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Clinical communication

ACUTE HEAPTOZOONOSIS CAUSED BY *HEPATOZOON CANIS* IN DOGS IN SRI LANKA

Ushani Atapattu, D.R. Anuruddhika Dissanayake, I.D. Silva, D.G.S.S. Bulumulla, N.G.D.A.K. Neelwala and Tharindu Wijekoon

Veterinary Teaching Hospital, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya

SUMMARY: Canine hepatozoonosis caused by *H. canis* is usually recognized as a mild clinical disease characterized by mild anaemia and lethargy. Complicated clinical manifestations of *H. Canis* infection have been occasionally observed in dogs. This clinical communication describes acute hepatozoonosis in five dogs characterized by neurological symptoms, ataxia or paresis, emaciation and anaemia.

BACKGROUND

Canine Hepatozoonosis is a tickborne disease caused by apicomplexan haemoprotozoan parasites of the Genus Hepatozoon. Two species of hepatozoons, namely Hepatozoon canis *(H. canis)* and *Hepatozoon americanum (H. amaricanum*) are known to infect dogs (Baneth *et al*., 2003). Of these two species, *H.canis* transmitted by the brown dog tick *Rhipicephalus sanguineus* is reported in Asian countries whereas *H. amaricanum* transmitted by *Amblyomma maculatum* is limited to Southern United States. Comparatively, latter is known to cause severe infection characterized by exostosis and myositis (Vincent-Johnson *et al*. 1997).

When first recognized in India in 1905, it was believed that *H. canis* only cause a milder disease resulting in anaemia and lethargy (Vincent-Johnson *et al*. 1997). However, it was later found out that certain factors including immunosuppression and co-infections can lead to severe clinical manifestations due to Hepatozoonosis. A number of nonspecific haematological and haemochemical changes including non-regenerative anaemia, thrombocytopenia, neutrophilia, hyperproteinaemia, hypoalbuminaemia, polyclonal gammopathy, and elevated serum creatine kinase (CK) and alkaline phosphatase (ALP) have been seen in dogs infected with *H. canis* (O'Dwye *et al., 2006*). The detection of capsule-like gammonts in the cytoplasm of the neutrophils in blood smears is routinely used to diagnose hepatozoonosis. Molecular diagnostic techniques, such as polymerase chain reaction (PCR) and sequencing are important for species identification.

In recent times, we have observed that the Hepatozoon causes emaciation, ataxia, paraplegia, circling movements, difficulty in prehension and severe muscle pain particularly among immunocompromised dogs. This communication discusses the clinical manifestations and prognosis of five dogs affected with hepatozoonosis.

CASE PRESENTATION

Five dogs aged between five months to seven years recently diagnosed with different disease conditions presented to Veterinary Teaching Hospital, Peradeniya with paresis, chronic emaciation, muscular pain, had Hepatozoon like gamonts (4-12%) in the peripheral circulation. Though *H.canis* is commonly known to cause clinically inapparent infection, the clinical manifestations, haematological parameters, serum alkaline phosphate (ALP) levels and response to treatment of those five patients were evaluated to identify the factors which complicated the clinical presentation. Signalements, clinical manifestations and the medical history of the five patients are given in Table 1.

Two of the five patients (patient 1 and 2) had been recently treated with immunosuppressive doses of corticosteroids. Third patient was on long-term antimicrobial therapy (over five months) and the fourth patient was on long term pancreatin. More than 4% (4-12%) of the neutrophils in peripheral blood contained capsule like gamonts (Figure 1). Multiplex polymerase chain reaction (PCR) was performed as described in Kledmanee *et al*. (2009) in order to determine whether the clinical complications were due to concurrent infection with *Ehrlichia canis* or *Babesia* species. Electrophoresis of PCR amplicons confirmed *Hepatozoon canis* as the Hepatozoon species observed in peripheral blood and the dogs were not concurrently infected with *E. canis* or *Babesia* (Figure 2).

Peripheral vestibular syndrome (due to *Malassezia pachydermatis*, *Otodectes cynotis*), myasthenia gravis and intervertebral disk disease were included as differential diagnoses. Peripheral vestibular syndrome was ruled out by the negative finding of parasites or fungi in cytological examination of the external ear canals. The possibility of myasthenia gravis was also ruled out by the negative response to oral neostigmine bromide test.

http://orcid.org/0000-0001-9902-2426

Table 1: Signalement, clinical manifestations and the clinical history of the patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age	4 years	16 months	7 years	3 years	6M
Breed	Dobermann	GS	GS	CB	CB
Sex	Male	Male	Male	Female	Male
Clinical manifestations					
Dysbasia /Paresis	tetraplegia	ataxia	ataxia	ataxia	paraplegia
Circling movements	-	+	+	+	-
Hypereasthesia	+	+	+	+	+
Difficulty in prehension	+	+	+	-	+
Pain	+	+	+	+	+
Chronic emaciation	severe	moderate	severe	severe	severe
Purulent eye discharges	+	+	-	+	+
Dyspnoea	severe	mild	mild	moderate	moderate
Diarrhoea	+	-	-	-	-
Previous diseases (Last two months)	IMTP	vestibular syndrome	chronic cystitis	EPI (2 months ago)	Babesisois

GS: German shepherd CB: Cross-bred IMTP: Immune mediated thrombocytopenia
EPI: Exocrine pancreatic insufficiency

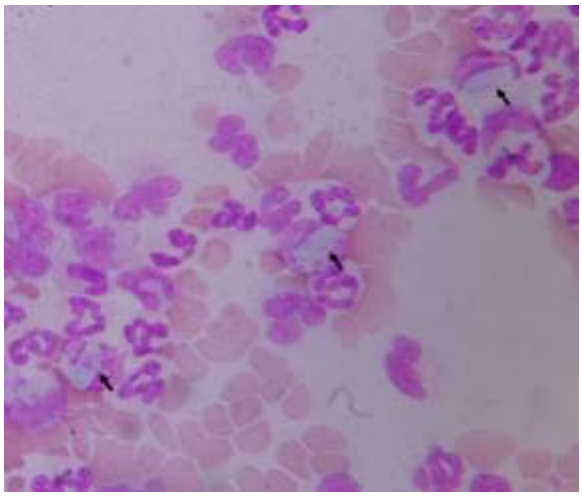

Figure 1: Capsule like Hepatozoon gamonts (black arrows)

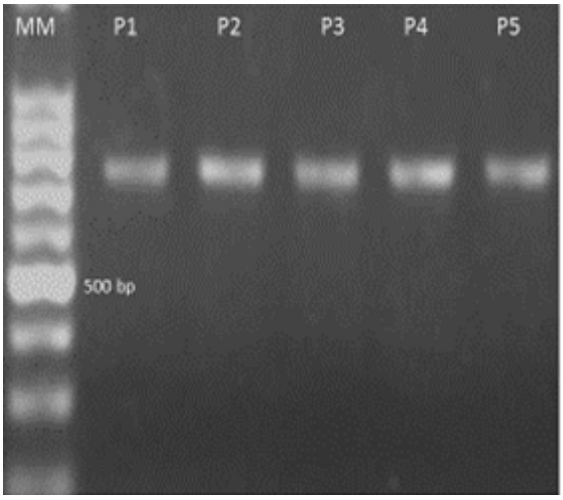

Figure 2: Agarose gel electrophoresis of *H. canis* present in the PCR amplicons (737 bp) 16s rRNA gene neutrophils

The total white blood cell counts were within normal range and no significant variations of differential WBC counts were detected. All five patients were moderately to severely anaemic with a mean PCV of 19.4 $\pm$ 2.5 (normal range 25-27) and mean haemoglobin of 6.98 $\pm$ 1.5 (normal range: 9-19). The decreased platelet counts were the most significant finding in all five patients. We compared the platelet counts of the hepatozoon affected patients with the breed and age matched healthy dogs (control). The platelet counts of the patients were significantly lower ($p < 0.05$) than that of the healthy dogs matched for the breed and age (control group) (Figure 3). Serum Alkaline Phosphatase (ALP) levels were moderately elevated in all five and ranged between 233-750 U/L (normal 20-157 U/L).

All patients were given two I/M doses of Imidocarb dipropionate (6.6mg/Kg) at two weeks intervals as the specific treatment for *H. canis.* All five patients showed significant improvement within first week of the treatment and recovered fully on completion of the treatment schedule.

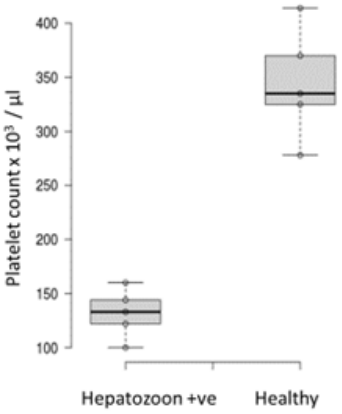

Figure 3: Comparative illustration of platelet counts of dogs affected with *H. canis* and healthy dogs (control)

DISCUSSION

Acute hepatozoonosis caused by *H. canis* has been reported in several Asian countries often characterized by anaemia, lethargy and chronic emaciation (Murata *et al*., 1991; Sakuma *et al*., 2009; O'Dwyer *et al*., 2006). Clinical signs observed in the patients of the present study are different to those due to presence of various neurological manifestations which were more commonly reported in infections caused *H. amaricanum*. However, Marchetti *et al*., (2009) has reported neurological signs and severe periosteal inflammation in a dog in Italy due to *H. canis*. A high level of parasitaemia was consistent in a majority of reported cases of *H. canis*. In contrary, the level of parasitaemia observed in our patients were low, probably because they were either immunosuppressed or chronically ill. It has been known that the pathogenesis and the severity of *H.canis* infection are usually influenced by immunosuppression or concurrent infections (Baneth and Weigler, 1997).

Radiographic examination of the appendicular bones of three patients did not reveal signs of periosteal inflammation which is a characteristic feature of *H. amaricanum* but observed occasionally in dogs with *H. canis* (Marchetti *et al*., 2009; Priya *et al*., 2004; Murata *et al*., 1991). Usually the increased ALP levels observed in the cases of *H. canis* are considered to be associated with the periosteal lesions. We also noted a moderate increment in serum ALP levels in these patients but it is important to determine the isoform of the ALP to identify the origin of the enzyme. Elevated serum ALP levels may originate from the liver (L-ALP) or bones (B-ALP). In addition, exogenous corticosteroids will increase the production of a unique form of ALP (C-ALP) in the liver (Fernandez and Kidney, 2007).The elevated ALP in two of the five dogs studied here may have an association with high doses of corticosteroids therapy in the past.

The neurological signs including circling movements, ataxia, paraplageia, tetraplegia and difficulty in prehension observed in these patients were difficult to explain. Difficulties in walking have been observed only in a few dogs infected with *H. canis* (Voyvoda *et al*., 2004) without confirmatory evidence for an association with the parasite. The limbs of the two patients who showed paraplegia and tetraplegia were stiff and extended and were painful on palpation. Therefore, it is most likely that the dysbasia and paresis were due to myalgia caused by parasitic cysts in muscle tissues, which would have been confirmed if serum creatinine kinase (CK) levels and aspartate aminotransferase (AST) levels were evaluated.

In consistent with the previous reports (Murata *et al*., 1991; Sakuma *et al*., 2009), we also noted some haematological changes such as low red blood cell counts, low PCV and low haemoglobin levels in the patients affected with *H. canis*. These changes could be mainly due to anaemia of chronic disease (ACD). It was not clear whether the cause of thrombocytopenia seen in all five patients was solely due to *H. canis* or another infection. Other haemoparasites including *Anaplasmaplatys* and *Mycoplasma haemocanis* as well as other infectious agents can cause thrombocytopenia in dogs, but we only tested the study patients for *Babesia* species and *Ehrlichia canis*.

Canine hepatozoonosis is usually treated with imidocarb dipropionate (5-6mg/kg) and the treatment needs to be repeated in 14 days until complete elimination of gamonts in peripheral blood smears (Baneth, 2011). Treatment with Doxycycline is stated as an alternative to imidocarb (Ezeokoli *et al*., 1983). In addition to the specific treatment, management of the clinical case should be done accordingly to achieve a good prognosis.

In contrary to the popular belief that the *H.canis* only causes mild clinically inapparent infection, the organism may cause clinically significant conditions particularly in immunocompromised cases. Most often these conditions may go undiagnosed and hence untreated due to non-specific clinical signs, lack of diagnostic facilities and lack of knowledge. Inclusion of Hepatozoonosis in the differential diagnoses in clinical cases with aforementioned manifestations will aid in diagnosis of the condition.

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