CASE REPORT

BABESIOSIS IN MALAYAN SUN BEAR *(HELARCTOS MALAYANUS)*

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ABSTRACT. A male sub-adult Malayan sun bear confiscated from a household in Klang Valley was presented for evaluation of gum paleness with no other clinical signs. Diagnosis of babesiosis was made based on blood smear examination. The haematology and biochemistry results revealed slight anaemia, thrombocytopenia and elevation of muscle enzyme levels. The sun bear was treated with Berenil® (diminazene aceturate) at 2 mg/kg by intramuscular injection, three times at one-week intervals. Resampling after two months showed no presence of the protozoan parasites and all blood parameters were within normal ranges. It was concluded that the treatment option is useful for babesiosis in Malayan sun bears. Treatment of infected bears is very crucial to avoid mortality as infection may flare up under stressful conditions.

Keywords: Case report, sun bear, babesiosis

INTRODUCTION

Babesiosis is a disease caused by intraerythrocytic protozoan blood parasites of the genus *Babesia* (Carter, 2015; Alvarado-Raybak 2016). Morphological determination of the *Babesia* species under microscope required vast experiences. For *B. bigemina*, paired merozites measure 2.5 to 3.5 µm of diameter; *B. bovis* merozoites measure 1.5 to 2 µm of diameter; *B. divergens* merozoites measure 1.5 to 0.4 µm of diameter; *B. canis* merozoites measure 3 to 5 µm of diameter and *B. gibsoni* merozoites measure 1 to 3 µm (Irwin, 2009; Mosqueda *et al.* , 2012; Carter, 2015). Over the years, more than 100 species of *Babesia* have been identified in numerous domestic animals and wildlife (Chauvin *et al.*, 2009; Yabsley *et al.*, 2013; Alvarado-Raybak, 2016).

First case of Babesiosis in wild Hokkaido brown bear (*Ursus arctos*) in Japan was reported by Jinnai *et al.* (2010). In this case report, new *Babesia* species (*Babesia* sp. UR1) was found circulating in nature in wild brown bears. Ikawa *et al.*, (2011) also documented the first case of *Babesia* sp. in wild Japanese black bears (*Ursus thibethanus japonicus*) which is closely related to the *Babesia* sp. imitative from racoons in Japan and the U.S.A. In addition, wild American black bear (*Ursus americanus*) in New Jersey, U.S.A also reported to inhabit these piroplasms (Shaw *et al.*, 2015). Until recently, there have been no reports on Babesiosis in Malayan sun bears (*Helarctos malayanus*) either in captivity or in the wild.

The most common route of *Babesia* sp. transmission is via tick-borne (Yabsley *et al.*, 2013; Carter, 2015; Alvarado-Raybak, 2016).

Non-vectoral transmission has also been documented, including from transfusion of infected blood, intrauterine infection and vertical transmission (de Vos *et al.*, 1976; Fukumoto *et al.*, 2005; Birkenheuer, 2012; Joseph *et al.*, 2012). Clinical manifestation of infection reported in domestic animals consists of fever, pallor, anaemia, anorexia, listlessness, jaundice and weight loss. In the later stages, hemoglobinemia and haemoglobinemia occur due to extensive lysis of erythrocytes. Hence, clinically, babesiosis may be confused with other diseases causing the same clinical features such as theileriosis, rickettsial disease, autoimmune haemolytic anaemia and immune-mediated thrombocytopenia (Carter, 2015; Vishwakarma and Nandini, 2019). In animals, the acute infection generally develops more than a week (Carter, 2015). Wildlife species is recognised to be a reservoir for zoonotic *Babesia* thus, there is increasing risk of zoonoses due to various factors such as increase interaction between human-wildlife conflicts, increase immunosuppression, habitat encroachment and changes in the environment (Penzhorn, 2006; Yabsley *et al.*, 2013). To our knowledge, this is the first report of *Babesia* species detected from Malayan sun bear through blood examination.

CASE REPORT

A male sub-adult Malayan sun bear approximately 15 kilogram was presented with paleness of gums with no other clinical signs, and the animal was bright and alert at the time of admission. The animal was confiscated from a household in Klang Valley as Malayan sun bears were listed under a totally protected species under the Second Schedule of the Wildlife Conservation Act 2010 (Act 716), which did not allow them to be kept as a pet.

For a complete health examination, the bear was fasted for 24 hours and anaesthetised using a combination of zoletil HCl (Zoletil®, Virbac, New Zealand, 3 mg/kg) and xylazine (Ilium Xylazil®, Troy Laboratories, Australia, 1 mg/kg) administered intramuscularly via tele-inject gun. Once anaesthesia was obtained, full physical examination was done and found no other clinical signs except pale mucous membranes. Thus, blood samples from the femoral vein were collected for further diagnosis. At that point of time, pallor was suspected due to nutritional deficiencies.

The diagnosis of infection was based on demonstration of *Babesia* sp. on thin blood smear (Parasitology Lab, Faculty of Veterinary Medicine, Universiti Putra Malaysia). The haematology results revealed a PCV of 0.29 L/L (normal range 0.30–0.50 L/L), haemoglobin of 94 g/L (normal range 107–167 g/L) indicating anaemia, slight thrombocytopenia 89.4 $\times$ 10^9/L (normal range 119–1100 $\times$ 10^9/L), and slight elevation of creatinine kinase with a value of 675 U/L (normal range <634 U/L). (Reference value from Haematology and Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia).

Following diagnosis, treatment with diminazene aceturate (Berenil® RTU, 7%, MSD) at a dosage of 2 mg/kg administered intramuscularly, three times at one-week intervals was started. Vitamin B complex and iron supplement was given once as a

supportive treatment at dosage of 15 mg/kg by intramuscular injection. After treatment was completed, the colour of mucous membrane returned to bright pink. Re-examination after 90 days post-treatment revealed no relapse of infection indicated by negative protozoan parasites on blood examination and all blood parameters were within the normal range.

DISCUSSION

In this case, diagnosis of babesiosis was made based on demonstration of protozoan parasites within erythrocytes on thin blood smears. Within 5 years (2015 to 2020), a total of 18 wild caught and 46 rescued Malayan sun bears were examined for the presence of blood parasites through blood examination and only 1 case were positive for blood protozoa (1.6%). Severity of infection is associated with wide variation of clinical signs along with variable clinic-pathological abnormalities comprises of haemolytic anaemia, neutrophilia, lymphopenia, moderate to severe thrombocytopenia, elevation of bilirubin ALT, AKP, urea and creatinine (Gonde *et al.* , 2017). This is related to the degree of parasite replication in the erythrocytes with subsequent cell lysis and also influenced by the host immunological responses (Ettinger and Feldman, 2005; Singla *et al.*, 2014). In this case, slight changes in the haemato-biochemical result along with minimal clinical signs indicate a non-severe infection.

Microscopic detection method is very useful in acute cases, as the number of parasites is higher in circulation (Kjemptrup *et al.*, 2000; Gonde *et al.*, 2017). Furthermore, this method was chosen due to low cost and faster way to identify parasites, although lack of sensitivity and specificity. However, in chronic mild infection and subclinical host, this method is not suitable. Thus, polymerase chain reaction (PCR) have become an important diagnostic method due to their high sensitivity and specificity to detect low numbers of circulating parasites (Schwint *et al.*, 2009, Mosqueda *et al.*, 2012) although access for routine clinical diagnosis is restricted to few laboratories. Regrettably, detection and species identification was not done in this case as the primary goal is to focus on the treatment. In the centre, the bear was kept individually along with 10 bears in separate enclosures. All the rescued bears were to undergo rehabilitation and rewilding programmed up to 3 years before being released back into the wild. Thus, for bears detected positive for protozoan parasites, it is critically important to begin treatment as soon as possible because infection may flare up and be life-threatening under stressful conditions such as confinement, crowding, capture and transportation. Moreover, spillage of infection into wild populations may happen if relapses occur in an animal in a reintroduction programme.

Unfortunately, clinical dosage of anti-piroplasm in the Ursidae family has not been determined. Thus, treatment in wildlife is established by extrapolating information from related species of domestic animals. A variety of drugs have been used to treat babesiosis in the past, but diminazene aceturate is still in common use (Irwin, 2009; Carter, 2015; Vishnurahav *et al.*, 2017). Manufacturer recommendation for

babesiosis treatment using diminazene aceturate is 5 to 10 ml for 100 kg body weight, equivalent to 3.5 to 7 mg/kg. Schoeman (2009) reported the effectiveness of diminazene aceturate to treat babesiosis in dogs at a dosage of 3.5 mg/kg administered subcutaneously or intramuscularly. However, administration of diminazene aceturate has negative side effects in dogs that appear to be dose-related, such as CNS toxicity, and occurrence is higher with repeated administration (Miller *et al.*, 2005). Therefore, the drug must be used with extra caution in wildlife species. The mechanism of action of Berenil® towards the parasites consists of disruption of kinetoplast replication and function, complete unfolding and inhibition of DNA replication. A study by Kuriakose *et al.* (2012) showed that Berenil® also modulates the host immune response. The successful treatment of piroplasms in animals is very challenging; improvement of clinical signs occurs but a true clearance of infection is rarely achieved (Irwin, 2009). Hence, re-testing affected animals is very important to ensure elimination of parasites in peripheral blood. Supportive treatment such as anti-inflammatory drugs, tick removal, iron preparation, dextrose, vitamin B complex and fluid therapy may be necessary based on the severity of clinical babesiosis. Blood transfusion may be life-saving in very anaemic animals (Carter, 2015; Zintl *et al.*, 2003; Kuttler *et al.*, 1981). In this case, the only supportive therapy given was iron and vitamin B complex due to the mild state of infection.

Prevention of babesiosis, as with any tick disease, vector controls such as the use of insecticide on animals (fipronil spray, ivermectin injection) and environment, bush clearing and use of prophylactic drugs can be implemented (Suarez *et al.*, 2011; Khan *et al.*, 2018; Pfeffer *et al.*, 2018).

CONCLUSION

In this case, treatment with Berenil® (diminazene aceturate) at dosage of 2 mg/kg three times at one-week intervals was successful to treat *Babesia* sp. infection in a Malayan sun bear. Complete recovery of the affected animal allowed successful reintroduction programmes to the wild. In the future, it is suggested to include a vector-borne infection screening programme as one of the screening activities.

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