

EXPERIMENTAL *Trypanosoma Evansi* INFECTION IN ALBINO MICE – A HISTOPATHOLOGICAL STUDY

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ABSTRACT. Histopathological changes were studied in Swiss albino mice (N:36) which were challenged with the South Indian local strain of *Trypanosoma evansi*. Each animal was infected with 5×10^5 trypanosomes intraperitoneally. The animals were examined daily for development of clinical signs and infection status by wet blood-films made from the tail veins. The infected mice were dull and depressed from two days post-infection (DPI) onwards. Systematic post-mortem examination of the infected mice was performed and pathological changes were recorded. The different tissue samples were collected in 10% formalin and were used to study the histopathological changes. Post-mortem examination from 3-4 DPI (the maximum period of observation) revealed splenomegaly, hepatomegaly, marked congestion of lungs, presence of fluid in peritoneal cavity. Histopathologically, heart muscles showed hyaline degenerative changes and haemorrhages. Liver parenchyma revealed congestion of central vein and sinusoids, binucleated hepatocytes and fatty change of hepatic cells. Thickening of interstitial space with mononuclear infiltration, areas of collapse, areas of emphysema, edema and dilated

and congested blood vessels were the histopathological changes noticed in the lungs of the infected mice. In the spleen, giant cells aggregation, hyperplasia, thickening of capsule and trabecule were the changes indicating irreversible degeneration. The affected kidney showed inter-tubular hemorrhages in the cortex, medullary hemorrhages, congested glomerulus, atrophied glomerulus, desquamated tubular epithelium and disruption of renal tubules at some places.

Keywords: histopathology, mice, *Trypanosoma evansi*

INTRODUCTION

Out of all the pathogenic trypanosomes, *Trypanosoma evansi* has the widest host range and worldwide geographical distribution. *T. evansi* is also highly pathogenic to laboratory animals (rat, mice and rabbit) (Sivajothi *et al.* 2013a). 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 changes develop in the organs either due to cellular damage caused by toxicants released by the parasite, or

due to immunological reactions. Though *T. evansi* is a haemoprotozoa, visceral forms have been reported in heart, optic lobes, cerebrum, liver, kidney and lungs (Bal *et al.*, 2012). Haematological abnormalities and different prevalence status with different diagnostic tests was recorded previously in the Andhra Pradesh (Sivajothi *et al.*, 2012 and Sivajothi *et al.*, 2014a). Pathology of the disease by different species of trypanosomes in laboratory animals has been documented (Virmani *et al.*, 2004 and Sivajothi *et al.*, 2013b), yet the information on pathology of the visceral organs in mice induced by south Indian isolate of *T. evansi* is very little. Hence, the present investigation was intended to study pathology of the visceral organs in mice induced by 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 albino mice through serial passages. Thirty-six albino mice were maintained in a fly-proof house with clean and hygienic environment. Mice were kept on *ad libitum* feed and filtered water was provided to these animals throughout the experimental study. Thirty-six mice were infected with 5×10^5 trypanosomes intraperitoneally. Twelve mice were kept as a control group. The blood of infected mice was collected by tail clipping and examined daily from the second day of

post-inoculation. After four (maximum) days of infection, mice were sacrificed and detailed post-mortem examination was carried out and representative tissue samples from liver, lungs, heart, kidneys and spleen were collected and preserved in 10% formalin for histopathological examination. These tissues were processed by routine conventional methods and sections were cut at 4 μ thickness and stained with haematoxylin and eosin stain (Sivajothi *et al.*, 2014b).

RESULTS

The infected mice were dull and depressed from two DPI onwards. Examination of wet blood films (WBF) revealed the presence of motile trypanosomes after two DPI. Post-mortem examination from 3-4 DPI (the maximum period of observation) revealed splenomegaly, hepatomegaly (Figure 1), marked congestion of lungs, presence of fluid in peritoneal cavity. Histopathologically, heart muscles showed hyaline degenerative changes and haemorrhages (Figure 2). Liver parenchyma revealed congestion of central vein and sinusoids, binucleated hepatocytes and fatty change of hepatic cells (Figure 3). Thickening of interstitial space with mononuclear infiltration, areas of collapse, areas of emphysema, edema and dilated and congested blood vessels were the histopathological changes noticed in the lungs of the infected mice (Figure 4). The affected kidney showed inter-tubular hemorrhages in the cortex, medullary



Figure 1. Post-mortem examination from 3-4 DPI (the maximum period of observation) revealed splenomegaly and hepatomegaly.

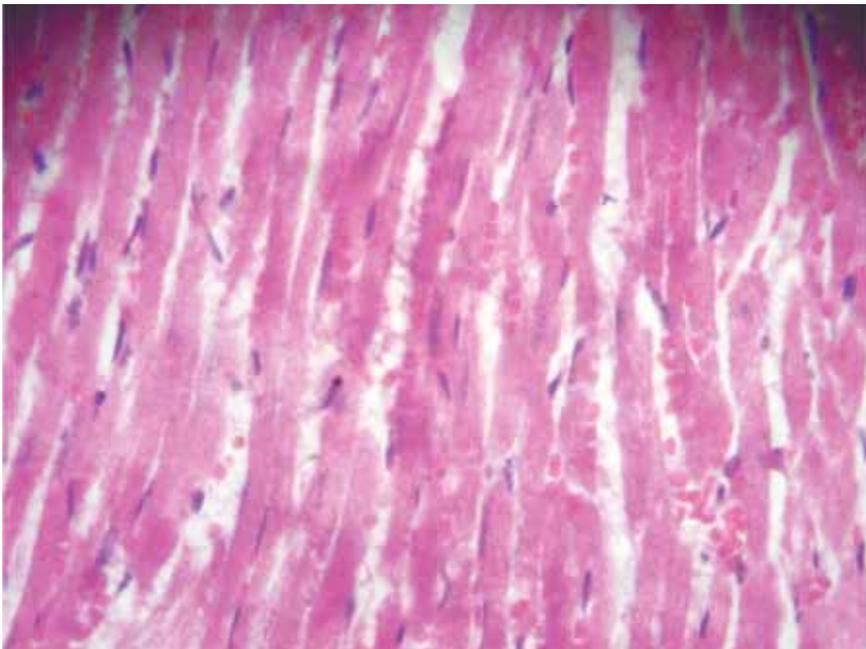


Figure 2. Heart muscles showing hyaline degenerative changes and haemorrhages.

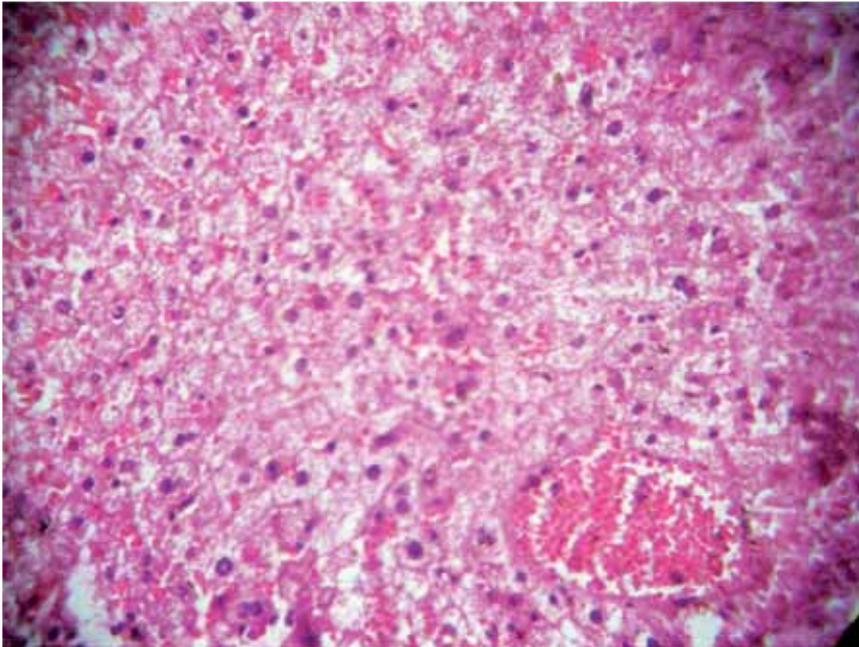


Figure 3. Liver parenchyma showing congestion of central vein and sinusoids, binucleated hepatocytes and fatty change of hepatic cells.

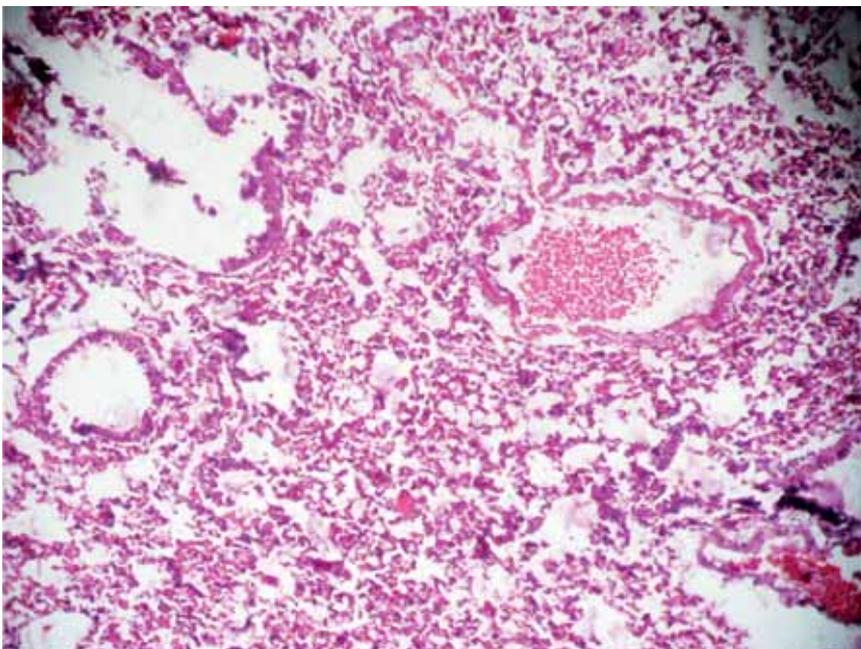


Figure 4. Lungs showing thickening of interstitial space with mononuclear infiltration, areas of collapse, areas of emphysema, edema and dilated and congested blood vessels.

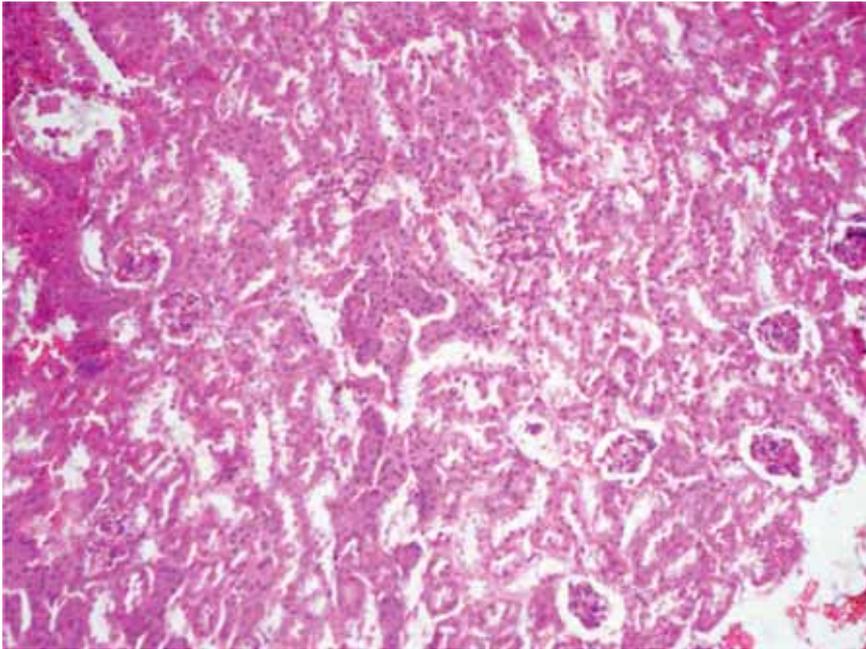


Figure 5. Kidney showed inter-tubular hemorrhages in the cortex, medullary hemorrhages, congested glomerulus, atrophied glomerulus, desquamated tubular epithelium and disruption of renal tubules at some places

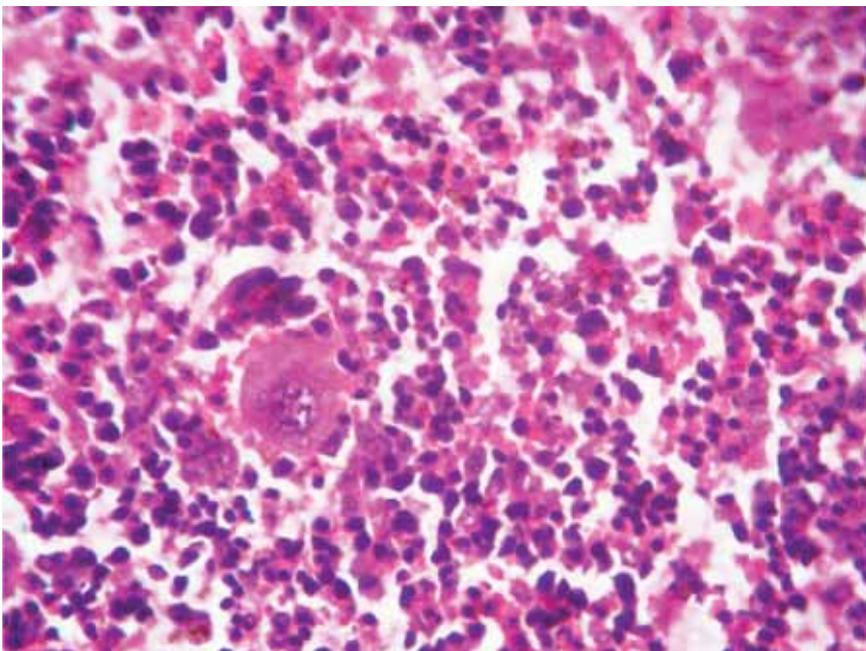


Figure 6. The spleen showing giant cells aggregation, hyperplasia, thickening of capsule and trabecule.

hemorrhages, congested glomerulus, atrophied glomerulus, desquamated tubular epithelium and disruption of renal tubules at some places (Figure 5). In the spleen, giant cells aggregation, hyperplasia, thickening of capsule and trabecule were the changes indicate irreversible degeneration (Figure 6).

DISCUSSION

The clinical signs and gross lesions in the present study were gained the support from Virmani *et al.*, (2004). They observed clinical signs were dull and depressed mice from two DPI till four DPI. Splenomegaly, marked congestion of lungs, petechiae on the serous surfaces and liver were the gross lesions observed. According to Tizard (1998), diseases caused by trypanosomes induce the formation of high levels of systemic antigen antibody immune complexes, and their consequent deposition in the heart, liver, brain and kidneys may possibly play a role in tissue damage. However, some reports indicated that trypanosomes can cause tissue inflammation directly as a result of the infection (Damayanti, 1993). Enlargement of spleen might be due to increased activity of mononuclear phagocytic system resulting in destruction of trypanosomal antigen coated RBCs. The RBCs destruction was corroborated by hemosiderosis of the spleen. Splenomegaly followed by hyperplasia and hypersplenism are very much pronounced as the disease progressed (Singla *et al.*, 2001).

Histopathologically, heart muscles showed hyaline degenerative changes and haemorrhages. The parasite utilizes glucose and oxygen for its growth and multiplication resulting in depletion of these metabolites leading to changes in the host. Further developmental changes are either due to toxins released by the parasite or to immunological reactions (Bal *et al.*, 2012). The degenerative changes in heart may be due to anemia and hypoglycemia.

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 such as necrosis and haemorrhages within the sinusoids of the liver with fatty degeneration in hepatic cells of the bandicoot rat infected with *T. evansi* were also noticed by Biswas *et al.* (2001). Suryanarayana *et al.*, 1986 observed congestion, haemorrhages and fatty degeneration of hepatocytes and concluded that it may be due to hypoglycemia leading to starvation of the cells and anoxia due to anaemia in *T. evansi* infected animals. Damayanti *et al.*, 1994 noticed congestion

in the liver following necropsy in goat and buffalo infected with *T. evansi*. Congestion observed in the present study was also in agreement with findings of Virmani *et al.* (2004).

Thickening of interstitial space with mononuclear infiltration, areas of collapse, areas of emphysema, oedema and dilated and congested blood vessels were the histopathological changes noticed in the lungs of the infected mice. Congestion and edema of lungs were mainly due to inflammatory response of the lungs to the parasite resulting in vasodilatation and exudation. Histopathological changes in lungs supported the results of Virmani *et al.*, 2004 who observed alveolar oedema, congestion of alveolar blood vessels and massive areas of haemorrhages along with focal areas of infiltration with polymorphs. Ngeranwa *et al.* (1993) observed marked cellular infiltration in lungs of small African goats.

The affected kidney showed inter-tubular hemorrhages in the cortex, medullary hemorrhages, congested glomerulus, atrophied glomerulus, desquamated tubular epithelium and disruption of renal tubules at some places. Pulmonary congestion and chronic interstitial nephritis developed by infected mice may be due to immune complex deposition and complement cascade reaction (Tizard, 1998). In the spleen, giant cells aggregation, hyperplasia, thickening of capsule and trabecule were the changes indicative of an immunological response by the infected mice. Virmani *et al.*, 2004

observed that the spleen was haemorrhagic with pronounced haemosiderosis and RE cell proliferation. It has been reported that changes in kidneys are mainly due to toxins produced the parasite and accumulation of immune complexes which impair the structure and function of the kidney (Ngeranwa *et al.*, 1993).

Initial changes in the spleen may be due to immediate hypersensitivity to *T. evansi*. Biswas *et al.* (2001) noticed the haemorrhages, congestion, absence of germinal centres, hemosiderosis, increase in follicular cells, focal necrosis and formation of giant cells due to aggregation of histiocytes during the progression of the disease. Suryanarayana *et al.* (1986) observed that the stimulation given by the presence of *T. evansi* or their toxic metabolites result in varying degrees of anaemic anoxia, which may induce splenic damage. Reduced proliferative activity followed by increase in the number of macrophages and multinucleated giant cells with severe disruption in the splenic architecture.

It was evident in this study that *T. evansi* was able to invade all the visceral organs examined. Previous work had also shown that *T. evansi* was capable of invading these organs. Similar observation was also been made by Lawal *et al.* (2007) in experimentally *T. brucei* infected rats. The invasive ability of *T. evansi* therefore, seems to be in line with that of the *brucei* group, to which *T. evansi* belongs (Losos, 1980). However, despite the fact that the organs enhance multiplication

of the parasites, they certainly provide the mechanism for destroying them as evidenced by the presence of numerous degenerated forms. The mononuclear phagocytic system might be responsible for the attack on the parasites. *T. evansi* is highly pathogenic to laboratory animals as observed in the previous studies also (Sivajothi *et al.*, 2014b). Consumptions of oxygen by trypanosomes for their multiplication lead to hypoxic state as a result of which animal tissues are deprived of oxygen and it results in degenerative changes in all the vital organs.

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