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Trypanosoma evansi causes thyroxin imbalance with biochemical alterations in wistar rats

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ABSTRACT

Animals affected with *Trypanosoma evansi* show rare serum hormonal disturbances. One of the important hormones for livestock is thyroxin, and the level of thyroxin may be reduced during the *T. evansi* infection. The objective of the study was to investigate thyroxin level during experimentally induced *T. evansi* infection in Wister albino rats. Wistar albino white rats (n=12) were challenged with the local strain of *T. evansi* (at $5x10^5$ trypanosomes/animal subcutaneously). At the high parasitemia, blood was collected from the rats, and serum was separated, which was subjected for biochemical evaluation. Decreased total serum thyroxin (2.91 ±0.04 µg/dL) and free thyroxin (1.30 ±0.05 ng/dL) levels *($p<0.01$)* were recorded in *T. evansi* infected rats as compared to the control group of rats. Along with lowered thyroxin levels, decreased levels of total erythrocyte count, packed cell volume, hemoglobin, total leucocyte count, total serum proteins, albumin and glucose levels were recorded. On the other hand, significant increase *($p<0.01$)* in cholesterol, blood urea nitrogen, serum alkaline phosphatase, serum aspartate aminotransferase, and serum alanine aminotransferase levels were observed. Thus, it is concluded that trypanosomiasis induces stress on rat, which have direct effect on thyroid hormone.

Keywords

Anemia, Biochemical alterations, India, Rats, *Trypanosoma evansi*, Thyroxin

ARTICLE HISTORY

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INTRODUCTION

Trypanosoma evansi causes a disease known as '*surra*' in numerous mammalian hosts. In Indian subcontinent, the disease is a constant threat to livestock productivity. Trypanosomiasis in animals occurs both in chronic and acute forms. The chronic form is most common and may present in association with secondary infections due to immunosuppressant caused by *T. evansi* infection. Clinical signs recorded as emaciation, intermittent fever, anemia, lacrimation, corneal opacity, diarrhea, and edema of the dependent parts. Detection of *T. evansi* in the blood is difficult because parasitemia is intermittent (Choudhary and Iqbal, 2000). The thyroid gland is one of the endocrine organs affected during trypanosomiasis. Chronic infection could impair the function of thyroid gland and recorded hypothyroidism associated with decrease in triiodothyronine (T_3) and thyroxin (T_4) levels in *T. evansi* infected camels (Al-Garawi et al., 2001). Prevalence of *T. evansi* was reported in India with different diagnostic tests in different animals (Sivajothi et al., 2012, 2013a, 2014a). Histopathological changes along with pathological changes in experimental lab animal's infection with *T. evansi* were also reported (Sivajothi et al., 2013b, 2014b). Studies regarding the lower thyroxin levels were recorded in goats, camels, zebu cattle affected with trypanosomiasis (Al-Garawi et al., 2001; Fatihu et al., 2009). However, few studies were conducted on the hematological and biochemical alterations associated with thyroxin levels variations in experimental *T. evansi* infected rats. Hence, the aim of the present study was to know about the hemato-biochemical and thyroxin changes associated with the local strain (South Indian) of *T. evansi* infection in experimentally infected rats in Andhra Pradesh.

MATERIALS AND METHODS

Present study was conducted on 12 healthy (9 males and 3 females) adult Wistar albino rats weighing more than 200 gm. Rats were kept in mosquito free cages with 12:12 h interval light/dark cycle throughout the experimental period with provision of *ad libitum* feed and water. The approval of the institutional animal ethics committee permission was obtained prior to commencement of the experiment (Letter No: 221, Dt: 01.06.2009). After 10 days of acclimatization the animals were randomly divided into two groups. The experimental rats were tested for gastrointestinal and blood parasites and were treated with fenbendazole and ivermectin dosed at 5 mg/kg body weight and 200 µgm/kg bwt respectively, as per the report of Takeet and Fagbemi (2009).

The virulent strain of *T. evansi* was isolated from a cattle suffering from clinical surra. The strain was maintained *in-vivo* in Wistar albino rats through serial passages. The rats were randomly assigned into two groups: one included twelve animals (infected group), the other six were in control group. Each animal was infected with $5x10^5$ trypanosomes subcutaneously. The blood of infected rats was collected by tail clipping and examined daily for the presence of *T. evansi* organisms from the second day of post-inoculation **(Figure-1** and **2).** The infected rats with high parasitemia were anesthetized with thiopental sodium (dosed at 45 mg/kg bwt, IV) and blood was collected directly from the heart in vials containing heparin dosed at 30 IU/mL of blood. Blood collected from all the rats was used for the estimation of total erythrocyte count (TEC), total leucocyte count (TLC) and packed cell volume (PCV), hemoglobin (Hb) and differential leucocyte count (DLC) as per the procedures mentioned by Benjamin (2001). Blood was collected from all rats into sterile test tube and was allowed to clot. The serum was collected and stored at -20^{0}C and was used for the estimation of total serum proteins, glucose, bilirubin, blood urea nitrogen (BUN), serum alkaline phosphatase (ALP), serum aspartate aminotransferase (AST), and serum alanine aminotransferase (ALT), (M/S Excel Diagnostics Ltd) using kits. Total T_4 and free T_4 levels were estimated by the enzyme-linked immunosorbent assay (ELISA) (as per manufacture's procedure) (**Figure 3**) using a kit obtained from United Biotech Inc. The data in respect of each parameter was tabulated and statistical analyses were carried out by using Statistical Package for Social Sciences (SPSS) using Student t-test. *P*-value more than 0.05 considered as non-significant, *p*-value of 0.05 or lower was considered to be significant and *p*-value lower than 0.01 considered as highly significant.

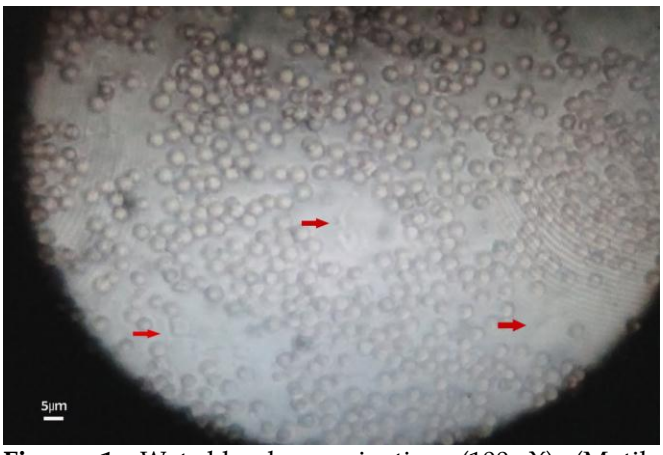

Figure 1: Wet blood examination (100 X) (Motile *Trypanosoma evansi* organism indicated by arrow).

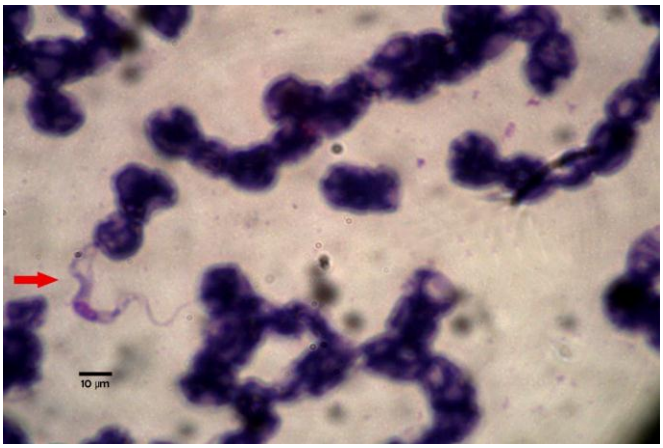

Figure 2: *Trypanosoma evansi* in stained blood smears (indicated by arrow) (1000X).

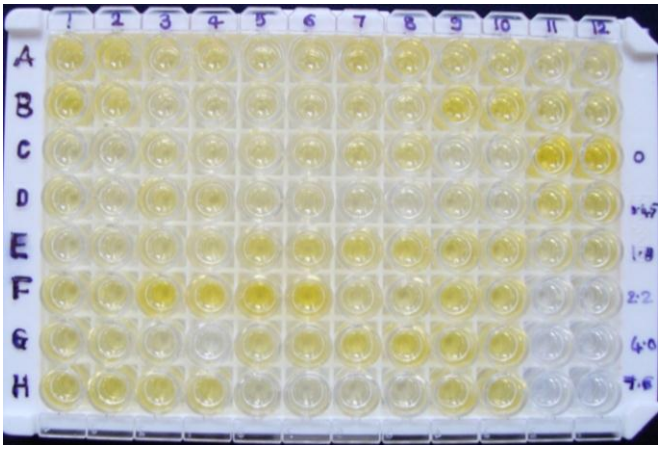

Figure 3: Estimation of thyroxin levels by using the Indirect ELISA (indicated by color formation).

Table 1: Mean haematological values of control and trypanosome affected rats (Mean±S.E).

Parameters	Control group (n=6)	Trypanosoma infected rats (n=12)	t-test	*p*-value
Hb (g/dL)	15.48 ±0.18	12.17 ±0.11	15.36**	0.0000
PCV (%)	46.50 ±0.85	38.90±15.48	6.39**	0.0000
TEC x10^6/cumm	7.47±0.12	6.22 ±0.46	8.27**	0.0000
TLC /cumm	11297±334.34	9798.40 ±71.86	3.94**	0.0010
% Neutrophils	18.28±0.24	23.33±0.98	3.80**	0.0025
% Lymphocytes	76.36±0.82	70.12±0.65	4.95**	0.0003
% Monocytes	2.66±0.77	3.12±0.13	3.03 NS	0.0991
% Eosinophils	2.54±0.12	3.43±012	1.79NS	0.104

NS – Non Significant *(p>0.05)*
* Significant *(p<0.05)*
* * Highly Significant *(p<0.01)*

Table 2: Serum biochemical changes in control and trypanosome affected rats (Mean±S.E).

Parameters	Control group (n=6)	Trypanosoma infected rats (n=12)	t-test	*p*-value
Total protein (g/dL)	6.81 ±0.10	6.57 ±0.08	1.80 NS	0.0852
Albumin (g/dL)	3.87 ±0.10	3.10 ±0.10	5.26**	0.0000
Globulin (g/dL)	2.83 ±0.09	3.17 ±0.04	3.06**	0.0057
Glucose (mg/dL)	122.92±4.80	82.17±4.2	6.37**	0.0000
SGOT (AST) (U/L)	82.92±2.7	114.08±5.9	4.73**	0.0001
SGPT (ALT) (U/L)	31.33±1.32	54.83±2.81	7.56**	0.0000
Creatinine (mg/dL)	0.75±0.02	1.12±0.07	4.66**	0.0001
BUN (mg/dL)	23.25±1.40	37.00±2.54	4.72**	0.0001
Total T_4 (µg/dL)	3.21 ±0.05	2.91 ±0.04	3.98**	0.0001
Free T_4 (ng/dL)	1.58±0.04	1.30 ±0.05	3.94**	0.0001

NS – Non Significant *(p>0.05)*
* * Highly Significant *(p<0.01)*

RESULTS AND DISCUSSION:

During the early stage of infection, *T. evansi* infected rats showed clinical signs like varying degrees of emaciation, dehydration, mucopurulent nasal discharges, and pasted perineum. Clinical signs such as roughened hair coat, more dulled appearance and recumbence were noticed during fifth day of infection. Severe anemia was the main hematological finding. The mean TEC, PCV, Hb and TLC values reduced significantly. Leucopenia associated with neutrophilia and lymphocytopaenia was noticed. Total serum proteins and glucose levels were decreased significantly *(p<0.01)* and there was significant increase *(p<0.01)* in cholesterol levels, bilirubin, creatinine, BUN, AST, and ALT values. Reduction in the total T_4 and free T_4 were recorded. Mean hematological and biochemical values in rats of experimental groups are mentioned in **Table-1** and **2.**

The clinical signs observed in rats experimentally infected with *T. evansi* in the present study were in agreement with the report of Dargantes et al. (2005). Decreased values of TEC, Hb and PCV found in the study might be due to increased susceptibility of red blood cell membrane to oxidative damage. Similar observations were made by Hilali et al. (2006). In the present investigation, anemia was the most consistent finding which was in accordance with the findings of Takeet and Fagbemi (2009) and Sivajothi et al. (2014c). Etiology of this anemia is complex, but the most important factor is said to be hemolysis based on a reduction in red cell mass and life span. Anemia caused by mechanical injury to erythrocyte occurred by the lashing action of the powerful locomotory flagella and microtubule reinforced bodies of the millions of the organisms during parasitemia.

Leucopenia associated with neutrophilia and lymphocytopenia were noticed. Leucopenia could have been the result of immune suppression, which usually co-exists with trypanosomiasis. The leucopenia is attributed to reduce myelopoiesis. These results were in consonance with the experiments of Hilali et al. (2006). Another experiment on goats opposed the mentioned results and stated that leucocytosis was not a reliable indicator of infection (Dargantes et al., 2005). In infected camels, Choudhary and Iqbal (2000) noted a significant decrease in lymphocyte with a visible increase in leucocytes and neutrophils.

The decrease in total serum protein level observed in this study was consistent with the findings of Sadique et al. (2001) who reported decreased in total protein in

cattle infected with *T. congolense*. Protein levels usually drop in trypanosome infections as a result of depressed albumin levels. The increase in protein levels during the chronic phase of the infection is usually due to the increase in globulin levels. This may happen as a result of the immune response by the animals to the *T. evansi* infection (Lushaikyaa et al., 2011). The result of hypoglycemia was in consonance with the findings of Takeet and Fagbemi (2009). Excessive utilization of the blood glucose by trypanosomes for their metabolism has been thought to account for the hypoglycemia observed during trypanosomiasis. However, increased metabolic rate caused by fever and hepatocyte degeneration could also be a reason for hypoglycemia in trypanosomiasis. In the present study, raised levels of serum creatinine were in accordance with the findings of Adejinmi and Akinboade (2000). This was associated with damage to host tissues as well as renal and hepatic malfunction (Abenga and Anosa, 2005). Elevation in the BUN level in the present study was in accordance with the observation reported by Abenga and Anosa (2007). The elevated values of ALT and AST enzymes might be due to tissue breakdown (necrosis) and inflammation in the host, particularly of the liver, heart, muscle and kidney. Another possibility of increased levels of ALT and AST enzymes was the lysed trypanosomes at different stages of the infection.

The possibility of primary hypothalamic pituitary dysfunction during trypanosomiasis has been indicated by hormonal imbalance related to thyroid hormonal pathway. Similar findings were previously reported in different species of the animals suffering with trypanosomiasis. Fatihu et al. (2009) reported that Zebu cattle infected with *T. vivax* showed a decrease in the level of T_4 (Fairouz et al., 2007). Al-Garawi et al. (2001) reported that the decreased T_3 and T_4 and TSH blood levels could be due to *T. evansi* infection in camels.

CONCLUSION

Present research reports the rare finding of reduction in the total T_4 and free T_4 levels in *T. evansi* infected rats along with other hemato-biochemical parameters. It is concluded that trypanosomiasis due to *T. evansi* in rats has influence on the thyroid hormones, which originates from severe stress induced by trypanosomiasis in rats. Treatment with trypanocidal drugs can protect from thyroid damage.

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