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Molecular detection of *Babesia* Spp by PCR in a spotted deer (*Axis axis*)

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Abstract
A fatal infection of *Babesia* spp in a spotted deer was confirmed by polymerase chain reaction. A two and half year-old male spotted deer was brought to the Veterinary Clinical complex, Veterinary College and Research Institute, Tirunelveli with a history of being rescued from the jungle for having intermittent seizures. Clinical examination revealed lateral recumbency with no response to stimuli, occasional convulsions with unilateral nystagmus on the right eye. Animal had involuntary dribbling of haemoglobinuria. The clinicopathological examination revealed neutrophilia and eosinophilia, elevated levels of blood urea nitrogen, bilirubin and reduced protein, blood glucose levels. Urinalysis revealed numerous RBCs with bilirubin crystals and few amorphous crystals. The whole blood sample was sent to molecular investigation by targeting 18S rRNA gene which showed amplification of *Babesia* Spp. in PCR at 619bp. Even though the animal showed some improvement in the treatment on second day but succumbed on third day.

Keywords: *Babesia* spp, haemoglobinuria, polymerase chain reaction, spotted deer

Introduction
Babesiosis is a common tick-borne haemoprotozoan disease prevalent in all domestic and wild mammalian hosts. Worldwide *Babesia* spp are primarily of veterinary importance, additionally some species are zoonotic and affects human health. Clinical babesiosis in free, ranging wild ruminants appears to be rare. Free-ranging wild animals may be considered reservoirs of an increasing number of pathogens with zoonotic potential (Kuiken *et al.* 2005 and Michel *et al.* 2014) [4, 6]. Piroplasmosis are becoming a recent threat even in wild animals due to their epidemiology and life of Ixodid tick vector as well as in the vertebrate host, especially the role of wildlife as reservoirs of infection (Yabsley & Shock, 2013) [14].
Evidenced the *Babesia bigemina* and *Babesia bovis* in white-tailed deer (*Odocoileus virginianus*) by molecular and serological testing methods. Recently, *Babesia microti*, *Babesia divergens*, *Babesia Odocoilei*, has been reported by Michael *et al.* (2014) in European and American continent in the various deer species like roe deer, rein deer, red deer, sika deer and white-tailed deer. Hilpertshauser *et al.* (2006) [3] conducted a survey for the occurrence of *Babesia* organism from the ticks of both domestic and wild ruminant by PCR technique in Switzerland. The threat in re-establishment of tick fever in wild animals is due to less of use of acaricides or acaricide resistant tick population in wild and free ranging animals (Miller *et al.* 2005) [7]. Zanet *et al.* (2014) [15] conducted a genetic analysis using the 18S rRNA gene for the identification of *Babesia* bigmeina in Roe deer and Red deer along with *Theilerial* spp in Italian Alps region. The *Babesia*l piroplasm causes fever, hemolytic anaemia, occasional hemoglobinuria and hypotensive shock leads to death of the animals (Radostits *et al.* 2000) [9].

Materials and Methods
From the Kalakkad mudanthurai tiger reserve area a two and half year-old brown male spotted deer weighing about 33 kg body weight was brought to the Veterinary Clinical complex, Veterinary College and Research Institute, Tirunelveli with a history of intermittent seizures.

Clinical examination revealed lateral recumbency (Figure 1) with no response to stimuli, occasional convulsions with unilateral nystagmus on the right eye and pale conjunctival mucus membrane. Blood samples were collected for the haemato-biochemical examination. Sample analysis was carried out in automated haematology (3part Celenium junior, Trivitron Pvt Ltd) and biochemical analyzer (Lab mate, Trivitron Pvt Ltd) using standard biochemical kits. Differential Leucocytes was counted manually in the monolayered area of the thin blood smear and blood picture were identified by the stained with Leishman-Giemsa cocktail stain.

Fig 1: Spotted deer-dull and lateral recumbency-lacerated wound on left mandible

The whole blood sample was sent to molecular investigation by Polymerase Chain Reaction. DNA was extracted from 200 µl of the EDTA-buffered whole blood using a commercially available DNA extraction kit (Qiagen, Germany) according to the manufacturer's instructions. The extracted DNA was stored at -20° C until further analysis. A 20 µl of polymerase chain reaction mixture was prepared which contains 10 µl of PCR Master mix (Taq 2X Master mix Red, 1.5 mM MgCl2, Ampliqon), 1 µl (10 pmol) of each *Babesia* genus specific forward and reverse primers, 3 µl of genomic DNA and 5 µl of nuclease free water. The primer set described by Azhahianambi *et al.* (2018) [2]. *Babesia* genus specific forward (Ba103F-5' CCAATCCTGACACAGGGAGGTAG-TGACA 3') and reverse (Ba721 R-5' CCCCAGAACCCAAAGACTTTGATTTCTCTCAAG 3') primers targeting partial 18S rRNA gene was selected. PCR amplification was performed in (Eppendorf Master cycler Nexus) Thermal Cycler. The cycling conditions for amplification of *Babesia* spp., organism was after an initial denaturation at 95°C for 3 min, 30 cycles of denaturation (94°C for 45 sec), annealing (60°C for 45 sec) and extension (72°C for 90 sec) and thereafter final extension (72°C for 10 min). The amplified PCR products were resolved in 1% agarose gel containing ethidium bromide and visualized under UV illumination of Gel Documentation system (Bio-Rad, USA).

Results and Discussion

The presented spotted deer was showing mixed clinical signs of hemoglobinuria for babesiosis and neurologic symptoms like nystagmus and convulsions. This might be due to the advanced stages of infection leads to cerebral form of babesiosis. The vital signs were subnormal in range, having a temperature of 32.4°C, 11 breaths /minute and bradycardia. The blood smear examination revealed neutrophilia and eosinophilia with absence of parasites due to subclinical form or advanced form of sequestration of parasites in brain microvasculature (Van de Maele *et al.* 2008) [13]. The serum biochemistry showed elevated levels of blood urea nitrogen, mild hyperbilirubinemia and hypoglycaemia, hypoproteinemia due to hepatic injury by the piroplasms. The animal had involuntary haemoglobinuria. Urinalysis revealed numerous RBCs with bilirubin crystals and few amorphous crystals (Ajith *et al.* 2017) [1].

The target sequence of 18S rRNA was isolated and amplified by using the *Babesia* genus specific forward (Ba103F-5' CCAATCCTGACACAGGGAGGTAGTGACA 3') and reverse (Ba721 R-5' CCCCAGAACCCAAAGACTTTGATTTCTCTCAAG 3') primers in conventional polymerase chain reaction. The amplified PCR product showed positive reaction by the appearance of band at 619 base pairs (Figure 2) in the agarose gel (Azhahianambi *et al.* 2018) [2] due to the non-availability of species-specific primer, the *Babesia* spp of spotted deer was not detected. *Babesia* spp were identified by Zanet *et al.* (2014) [15] and similar studies were carried out in Roe deer, Reindeer and Red deer (Ramos *et al.* 2010 and Mathieu *et al.* 2018) [10, 5]. The PCR is the best and reliable method of diagnosis for the infected animal which does not reveals the presence of piroplasms in blood smear examination (Mosqueda *et al.* 2012) [8].

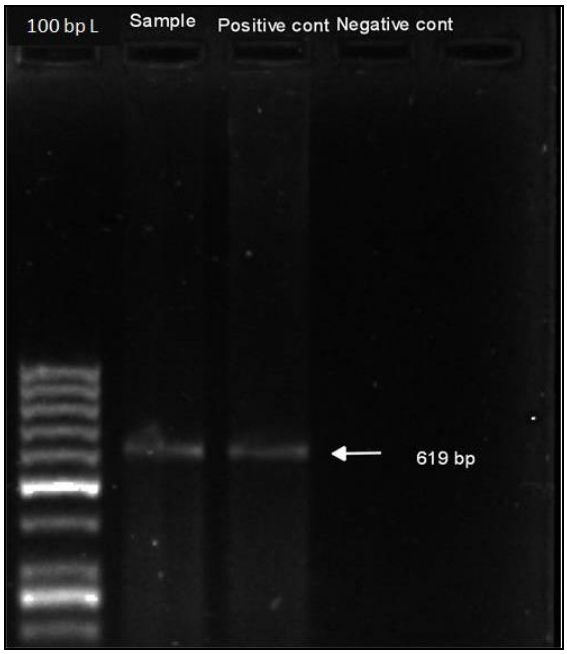

Fig 2: Spotted deer blood sample showing amplification of *Babesial* spp band at 619bp against the positive control product

The animal was treated with warmed ringers lactate and 10% dextrose solution and Inj. Diaminazine aceturate @ 2.5 mg /kg BW and Inj.Ceftriaxone Tazobactum @ 20 mg/kg for neutrophilic condition. The drug diaminazine aceturate is the worldwide due to its availability, efficacy and cost effectiveness (Taylor *et al.* 2015) [12]. In domestic animals, the combination therapy of diminazene aceturate and oxytetracycline found to be more effective (Saini & Sankhala, 2015) [11]. The animal showed some improvement on first two days, in spite of intensive therapy to the afflicted animal in advance stage, it succumbs to death on the 3rd day in the forest area.

The sensitivity and specificity of microscopical detection of *Babesia* organisms in the blood smears are limited to wild animals. Hence for the effective control and management of *Babesia* spp infection in both domestic and wild ruminants, there should be proper surveillance and diagnosis can be made using the molecular detection by PCR is much needed in the high time. This will also enhance the efficient control in domestic livestock population and in certain cases of human babesiosis infection. As wild animals, can be reservoirs of *Babesia* spp this article can provide valuable insights to a greater extent in study of tick-borne parasitic diseases in wild free-ranging deer in India.

Conclusion

Babesiosis remains a significant tick-borne disease affecting both domestic and wild mammals worldwide. Wild animals, such as deer, may serve as reservoirs for zoonotic *Babesia* species, complicating disease control. Molecular techniques like PCR are essential for accurate diagnosis, especially when blood smear examination is inconclusive. The presence of *Babesia* spp. in wild deer highlights their potential role in the epidemiology of tick-borne diseases and underscores the importance of surveillance and management strategies. Understanding the dynamics of *Babesia* infections in wildlife can aid in preventing outbreaks in domestic animals and reduce zoonotic transmission risks, contributing to improved animal and public health.

Conflict of Interest

Not available

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Not available

Reference

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