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CATT/*T.evansi* Antibody Levels in Patients Suffering from Pyrexia of Unknown Origin in a Tertiary Care Hospital in Kolkata

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ABSTRACT

'Trypanosomiasis' caused by *Trypanosoma evansi* ('Surra') is one of the most widely distributed vector borne diseases in India which mainly affects domesticated and wild animals such as camel, horses, pig and chronic diseases in cattle and buffaloes. In humans *T.evansi* infection has been documented in the last decade. The main objective of this study was to investigate seroprevalence of *T. evansi* antibody in patients suffering from pyrexia of unknown origin (PUO) using CATT (card agglutination test for trypanosomiasis)/ *T. evansi* test. This study was carried in a tertiary care hospital (Peerless Hospital and B.K.Roy Research centre) based in Kolkata, India. We have so far studied 30 cases of PUO with 50 control cases without pyrexia who attended the hospital for common surgical problems. In this study 2 cases (6.6%) of PUO showed positive results with CATT)/ *T. evansi* test while none of the 50 control cases showed positive results with this test. Although we cannot conclude that *T. evansi* infection may be a cause of PUO, however, this study creates a scope to investigate this further for the benefit of the mankind.

Keywords: Trypanosomiasis, *T.evansi*, CATT(card agglutination test for trypanosomiasis), PUO(Pyrexia of unknown origin)..

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INTRODUCTION

T.evansi is a pathogenic arthropod borne protozoan flagellated parasite. It causes 'Surra' in several animals such as cattle, buffalo, horse, camel, pig and deer [1]. It is the most widely distributed trypanosome which is affecting domesticated livestocks in Asia, Africa, South America and Central America. *T. evansi* belongs to the genus *Trypanosoma* of the family Trypanosomatidae. The first mammalian pathogenic species of *T.evansi* was discovered by Evans in 1880 during an investigation of a disease of horses known by people as 'Surra' (means low and rotten) [2]

Biologically *T.evansi* is similar to *T.equiperdum,* the causative agent of Dourine [3]. Morphologically it has terminal kinetoplast, an elongated nucleus and long free flagellum [4] which resembles the slender forms of trypanosomes like *T.brucei, T.gambiense* and *T. rhodesiense*. Total length of *T.evansi* varies between 15-34 µm. This parasite is covered by a dense protein layer called surface glycoprotein which acts as an immunogen and elicits formation of specific antibodies. *T.evansi* is incapable of infecting invertebrate vector because a part of its mitochondrial (Kinetoplast) DNA is lost [5, 6]. It multiplies by the process of longitudinal binary fission [7]. Multiplication of the parasites is restricted to the vertebrate host only as it does not undergo development in an intermediate host.

T.evansi is mainly transmitted by Haematophagous flies such as *chrysops, stomoxys Haematopota* and *Tabanus* [1, 4]. Surra in susceptible animals like cattle, camel, horses, buffalo is manifested by pyrexia which is associated with parasitaemia. The first sign of this infection is a localised swelling of the skin which is followed by fever, severe anaemia, oedema in the lower parts of the body, weight loss, loss of appetite etc. Infection occurs in horses, camels and dogs causing oedema and emaciation [8]. In case of donkeys, sheep and goats, infection is subclinical [9]. Cattle and buffaloes are considered reservoir host because no clinical signs seen in both the animals [10]. The reason of this clinical variability can be explained by the genetic diversity of the natural population. [11] .*T.evansi* infection in human is rare [12]. *T.evansi* is sensitive to human serum [13, 14]. This innate immunity against *T.evansi* infection is due to the trypanolytic factor Apolipoprotein L1 [15].

Diagnosis of this disease is based on the detection of parasites in the blood by haematological tests, metabolic products by biochemical tests and antibody levels by serological tests. It has also been reported that the disease causes immunodeficiency [16,17]. Chemotherapy and.chemoprophylaxis are the main methods of controlling Surra. Development of vaccine against *T.evansi* is difficult because the parasite alters surface antigen during the course of infection [18]. Drugs used for Surra are mainly Suramin, quinapyramine, Dimethylsulfate (Antryside), quinapyramine methyl sulphate, quintrycide, Diminazene aceturate,etc. As the disease has recently been reported in human beings we felt it is important to look into its possible causative role in patients suffering from PUO.

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MATERIALS AND METHODS

Study area

This study was conducted in a tertiary care hospital (Peerless Hospital and B.K.Roy Research centre) based in Kolkata, India.

The patients

The patients who were included in this study after taking consent were either suffering from PUO (test group, 30 cases) or came to the hospital for treatment of common surgical problems of hernia, hydrocele, fibroadenoma breast etc. without any evidence of pyrexia or any infectious aetiology (control group, 50 cases). In the control group 27 (54%) cases were males and 23(46%) cases were females. 5 cases (10%) were below 20 years of age, 14 cases (28%) were in between 20-40 years of age ,16 cases (32%) were in between 41-60 years og age and 15 cases (30%) were more than 60 years og age.

In the test group 17 (56.7%) cases were males and 13 (43.3%) cases were females. 3 cases (10%) were below 20 years of age, 14 cases (46.7%) were in between 20-40 years of age, 10 cases (33.3%) were in between 41-60 years of age and 3 cases (10%) were more than 60 years of ages. The demography and clinical history of these patients were recorded in a proforma prepared by us. Only those PUO cases were included in this study in whom brucellosis, tuberculosis and kalaazar were excluded which are the common causes of PUO in this study area. Case history of the two CATT/*T .evansi* Positive patients has been provided below :

1 .A 39 years old businessman residing in Rajshahi ,Bangladesh was reported with fever and fatigue for more than three weeks with no history of joint pain and low back pain. Fever was not associated with night sweat. This was followed by the onset of severe headache also.

2 .A 58 years old businessman residing in Dafarpur, Raghunathganj , West Bengal was reported with history of high fever of four weeks duration with joint pain. There were no associated low back pain and fatigability and night sweat.

Reagents

Freeze dried suspension of purified, fixed and stained *T. evansi* of (VAT) RoTat 1.2 sodium azide (0.1%) as preservative was used as antigen. During the test reconstitution of this antigen was done with phosphate buffered saline (pH 7.2) and known positive control and negative controls were used with each test lot.

Methods

25µl of diluted serum collected from a patient was mixed with 45µl of antigen suspensions on a white coloured card. After mixing and spreading, the card was rotated for five minutes at ~70 rpm. A positive reaction was indicated by granular deposits visible to the

naked eye. Agglutination reactions were interpreted as ± , +, ++, +++ representing weakly positive, positive, strongly positive, very strongly positive reactions respectively. However, in this study weakly positive cases were considered as negative as this type of reaction was commonly found (50%) in brucellosis cases which is also an important cause of PUO. We had previously studied 10 brucellosis cases with this test and 5 cases showed weakly positive reactions. .

RESULTS

In the test group CATT/*T.evansi* test was positive in two cases (6.6%; both of them were strongly positive, ++), while none of the control group showed positive reaction with this test. As we mentioned earlier that we considered weakly reaction as negative, we considered 9 (30%) test group patients and 15 (30%) control group patient as negative who showed weakly positive (±) reactions in this study.

Table 1: CATT/*T.evansi* result in control group and Test group patients

Total no of Human blood sample	CATT/*T.evansi* result			
	Very strongly positive +++(%)	Strongly positive ++(%)	Weakly positive* ±(%)	Positive +(%)
Control group (50)	0(%)	0(%)	15(30%)	0(0%)
Test group (30)	0(%)	2(6.66%)	9(30%)	0(0%)

*weakly positive reaction was considered as negative reaction.

DISCUSSION

Trypanosomiasis [19] caused by *T.evansi* (Surra) is very common in domestic and wild animals in India. 'Surra' is mostly prevalent in Northern India but prevalence of Surra from Southern and Eastern India has also been reported earlier. This disease causes remarkable economic losses to the livestock owners and due to that there has been many studies on the prevalence of *T.evansi* in several animals in India. Although *T.evansi* is not infective to human but still few cases of Human trypanosomiasis caused by *T.evansi* were reported in India. The first Human trypanosomiasis caused by *T.evansi* were reported at the state of Maharastra in India[20]. Nineteen atypical human trypanosomiasis are reported and out of nineteen cases eight were from India. Out of 8 cases 3 cases of human trypanosomiasis caused by *T.evansi* were reported in India.

The purpose of this study was to investigate the possible occurence of *T.evansi* in the patients blood serum using CATT and the result clearly demonstrated that the used teachnique detected *T.evansi* in the patients serum with PUO symptoms.. In this study no *T.evansi* were detected in the fifty group 1 patients serum sample & this can be explained by the presence of trypanolytic factor in the patients serum sample. The present work detected *T.evansi* in the group 2 patients serum. Although *T.evansi* infection in rare in human but still the study showed two CATT positive serum sample among the thirty patients with PUO symptoms serum. This can be explained on the basis of some reasons such as (1) lack of

trypanolytic factor in the infected patients. (2) Parasite mutated to form which can resist the trypanolytic factor in human serum. (3) Frequent vector transmission of parasites to human.

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