



## Histopathological Observations in Rabbits Experimentally Infected with *Trypanosoma evansi*

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### Abstract

New Zealand white rabbits (n=6) were challenged with the South Indian local strain of *Trypanosoma evansi*. Each animal was infected with  $5 \times 10^5$  trypanosomes subcutaneously. Animals were daily examined for the development of clinical signs using wet blood-films sampled from the ear veins. Clinically, intermittent pyrexia, undulating parasitaemia, anorexia and emaciation were predominant. Three months post infection, rabbits were sacrificed, detailed postmortem examination was carried out and representative tissue samples were fixed. Gross pathological changes including paleness of visceral organs, gelatinization of fat, congested and oedematous lungs, mucoid enteritis, hepatomegaly and splenomegaly were noticed. Histopathologically, internal organs elucidated clear changes consisted of severe hepatic fatty change, pulmonary congestion with thickened interstitial spaces and emphysema, degeneration of the renal epithelium associated with cystic tubular formation and congested red pulp.

**Keywords:** Rabbits; Histopathology; *Trypanosoma evansi*

### Introduction

*Trypanosoma evansi*, a unicellular haemoflagellate, is known to cause trypanosomosis in domestic and wild ruminants and carnivores (Da Silva *et al.*, 2012). The incidence and severity of the disease vary according to the strain virulence and the host species (de Menezes *et al.*, 2004). In Indian sub-continent, the disease is a constant threat to productivity (Laha and Sasmal, 2007). Although pathology of the disease by different species of trypanosomes in domestic and laboratory animals has been well known (Abdul-Majeed *et al.*, 2007), yet the information on the visceral organs in rabbits as a result of the infection caused by the South Indian isolate of *T. evansi* is very meager. Hence, the present investigation was intended to study pathology of the visceral organs in rabbits infected with the South Indian (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 (Takeet and Fagbemi, 2009). The blood of infected rats was collected by tail clipping and daily examined beginning from the second day post inoculation (PI). Infected rats showing a high parasitaemia were anaesthetized with thiopental sodium, and blood was collected directly from the heart into heparinized tubes. Six New Zealand White rabbits were maintained in fly proof house with under hygienic conditions. Rabbits were given sterilized water and feed ad libitum. Each animal was subcutaneously inoculated (Tewari *et al.*, 2009) with a dose of  $5 \times 10^5$  trypomastigotes. Animals were daily examined for the development of clinical signs using wet blood-films sampled from the ear veins. Clinically, intermittent pyrexia, undulating parasitaemia, anorexia and emaciation were predominant. Three months post infection,

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rabbits were sacrificed, detailed postmortem examination was carried out and representative tissue samples were fixed in 10% neutral-buffered formalin for histopathology (Tewari *et al.*, 2009). Specimens were routinely processed by embedding in paraffin, sectioned at 4 µm thickness and stained with haematoxylin and eosin.

Ethical permission was taken by the Institutional Animal Ethics Committee (IAEC) of the S.V.V.U.

## Results

Examination of wet blood films (WBF) revealed

the presence of motile trypanosomes after 30 days PI. During the early stage of infection, *T. evansi*-infected rabbits showed clinical signs consisted of varying degrees of emaciation, dehydration, mucopurulent oculonasal discharges and pasted perineum. In chronic infection, clinical signs included roughened hair coat, more dulled appearance, recumbence and fluctuating pyrexia. Moreover, two animals also showed signs of corneal opacity and blindness, and remained until the end of the experiment. Gross pathological changes including paleness of visceral organs, gelatinization of fat, congested and oedematous lungs, mucoid enteritis, hepatomegaly and splenomegaly were noticed. Mi-

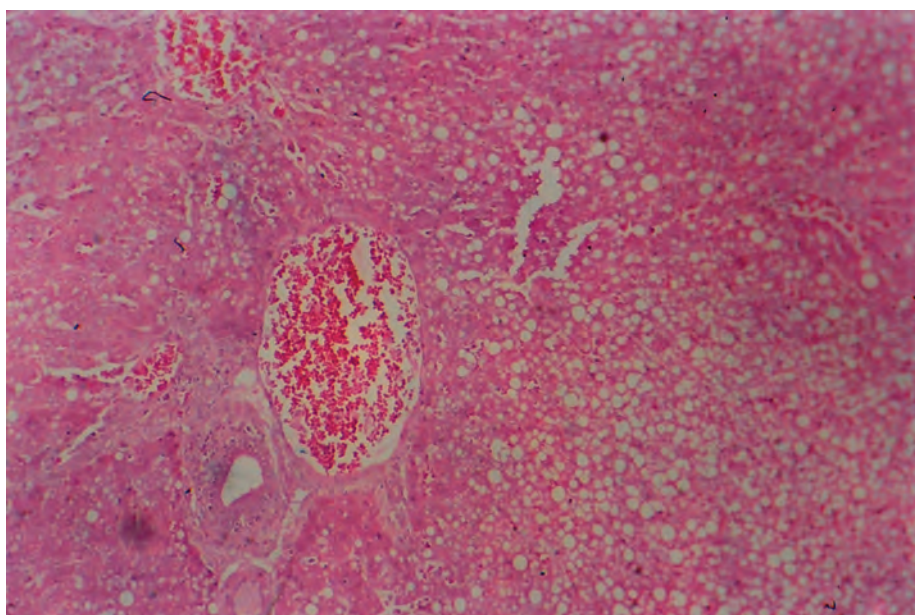


Fig.1. Liver of *T. evansi* infected rabbit.

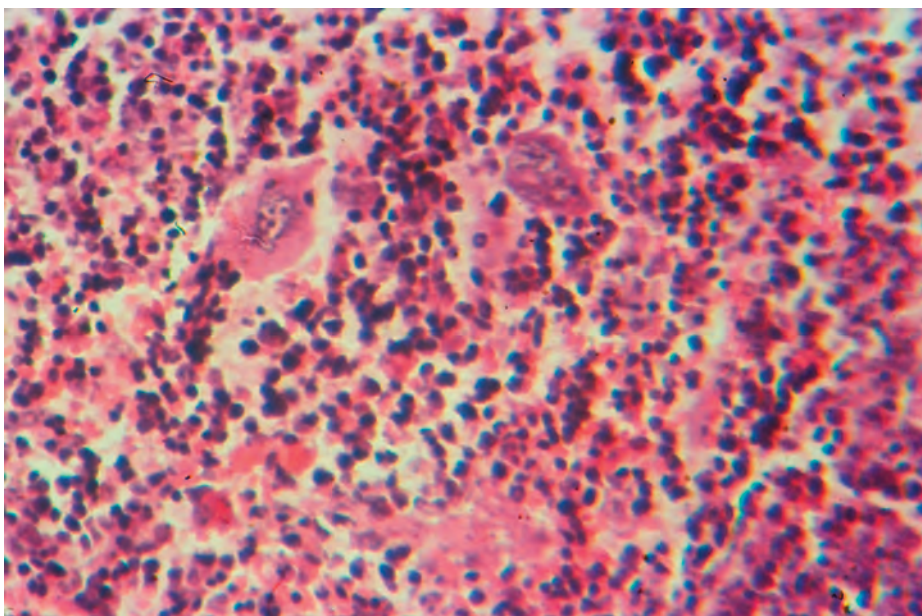


Fig..2: Spleen of *T. evansi* infected rabbit.



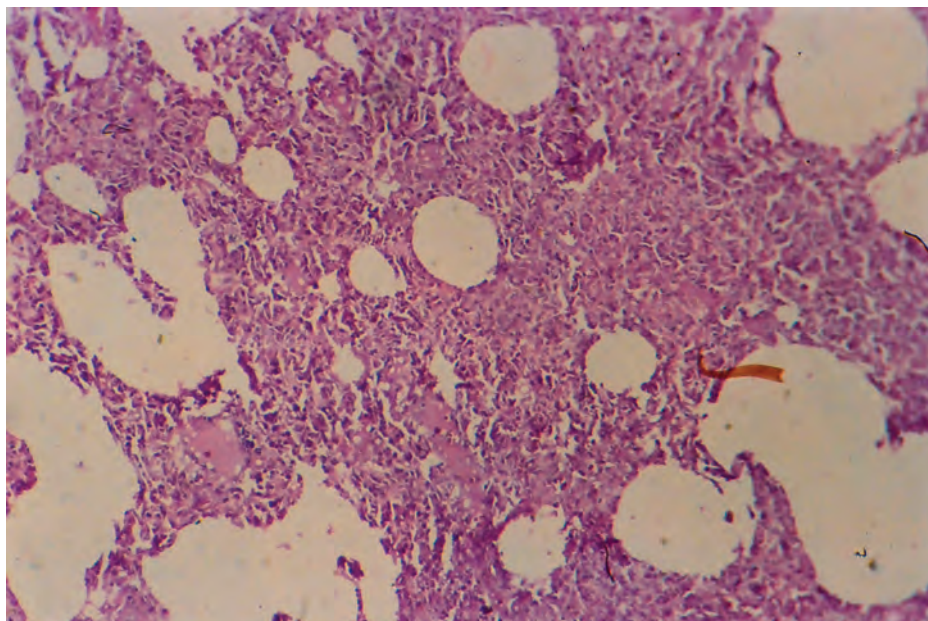


Fig. 3. Lung of *T. evansi* infected rabbit.

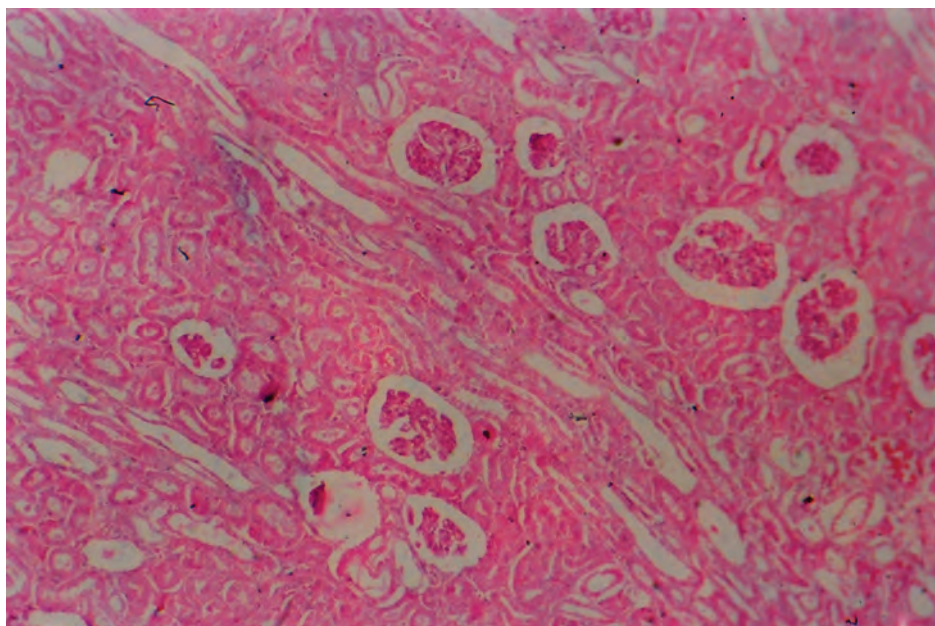


Fig.4. Kidney of *T. evansi* infected rabbit

croscopically, liver revealed severe fatty change, focal areas of dilated and congested sinusoids, congested blood vessels and periportal infiltration of mononuclear cells (Fig.1). Mild depletion of cells in the white pulp and engorgement of the red pulp with RBC were noticed in spleen (Fig.2). Lung revealed congestion, oedema, emphysema, thickened interstitial space with mononuclear cell infiltration, thickened blood vessels, desquamated epithelial cells, (Fig.3). Intertubular haemorrhages in both cortex and medulla, atrophied and cystic glomerulus, intertubular edema, coagulative necrosis of

tubule epithelial cells, desquamated tubular epithelial cells and cystic tubule formation were observed in kidneys (Fig.4).

## Discussion

*Trypanosoma evansi* is highly pathogenic to laboratory animals (mice, rats and rabbits) (Biswas *et al.*, 2001). The parasite utilizes glucose and oxygen for its growth and multiplication resulting in depletion of these metabolites leading to degenerative changes in the host. Further developmental changes

are either due to toxins released by the parasite or to immunological reactions (Bal *et al.*, 2012).

Clinical signs with associated gross lesions revealed from the current investigation coincided with those reported by Brown *et al.* (1977); Damayanti *et al.* (1994); Audu *et al.* (1999); Chandra *et al.* (1999); Taiwo *et al.* (2003) and Dargantes *et al.* (2005) who observed anaemic mucous membranes, depression, weakness, refusal to walk, loss of appetite and emaciation associated with serous atrophy of fat, hydropericardium, petechial to larger haemorrhages in the pericardium, pneumonia, congested and enlarged liver and spleen in *T. evansi*-infected Indonesian buffalo, *T. brucei* and *T. congolense*-infected albino rats, *T. evansi*-infected Yankasa sheep, experimental *T. evansi*-infected rabbits, *T. congolense* and *T. brucei*-infected sheep and *T. evansi*-infected goat, respectively. Enlargement of spleen might be due to increased activity of phagocytic system in elimination of trypanosomes.

Microscopically, the liver revealed severe fatty change, focal areas of dilated and congested sinusoids, congested blood vessels and periportal infiltration of mononuclear cells. Fatty changes in liver, manifested by lipid accumulation inside hepatocytes due to tissue hypoxia resulted from the present anemia and vascular damage Derakhshanfar *et al.* (2010). Fatty degeneration followed by necrosis of hepatocytes was considered the common cytological findings associated with *T. evansi* infection as a result of nutritional impairment and the asphyxia (Uche and Jones, 1992). Histopathological changes observed in liver were in consonance with Abdul-Majeed *et al.* (2007). Histopathological changes such as necrosis and haemorrhages within the sinusoids of the liver fatty degeneration in hepatic cells of the bandicoat rat infected with *T. evansi* were detected by Biswas *et al.* (2001). Hepatomegaly observed in the present study was in agreement with Brown *et al.* (1977) and Dargantes *et al.* (2005). Damayanti *et al.* (1994) revealed congestion in the liver following necropsy in goat and buffalo infected with *T. evansi*. Congestion and necrosis observed in the present study were also in went parallel findings of Brown *et al.* (1977) and Taiwo *et al.* (2003).

Mild depletion of cells in the white pulp and engorgement of the red pulp with RBC were noticed in spleen. Among the lymphatic tissues, spleen is the most important organ that serves as a first line

of defense mechanism. Formation of granulomatous lesions due to aggregation of histocytes may be considered the initial splenic response to *T. evansi* challenge (Uche and Jones, 1992).

Lung revealed congestion, oedema, emphysema, thickened interstitial space with mononuclear cell infiltration, thickened blood vessels, desquamated epithelial cells with a period.

Histopathological changes in lungs supported the findings of Chandra *et al.* (1999) who observed emphysema and compensatory atelectasis in *T. evansi*-infected rabbits. Ngeranwa *et al.* (1993) observed marked cellular infiltration in lungs of small African goats.

Intertubular haemorrhages in cortex and medulla, atrophied and cystic glomerulus, intertubular edema, coagulative necrosis of tubule epithelial cells, desquamated tubular epithelial cells and cystic tubule formation were observed in kidneys. Toxins liberated by the parasites are likely to impair the function of the kidney. In such cases, the renal cast formation as well as granulomatous lesions indicated non-functional kidney especially at the late stage of *T. evansi* infection (Uche and Jones, 1992). Pulmonary congestion and chronic interstitial nephritis developed by infected animals may be due to immune complex deposition and complement cascade reaction (Tizard, 1998). Histopathological changes observed in spleen, heart, lungs, liver and kidneys in the present study are similar to those described by Chandra *et al.* (1999) and Takeet and Fagbemi (2009).

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