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Research Article

Clinico-Molecular Confirmation of Theileriosis in Captive Sambar Deer (*Rusa unicolor*) and Spotted Deer (*Cervus axis or Axis axis*) in India

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Abstract

Theileriosis is caused by a haemoprotozoan of the genus *Theileria*. The present study reports theileriosis in captive sambar deer (*Rusa unicolor*) and spotted deer (*Cervus axis* or *Axis axis*) in Bannerghatta Biological Park, Bengaluru. Postmortem examination revealed severe anemia, gelatinization of body fat, lymph node enlargement all over the body, pale spleen with accumulation of straw-colored fluid in the abdominal and thoracic cavity. In few animals, hemorrhages with punched out ulcers were observed on abomasal mucosa. Hematological profiling of infected animals showed characteristic indicators of anemia, hypoproteinemia, thrombocytopenia, low hemoglobin and packed cell volume, lower white blood cell counts reflecting lymphocytopenia with lower erythrocyte counts. Liver specific enzymes estimation indicated biliary or hepatocellular injury, while total bilirubin level was increased reflecting hemolysis and impaired liver clearance. Additionally, serum total protein level was decreased mainly due to hypoalbuminemia. These alterations collectively suggest compromised liver function, often secondary to systemic effects of theileriosis, such as hemolysis and inflammation. Theilerial schizonts were observed in erythrocytes and leucocytes in blood smear. For further confirmation, DNA isolated from blood was subjected to PCR and found positive for *Theileria* spp. The treatment was done for all the deer in the safari with tetracycline powder, tick control measures using ivermectin suspension along with administration of liver tonic. The animals responded to the treatment with reduction in mortalities.

Keywords: *Captive; PCR; Sambar Deer; Spotted Deer; Theileriosis*

Introduction

Theileriosis is popularly known as Corridor disease (CD), or East coast fever (ECF) of domestic cattle around the world. *Theileria* (next to *Babesia* or like *Babesia*), an Apicomplexan member belonging to the order Piroplasmida affects domestic and wild animals [1]. Piroplasmosis is generally caused by the parasites belonging to the genera *Babesia* and *Theileria* transmitted by ticks that infect humans, livestock including wild animals. The traditional classification of *Theileria* and *Babesia* spp. is based on differences in their morphology, host specificity, and mode of transmission by a tick vector [2]. *Theileria* is an obligate hemoprotozoan parasite and a tick-borne pathogen.

Theileriosis produce clinical signs and pathological manifestations like fever, chronic anemia, anorexia, jaundice, pyrexia, decreased milk production, weight loss, lymph node swelling, and leukopenia [3]. Treatment for theileriosis has not yet been established; however, buparvaquone is considered a promising drug [4].

The diagnostic methods employed for theileriosis are classified into 3 main categories i.e. conventional methods, serological methods and molecular methods. Conventionally, observation of specific clinical signs and blood smear examination is used for theileriosis diagnosis along with lymph node biopsy, but this method is not sensitive and cannot be used in carrier animals [5]. On Giemsa or Wright staining, macroschizonts can appear round, oval, or pear-shaped. Giemsa staining further cannot fruitfully differentiate between *Theileria* species which are mostly non-pathogenic and carrier animals may remain unnoticed. Researchers have compared polymerase chain reaction (PCR) with microscopic examination and found that pathogenic and non-pathogenic protozoa cannot be differentiated by simple microscopic examination [6]. Serological methods, such as the immunofluorescent antibody test even though can be used, they prove effective in recovered animals. However, PCR is an effective tool for the detection of protozoa even the quantity of DNA is very low that has been much more sensitive and specific than other techniques [7]. The specificity and sensitivity of such molecular methods are better as compared to conventional and serological methods [6] more specifically in low infectivity. This report presents theileriosis in captive sambar deer (*Rusa unicolor*) and spotted deer (also known as chital deer and axis deer) (*Cervus axis* or *Axis axis*) in Herbivorous Safari of Bannerghatta Biological Park, Bengaluru, Karnataka, India.

Materials and Methods

Clinical examination

Mortality was reported in sambar deer and spotted deer in Herbivorous Safari. The affected deer exhibited clinical signs like high fever, dull, depressed anorexia, weakness, severe tick infestation (Figure 1) and few succumbed. Mortalities continued for up to 10 days; one or two animals died per day from both the species.

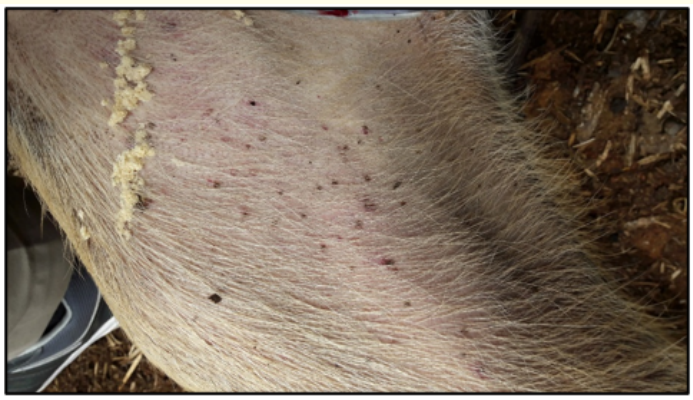

Figure 1: *Sambar deer showing severe tick infestation.*

Collection of blood

The site was visited, EDTA blood samples were collected from the diseased and heart blood from the dead animals for further analyses of hematological and biochemical parameters.

Blood smear examination

Blood smears were prepared, fixed with methanol and stained with Giemsa's stain followed by microscopic examination.

Necropsy examination

Systemic postmortem examination of dead deer was conducted and appropriate morbid tissue samples were collected in 10% neutral buffered formalin for laboratory examination.

Polymerase chain reaction (PCR)

To rule out the involvement of any septicemic conditions, DNA was isolated from blood samples and subjected to PCR for *Pasteurella* spp. To confirm the theilerial organisms, DNA was extracted from EDTA blood sample using HiPurA® Blood Genomic DNA Miniprep Purification Kit (Himedia) and PCR was performed using *Theileria*-specific primers (forward 5′-CCTGAGAAACGGCTACCACATCT-3′ and reverse 5′GGACTACGACGGTATCTGATCG-3). HiMedia's 2X PCR TaqMixture was used that is a premixed, ready-to-use solution containing Taq DNA polymerase, dNTPs, $MgCl_2$ and reaction buffers at optimal concentrations for efficient endpoint PCR amplification of DNA templates in the range of 0.1 - 3 kb. PCR reaction of 25 µl with HiMedia's 2X PCR TaqMixture 12.5 µl, forward primer 1 µl, reverse primer 1 µl, DNA template 4 µl, nuclease-free water 6.5 µl was performed. Thermal cycling conditions included an initial denaturation step of 5 minutes at 94°C, 32 cycles of denaturation at 94°C for 45 seconds, annealing at 54°C, extension at 72°C, a post-elongation or final extension of 5 minutes at 72°C. Following the amplification, electrophoresis using ethidium bromide stained 2% agarose gel was performed for 30 minutes at 100V. Gel documentation system was used to obtain images and to confirm the positive bands.

Results and Discussion

During postmortem examination all the spotted deer showed pale mucous membranes indicative of severe anemia (Figure 2), accumulation of straw-colored fluid in the abdominal and thoracic cavity with gelatinization of body fat (Figure 3), lymph node enlargement all over the body, pale spleen. In few animals, hemorrhages were evident on lungs and abomasal mucosa with punched out abomasal ulcers (Figure 4). Presence of such punched out abomasal ulcers is reportedly the pathognomonic lesion of theileriosis. Hemorrhagic lesions created a suspicion for any septicemic conditions.

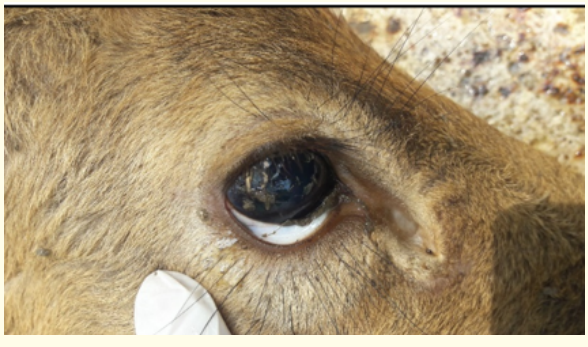

Figure 2: *Spotted deer showing pale ocular mucous membranes indicative of anaemia.*

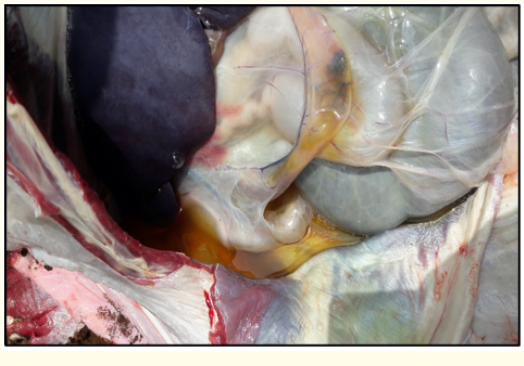

Figure 3: *Spotted deer carcass with gelatenous fluid occumulation in body cavities with gelatinization of body fat.*

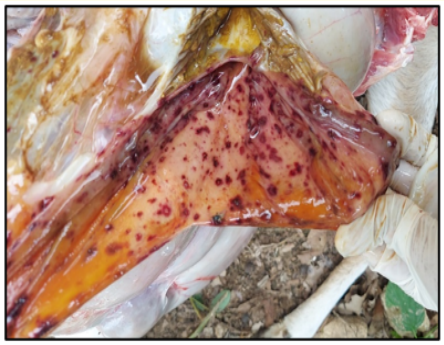

Figure 4: *Spotted deer abomasum showing punched out mucosal ulcers and hemorrhages*

Laboratory examination of blood smears from spotted deer fixed with methanol and stained with Giemsa's stain and observed under oil immersion microscope revealed the presence of theilerial intermediate forms in erythrocytes known as theilerial schizonts (Figure 5) that are basophilic in nature and small dot-like structures present in the RBCs.

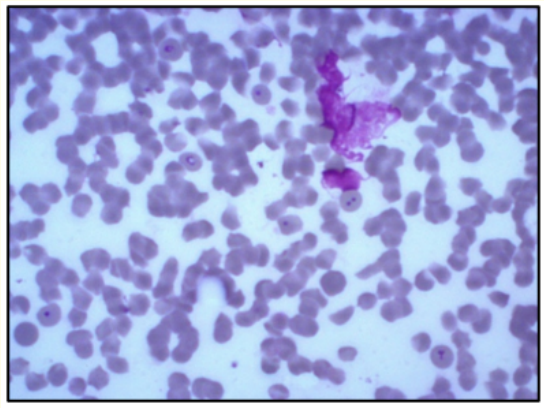

Figure 5: *Giemsa-stained blood smear from spotted deer revealing schizonts in the RBCs.*

Histopathology of lymph nodes showed depleted lymphocytic population in germinal center of secondary lymphoid follicle.

Blood sample analysis for hematological parameters revealed anemia and thrombocytopenia. The erythrocyte value ranged from 3.5 - 5.0 $\times 10^6/\mu L$ (Normal: 5-8$\times 10^6/\mu L$), while hemoglobin level decreased to 6 - 8 g/dL (Normal: 8 - 15 g/dL) and packed cell volume (PCV) was reduced to 15 - 25% (Normal: 24 - 46%) reflecting anemia. White blood cell (WBC) counts declined to 4-6$\times 10^3/\mu L$ (Normal: 6-12$\times 10^3/\mu L$) particularly due to lymphocytopenia. Thrombocytopenia was evident with platelet counts below 150$\times 10^3/\mu L$ (Normal: 200-800 $\times 10^3/\mu L$).

Serum biochemistry comprising liver function tests often revealed biochemical alterations indicating hepatic stress/damage/ hepatopathy. Serum levels of liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were elevated. AST was elevated to 150-250 IU/L (Normal: 60-125 IU/L), while ALT though less liver-specific in ruminants, also increased slightly ranging from 40-60 IU/L (Normal: 15-40 IU/L). Gamma-glutamyl transferase (GGT), a more liver-specific enzyme was elevated to 40-

70 IU/L (Normal: 10-35 IU/L), indicating biliary or hepatocellular injury. Total bilirubin level was increased to 1.2-2.5 mg/dL (Normal: 0.1-0.5 mg/dL) indicating hemolysis and impaired liver clearance. Additionally, serum total protein level was decreased to 5.0-6.5 g/dL (Normal: 6.5-8.0 g/dL), mainly due to hypoalbuminemia with albumin level dropping down to 2.0-3.0 g/dL (Normal: 3.0-3.5 g/dL). These alterations collectively suggested compromised liver function often secondary to systemic effects of theileriosis, such as hemolysis and inflammation.

The expected amplicon size was 592 bp (Figure 6) that was observed in all the deer blood samples. The DNA was found positive for *Theileria* spp and negative for *Pasteurella* spp.

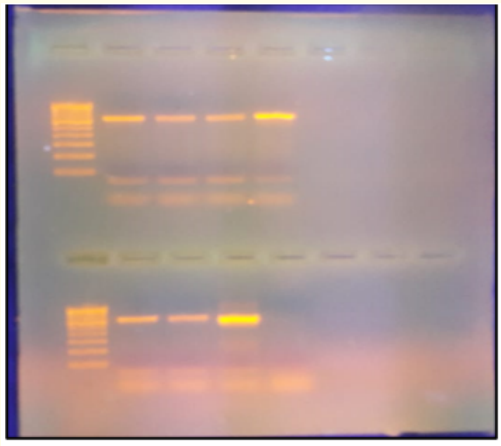

Figure 6: *Different deer blood samples showing positivity for Theileria spp with 592bp amplicon. Upper Lane: Lane 1: 100 bp DNA Ladder, lane 2, 3, 4, 5 are samples. Lower lanes: Lane 1: 100 bp DNA Ladder, lane 2, 3 are samples, lane 4 and 5 are positive control and negative control, respectively.*

These collective diagnostic methods further guided the path towards the treatment protocol to save the precious deer population. After discussion with the health committee experts, all the live deer in the safari were treated with tetracycline powder for 10 days at the dose rate of 10 mg/kg body weight in the feed along with liver tonics in the feed for 10 days. Tick control was done by ivermectin suspension in the feed in alternative day for 3 times. The animals responded to the treatment and mortalities were reduced after 1 week.

Studies on the detection and identification of *Theileria* spp. have been conducted in many countries. In Spain, a study was conducted on the prevalence of *Theileria* in roe deer (*Capreolus capreolus*) [8]. In Brazil, the occurrence of *Theileria* spp. in brown brocket deer (*Mazama gouazoubira*) and marsh deer (*Blastocerus dichotomus*) has been reported [9]. Phylogenetic analyses of the genetic diversity [10], prevalence, and molecular characterization of *Theileria* spp. in sika deer were conducted in Japan [11]. In China, studies on *Theileria* infection using molecular methods for the diagnosis in sika deer [12] and on the prevalence of *Theileria* in goats, sheep, and cattle [13] have been conducted. In wildlife, studies have been conducted on tick-borne pathogens including *Theileria* in Korean water deer (*Hydropotes inermis argyropus*) [14,15].

Theileriosis caused by *Theileria cervi* in a white-tailed deer (*Odocoileus virginianus*) has been reported [16] using PCR and sequencing of the V4 variable region of the 18S rRNA gene from paraffin-embedded section of lung. Haus., *et al*. [17] have examined 21 neonatal white-tailed deer from Delaware, USA and identified six fawns with *Theileria* organisms or suspected infection. A survey of piroplasms in white-tailed deer in the southeastern United States was carried out by researchers using the PCR-RFLP method [18]. *Theileria* sp.

was found in 57% of 1630 white-tailed deer killed in Texas [19]. Several studies on tick-borne diseases have been conducted across the globe owing to their pathological and zoonotic significance [20,21]. Climate change and decreased environment-related diversity have enhanced the infection rate of tick-borne diseases [14].

Conclusion

The study revealed the presence of theilerial schizonts in erythrocytes of spotted deer. Histopathology of lymph nodes showed depleted lymphocytic population in germinal center of secondary lymphoid follicle. Blood sample analysis for hematological parameters revealed anemia and thrombocytopenia. Serum biochemistry comprising liver function tests often revealed biochemical alterations. The expected amplicon of 592bp was amplified in all the deer blood samples in PCR. Although this study did not specifically identify ticks infesting deer they could be identified in the blood samples of the sambar and spotted deer, further research is needed regarding ticks in deer that are presumed to transmit this pathogen.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Volume 22 Issue 7 July 2026