Indian Journal of Animal Sciences **86** (4): 372–375, April 2016/Article
https://doi.org/10.56093/ijans.v86i4.57690

Epidemiological, clinical, haemato-biochemical and therapeutic evaluation of canine trypanosomosis in Mumbai

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Received: 18 March 2015; Accepted: 1 September 2015

ABSTRACT

Evaluation of 20 confirmed clinical cases of canine trypanosomosis caused by *Trypanosoma evansi* in Mumbai from June 2012 to May 2013 was done to compile the basic information on parasitological, epidemiological, clinical, clinico-pathological and chemotherapeutic aspects. The disease was predominantly found in the localities having substantial equine and bovine population thus underlining the potential source for canine cases. Molecular analysis of few representative samples also confirmed the ability of the organisms to crossover from one species to other species of host. The disease was characterized by clinical signs such as depression, anorexia, anaemia, fever, splenomegaly, hepatomegaly and oedema of legs and paraclinical findings such as low levels of TEC, Hb and PCV indicating anaemic trend, elevated levels of alkaline phosphatase, AST, ALT and hyperglobulinaemia associated with reversing of albumin: globulin ratio. Hypoglycaemic trend noted in the study was found positively correlated with intensity of parasitaemia. Blood glucose levels were inversely related to degree of parasitaemia. The chemotherapeutic options, diminazeneaceturate and antrycideprosalt although cleared parasitaemia but failed to provide complete recovery, as 50% of cases eventually died in spite of specific chemotherapy suggesting some secondary involvement in the disease entity.

Key words: Canine, Clinico-pathology, PCR, Treatment, *Trypanosoma evansi*

Canine trypanosomosis in India, primarily caused by *Trypanosoma evansi*, is adequately defined in cattle, buffaloes, camel and horses (Birajdar 2007, Chaudhary *et al*. 2008) such as covering different aspects like pathogenesis, clinical entity, paraclinical findings, diagnosis, treatment and even vaccine development. However, the information regarding canine trypanosomosis in Indian literature is far from adequate and most of the references are based on case reports describing clinical signs and chemotherapy. Thus, there is need to compile the information by studying number of cases from the same region, which might contribute significantly to bridge existing knowledge gap in the literature. This study was therefore conducted on dogs naturally infected with *Trypanosoma evansi* in Mumbai region with these broad objectives in mind.

MATERIALS AND METHODS

Canine cases admitted to different veterinary clinics with clinical signs varying from intermittent fever, weakness, anorexia, anaemia, dehydration, oedema of throat and/ or legs, jaundice and nervous signs from June 2012 to May 2013 were included.

Total 20 cases were encountered of varying ages (7 months to 14 years) and breeds (Non-descript, Cocker spaniel, Labrador, Boxer and German shepherd). The epidemiological findings on 20 confirmed cases included observations on influence of age, breed, sex, location, habitats and food habits. In order to ascertain the link between large ruminants (cattle and buffaloes) which are reported to be the reservoir hosts and canine population, few of the positive blood samples were subjected for PCR to amplify VSG gene (Migri 2011) and the products were sent for gene sequencing. The sequences obtained were analyzed and curetted using Biology Workbench 3.2. The curetted sequences were submitted to search their similarity in NCBI database using BLAST tool.

Blood smears were stained by field stain (Fleck and Moody 1993) and measurements of the haemoflagellates were obtained by using micrometry technique (Rathore and Senger 2005). The intensity of parasitaemia was arbitrarily judged by counting number of trypanosomes per oil immersion field and the cases were divided into three groups *viz.* mild (up to 5), moderate (6–10) and heavy (more then 11).

Clinical signs of each case were noted and physical examination was performed in detail to record rectal

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temperature, respiratory rate, colour of visible mucus membrane and enlargement of liver, spleen and palpable lymphnodes. Blood samples were collected on the day 0 (before treatment), day 2 and day 10 post treatment. Blood samples were processed for CBC (complete blood count) and serum samples for biochemical analysis.

These cases were divided into 2 groups, in which group 1 (14 cases) and group 2 (5 cases) were treated with single doses of antrycideprosalts @ 6 mg/kg body weight, subcutaneously and diminazeneaceturate @ 5 mg/kg body weight, deep intramuscularly, respectively along with supportive therapy which included intravenous fluids, liver tonics and B complex. A few dogs were also administered with corticosteroids. Efficacy of chemotherapy was assessed on the basis of parasitaemia, clinical evaluation and haemato-biochemical profiles.

The data generated during the study were subjected to paired t test, as per the method described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Peripheral blood smears of 20 cases of canine trypanosomosis revealed haemoflagellates measuring 17 to 34µ (average 25.5µ) in length and 2 to 4µ (average 2.9µ) in width. Recurrent flagellum emerging from sub terminally placed kinetoplast showed distinct undulating membrane and free portion. Nucleus was located in the centre (Fig. 1). The morphometric analysis suggested that the haemoflagellates belonged to *Trypanosoma evansi*. To ensure the taxonomic identification, all the 10 samples revealed a distinct band of 391 bp after electrophoresis of the PCR product (Fig. 2). Further,nucleotide sequence of 2 PCR products was blasted with NCBI Gene bank (Accession No.: EF495337.1, AF317914.1, AB259839.1 and JX888091.1) which revealed 98–100% homology with *Trypanosoma evansi*. Thus, it was confirmed that *Trypanosoma evansi* encountered in the study is a euryxenous parasite having potential to infect cattle, buffalo and horses.

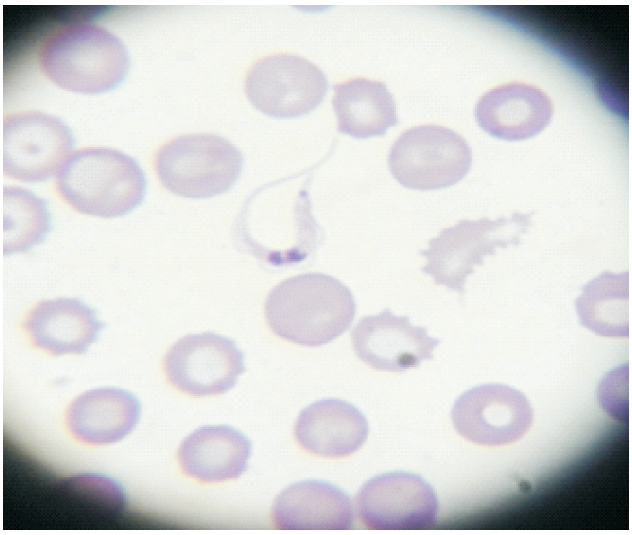
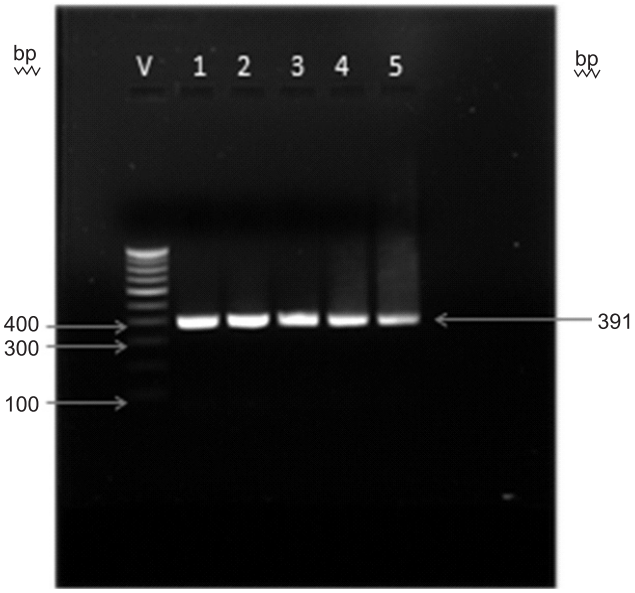


Fig. 1. Morphologic analysis of *Trypanosoma evansi.*



Lane: V: 100 bp DNA ladder, 1: 2, 2: 6, 3: 9, 4: 13, 5: 16

Fig. 2. PCR amplification of VSG gene of *Trypanosoma evansi* from blood samples (391bp).

Among the 20 cases encountered, 14 (70%) were local non-descript dogs and remaining 6 (30%) belonged to Labrador (2), Boxer (2), German shepherd and Cocker spaniel. As regards the gender, 13 (65%) of the infected dogs were males and remaining 7 (35%) were female. The infection was predominantly seen in adult dogs above five years of age (15 cases - 75%) and only one dog (5%) below one year of age was found infected with *T. evansi.* The remaining four (20%) infected dogs aged between 1–5 years.

The analysis of clinical signs and observations of physical examination revealed lethargy (95%), anorexia (80%), anaemia (80%), fever (65%) and splenomegaly

Table 1. Level of parasitaemia in dogs infected with *T.evansi* and correlation between intensity of parasitaemia and blood glucose levels

Intensity	No. of cases	Average load per oil immersion	Maximum load	Blood glucose level (Avg.) mg/dl	Percent of hypoglycaemia cases (%)
Low	11 (55%)	2.09	05	52–103 (69.64)	81.82
Moderate	05 (25%)	6.60	10	32–97 (60.8)	80.00
Heavy	04 (20%)	22.50	42	42–76 (55.75)	100
Total	20	7.3	42	32–103 (64.65)	85

Table 2. Mean±SE values of haematological observations in *Trypanosoma evansi* infected dogs before and after treatment with diminazene aceturate and antrycideprosalts

Parameter	Diminazene aceturate*		Antrycideprosalts#	
	Pre-treatment	Post-treatment (10^{th} day)	Pre-treatment	Post-treatment (10^{th} day)
TEC×10^6/cumm	4.93±0.69	5.85±0.72	3.68±0.27	4.27±0.47
Hb g%	10.92±1.89	13.02±2.13	8.2±0.77	9.53±1.2
PCV %	33.2±5.28	40.22±6.39	25.12±1.97	28.83±3.25
MCV fl	66.91±3.2	67.93±2.85	69.53±2.01	67.5±1.16
MCHC g/dl	32.71±1.08	32.26±0.47	31.82±1.06	32.77±0.92
Platelets×10^5/cumm	93000±35085.6*	215750±19093*	1,34,666±51,184*	3,29,222±55,498*
Glucose mg/dl	67.75± 9.98*	81.5±10.03*	66.33±5.57*	82.66±6.93*

*Significant ($P<0.05$) at t crit (3.18); # significant ($P<0.05$) at t crit (2.306).

Table 3. Mean±SE values of biochemical observations in *T. evansi* infected dogs before and after treatment with diminazene aceturate and antrycideprosalts

Parameter	Diminazene aceturate		Antrycideprosalts	
	Pre-treatment	Post-treatment (10^{th} day)	Pre-treatment	Post-treatment (10^{th} day)
ASTIU/L	126±46.83	22.3±4.56	132±46	49±6.77
ALTIU/L	65.5±11.93	27.55±5.07	66.78±19.25	91.2±10.12
ALPIU/L	142.5±36.9	131.375±24.55	642.89±186	735±261
Total proteing/dl	6.9±0.8	7.02±0.59	6.74±0.5	6.75±0.32
Albuming/dl	2.075±0.165	3.125±0.45	1.97±0.2	2.14±0.11
Globuling/dl	4.82±0.73	3.9±0.7	4.75±0.3	4.61±0.3
A/G	0.4±0.05	0.9±0.304	0.42±0.04	0.48±0.03
BUNmg/dl	13.175±0.82	14.76±3.84	29.29±8.06	17.43±1.54
Creatininemg/dl	0.9±0.21	1.16±0.08	2.03±0.7	0.95±0.15

(65%) as the predominant features followed by dehydration (55%), dyspnoea (45%) hepatomegaly (45%), and oedema (40%) with pitting on pressure on legs. This clinical picture tallies with the observations of Garud *et al.* (2009) and Tresamol *et al.*(2013). Other clinical findings viz. lymphadenopathy (25%), erythematous skin lesions (10%) and jaundice (5%), observed in the present study were also recorded by Aquino *et al*. (1999) and Garud *et al*. (2009). Although corneal opacity reported by Gunaseelan *et al.* (2009) and Tresamol *et al.* (2015) in dogs with *Trypanosoma evansi* infection in Bikaner, Bareilly, Mannuty, Jaipur and Chennai respectively. But in the present study, none of the infected dogs showed corneal opacity.However, 2 cases revealed excessive lacrimation, 1 of which also shown prolapse of third eyelid. Surprisingly, laryngeal oedema with change in voice and drooling of saliva, which is described as classical signs of the disease in dogs (Soulsby 1982, Gill, 1991, and Garud *et al. 2*009), was evident in only one case in the present study. History records of these dogs also revealed digestive disturbances (10%) such as diarrhoea (5%) and vomiting (10%).

From Table 1 it is evident that 55% of the cases detected had mild level of parasitaemia, 25% cases had moderate and 20% cases had heavy level of parasitaemia. The minimum and maximum load of the haemoflagellate per oil immersion field of peripheral blood smear was one and 42 respectively, with an average of 7.3. Among 20 positive cases, 17 (85%) dogs showed blood glucose lower than the reference range indicating hypoglycaemic status. There is positive correlation between level of parasitaemia and degree of hypoglycaemia. Birajdar (2007) also noted similar type of correlation in buffaloes. However, there is no previous reference to confirm this trend in dogs. Thus, blood glucose estimate can be taken as good indicator of the disease since diabetes mellitus is not as common metabolic condition in dogs.

Out of 20 confirmed cases, 80%, 90% and 90% dogs showed TEC, Hb and PCV levels below the reference range indicating anaemia. Out of 18 cases, anaemia was characterized as normocytic (88.9%) with either normochromic (72.2%) or hypochromic (27. 8%) type (Hosseininejad *et al*. 2007 and Howes *et al*.2011). Leucocytosis was observed in 20% dogs and leucocytopenia was seen in 25% dogs. Thrombocytopenia was revealed in 17 (85%) dogs. Similar trend of low platelet count was also recorded by De La Rue *et al*. (1997).

Only one dog revealed higher values of total, direct and indirect indicating jaundice. The discrepancy in the findings could be attributed to the extent of immunostimulation and longevity of the illness. As regards protein estimation, hyperproteinaemia was noticed in 40% dogs; while in 30% hypoproteinaemia was evident. Interestingly, hyperproteinaemia in all the eight cases was apparently due to hyperglobulinaemia. Further in 85% cases, albumin:globulin ratio was reversed.These findings clearly indicated immunostimulation of the dogs by trypanosomal

surface antigens.Howes *et al.* (2011) categorically stated that hyperglobulinaemia and reversing of albumin: globulin ratio as important clinico-pathological findings of canine trypanosomosis.

Among 20 positive cases, higher levels of alkaline phosphatase (100%), AST (85%) and ALT (70%) was observed. Aquino *et al.* (2002) also witnessed a similar trend in experimentally induced trypanosomosis in dogs. Hosseininejad *et al.* (2007), however, did not find any deviation in the levels in three crossbred dogs naturally infected with *Trypanosoma evansi*. Five dogs (25%) revealed higher levels of BUN and serum creatinine and four out of these dogs succumbed to the infection and could not survive. It might be due to glomerulo-nephritis because of deposition of antigen antibody complexes in the glomeruli. Necropsy of two dogs that died during the study revealed glomerulonephritis.

All the dogs treated with antitrypanosomal drugs showed clearance of the haemoflagellates from blood circulation. The dogs which died immediately (within 10 days) and subsequently also showed clearance of the parasites. Similar situation of clearance of the organism, but death of the infected dog was also confronted by Defontis *et al.* (2012). Thus on the basis of parasitaemia, the efficacy of both the compounds was found to be 100%.

As regards symptomatic relief after specific chemotherapy, in diminazene treated group (5 cases), four cases recovered clinically and one dog died 12 days post treatment. Thus, the efficacy was 80%. As regards antrycideprosalt treated group (14 dogs), only six out of 14 dogs showed clinical recovery indicating efficacy of only 42.85%. Mortality rate in this group was 57.14% (8 cases). Varshney (2005) has reported 30% mortality of clinical cases of canine trypanosomosis. Early diagnosis of canine trypanosomosis is invariably missed and it could be the contributing factor along with prolonged corticosteroidthearpy for fatal sequelle and immunosuppressing effect of the organism.

The parameters denoting anaemic trend such as TEC, Hb, MCV and MCHC showed striking improvement in the values (Table 2) although the differences in pre and post treatment collections were statistically nonsignificant in both the treatment groups. However thrombocyte counts before and after treatment showed statistically significant difference ($P<0.05$). There was also improvement in blood glucose levels in both the treatment groups and the differences were statistically significant ($P<0.05$) in both the groups. Increase in blood glucose after the treatment was obviously due to disappearance of trypanosomes from blood stream that could utilize blood glucose for their asexual reproduction.

In biochemical analysis, noticeable improvement was shown only in SGOT while other parameters showed inconsistent results (Table 3). There is no in depth information about biochemical profiles of *Trypanosoma evansi* cases before and after treatment in dogs to evaluate findings of this study.

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