiosis caused by *B. bigemina* in a cross bred cattle maintained in a farm of hilly region of India was diagnosed by both conventional parasitological and molecular techniques is being reported through this communication.

A cross-bred cow (No. 33) aged four years, maintained in the dairy farm of ICAR research complex for NEH Region, Umiam, Meghalaya, in its 3 months of lactation (first lactation period) was found suffering from high rise of temperature (106.4^{0}F), haemoglobinurea, anorexia, decrease milk production, anaemia and diarrhoea.

Blood sample of this cow was collected from jugular vein in sterile condition on the day of acute stage of infection, 48 hours post treatment and then at an interval of 3 days up to a period of 21 days PT for diagnosis of the

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infection as well as to monitor the infection. The body of the cow was thoroughly searched for presence of ticks, if any.

For diagnosis of infection by conventional parasitological technique, at least four blood smears were prepared each time and stained by Giemsa stain as per standard methods. For diagnosis of infection by molecular techniques, parasite DNA from the blood was extracted using DNA extraction kit (Genei™ Blood Genomic DNA purification kit, Genei Bangalore, India) as per manufacturer instruction. Agarose gel electrophoresis based method was used to analysis the extracted DNA. PCR was done using *B. bigemina* specific primers: Forward GFU5 (F) TGG CGG CGT TTA TTA GTT CG and Reverse GFU6 (R) CCA CGC TTG AAG CAC AGG A , as described by Guido *et al.* (2002) and cited by Durrani and Kamal (2008) . The expected amplicon size in positive case is 1124bp.The PCR was set up in 50 µl reactions and amplification done in a thermal cycler. For *B. bigemina* specific primers, reaction mixture consisted of 10 µl of 5X Colourless PCR buffer (Promega), 4 µl of $Mgcl_2$ (25mM) (Promega), 1 µl of 10mM dNTP (Promega), 1 µl of Forward Primer (20µM) (GCC), 1 µl of Reverse Primer (20µM) (GCC), 0.25 µl of Taq Polymerase (5U/ µl) (Promega), 2 µl of Template DNA and 30.75µl of autoclaved triple-distilled water. The cyclic conditions were as followed: Initial denaturation at 94°C for 2 minutes, denaturation at 94 °C for 30 second, annealing at 55°C for 30 second, extension at 72 °C for 1minute. From denaturation to extension, 40 cycles completed and then a final extension set at 72 °C for 5minutes. Seven microliters of the PCR product were electrophorized on a 0.8% agarose gel in Tris Boric acid EDTA (TBE) as running buffer (2 hours at 80 V) with 1kb DNA marker as size marker. The gels were stained with ethidium bromide (0. 5µg/ml) and analyzed on a UV transilluminator to visualize the expected size (1124 bp) product and taken the photographs.

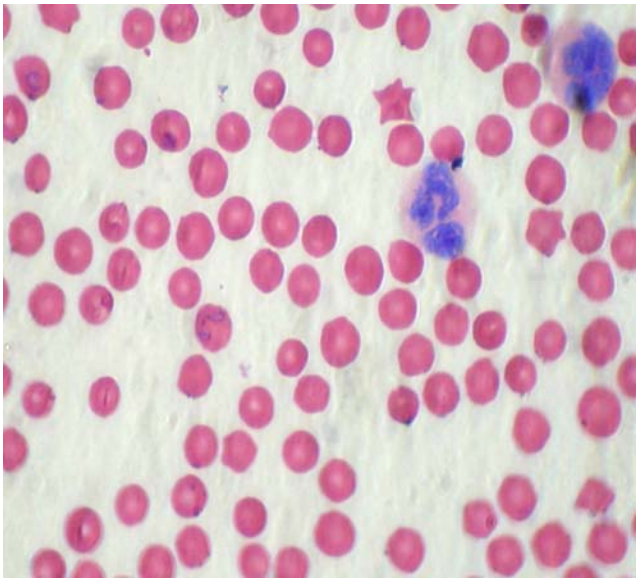

FIG.1: *Babesia bigemina* in RBC of Giemsa stained blood smears.

After confirmative diagnosis, the cow was treated with a single dose of 4,4' Diamidine diazoamine benzene diaceturate (Berenil) by deep intra muscular injection @ 3.5 mg/ kg body weight with supportive treatment.

The examination of Giemsa stained blood smears revealed the presence of pear shaped organism remained in pair with acute angle between them, which were indistinguishable from *B. bigemina* (Fig-1). The photograph of amplified products after PCR is presented in Fig-2. An 1124 bp size band specific for *B. bigemina* could be observed from this figure. No infection was detected after a period of 48 hours of treatment onwards either in Giemsa stained blood smears or by molecular diagnosis by PCR. After a period of 24 hours PT febrile stage disappeared, urine became straw coloured and increased appetite. After 48 hours PT onwards, the colour of urine became normal. Ticks were collected from the body of the infected animals and identified as *Boophilus microplus*.

From the clinical symptoms it was suspected that the cow was suffering from Babesiosis. After examination of Giemsa stained blood smears and molecular diagnosis using PCR, the diagnosis was confirmed as *B. bigemina* infection. An expected PCR product of 1124 bp was observed after using *B. bigemina* specific primers, further confirmed

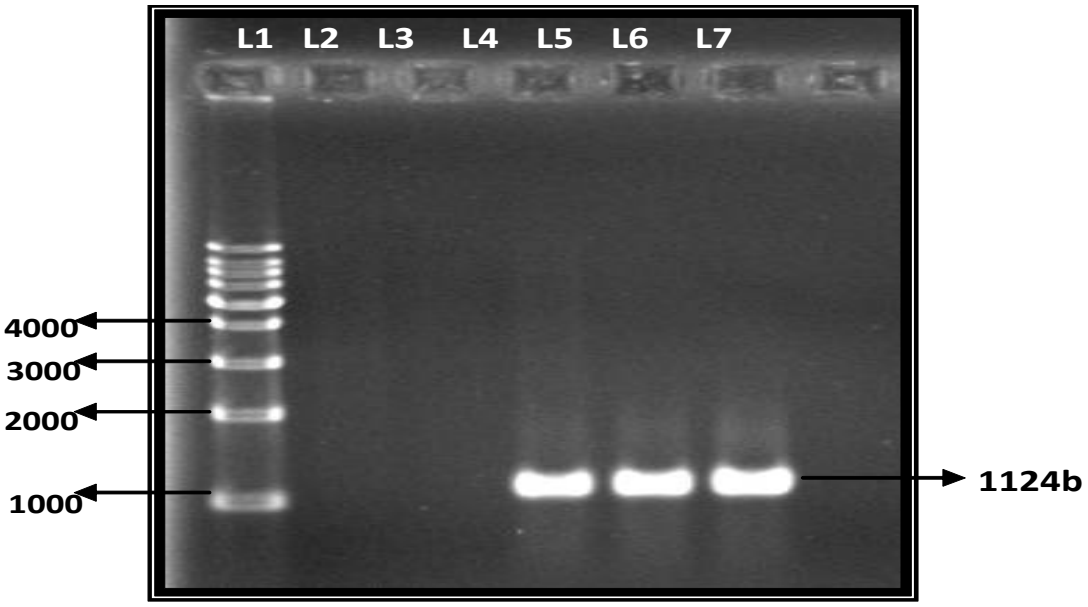

FIG. 2: Electrophoresis gel (0.8% agarose, stained with ethidium bromide), showing lanes from left to right : Lane 1- 1kb DNA ladder, Lane 2- Negative after 9 days PT, Lane 3- Negative after 48 hours PT, Lane 4 to Lane 6- PCR product showing positive during infection (1124 bp), Lane 7 - Negative control sample contains water and no template DNA.

that the parasite was *B. bigemina*. The clinical signs, hypoagalactia and successful treatment with Berenil as observed in the present study, also observed earlier in India (Banerjee *et al.,* 2005; Wadhwa *et al.,* 2008) The presence of *B. microplus* ticks in *B.bigemina* infected animals reported earlier (Wadhwa *et al.,* 2008). The important factors of presence of *B. bigemina* in this hilly region might be due to the spread of infected ticks under favourable climatic conditions, which has been changed in Meghalaya as a whole due to global warming which results in the spread of the distribution areas of the ticks and Babesiosis. This may be considered as a first report of its kind as per diagnosis of B*. bigemina* infections in cross bred cow using both conventional and molecular methods are concerned.

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