ATYPICAL CHRONIC CANINE TRYPANOSOMOSIS: CLINICO-PARASITOLOGICAL AND HEMATO-BIOCHEMICAL APPRAISAL

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ABSTRACT. This report describes atypical chronic trypanosomosis in a three year male Spitz dog. Fever, lethargy and anorexia were the early presenting signs without any hemato-biochemical abnormality. Peripheral blood smear examination was non-diagnostic on three consecutive times. *Trypanosma evansi* was confirmed in the Leishman stained thin blood smears (moderate parasetemia) on fourth parasitological examination. Biochemical profile showed a remarkable elevation in total serum bilirubin (6.7 mg%) and activities of alanine amino transferase (ALT) (950 IU/L) and alkaline phosphatase (AKP) (1050 IU/L) after a month. Anemia, leucopenia, neutropenia, lymphopenia and thrombocytopenia suggestive of bone marrow depression appeared by about 73 days of presentation of case. A rapid complete clinical recovery occurred within a week after treatment with quinapiramine sulphate and chloride combination @ 3.5mg/kg bwt. Hemoglobin, leucocyte and thrombocyte count improved within six days, however, liver enzyme activity normalized slowly over three months.

Keywords: anemia, chronic trypanosomosis, dog, hepatitis, leucopenia, thrombocytopenia.

INTRODUCTION

Though canine trypanosomosis can be mainly categorized into American trypanosomosis and African trypanosomosis (Barr *et al*., 1991; Castanera *et al*., 1998; Matete 2003; Kjos *et al*., 2008). However, it was established that dogs are susceptible to almost all the species of trypanosomes *viz*. *Trypanosoma evansi, T. cruzi, T. brucei, T. rhodesiense, T. gambiense, T. congolens*e (Eloy & Lucheis 2009; Jones *et al.,* 2000). *Trypanosoma evansi* is the only pathogenic species reported from the dogs in India (Juyal *et al.,* 2005). The disease caused by *T. evansi* known as 'Surra', is widely distributed in India infecting camels, cattle, horses, dogs and rodents, and spreads mechanically by arthropod vectors mainly Tabanid flies (Herrera *et al.,* 2004; Singh & Singla, 2013). Dogs were thought to be infected by eating fresh meat, blood, offal or bones. Once infected, a trypomastigote form of protozoan parasite enters the bloodstream directly or through the lymphatics. It

multiplies sub-clinically by escaping the immune system and spreading throughout the body. The primary clinical signs in dogs are non-specific including anorexia, weight loss and intermittent fever; edema of the face, genitalia and subcutaneous tissues; purulent nasal and ocular discharge; orchitis in males; and weakness (Colpo *et al*., 2005). As the clinical signs are variable, the disease cannot be diagnosed with certainty except through detection of parasites by microscopic examination of blood or by various serological or molecular techniques.

Here, we want to place on record a parasitologically confirmed and successfully cured case of a chronic atypical canine trypanosomiasis. The diagnosis, hematological, and biochemical parameters along with the successful treatment with quinapyramine sulphate and chloride is discussed.

MATERIALS AND METHODS

A three-year-old, home-confined, male Spitz dog was presented for lethargy and decreased appetite. On physical examination, rectal temperature was elevated at 104.0 °F, respiration rate was 42 bpm and the pulse rate was 130 bpm. Examination of mucous membranes and abdominal palpation did not reveal any apparent abnormality. Hematology and biochemical profile was unremarkable on the day of first presentation (20 October, 2015). Peripheral blood smear examination was negative for haemoprotozoa. Treatment of the dog was attempted with ampicillin sodium @ 15 mg/kg and B vitamins. The dog was again presented after about a month (28th November) for recurrence of fever, vomiting and lethargy. This time physical examination showed fever 104.5 °F and elevated pulse (145 bpm) and respiration rate (45 bpm). There was remarkable leucocytosis due to neutrophilia and lymphopenia with normal platelet count. Blood biochemical profile showed altered liver functions (Table 2). Peripheral blood smear examination was negative for haemoprotozoa. There was no evidence of any exposure to toxicant or hepatotoxic drug or any clinical sign of infectious cause of hepatitis. The dog was treated for non-specific hepatitis with ampicillin (10 mg/kg bwt), liver extract and fluids for two weeks without any clinical response. Thereafter, acute hepatitis progressed to chronic form and the dog developed anemia, leucopenia and thrombocytopenia (Table 1). Liver function tests showed a gradual decline in liver enzyme activities and near normal total bilirubin (29th December, 2014). Ultrasound of abdomen showed hepato and splenomegaly with normal echotexture. Based on the clinical signs, hemogram (thrombocytopenia), ultrasonographic findings and epidemiology, the dog was tentatively suspected for chronic ehrlichiosis and treated with doxycycline (10 mg/kg) for a week. There was no clinical response to treatment and animal developed vomiting and hemorrhagic enteritis (January 13, 2015). Fecal examination twice was negative for ova/cyst. Blood film examination (Leishman stain thin blood film), now on fourth time (January 13, 2015) confirmed the presence of *Trypanosoma evansi* infection. Blood smear examination also revealed hypochromic normocytic anemia, severe toxic changes

in neutrophils with aggregates of platelets. The dog was treated with quinapyramine sulphate 3.5 mg/kg bwt sc in two divided doses at 12 hour interval (January13, 2015). The dog showed clinical recovery within a week. Hemoglobin, leucocytes count and thrombocytes count improved within six days, without any additional treatment, however liver enzymes normalised only after a month. Follow up was after 10 months with no relapse of clinical signs.

DISCUSSION

This clinical report describes a clinical course of 73 days in natural infection of *T. evansi* in a dog. Fever was the first presenting clinical sign. Clinical evidence of acute hepatitis was evident after 30 days. Anemia, leucopenia, thrombocytopenia accompanied by haemorrahagic gastro-enteritis appeared after 70 days. Initial clinical signs of chronic fever, lethargy were non-specific as has earlier been observed in clinical cases

Table 1. Temporal changes in hematology of a dog suffering from chronic trypanosomosis

Parameters	Oct. 20	Nov. 28	Dec. 2	Dec. 13	Dec. 29	Jan. 2	Jan. 13
Hb (gm/l)	161	145	89	112	126	129	72
PCV (l/l)	0.48	0.39	0.23	0.29	0.33	0.33	0.20
TEC (10^{12}/l)	7.32	7.21	4.40	5.29	6.29	6.43	3.77
TLC (10^9/l)	11.0	45.4	32.7	12.9	9.72	5.30	2.46
Neutrophils (10^9/l)	9.29	44.4	26.8	10.9	7.78	4.24	1.97
Lymphocytes (10^9/l)	1.55	0.91	3.27	2.06	0.97	1.06	0.49
Monocytes (10^9/l)	-	-	-	0.06	0.19	-	-
Eosinophils (10^9/l)	0.221	-	2.62		0.78	-	-
Platelets (10^9/l)	269	209	223	408	98	127	0.36

Table 2. Temporal changes in plasma biochemical profile of a dog suffering from chronic trypanosomosis

Parameters	Oct. 20	Nov. 28	Dec. 2	Dec. 13	Dec. 29	Feb. 6	Apr. 6
T. Bil (µmol/l)	8.55	109.4	64.5	17.1	12.0	3.42	5.13
ALT (IU/L)	-	950	405	192	127	390	43
AST(IU/L)	15					32	07
ALKP(IU/L)	75	1050	1398	1178	666	625	70
BUN (mmol/l)	3.20	6.04	-	-	-	6.76	-
Creatinine (µmol/l)	123.8	123.8	-	-	-	159.1	-

(Juyal *et al*. 2005; Colpo *et al*., 2005). More specific clinical signs of corneal opacity, keratitis, lymphadenopathy did not appear throughout the course of disease.

In this case, chronic fever was the most prominent and consistent clinical sign accompanied by other attendant signs of lethargy, vomiting and dysentery. The diagnosis was however, delayed by more than 10 weeks by blood film examination. It may be deduced from this case report that trypanosomosis should form a part of differential diagnosis of fever of unknown origin in dogs of the regions endemic for this infection. This case took a longer time for confirmatory diagnosis by parasitological examination as the case was negative on three consecutive blood smear examination. This may be due to low undetectable peripheral parasitaemia. From the result of this case, it may be suggested that more sensitive serological tests are advised for early diagnosis and management of chronic trypanosomosis.

Biochemical evidence of acute severe hepatopathy was an uncommon finding noticed in this case. There are few reports of elevation of serum liver enzyme activities in experimental infection with *T. evansi* (Aquino *et al.,* 2002) and *T. brucei* in dogs (Nwoha *et al.,* 2013). However, the elevation in liver enzymes was subtle (3-5 folds) as compared to this naturally infected dog (>10 folds). It is believed that the trypanosomosis caused by *T evansi* as the cause of acute hepatitis has not been described in dogs (Watson & Bunch 2013). It may be proposed from this case report that in dogs where etiology of hepatits is obscure, *T. evansi* should also be considered for differential diagnosis in endemic areas.

Anemia, leucopenia, and thrombocytopenia suggestive of bone marrow suppression, observed as a late manifestation in this dog, was an uncommon observation. Evidence of bone marrow suppression is believed to be rarely reported in natural or experimental infection of *T. evansi* (Garcıa *et al.,* 2006; Rjeibi *et al*., 2015). A similar effect on bone marrow was observed with *T. cruzi* infection in mice (Marcondes *et al.,* 2000). Aquino *et al*. (2002) recorded anemia, leucopenia and neutropenia after 2 to 5 weeks of experimental infection of *T. evansi* in dogs. Rajeibi *et al.,* 2015) observed anemia (6.8 g/dl) and severe thrombocytopenia (5000/µl) without any other change in hemogram in natural infection. Hosseininejad *et al.* (2007) reported non-regenerative anemia with low packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. It may, therefore, be summarised that like clinical signs, hematological changes in canine trypanosomosis are variable. This variation in pathological effects may be due to strain variations (Bal *et al.,* 2012).

An important observation in this dog was that source and transmission of infection was difficult to explain as the dog was confined to home in a city with no contact or proximity to more susceptible bovine or equine species. Probable mode of transmission of *T. evansi* through contaminated meat, offals or bones was also unlikely as the dog was primarily on a vegetarian diet mixed occasionally with cooked chicken meat. It may be stated that

the possible mode of transmission may be through insect vectors.

Previous clinical reports on chronic trypanosomosis described keratitis as the most common presenting signs with minimal systemic signs (Juyal *et al.,* 2005). Increased body temperature with increased pulse rate and respiration rate during febrile phase in infected dogs was also observed by Galhotra (1986) and Gunaseelan *et al.* (2009). Rani and Suresh (2007) reported *T. evansi* organism in peripheral blood with history of inappetence, dullness and persistent fever for five days. As evident from this report, diagnosis of a chronic form of disease may also be difficult as blood examination trice failed to diagnose the disease. The findings in this case also emphasised the need for serological diagnosis in chronic form of the disease for early diagnosis.

The objective in this case was to achieve fully curative or "sterilising" treatment, which requires the use of chemoprophylactic drugs, such as quinapyramine group of drugs (Lopes *et al.,* 1997). Quinapyramine sulphate and chloride is quite efficient and the chemoprophylactic effect can last up to 4 months (Finelle, 1973). In addition to therapeutic control, more generally, preventing infection is an important part of disease control, especially for highly susceptible species, such as horses and dogs. After treatment, all clinical signs disappeared, biochemical and hematological parameters returned to normal levels, allowing us to conclude that this new protocol tested was effective to cure of this disease in dogs.

Although the disease was severe and chronic with marked hematological and biochemical changes, clinical recovery was rapid and complete without any supportive therapy, suggesting single dose of quinapyramine as an effective therapy. Hepatic functions were slow to normalise as was earlier recorded in dogs (Howes, 2011). Although diminazine aceturate is one of the commonly used chemotherapeutic agent for trypanosomosis in cattle, buffalo, pig, sheep and camels (Singla *et al*., 2008). There were reports that treatment failure and relapse occured with a single dose of diminazine aceturate (Colpo *et al*., 2005; Da Silva *et al.,* 2008; Tuntasuvan *et al.,* 2003) and for effective treatment, a five-dose treatment protocol was effective (Howes, 2011). It may, therefore, be suggested that quinapyramine as a single dose is an effective treatment as no recurrence of infection occurred after two years.

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