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Pathological studies on experimental infection of buffalo isolate of *Trypanosoma evansi* infection in Swiss albino mice

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Abstract
The study was conducted to evaluate the pathological changes of *T. evansi* infection in Swiss albino mice inoculated with buffalo isolate. Fifteen Swiss albino mice were used in present experiment divided in two groups consisting of six mice in each group. Group I is considered as a negative (control) group which did not receive any infection. Group II is considered as positive group were inoculated with 1×10^4 trypanosomes. Systematic necropsy examination of Group I healthy control mice and Group II infected mice was conducted and histopathological changes were recorded. A part of tissue sample was collected from each visceral organ in 10% NBF for further studies. Gross post mortem examination showed enlargement of spleen and liver. Histopathological tissue section showed presence of clump of *T. evansi* organisms in brain, lungs, heart, liver, spleen and kidneys. Microscopic examination of brain showed congestion of blood vessels containing trypanosomes. Lung showed congestion haemorrhage, emphysema and broncho-interstitial pneumonia. Heart revealed haemorrhage, congestion and granular appearance of cytoplasm with presence of trypanosome organisms. Liver section showed congestion of blood vessels, hepatocytes were swollen with diffused vacuolar degenerative changes. Spleen revealed haemorrhage in the white pulp with presence of megakaryocytes. Kidney revealed cortico medullary haemorrhage with varying degree of congestion with presence of large number of trypanosome organisms in between the red blood cells.

Keywords: *T. evansi*, Mice. parasitemia, megakaryocytes

Introduction
Trypanosoma evansi is a unicellular, flagellated hemoparasitic protozoan disease that causes surra in domestic and wild animals. The clinical signs are characterised by anaemia, emaciation, immunosuppression, corneal opacity, nervous symptoms and paralysis, which lead to a mortality rate of up to 70% (Da Silva *et al.* 2019) [3]. The disease is endemic in India and has an economic impact on livestock production, especially cattle. The highest prevalence of 42.2% was recorded in West Bengal. In Karnataka, cattle showed a high prevalence of 34.8% followed by buffalo, horses and donkey with 13.0, 2.5 and 5.2%, respectively. Whereas the prevalence of surra in camels in Rajasthan was 19.9% (Sengupta *et al.* 2016) [11]. The disease transmitted by blood sucking hematophagous flies such as tabanids and *Stomoxys* also by vampire bats, iatrogenic and pre-oral. It is an emerging zoonotic disease (Rani *et al.* 2022) [7] and notifiable disease by the World Organization for Animal Health from 2020.
The diagnosis by routine blood smear examination is challenging due to fluctuating Parasitemia and the limitation of the immunological tests for trypanosomosis, the diagnosis is found much promising by molecular assays which can detected 0.02 trypanosomes per ml (Rudramurthy *et al*. 2013) [8]. The disease is controlled by trypanocidal drugs, *viz.*, suramin, melarsomine hydrochloride, phenanthridinium salts, diminazene aceturate and quinapyramine salts. The efficacy of antitrypanosomal drugs and the pathogenicity of surra depend on the virulence of *T. evansi*, isolate type. Hence, the objective of the present study was to evaluate the pathological changes caused by the buffalo strain of *T. evansi* in experimentally inoculated Swiss albino mice.

Materials and Methods
Source of the *Trypanosoma evansi* isolate

The cryopreserved buffalo isolate of *T. evansi*, procured from Parasitology laboratory, National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru, India.

Experimental infection in mice

After the approval by the Committee for the Purpose of Control and Supervision of Experiments on Animals, fifteen healthy Swiss albino male mice weighing 28 to 30 grams were procured from authorised vendors and maintained with ab libitum food and water.

Revived *T. evansi* organisms

The revived *T. evansi* organisms were inoculated to three healthy mice. After attaining the peak Parasitemia on 7th day of post infection the blood was collected by tail bleeding in 1ml Alsever's solution. The inoculum dose of 1 x 10^4 was calculated by hemocytometer.

Experimental groups

The experimental study consists of two groups with six mice in each. Group I mice served as negative control, which did not receive inoculum dose of *T. evansi*. Group II mice were inoculated with 1× 10^4 organisms intraperitoneally (IP) and Parasitemia level was checked at 24 hours of interval by tail bleed.

Detection of Parasitemia level

The thin, methanol-fixed blood smear was prepared and stained by Giemsa at a 2:8 ratio and examined under higher magnification (100X). The Parasitemia was graded as "0" for no organisms, "+" for 1 to 2 trypanosomes per microscopic field, "+ + "for 2 to 4 parasites, "+ + + " for 4 to 6 trypanosomes per microscopic field, "+ + + + " for 8 to 10 organisms per field, and "M" for more than 10 organisms per field, indicating massive infection.

Necropsy studies

The organs such as brain, lung, heart, liver, spleen and kidney of mice that died due to infection were examined and stored in 10.0% neutral buffered formalin (NBF). After sacrificing mice with is of lurane, tissue samples from Group I were collected for comparison.

Histopathological studies

The tissue samples of five micron were examined for histopathological changes as per standard procedure (Suvarna *et al.* 2013) [13].

Results and discussion

The Group II which received 1 x 10 4 inoculum of *T. evansi*, showed '0' degree of Parasitemia at 24 hrs post infection, '+ +' degree of Parasitemia at 48 hrs post infection, '+ + +' degree of parasites at 72 hrs post infection, '+ + + +' degree of parasites at 96 hrs post infection and "massive" infection at 120 hr which led to death. In similar studies on mice, Sawitri and Damayanti (2021) [9] observed massive Parasitemia at 96 hours post infection with the Indonesia Bang 87 isolate of *T. evansi* and considered the isolate to be virulent, whereas the Pml 287 isolate showed fluctuating Parasitemia where peak Parasitemia was recoded on the 6th, 8th and 14th day of post infection, which sustained up to the 24th day and thereby concluded the isolate to be of low virulent. Similarly, *Bal et al*. (2012) [1] recorded peak Parasitemia on 6th day of post infection with cattle isolate.

The infected group showed clinical signs of dullness, shivering and huddling at one corner of the cage, convulsions, muscle tremors and grinding of teeth and death on the 5th day of post infection. Similar observations were recorded by Bal *et al.* (2012) [1] in mice inoculated with cattle isolate. According to Taylor *et al*. (1999) [14], clinical signs are caused by TNF-alpha, which involved in Parasitemia and pathology such as anaemia, CNS damage, fever, and emaciation. Utilisation of tryptophan by trypanosomes in the brain has been related to functional disturbances of the CNS and is due to the production of phenyl pyruvate and indole-3-ethanol.

The gross examination of dead mice revealed severely enlarged spleen, congested and enlarged liver, slightly oedematous and congested brain, swollen and congested kidney (Fig a). Similarly, Sivajothi *et al.* (2015) [12], documented splenomegaly, hepatomegaly, congestion of the lung along with presence of peritoneal fluid in experimentally infected mice with a South Indian isolate of *T. evansi*. Ngeranwa *et al.* (1993) [6] recorded in *T. evansi* infected goats with dehydration emaciation and oedema of lower limbs, along with splenomegaly, hepatomegaly, flabby heart, oedematous brain and congested blood vessels. Whereas Sawitri and Damayanti (2021) [9], did not reveal gross lesions in mice inoculated with virulent Indonesian isolate and Garab *et al*. (2017) [4] did not observe gross lesions in the acute stage of *T. evansi* infection in donkeys. The absence of gross pathological changes in acute trypanosomosis, as suggested by Sawitri *et al.* (2016) [10] was due to the failure of the immune response, characterised by elevated proinflammatory cytokines. Whereas, Sivajothi *et al.* (2015) [12] revealed the cellular damage in *T. evansi* infection was due to the release of toxicants by the parasites or probably due to immunological reactions.

Histopathological lesions

The pathological changes in *T. evansi* infection are varies with host and isolate type (Sawitri *at al.* 2016) [10].

Brain

In the present study, the section of mice brain that died from *T. evansi* infection on 5th day of post inoculum showed a vascular appearance and congestion of blood vessels which contained innumerable organisms (Fig b & c) and succeeded in entering the CNS by crossing the blood brain barrier. Wolburg *et al*. (2012) [18] hypothesised that trypanosomes passed directly through the leptomeningeal vessel, which is more permeable to macromolecules than brain parenchymal vessels. The lack of astrocyte-derived parenchymal basement membrane in the meningeal vessels predicted to facilitate the passage of trypanosomes into the meninges and subarachnoid space.

According to Lonsdale and Grab (2002) [5], trypanosomal hydrolases such as proteases are potential mediators for cause of pathology in trypanosomosis and proteolytic enzymes such as trypanopains and oligopeptide B released by the organisms which are found on their surface are responsible for pathology. Since, the tryptopains and oligopeptide B degrade the immunoglobulins and peptide hormones, which distorts the intercellular protein junction, intercellular matrix and basement membrane. The presence of trypanosomes in the host environment results in the migration of defence cells to the infected area and generates inflammatory responses with the concomitant release of many cytokines and proteases, therefore, the interaction leads to the degradation of the

intercellular matrix, thereby the parasites penetrate through the brain tissues. Similarly, Bal *et al.* (2012) [1], observed congested blood vessels in the brain section of mice with swarming cattle isolate of *T. evansi.*

In contrast, the findings of Virmani *et al.* (2004) [17] who infected rats with camel isolates of *T. evansi*, also revealed congested blood vessels of the brain and perivascular cuffing of lymphocytes but did not find trypanosomes in the brain section, but demonstrated the organisms only in the liver and kidney, which suggested that the camel isolate has failed to cross the BBB. Whereas in another study on camel isolate by Ngernawa *et al.* (1993) in an experimental East African goat, the presence of trypanosomes was revealed in the brain section and also recorded oedema of the brain with enlarged blood vessels and micro thrombi in capillaries, which is in similar to the present observation.

Lung

The microscopic lesions of lungs showed severe congestion, haemorrhage, proliferation of Bronchial Associated Lymphoid Tissue (BALT), emphysema, pneumonia, thickening of alveolar wall with RBC and inflammatory cells, disruption of bronchial lining epithelium, mild degree of oedematous fluid in alveolar lumen, congested blood vessels showed presence of large number of trypanosomes (Fig d & e). Similarly, Sivajothi *et al.* (2014) [19] observed areas of collapse, emphysema, oedema and congested blood vessels in infected mice with cattle isolate and also similar lesions were observed by Virmani *et al.* (2004) [17] in inoculated rats with *T. evansi* from a heterologous host. Sawitri and Damayanti (2021) [9] documented lung lesions in the infected mice that were inoculated with Indonesian isolates of *T. evansi*. Similarly, Bal *et al.* (2012) [1] documented congestion, oedema, vasodilatation and exudation in the lung, which might be due to an inflammatory response by *T. evansi* infection in Swiss albino mice.

The presence of inflammatory cells has a minimal role in protecting the host from trypanosome infection because, within a short incubation period, the organisms multiplied exponentially to infinite numbers and occupied major volume of the plasma. There is possibility that the metabolites of trypanosomes would have caused disruption of the bronchial epithelium.

Heart

In heart sections revealed myocardial degeneration, oedema of cardiac myofibers, with granular appearance of cytoplasm. A mild degree of haemorrhage and congestion with presence of trypanosomes in between the RBC's were observed. Interspersed between the swollen myofibers, showed presence of few highly eosinophilic fibres with condensed to absence of nuclei (Fig f & g). The observation was similar to Ngeranwa *et al.* (1993) [6] where the changes in the heart revealed myocardial degeneration and intramuscular oedema in experimental inoculated goats. Where Virmani *et al.* (2004) [17] documented similar changes in infected albino rats and Sivajothi *et al.* (2014) [19] reported similar histopathological changes in the hearts of infected rats with cattle isolate.

Liver

The liver sections of mice revealed congestion of blood vessels and sinusoids showed with teeming number of trypanosomes in between the RBC's. The hepatocytes were swollen with diffused vacuolar degenerative changes due to anoxia and hypoglycaemia. A mild degree of bile duct proliferation was also evident, along with the infiltration of inflammatory cells (Fig h & i). Similar lesions were noticed by Bal *et al.* (2012) [1], in Swiss albino mice inoculated with cattle isolate and also Sivajothi *et al.* (2015) [12], observed similar lesions in infected rats. Similarly, in experimentally infected *T. evansi* infection in small East African goats, Ngeranwa *et al.* (1993) [6] revealed haemorrhage and an area of oedema containing pink coloured fluid with increased inflammatory exudate in central veins. In accordance with the present finding, Virmani *et al.* (2004) [17], recorded congestion, hepatic sinusoids, haemorrhages and Von Kupper cells, along with increased mononuclear cell infiltration, in the liver sections in rats infected with camel isolate. Whereas, similarly, recorded in mice with non-suppurative hepatitis, bile duct proliferation and the presence of clumps of trypanosomes.

Spleen

The spleen is an important part of the lymphatic and immune systems. It filters the blood and removes cellular waste and damaged blood cells, it's an important organ that produces WBCs and antibodies against various infections. In the present study, the histopathological changes recorded in the spleen showed depletion of lymphocytes in the white pulp, giving a punched-out appearance of a splenic follicle, giant cell aggregation, haemorrhage in the white pulp, and the presence of megakaryocytes (Fig j & k). Documented in Bandicoot rats suffered from *Trypanosoma* infection, revealed haemorrhagic lesions, congestion, haemosidirosis increase in follicular cells and necrosis. In present findings, haemosidirosis was not appreciated and probably the erythrocytes were not destroyed in the spleen, which indicates the metabolites of trypanosomes would have caused major splenic tissue damage. Due to infinite number of trypanosomes in blood have utilized oxygen and blood nutrients and also the major occupancy of organisms in the blood vessels which inturn would have led to anoxic condition of spleen and also other organs.

Similar observations were documented by Sivajothi *et al.* (2014) [19] in infected mice with cattle isolate, recorded giant cell aggregation, hyperplasia, capsular thickening and irreversible degeneration. Whereas Virmani *et al.* (2004) [17] revealed depopulation of lymphocytes in white pulp, area of abscess, inflammatory exudate in sinuses, deposition of haemosiderin in goats which survived up to 88th days of post inoculum. According to Uche and Jones (1992) [16] *Trypanosoma evansi* will produce immediate type of hypersensitivity reaction which might be the cause of initial changes in the spleen. Suggested multiple necrotic foci in lung, spleen and liver during post mortem is due to secondary bacterial infection and immunodepressive condition caused by trypanosomosis. Tizard *et al.* (1998) [15] revealed the aggregation of giant cells in spleen, thickening of capsule, hyperplasia is mainly due to immunological response initiated in the infected animals.

Kidney

The infected kidney section revealed cortico medullary haemorrhage of varying degree with congestion, the renal tubules showed tubular degeneration varying from cloudy swelling to hydropic degeneration, the tubular lumen was reduced due to swelling of tubular lining epithelial cells and in multiple area tubular lumen was filled with pink coloured

cellular debris. The congested blood vessels showed presence of large number of trypanosome organisms in between the RBC's (Fig l & m). similar lesions were observed by Sivajothi *et al.* (2014) [19] in experimentally infected mice with different cattle strains. Whereas, Ngeranwa *et al.* (1993) [6] revealed loss of architecture in kidney sections of the infected goat, which might be due to toxicants produced by the parasites and the accumulation of immune complexes in the kidney.

Conclusion

The infected mice with the buffalo isolate of *T. evansi* showed massive Parasitemia, neurological symptoms before succumbing to death on the fifth day after infection. The gross lesions showed hepatomegaly, splenomegaly and congestion of the brain. The histopathological studies revealed proliferation of Bal T, diffused vacuolar degenerative changes of liver, depletion of lymphocytes in the white pulp of the spleen and tubular degeneration of the kidney, with the presence of *T. evansi* organisms in the blood vessels of all the sections of the visceral organs. In the present, histopathological observations in infected mice suggest early detection of *T. evansi* and appropriate treatment with trypanocidals are required to avoid fatal consequences.

Fig 1: (a) Splenomegaly and hepatomegaly in experimentally infected mice

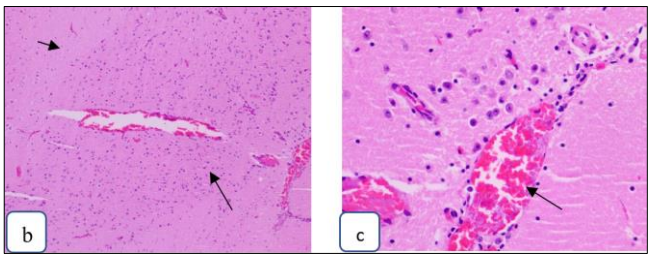

Fig (b, c): The section of brain showing congestion of blood vessels, with mild vacuolation (H&E X100), Presence of trypanosomes in blood vessels with red blood cells. (H&E X 1,000)

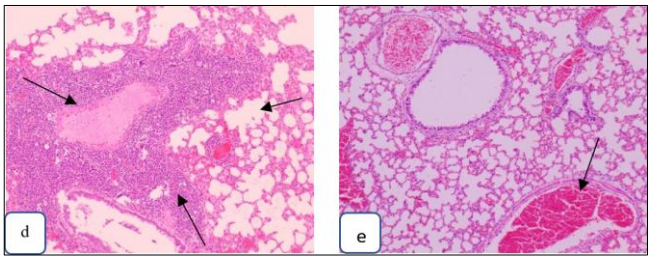

Fig (d, e): The section of lung showing hyperplasia of BALT, emphysema and congestion (H&E X100) Presence of *T. evansi* in blood vessels the lumen (H&E × 1,000)

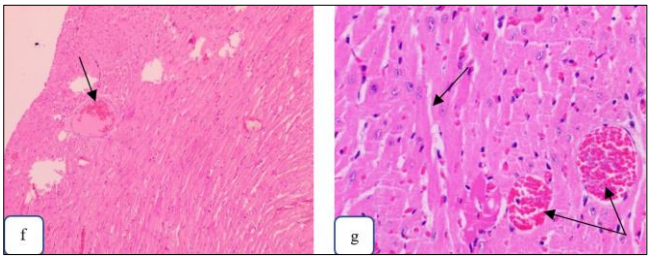

Fig (f): The section of heart showing haemorrhage, congestion and oedema (H&E X100)

Fig (g): Swollen myocardial tissue with presence of trypanosome in the blood vessel (H&E X 1000)

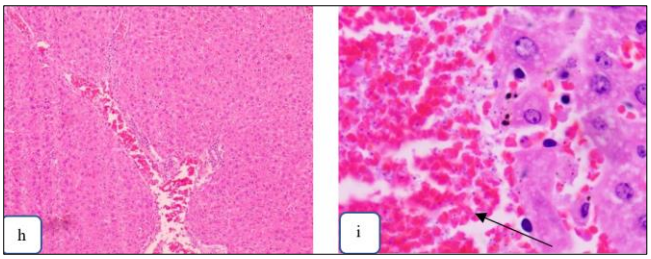

Fig (h, i): Liver section of mice showing congestion, haemorrhage and mild decrease of duct proliferation (H&E X100) Presence of trypanosomes in blood vessels along with red blood cells in the lumen (H&E X1000)

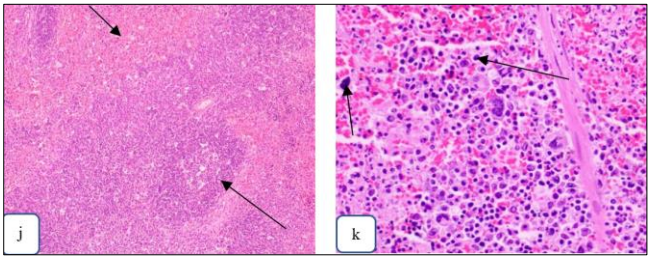

Fig (j, k): section of spleen showing haemorrhage (H&E X 100), depletion of lymphoid cells in white pulp along with giant cells (H&E× 400)

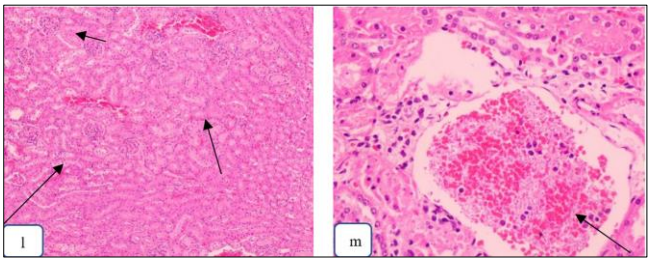

Fig (l, m): Kidney section of mice showing congestion, coraticular haemorrhages with swollen renal tubules (H&E X100), presence of trypanosomes in blood vessels along with red blood cells in the lumen (H&E X1000)

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