Haemato-biochemical and thyroxin status in *Trypanosoma evansi* infected dogs

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Abstract In one year period of study, dogs with inappetence, fever, ocular discharges, dullness, enlarged lymph nodes were screened for the presence of haemoprotezoans at College Hospital of College of Veterinary Science, Tirupati. Wet blood film examination and stained blood smear examination was done to confirm the condition. Peripheral blood smears of dogs revealed the presence of Trypanosomes. *Trypanosoma evansi* was confirmed based on the morphology and measurements of the organisms in the stained blood smears. Haematology revealed decreased total erythrocyte count, packed cell volume, haemoglobin and total leucocyte count values. Total serum proteins, albumin and glucose levels were decreased significantly ($P < 0.01$), but a significant increase ($P < 0.01$) in cholesterol, blood urea nitrogen, serum alkaline phosphatase, serum aspartate aminotransferase and serum alanine aminotransferase levels were observed. Decreased total T_4 and free T_4 were also observed in the *T. evansi* infected dogs.

Keywords Anemia · Dogs · *Trypanosoma evansi* · Thyroxin

Introduction

Trypanosoma evansi has a wide host range in most domestic and wild animals. The incidence and the severity of the disease vary with the strain of the parasite as well as the species of host affected (Sivajothi et al. 2013a). Equines, camels, dogs, deer and Asian elephants are more frequently diagnosed with the disease than buffaloes and cattle. In dogs, an acute and fatal type of disease is usual and death possibly occurs in two to four weeks of infection (De Menezes et al. 2004). The disease may occur in both acute and chronic forms, but generally the chronic form is more common (Soulsby 1982). The acute form of the disease is usually fatal within a few weeks, but the chronic form lasts for years and is characterized by anemia, emaciation, recurrent fever, edema, conjunctivitis, lacrymation, enlargement of the lymph nodes, and abortions (Gutierrez et al. 2005). The thyroid gland is one of the endocrine organs, which is affected during *Trypanosomiasis* (Abebe and Eley 1992). Chronic infection impairs the function of thyroid gland in goats as defined by considerable plasma thyroxin (T_4) decrease (Mutayoba et al. 1988). *T. evansi* infection in camels causes hypothyroidism associated with decrease in triiodothyronine (T_3) and T_4 (Sazmand et al. 2011). Prevalence of *T. evansi* was reported in India with different diagnostic tests in different animals (Sivajothi et al. 2012, 2013a, 2014a). Haematological, biochemical alterations along with the pathological changes in lab animal's experimental infection with *T. evansi* were also reported (Sivajothi et al. 2013b; 2014b). But, no study was conducted on haematological and biochemical alterations with thyroxin levels in naturally *T. evansi* infected dogs. So, the present study was taken to know about the haemato-biochemical and thyroxin changes associated with the local strain of *T. evansi* infection in dogs to formulate a better therapeutic regimen.

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Materials and methods

Present study was carried out on the dogs referred to the College Hospital of College of Veterinary Science, Tirupati. During the one year period (2009 to 2010) of study dogs showing the clinical signs of inappetance, dullness, pyrexia, corneal opacity, bilateral ocular discharges, emaciation and enlarged lymph nodes were screened for the haemoprotozoans. From these dogs peripheral blood was collected initially for wet blood film examination followed by stained smears examination. Wet blood films examination was done by placing a droplet of blood on a clean microscope slide and covering with a cover-slip (22 mm). The blood was examined microscopically at total magnification approximately 50–100 fields per slide. *Trypanosomes* were recognized by their movement among the red blood cells. Stained smears were examined at high magnification with oil immersion. Approximately 50–100 fields of the stained thin smear were examined, with a oil-immersion objective lens, before the specimen is considered to be negative. Even after a trypanosome has been detected, approximately 20 extra fields are investigated to determine if more than one species is present (O.I.E. 2008; Sivajothi et al. 2013a). Blood and serum were also collected from ten apparently healthy dogs in the age group of 2–7 years from among the dogs presented to College Hospital for general check-up and vaccination which served as normal group. Blood samples were collected into separate vials without EDTA for serum collection. Serum samples were collected in sterile vials. Few drops of 1:10,000 sodium azide solution was added to the serum samples and stored at -20°C until use. Blood was collected in 10 % EDTA solution was used for estimation of packed cell volume (PCV), total leucocyte count (TLC), total erythrocyte count (TEC) and haemoglobin (Hb). The peripheral blood smears were stained by Leishman's stain to study the differential count (DLC) by battlement method following which, the absolute counts were also calculated. Serum biochemical parameters studied included glucose, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatase (ALP), blood urea nitrogen (BUN), total protein, serum albumin and total cholesterol by using Span diagnostics Ltd. Kits. Total thyroxine (tT_4) and free thyroxine (fT_4) levels were estimated by enzyme-linked immunosorbent assay (ELISA) (as per manufacture's procedure) by using a kit obtained from United Biotech Inc. Statistical analysis was done by using Student's *t*-test (Reddy et al. 2014).

Results

Based on the clinical and laboratory findings ten dogs were identified that they were suffering with *T. evansi* infection.

All the dogs exhibited high rise of temperature (more than 104.2°F), dullness, enlarged lymph nodes, congested conjunctival mucus membranes, bilateral lacrimation, emaciation and also two dogs had unilateral corneal opacity (Fig. 1). Out of ten dogs six dogs revealed motile *Trypanosoma* organisms in the wet blood film examination (Fig. 2, Marked with round area). Remaining four dogs had *Trypanosoma* organisms in the stained blood smears. Stained smears of positive dogs were examined for confirmation of species trypanosomes. *Trypanosoma evansi* was confirmed based on the morphology and measurements of the organisms in the stained blood smears (Fig. 3). Mean haematological and biochemical values of apparently healthy and infected dogs were given in Tables 1 and 2.

Discussion

Stained blood smears revealed the presence of *Trypanosoma* organisms which contain the following morphology. Organisms have medium sized fusiform shaped body ($20\text{--}32\text{ }\mu\text{m}$), centrally placed nucleus, well developed undulating membrane, free flagellum and subterminal kinetoplast placed at posterior end. Based on the morphology of organism they were confirmed as *T. evansi* (Soulsby 1982). The mean TEC, PCV and Hb were reduced significantly ($P < 0.01$) as compared to healthy dogs, indicating anemia. The patho physiology of anaemia in *Trypanosomiasis* is complex and it is due to mechanical injury to erythrocyte, occurs by the lashing action of the powerful locomotory flagella and microtubule reinforced bodies of the millions of the organisms during parasitaemia. Severity of anaemia usually reflects the intensity and duration of parasitaemia (Sivajothi et al. 2013b). Gunaseelan et al. (2009) reported that anemia was the main

Fig. 1 *Trypanosoma evansi* infected dog with dullness and emaciation

haematological findings of *T. evansi* in dogs. The present leucopaenia was characterised by neutropaenia, eosinophilia and lymphocytosis that agrees with findings by

Fig. 2 *Trypanosoma evansi* in wet blood film examination (400 X)

Fig. 3 *Trypanosoma evansi* in stained smears (1000X)

Allam et al. (2011). They reported that leucopenia could have been the result of immune suppression, which usually co-exists with trypanosomosis. Monocytosis was a consistent finding in trypanosomosis and had been reported in *T. evansi* infection of camels (Anosa 1988). Eosinophilia is associated with immediate-type hypersensitivity reactions. Lymphocytosis is due to generalized lymphoid tissue hyperplasia, characteristic of the acute phase of the disease, during which period, lymph nodes and spleen are remarkably reactive while in chronic infection, the immune system becomes depleted of lymphoid cells (sulaiman and Adeyemi 2010). Sivajothi et al. (2014c) was observed decreased TEC, PCV, Hb and TLC values with neutrophilia, eosinophilia and lymphocytopaenia in cattle naturally infected with *T. evansi*.

Total serum proteins, albumin and glucose levels were decreased significantly ($P < 0.01$). But there was a significant increase ($P < 0.01$) in cholesterol, BUN, ALP, AST and ALT values. Orhue et al. (2005) and Kjos et al. (2008) reported hypoproteinemia, hypoalbuminemia, anemia, decrease PCV and thrombocytopenia. Reduction in the serum albumin levels suggested hepatic damage, while the increase in globulin levels might have been due to enhanced antibody production in this study. Hypoglycemia is in consonance with the findings of Gunaseelan et al. (2009) and this might be due to excessive utilization of blood glucose by the parasites for their metabolism (Anosa 1988). However, increased metabolic rate caused by fever and hepatocyte degeneration could also be a reason for hypoglycemia in trypanosomiasis (Cadioli et al. 2006). In the present study, increase in cholesterol levels agrees with Abenga and Anosa (2007) who also reported an increase in cholesterol levels in *Trypanosoma brucei gambiense* infected vervet monkey. Hypercholesteremia has been associated with hepatic malfunction resulting from impairment of liver lipid metabolism (Adejinmi and Akinboade 2000). Elevated BUN levels of the present study is in consonance

Table 1 Mean haematological values of apparently healthy and *T. evansi* infected dogs (Mean $\pm$ S.E)

Parameters	Control group (n = 10)	Trypanosoma infected dogs (n = 10)	t test	P
Hb (g/dl)	13.03 $\pm$ 0.42	9.30 $\pm$ 0.39	7.08**	0.000
PCV (%)	39.29 $\pm$ 2.14	28.17 $\pm$ 6.51	4.29**	0.001
TEC $\times 10^6$ /cumm	6.51 $\pm$ 0.19	5.08 $\pm$ 0.12	5.92***	0.000
TLC/cumm	9334.29 $\pm$ 81.34	8720 $\pm$ 71	2.67*	0.021
Neutrophils/cumm	6698.06 $\pm$ 244.96	5703 $\pm$ 295	2.6**	0.024
Lymphocytes/cumm	2417.37 $\pm$ 119.77	2859 $\pm$ 119	2.60*	0.024
Eosinophils/cumm	135.57 $\pm$ 22.11	343 $\pm$ 72.2	2.94*	0.013
Monocytes/cumm	68.36 $\pm$ 17.83	349 $\pm$ 42.58	6.43***	0.000

NS non significant ($P > 0.05$)

* Significant ($P < 0.05$)

** Highly significant ($P < 0.01$)

Table 2 Serum biochemical changes in apparently healthy and *T. evansi* infected dogs (Mean $\pm$ S.E)

Parameters	Control group (n = 10)	Trypanosoma infected dogs (n = 10)	t test	P
Total protein (g/dL)	6.81 $\pm$ 0.22	6.18 $\pm$ 0.15	2.36*	0.037
Albumin (g/dL)	3.25 $\pm$ 0.04	2.47 $\pm$ 0.10	7.53***	0.000
Globulin (g/dL)	3.56 $\pm$ 0.20	3.88 $\pm$ 0.19	1.16 ^{NS}	0.271
A:G ratio	0.85 $\pm$ 0.11	0.64 $\pm$ 0.05	1.63 ^{NS}	0.130
Glucose (mg/dL)	120 $\pm$ 6.22	55 $\pm$ 3.26	8.88***	0.000
Total cholesterol (mg/dL)	84.57 $\pm$ 6.81	179.32 $\pm$ 22.14	2.23***	0.030
SGOT (AST) (U/L)	27.71 $\pm$ 2.00	68.33 $\pm$ 4.23	9.11***	0.000
SGPT (ALT) (U/L)	18.71 $\pm$ 1.56	66.67 $\pm$ 6.5	7.69***	0.000
ALP (U/L)	51.29 $\pm$ 1.7	92.33 $\pm$ 2.45	14.11***	0.000
BUN (mg/dL)	16 $\pm$ 0.95	27.83 $\pm$ 1.32	7.40***	0.001
Total T4 (μ g/dL)	2.69 $\pm$ 0.34	2.47 $\pm$ 0.08	2.55*	0.027
Free T4 (ng/dL)	1.57 $\pm$ 0.07	1.11 $\pm$ 0.07	4.36**	0.001

NS non significant ($P > 0.05$)

* Significant ($P < 0.05$)

** Highly significant ($P < 0.01$)

with the observation of Abenga and Anosa (2007) which could be due to renal damage. The elevated levels of ALT and AST enzymes are in agreement with earlier reports of Taiwo et al. (2003), which may be due to tissue breakdown and inflammation in the host, particularly of the liver, heart, muscle and kidney (Taiwo et al. 2003; Takeet and Fagbemi 2009). In the present study, a significant reduction in the concentrations of total T₄ and free T₄ was noticed as compared to healthy dogs. However, even the decreased levels were within the normal range and no clinical signs of hypothyroidism were seen in the dogs. It was shown that trypanosomal infection rapidly impairs function of the thyroid gland in goats experimentally infected with *Trypanosoma congolense* (Mutayoba et al. 1988) and induces hypothyroidism in cattle (Abebe and Eley 1992). Mechanisms by which trypanosomes affect the thyroid functions are not completely understood. However, in the infected calves release of cytokines could inhibit the thyroid hormone production (Kahl et al. 2002). Bartalena et al. (1998) indicated that all steps of thyroid hormones synthesis may negatively be affected by cytokines. Hence, *T. evansi* infection may also have to be considered as non thyroidal illness affecting thyroxin levels. Sazmand et al. (2011) also recorded the reduction in T₄ and T₃ concentration levels in sub clinically affected dromedary camels with *T. evansi* in Iran.

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