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Case Report

Case report: Babesiosis in captive Chinkara deer (*Gazella bennettii*) at Bahawalpur, Pakistan

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Abstract

Gazella bennettii also known as Chinkara is an endangered species. Two Chinkara were brought to the University Teaching Hospital of the University College of Veterinary & Animal Sciences, Bahawalpur. Clinical examination revealed lethargy, progressive weight loss, subnormal body temperature, sunken eyes, congested conjunctiva, rapid abdominal respiration, and anemia. The babesiosis was detected on the basis of intra erythrocytic protozoa in Giemsa stained blood smears. Furthermore, molecular identification was conducted by targeting V4 region of 18S rRNA gene. Grossly, lungs were the most severely affected organs observed in the study. The condition was successfully treated with imidocarb dipropionate (Imipro®, 12%, Selmore Pharmaceutical, Pakistan) at dose rate of 3 mg/kg intramuscularly once a daily dose on days 1, 3, 6, 9, 15 and 21 along with supportive treatment. This study is the first one to report babesiosis in captive Chinkara deer.

Keywords: Babesiosis, Chinkara gazelle, Case report, Molecular detection

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Introduction

The Indian gazelle, *Gazella bennettii* also known as "Chinkara" due to its alarming voice "Chink-Chink" found in *Thar* desert of Sindh (Pakistan) and India (Chaudhary and Jakher, 2012). Chinkara is thought to be resistant against certain diseases (Gaydos et al., 2002). Babesial parasites have a worldwide distribution and infect a wide range of vertebrate hosts (Zanet et al., 2014). It has been reported that the *Babesia odocoilei* cause babesiosis in deer and other cervids (García-Vázquez et al., 2015). *Babesia bigemina* has perhaps the greatest potential for infecting wild ruminants. *Rhipicephalus* and *Boophilus* species of ticks mostly associated in the transmission of disease that preferentially feed on cattle and other ungulates (de León et al., 2010).

The traditional methods for identification of babesial parasites involves microscopic examination of Giemsa stained blood smears. However, highly specific and sensitive method for the piroplasms diagnosis is through PCR. Mostly, V4 hypervariable region of the 18S rRNA gene is targeted for identification (Altay et al., 2008).

Babesiosis is effectively treated by a large number of babesicidal compounds, but it is claimed that

imidocarb dipropionate (*N*,*N*′-bis[3-(4,5-dihydro-1H-imidazol-2-yl)phenyl], steadily clears the parasites when administrated intramuscularly or subcutaneously (Chaudhry et al., 2014). The present study is the first one to describe babesiosis in captive Chinkara gazelles. This study also demonstrates the therapeutic efficacy of immidocarb dipropionate in infected Chinkara.

Material and Methods

History and clinical examination

Two Chinkara males, approximately 9 and 10 years of age were brought to the University Veterinary Teaching Hospital of the University College of Veterinary & Animal Sciences, Bahawalpur. One with the history of lateral recumbency, anorexia and weight loss. The clinical examination revealed a subnormal body temperature 98°F, sunken eyes, congested conjunctiva, depressed mentation, rapid abdominal respiration and open-mouth breathing. The second individual had a medical history of anemia, anorexia and weakness. Both animals had delayed capillary refill time (>4 s). The farm had the history of sporadic, but extensive mortality (>30 Chinkara) since last 8 months. The animals, that died shown anorexia, progressive loss of weight and prostration prior death.

Parasitological identification

For hemoparasitic studies, blood samples were taken aseptically from the jugular vein in EDTA coated vacutainers and transported to laboratory at 4 ºC. Thin blood smears were prepared immediately after taking the blood samples with EDTA and were fixed in methanol for 5 min and stained in 5% Giemsa stain for 30 min, and examined with an oil immersion lens at a total magnification of 100X under the microscope for the of presence Babesia piroplasms (OIE 2014). Gene-Jet Genomic DNA Purification Kit (Thermo Fischer Scientific) was used for the DNA extraction with modifications for mammalian blood DNA isolation. Nano Drop ND-2000 UV-Vis spectrophotometer was used to check the purity and concentration of the DNA. Molecular identification was done by using a set of primers that target 430 bp portion of the V4 region of 18S rRNA gene of *Babesia*. Forward primer 5'-GACACAGGGAGGTAGTGACAAG-3' and the reverse primer 5'-CTAAGAATTTCACCTCTGACAGT-3' were used as previously described by Georges et al. (2001). The PCR reaction conditions were followed according to the protocols described by Altay et al., (2007). PCR products were then electrophoresed in 2% agarose and visualized on gel documentation system in UV rays (260nm).

Postmortem examination

The 10-year-old Chinkara was slaughtered to study the etiology of sporadic death in herd. Organs were observed in-situ for any abnormality. Swab from nasal cavity, lungs, liver, spleen and kidneys were taken aseptically and cultured on nutrient agar, MacConkey agar and Sabouraud dextrose agar for 48 hours at 37°C to rule out any bacterial or fungal infection.

Therapeutic trail

Chinkara was treated with supportive care and an antiprotozoal, imidocarbdipropionate (Imipro®, 12%, Selmore Pharmaceutical, Pakistan) at 3 mg/kg intramuscularly s.i.d. on days 1, 3, 6, 9, 15 and 21.

Results and Discussion

Hemoparasitic study of both animals showed the presence of babesial parasites. (Fig. 1) in blood smears.

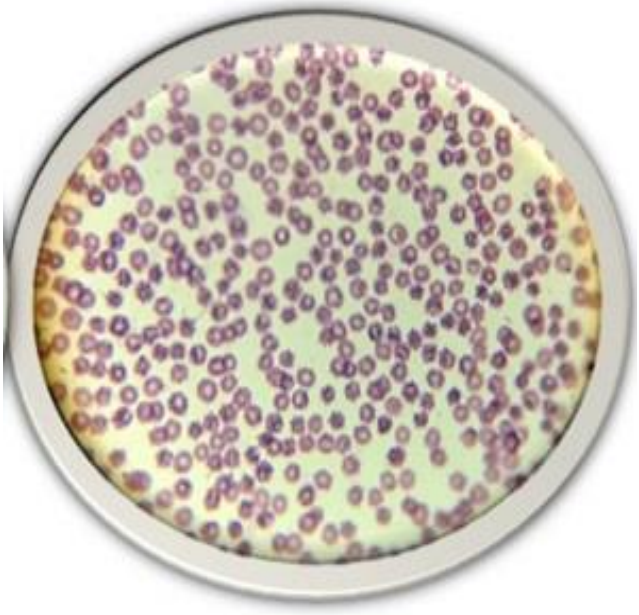

Figure 1. Babesial parasite in the blood smears of infected *Chinkara* deer. (40×)

Furthermore, PCR assay amplified a fragment 430 bp from V4 hypervariable region of 18S rRNA gene of *Babesia*. Results of molecular identification are in accordance with the studies of Altay et al., (2008), Altay et al., (2007) and Georges et al., (2001) who used the same set of primers for the identification of babesial parasites. Figure 2, shows agarose gel electrophoresis the PCR-amplified products.

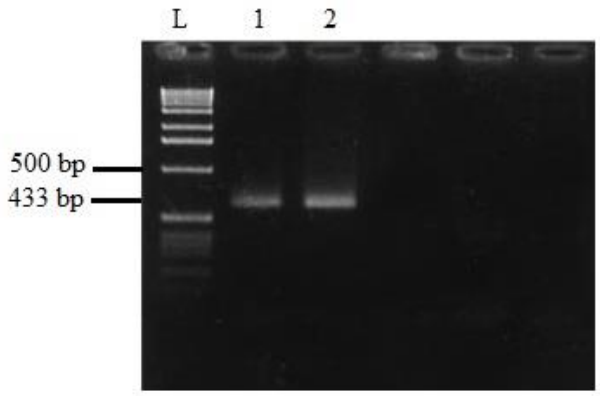

Figure 2. Agarose gel electrophoresis of. L: Molecular ladder of 100 bp. Lane 1 and 2 shows the amplified PCR products.

Clinically, the prolonged capillary refill time may be the suggestive of anemia and dehydration which may be the result of pathogenesis of babesial parasites (Teglas et al., 2005). Postmortem examination of the slaughtered Chinkara predominantly reveled severe pneumonia. Furthermore, pulmonary congestion and hepatization could also be noticed. Microbiological cultures did not reveal involvement of any pathological agent. Therefore, the involvement of the lungs could be considered as direct pathological lesion caused by the piroplasmosis. These findings are supported by the observations of Rahbari et al., (2008), whose reported the severe involvement of kidneys and lungs in sheep experimentally infected with *Babesia*. Hoby et al., (2007) also reported interstitial and alveolar pulmonary edema in free-ranging chamois infected with *Babesia*.

The second individual was continued with the antiprotozoal therapy. Blood samples were collected for hemoparasitic examination at the end of treatment, blood smear slides revealed no parasites. The result shows that Imidocarb dipropionate is an effective therapeutic agent against *Babesia* in chinkara gazelles. Hashemi-Fesharki and Esmaeilnia, (2016) reported that the Imidocarb dipropionate chunks the entrance of inositol into the erythrocytes, resulting in 'starvation' of the babesial parasite. Later on, upon screening, hemoparasitic examination revealed that all the animals at farm were infected with subclinical piroplasmosis. Chinkara is an endangered species and it has been reported previously with other pathogenic agents, i.e. Khan et al., (2008) reported enterotoxemia, Mukherjee et al., (2015) observed *Mycobacterium tuberculosis*, Ortiz et al., (2000) reported *Trichostrongylus probolurus* while Kachhawaha and Singh, (2010) studied *Hypoderma lineatum*. However, to the best of our knowledge this study is the first to report babesiosis in Chinkara gazelles. Nonetheless, the specie identification, hemato-biochemical pathology of the disease still needs to be addressed. The clinical observations, postmortem and laboratory findings presented in this report warrants further investigations to search out the role of babesiosis in mortality of Chinkara and to make recommendations for its control in similar areas.

Conclusion

It could be concluded from present ease report that babesiosis is prevalent in Chinkara deer in Pakistan and can cause pathological effects in the host. Furthermore, imidocarb dipropionate is an effective therapeutic agent against babesiosis in chinkara deer.

Contribution of Authors

Riaz MT: First handled the clinical case and collected Samples and Manuscript Writing
Ahmad W: Performed lab work for diagnosis
Mehmood K: Literature search
Rehman T: Collected data
Abbas A: Literature review & write up
Salam MMU: Data Interpretation
Shafi J: Write up & Proof reading
Hussain Z: Write up, final reading and approval

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