Haematological and biochemical changes in experimental *Trypanosoma evansi* infection in rabbits

S. Sivajothi · V. C. Rayulu · B. Sudhakara Reddy

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Abstract New Zealand white rabbits (N = 4) were challenged with the local strain of *Trypanosoma evansi*. Each rabbit was infected with 5×10^5 trypanosomes subcutaneously. The infection was characterized by intermittent pyrexia, undulating parasitaemia, anorexia and emaciation. The infected rabbits were examined daily for development of clinical signs and infection status by wet blood-films made from the ear veins. Thick and thin blood smears were also examined daily until the end of the experiment for description of blood cells. Differential leukocyte count (DLC) was also done. The parasite was observed in the blood during the acute phase only. Leukocytosis in the acute phase followed by leukopenia during the chronic phase was recognized. Haematological studies revealed reduced TEC, Hb and PCV. The main changes in the erythrocytes were macrocytes, hypochromic cells, Howell–Jolly bodies, target cells, stomatocytes and burr cells. Serum chemistry revealed hypoproteinemia, hypocholesterolaemia, hypoglycemia, hyperbilirubinemia, elevated creatinine, BUN, increased AST and ALT.

Keywords Rabbits · Haemato-biochemical changes · *Trypanosoma evansi*

Introduction

Trypanosoma evansi causes a disease known as ‘surra’ in numerous mammalian hosts. The incidence and the

severity of the disease vary with the strain of the parasite as well as the species of host affected. In Indian subcontinent, the disease is a constant threat to livestock productivity. Although haemato-biochemical changes of the disease by different species of trypanosomes in domestic and laboratory animals has been well documented (Takeet and Fagbemi 2009), yet the information on haemato-biochemical changes in rabbits induced by *T. evansi* is meager. Hence, the present investigation was intended to study haemato-biochemical changes in rabbits induced by local isolate of *T. evansi*.

Materials and methods

The virulent strain of *T. evansi* was isolated from a cattle suffering from clinical surra. The strain was maintained in vivo in whistar rats through serial passages. The blood of infected rats was collected by tail clipping and examined daily from the second day of post-inoculation. The infected rats with high parasitaemia were anaesthetized with thio-pental sodium and blood was collected directly from the heart in vials containing heparin @ 30 I.U. per ml of blood. Six New Zealand white rabbits were maintained in fly proof house with clean and hygienic environment throughout the experimental period for 12 weeks by taking necessary precautions. Rabbits were kept on ad libitum feed and boiled filtered water was provided to these animals throughout the experimental study. The experimental rabbits were tested for gastrointestinal and blood parasites and were treated with fenbendazole and ivermectin at the rate of 5 mg/kg body weight and 200 µg/kg body weight respectively (Takeet and Fagbemi 2009). The rabbits were randomly assigned into two groups: one included four animals (infected group), the other two were in control

S. Sivajothi (✉) · V. C. Rayulu
Department of Veterinary Parasitology, Sri Venkateswara
Veterinary University, Proddatur, India
e-mail: sivajothi579@gmail.com

B. Sudhakara Reddy
College of Veterinary Science, TVCC, Proddatur, India

group. Each animal was infected with 5×10^5 trypanosomes subcutaneously. The animals were examined daily for development of clinical signs and the infection status was confirmed by the examination of wet blood-films made from the ear veins.

Blood collected in 10 % EDTA solution from all the rabbits was used for the estimation of total erythrocyte count (TEC), total leukocyte count (TLC) and packed cell volume (PCV) by microhematocrit method (Jain 1986) and Hemoglobin (Hb) by Sahli's method (Coles 1986). The blood smears were directly prepared and stained by Leishman's stain for differential leukocyte count (DLC) by Battlement method (Jain 1986) and for description of morphological changes in the blood cells. Blood was collected from all rabbits into sterile test tube and allowed to clot. The serum was collected and stored at 4 °C and was used for the estimation of total serum proteins (Transia biochemical Ltd), glucose (M/S Excel Diagnostics Ltd), bilirubin (M/S Excel Diagnostics Ltd), BUN (M/S Excel Diagnostics Ltd), AST (M/S Excel Diagnostics Ltd), ALT (M/S Excel Diagnostics Ltd) and creatinine (Kamineni life Sciences Pvt Ltd) using kits.

Results

During early stage of infection, *T. evansi* infected rabbits 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, recumbence and fluctuating pyrexia were noticed during third month of infection. Some animals were also showed the signs of corneal opacity and blindness, and these signs remained until the end of the experiment. Severe anaemia was the main haematological

finding. The mean TEC, PCV, Hb and TLC values reduced significantly. In the acute phase, leukocytosis due to lymphocytosis followed by leukopenia during the chronic phase was recognized. Macrocytes, hypochromic cells, Howell-Jolly bodies, target cells, stomatocytes and burr cells are the main abnormalities observed in the erythrocytes. In addition to these changes anisocytosis, poikilocytosis and hypochromic RBC were also observed. 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. Mean haematological and biochemical values in rabbits of experimental groups were given in Tables 1 and 2.

Discussion

The clinical signs observed in rabbits experimentally infected with *T. evansi* in the present study were in agreement with the reports of Chandra et al. (1999), Audu et al. (1999), Dargantes et al. (2005), Taiwo et al. (2003). Decreased values of TEC, Hb and PCV in the study might be due to increased susceptibility of red blood cell membrane to oxidative damage (Taiwo et al. 2003). Similar observations were made by Audu et al. (1999), Herrera et al. (2002), Hilali et al. (2006). In the present investigation, anaemia is the most consistent finding which is in accordance with Taiwo et al. (2003), Takeet and Fagbemi (2009). Morphological changes of erythrocytes observed in the study might be due to involvement of liver and the presence of burr cells was an indicator of renal failure (Abdul-majeed et al. 2007). Similar morphological changes were reported by Abdul-majeed et al. (2007), who observed the presence of anisocytosis, poikilocytosis, target cells,

Table 1 Hematological values in the experimentally *T. evansi* infected and control group of rabbits

Parameters	Control group of rabbits	Infected group of rabbits		<i>t</i> test	<i>P</i> value
		30 days DPI	60 days DPI		
TEC ($10^6/\text{mm}^3$)	3.95 ± 0.05	2.3 ± 0.16	1.2 ± 0.06	24.7**	0.000
Hb (g/dl)	9.8 ± 0.10	5.25 ± 0.42	3.65 ± 0.09	39.3***	0.000
PCV (%)	37.0 ± 1.00	28 ± 0.76	19.50 ± 0.9	11.2***	0.000
TLC ($10^9/\text{l}$)	10.50 ± 0.5	13.5 ± 1.2	17.0 ± 0.5	7.08**	0.002
DLC					
Basophils (%)	0	0	0		
Eosinophils (%)	0	0	0		
Lymphocytes (%)	61 ± 01	78 ± 1.59	90 ± 0.40	33.5***	0.000
Monocytes (%)	0	1	0		
Neutrophils (%)	40.5 ± 0.5	21.17 ± 1.84	10.25 ± 0.47	38.7***	0.000

NS non significant ($P > 0.05$)

* Significant ($P < 0.05$); ** highly significant ($P < 0.01$)

Table 2 Biochemical values in the experimentally *T. evansi* infected and control group of rabbits

Parameters	Control group	Infected group		<i>t</i> test	<i>P</i> value
		20 days DPI	60 days DPI		
TSP (mg/dl)	5.35 ± 0.05	2.32 ± 0.16	1.48 ± 0.1	24.7***	0.000
Cholesterol (mg/dl)	163.5 ± 1.5	183 ± 20.9	249 ± 5.5	10.2***	0.005
Serum glucose (mg/dl)	78 ± 2.0	70.3 ± 1.35	52.75 ± 1.1	12.2***	0.000
Bilirubin (µg/dl)	0.75 ± 0.02	0.89 ± 0.9	1.40 ± 0.14	3.0*	0.036
Creatinine (mg/l)	2.1 ± 0.1	10.6 ± 0.34	16.5 ± 0.25	37.1***	0.000
BUN (mg/dL)	14.05 ± 0.4	17.5 ± 1.1	21.1 ± 1.3	3.3*	0.027
ALT (µl)	47.8 ± 0.2	50.25 ± 1.9	63.38 ± 1.8	5.7**	0.004
AST (µl)	22.5 ± 0.5	48 ± 1.59	71.7 ± 1.75	18.6***	0.000

NS non significant ($P > 0.05$)

* Significant ($P < 0.05$); ** highly significant ($P < 0.01$)

macrocytes, Howell-Jolly bodies, Burr cells and stomatocytes in rabbits experimentally infected with *T. evansi* Iraqi strain. The studies of Silva et al. (1999) and Ogunsanmi et al. (1994) supported our results and denoted that the presence of macrocytic hypochromic cells in the acute stage and normocytic hypochromic cells in chronic stage. Emeribe and Anosa (1991) stated that the macrocytic cells shifted terminally to microcytic hypochromic cells, with evidence of a moderate anisocytosis and poikilocytosis of erythrocytes.

In the acute phase, leukocytosis due to lymphocytosis followed by leukopenia during the chronic phase was recognized. These results were in consonance with the experiments of Emeribe and Anosa (1991), Onah et al. (1996), Silva et al. (1999), Hilali et al. (2006). Another experiment on goats opposed the mentioned results and stated that leukocytosis 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 leukocytes and neutrophils. In other experimental infection of Norwegian lemmings with *T. lemmi*, the leukocyte counts remained the same (Wiqr 1978). The decrease in total serum protein level observed in this study is consistent with the findings of Sadique et al. (2001) who reported decreased in total protein in cattle infected with *T. congolense*. The results contradicts the observations made in sheep infected with *T. brucei* by Taiwo et al. (2003), who observed no change in the levels of total plasma proteins from pre-infected values at the initial stage of the infection, but in the later stage the levels increased significantly above pre-infection levels. 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 is as a result of the immune response by the animals to the *T. evansi* infection (Lushaikyaa et al. 2011).

In the present study, increase in cholesterol levels agrees with the finding of Diehl and Risby (1974), Abenga and Anosa (2007) who also reported increase in cholesterol levels in *T. brucei gambiense* infected rabbits and vervet monkey, respectively. In contrast, decreased cholesterol levels were reported by Robert and Clarkson (1977), Adamu et al. (2008). Hypercholesterolemia has been associated with hepatic malfunction resulting from impairment of liver lipid metabolism (Adejinmi and Akinboade 2000). The result of hypoglycemia is in consonance with the findings of Takeet and Fagbemi (2009), Taiwo et al. (2003). Hypoglycaemia has been reported in natural trypanosomosis in human and animals (Moon et al. 1968; Welde et al. 1974). Excessive utilization of the blood glucose by trypanosomes for their metabolism has been thought to account for the hypoglycaemia observed during trypanosomosis (Anosa 1988).

The increased levels of total plasma bilirubin in the present experiment support the earlier observations of Gow et al. (2007) who observed in dogs naturally infected with *T. congolense* had elevated level of bilirubin. The increase in bilirubin in rabbits is suggestive of haemolytic anaemia due to *T. congolense* (Takeet and Fagbemi 2009) or obstructive jaundice as previously reported in *T. brucei* infected rabbits (Arowolo et al. 1988). 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 is in consonance with the observation reported by Adejinmi and Akinboade (2000), Abenga et al. (2002), Abenga and Anosa (2007). The elevated levels of ALT and AST enzymes are in agreement with earlier reports of Taiwo et al. (2003), Adah et al. (1992), Omotainse et al. (1994) in various trypanosome-infected animals. The elevated values of ALT and AST enzymes may be due to tissue breakdown (necrosis)

Fig. 1 Blood film (a) macrocyte, (b) hypochromic RBC, (c) burr cell, (d) stomatocyte

Fig. 2 Blood film (a) Howell-Jolly bodies

Fig. 3 Blood film (a) target cell

and inflammation in the host, particularly of the liver, heart, muscle and kidney (Taiwo et al. 2003; Takeet and Fagbemi 2009). Another possibility of increased levels of ALT and AST enzymes is the lysed trypanosomes (Taiwo et al. 2003; Takeet and Fagbemi 2009) at different stages of the infection (Figs. 1, 2, 3).

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