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Comparative pathology of experimental infection with four isolates of *Trypanosoma evansi* in Wistar albino Rats

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ABSTRACT

In the present study, pathological changes due to experimental infection with four isolates of *T. evansi* were studied in rats to know the pathogenicity. Male Wistar albino rats (140) were divided in five groups of 28 rats each (4 isolate groups and 1 control group). Each rat was infected with 1×10^5 *T. evansi* from buffalo, dog, leopard and lion isolates and normal saline in control group by intraperitoneal route. Four rats were sacrificed on Day 1 (D1) to D7 post infection in each group and collected blood was for microscopic examination and tissues for pathology. Blood smear examination revealed presence of trypanosomes on D3, D4, D5 and D5 in buffalo, dog, leopard and lion isolates, respectively. All the *T. evansi* infected rats showed hypoglycaemia and increased blood urea nitrogen in serum. Rats showed clinical signs with dullness, lethargy and hunched back on D7. Liver showed vacuolar degeneration, congestion of blood vessels and infiltration of mononuclear cells on D7 in buffalo, dog, lion isolates, and no changes in leopard isolate infected rats. Kidney, spleen, lung and testis revealed pathological changes in buffalo and dog isolates. Trypanosomes were present in the endocardium of heart in buffalo isolate infected rats. Immunohistopathology revealed presence of trypanosome antigens in the blood vessels of liver, and spleen. Thus, based on trypanosome counts, serum glucose, blood urea nitrogen and pathological changes, there was variation between these isolates and revealed pathogenicity in ascending order as leopard, lion, dog and buffalo isolates in rats.

Keywords: Buffalo, Dog, Leopard, Lion, Pathology, Rats, *Trypanosoma evansi*

Trypanosoma (*T.*) *evansi*, haemoflagellate parasite causes great economic loss to the livestock farmers in India and affects many species of livestock and wild animals. It causes diseases in horse, camel, cattle, buffalo, dog and rarely in wild animals of India. The disease progression of trypanosomiasis depends on the host susceptibility, isolate variability, vector availability and favourable environmental conditions. Visceral forms of *T. evansi* have been reported in heart, optic lobes, cerebrum, liver, kidney and lungs, although it is a haemoprotozoa (Singla *et al.* 2001). Trypanosomiasis causes serious economic loss to dairy farmers from anaemia, loss of condition and its effect on reproduction. The projected economic loss due to trypanosomiasis in buffaloes in India was ₹ 2,459.1 crore and loss per animals was ₹ 286.2 (Narladkar 2018). The confirmatory diagnosis of surra is by way of animal inoculation and rats, mice are the laboratory animals of choice for this purpose (Virmani *et al.* 2004). *T. evansi* is also highly pathogenic to laboratory animals like rat, mice and rabbits (Singla *et al.* 2003). The isolation of *T. evansi* from wild animals is difficult and has been isolated from leopard and lion, as reported previously (Sengupta *et al.* 2010). The pathology of *T. evansi* infection using cattle isolate was studied in Swiss albino mice and observed changes consistent with infection and the presence of trypanosomes in most of the tissue sections (Bal *et al.* 2012). In our previous study, we compared the buffalo and dog isolate of *T. evansi* infected mice for pathology and cytokine gene expression in various organs and indicated variation between these two isolates in pathogenicity in mice (Krishnamoorthy *et al.* 2016). However, *T. evansi* isolates from herbivores and carnivores have not been compared so far to study the pathogenicity between these isolates. Studying the pathogenicity between different isolates of *T. evansi,* will help us to understand the variation between these isolates of *T. evansi* isolated from different animals (buffalo, dog, leopard and lion) in laboratory rodents. This information will help us to determine the variation between these isolates based on the host specificity in laboratory rodents by understanding the pathogenicity. No literature was available on the comparative pathology of experimental infection with different isolates of *T. evansi* from domestic and wild animals in laboratory rodents. Hence, the present study was undertaken to know the pathological changes induced by experimental infection with buffalo, dog, leopard and lion isolates of *T. evansi* in Wistar albino rats.

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MATERIALS AND METHODS

Experimental infection in rats: Male Wistar albino rats (140; inbred) approximately 8–12 weeks of age weighing 150–180 g and 10 adult rabbits of 16–20 weeks of age were procured from Veterinary College, Bengaluru and used in the present study. Rats and rabbits were housed in polypropylene cages and stainless steel cages, respectively in the Laboratory Animal House. The animals were fed with rodent and rabbit pellet feed for rats and rabbits, respectively along with purified water *ad lib*. The animal house room temperature and humidity were maintained at 23±2°C, and 50 to 70%, respectively. The animal experiments (rats and rabbits) were approved by Institutional Animal Ethics Committee (Registration No. 881/05/R/S/CPCSEA) of Indian Council of Agricultural Research-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI) and carried our as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India, New Delhi. The *T. evansi* isolates from buffalo, dog, leopard and lion, which were maintained in the Parasitology Laboratory, ICAR-NIVEDI, Bengaluru, India were used in the present study. These four isolates of *T. evansi* were propagated in mice as reported earlier (Sengupta *et al.* 2010) and purified using diethyl amino ethyl (DEAE) cellulose (DE.52-Whatman) column (Lanham and Godfrey 1970). Counting of the trypanosomes was done by using haemocytometer method as previously reported (Janeen *et al.* 1972). Wistar albino rats (140) were divided in to five groups (4 isolate groups and 1 control group), and each group consisting of 28 rats which were sacrificed four rats each from one to seven days (D1-D7) post infection with different *T. evansi* isolates. The dose of *T. evansi* for infection in Wistar albino rats was fixed based on the previous studies (Bal *et al.* 2012, Krishnamoorthy *et al.* 2016). Each rat in control group was given sterile PBS through intraperitoneal route and rats in four isolate groups were infected with *T. evansi* buffalo, dog, leopard and lion isolates by injecting with 1×10^5 trypanosomes by intraperitoneal route on D0 as described earlier (Bal *et al.* 2012, Krishnamoorthy *et al.* 2016). The rats were observed daily for the development of clinical signs till death/sacrifice. Four rats per group were sacrificed by using overdose of injectable anaesthesia (ketamine and xylazine) from D1 to D7 and control group rats were sacrificed at the end of the study.

Production of hyperimmune sera in rabbits: The hyperimmune serum in rabbits was raised as per the methodology described earlier (Sengupta *et al.* 2012, Sengupta *et al.* 2014). Ten adult rabbits of 8–12 weeks of age were divided into five groups each consisting of 2 animals per group. Hyperimmunization with the whole cell lysate of *T. evansi* of buffalo, dog, leopard, lion isolates were carried out in Group I, Group II, Group III and Group IV, respectively and Group V rabbits were used as healthy control. Rabbits in Group I to IV were initially immunized by giving subcutaneous injection of 500 µg of each antigen emulsified with Freund's complete adjuvant into multiple sites. Two weeks following the initial dose, four booster dose injections with 500 µg of each antigen emulsified with Freund's incomplete adjuvant were administered at weekly intervals. The blood was collected from the marginal ear vein of rabbits at the end of the period. Sera were separated by centrifugation at 2,000 rpm for 15–20 min. Both hyper immune sera and healthy control sera samples were stored at –20°C until further use.

Blood smear examination and trypanosome counts: Blood was collected from intracardiac route from all the rats after sacrifice during D1–D7. The thin blood smear was prepared on grease free glass slide and stained with Giemsa stain. The stained slides were examined under light microscopy and counted the trypanosomes per high power field. The trypanosome count was expressed as the number of trypanosomes per high power field.

Estimation of serum glucose and blood urea nitrogen (BUN): Serum was separated from the blood collected from all the rats after sacrifice during D1–D7 by centrifugation at 2,000 rpm for 15–20 min. Estimation of glucose by Glucose oxidase - peroxidase method (Trinder 1969) and blood urea nitrogen by method as described (Lattig 1964), by using commercially available kits (M/s Span Diagnostics Limited, Surat, India) and Semiauto analyzer, Biosystems (BTS 320).

Pathology: At sacrifice, gross examination of visceral organs was done and collected liver, kidney, spleen, lung, heart and testis were in 10% buffered formalin. The formalin fixed tissues were processed, embedded in paraffin, 5 µm tissue sections were prepared using rotatory microtome and stained with haematoxylin and eosin (Bancroft and Stevens 1996). After staining, the sections were dehydrated in increasing ethanol concentrations, mounted in DPX and slides were examined under light microscope for observing histological changes. The immunohistopathology was carried on the paraffin embedded tissue sections to detect the trypanosome antigens in the liver, kidney, spleen, lung, heart and testis. The tissue sections were deparaffinised, antigen retrieval was done by using heat induced sodium citrate buffer 10 mM, pH 6.0. The sections were coated with anti-*T. evansi* hyper immune serum which was raised in New Zealand White Rabbits by using the buffalo, dog, leopard and lion isolates as described earlier (Sengupta *et al.* 2014) was considered as primary antibody. The slides were kept at 4°C overnight for incubation in humidified chamber, and washed with phosphate buffer saline (PBS), pH 7.4. The slides were coated with secondary antibody raised against rabbit, i.e. Anti-rabbit horse radish peroxidase conjugate. The slides were washed with PBS and coated with peroxide substrate and chromogen 3, 3 diamino benzidine tetra hydrochloride (DAB). The slides were counter stained by haematoxylin for better visualization of the nucleus and the slides were examined under light microscopy for detection of antigen-antibody reaction as dark brown colour.

Statistical analysis: The blood smear trypanosome

counts, serum glucose and blood urea nitrogen levels in rats obtained during D1 to D7 was analyzed using Statistical Analysis System (SAS) software Enterprise Guide version 5.1 (SAS India limited, Mumbai) by using one-way analysis of variance (ANOVA) method as described earlier (Snedecor and Cochran 1980). The results were expressed as the Mean±SE (Standard Error) with significant difference at $P<0.05$ and confidence interval (CI) at 95% level.

RESULTS AND DISCUSSION

Trypanosome counts in blood smear: The details of trypanosome counts per high power field by blood smear examination during D1 to D7 for four *T. evansi* isolates are given in Fig. 1. The mean±SE of trypanosome counts were 7.75±1.30, 4.14±0.95, 2.36±0.69 and 3.54±1.02 in buffalo, dog, leopard and lion isolate infected rats, respectively. The blood smear trypanosomes counts in rats infected with buffalo, dog, leopard and lion isolates showed variation in the number of trypanosomes indicating the difference in the virulence pattern. The trypanosomes in blood smear were detected on D3 and D4 for buffalo and dog isolate of *T. evansi* in rats, which concurred with previous report of buffalo and dog isolates of *T. evansi* infected mice (Krishnamoorthy *et al.* 2016). The streaming of trypanosomes on blood smear examination on D7 in rat infected with buffalo isolate of *T. evansi* was observed and corroborated with previous studies of buffalo isolate in rats (Sengupta *et al.* 2010) and in mice (Krishnamoorthy *et al.* 2016). The high pathogenicity of *T. evansi* in rats with high mortality rates at day 5–6 post infection has been reported previously (Wolkmer *et al.* 2009). However, in our study, the rats become lethargic on D5, with high parasitemia in case of buffalo isolate and moderate parasitemia in dog and lion isolates, and low parasitemia in leopard isolate infected rats were observed on D6 and D7. However, no mortality was observed in the rats infected with four isolates of *T. evansi*. There was significant ($P<0.05$) difference in the trypanosome counts on D7 when compared to D6 and other days in all the groups infected with four isolates. The trypanosome counts in buffalo isolate infected rats revealed two folds increase when compared to dog and lion isolates, and three folds increase when compared to leopard isolate infected rats on D7. The trypanosome counts observed in this study concurred with the previous report of buffalo and dog isolate infected in mice, which showed increase in trypanosome counts and variation between these two isolates (Krishnamoorthy *et al.* 2016).

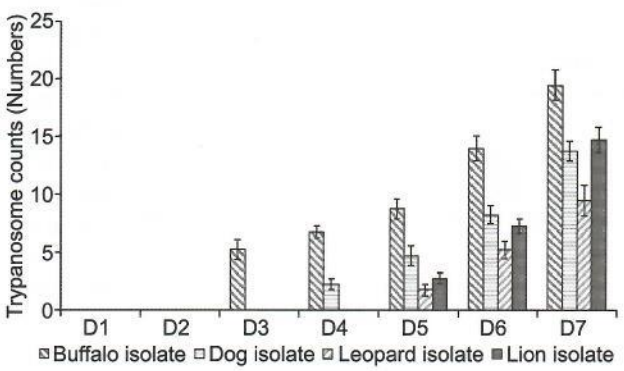

Fig. 1. Trypanosome counts by blood smear examination at different days after experimental infection with different isolates of *T. evansi* infection in rats.

Serum glucose and blood urea nitrogen: The serum glucose and blood urea nitrogen levels observed in the rats on different days after infection are depicted in Fig. 2. The mean±SE of serum glucose were 68.97±4.97, 76.12±3.37, 74.34±3.24 and 72.09±3.24 mg/dL in buffalo, dog, leopard and lion isolates infected rats, respectively. There was significant ($P<0.05$) decrease in the glucose levels from D2 in buffalo and dog, D3 in leopard and lion isolates infected rats when compared to the control and D1 rats. The serum glucose levels showed severe decrease in all the groups infected with four isolates of *T. evansi* and indicated the occurrence of hypoglycaemia in rats and concurred with previous report (Fatihu *et al.* 2008). This might be due to the increased requirement of glucose by *T. evansi* for its motility and movements in the blood vessels and depleting the glucose availability to the host. The mean±SE of serum BUN were 37.72±2.23, 31.50±2.12, 34.37±2.13 and 36.66±2.21 mg/dL in buffalo, dog, leopard and lion isolates infected rats, respectively. There was significant ($P<0.05$) increase in the BUN from D2 in buffalo, D1 in dog, D4 in leopard and D3 in lion isolates infected rats when compared to control rats. The serum BUN levels revealed severe increase in all the rats infected with four isolates and concurred with previous study (Fatihu *et al.* 2008). This

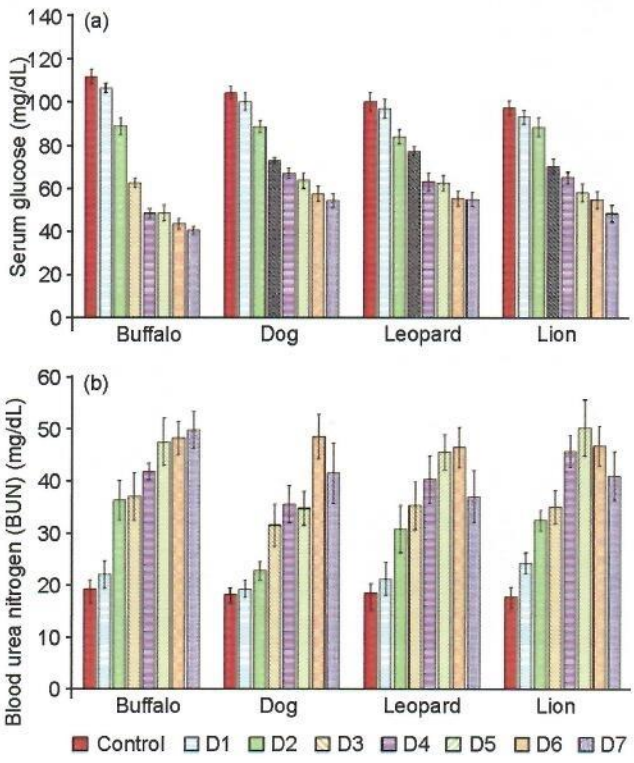

Fig. 2. Serum glucose (A) and blood urea nitrogen (B) levels at different days after experimental infection with different isolates of *T. evansi* in rats.

may be due to the damage to the kidney caused by the trypanosome antigens and concurred with the histopathological lesions in kidney observed in the present study.

Pathology: On clinical examination, the rats showed dullness, lethargy, hunched back on D7 in buffalo and dog isolates when compared to the leopard, lion isolates and control groups and concurred with previous reports in rats and mice (Virmani *et al.* 2004, Sengupta *et al.* 2010, Paim *et al.* 2011, Bal *et al.* 2012). The clinical signs were severe in rats infected with buffalo isolate when compared to other three isolates. The rats were examined grossly from D1 to D7 for all the four isolates infected rats and no abnormal changes in the visceral organs except splenomegaly with rounded borders in rats infected with buffalo and dog isolate on D6 and D7 was observed. This might be due to increased activity of mononuclear phagocytic system resulting from destruction of trypanosome antigen coated RBCs in spleen. Splenomegaly followed by hyperplasia and hypersplenism are very much pronounced as the disease progresses (Singla *et al.* 2001). The splenomegaly observed in the present study concurred with the previous reports (Singla *et al.* 2001, Virmani *et al.* 2004, Bal *et al.* 2012, Sivajothi *et al.* 2014). The presence or absence of histopathological lesions observed in the different isolates of *T. evansi* infected rats are given in Table 1. On histopathological examination, the changes observed in the rats infected with buffalo, dog, leopard and lion isolates of *T. evansi* are depicted in Figs 3, 4, 5 and 6, respectively. The control group of rats showed no observable changes and normal histological architecture in liver, kidney, spleen, lung, heart and testis when compared to the different isolates of *T. evansi* infected rats. Histopathological examination of liver showed vacuolar, ballooning degeneration and condensation of nucleus from D5 to D7 in buffalo isolate of *T. evansi* infected rats and

Table 1. Pathological changes observed in various organs in four isolates of *T. evansi* experimental infection in rats

Organ	*T. evansi isolates*			
	Buffalo	Dog	Leopard	Lion
	Histopathology lesions			
Liver	Present	Present	Absent	Present
Kidney	Present	Present	Present	Present
Spleen	Present	Present	Absent	Present
Lung	Present	Present	Absent	Absent
Heart	Present	Absent	Absent	Absent
Testis	Present	Present	Absent	Absent
	Immunohistopathology for Trypanosome antigen			
Liver	Present	Absent	Absent	Absent
Spleen	Present	Absent	Absent	Absent
Lung	Absent	Absent	Absent	Absent
Heart	Absent	Absent	Absent	Absent

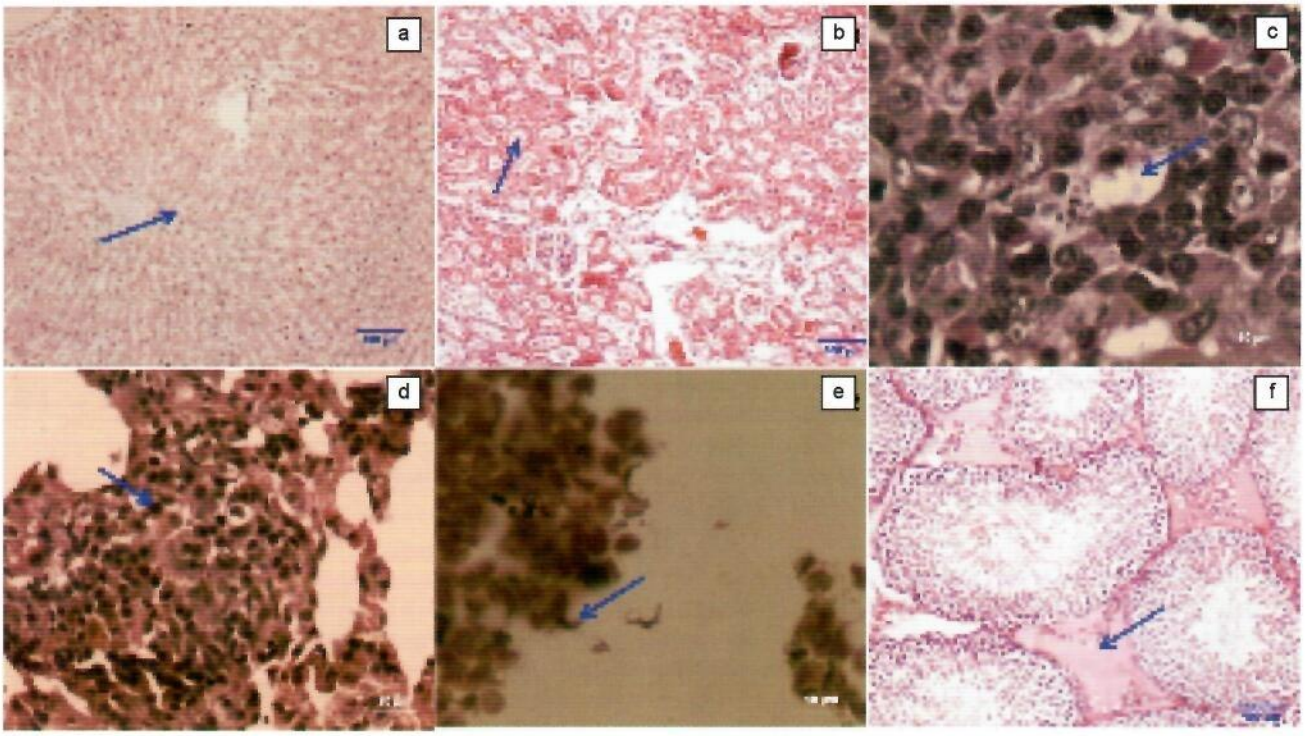

Fig. 3. Rat infected with buffalo isolate of *T. evansi*. Liver showing vacuolar degeneration (arrow) and loss of hepatocytes and condensed nucleus on D6 (a), Kidney showing tissue and epithelial cell debris in the lumen of the tubules (arrow) and mild degeneration of glomerulus on D7 (b), Spleen showing trypanosome (arrow) in between the lymphocytes along with loss of lymphocytes in the white pulp (c), Lung showing focal accumulation of plasma cells (arrow) in between the alveolar epithelial cells (d), Heart showing trypanosomes in the ventricular chamber along with red blood cells (arrow) with normal architecture of skeletal muscles (e), Testis showing severe interstitial edema (arrow) in between the seminiferous tubules containing the spermatozoa's in the lumen (f). Haematoxylin and Eosin stain, Scale bar=10 μm, 20 μm, 100 μm.

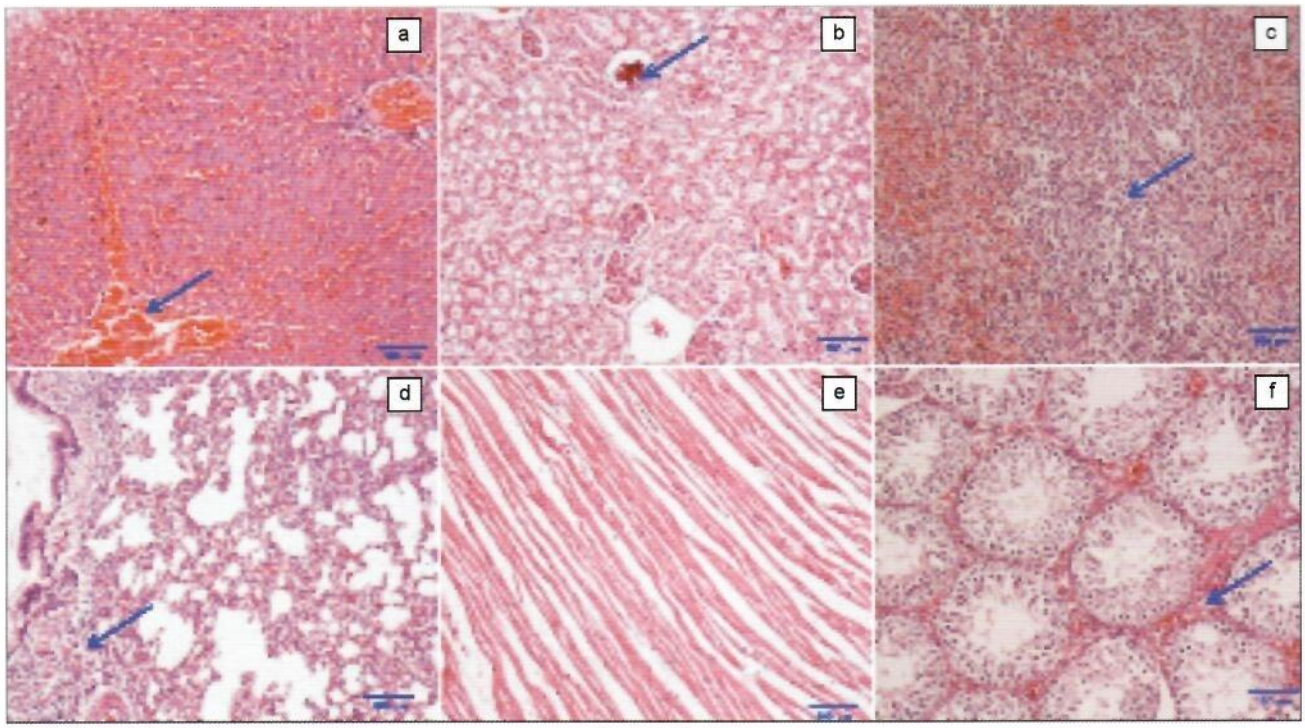

Fig. 4. Rat infected with dog isolate of *T. evansi.* Liver showing haemorrhages and severe congestion of blood vessels (arrow) and normal architecture of hepatocytes (a), Kidney showing mild degeneration of glomerulus (arrow), cells and epithelial debris in the lumen of the tubules (b), Spleen showing loss of lymphocytes (arrow) with empty spaces in white pulp (c), Lung showing infiltration of mononuclear cells (arrow) in between the alveolar epithelial cells (d), Heart showing normal architecture of skeletal muscles (e), Testis showing mild interstitial edema (arrow) in between the seminiferous tubules containing the spermatozoa's in the centre (f). Haematoxylin and Eosin stain, Scale bar=100 μm.

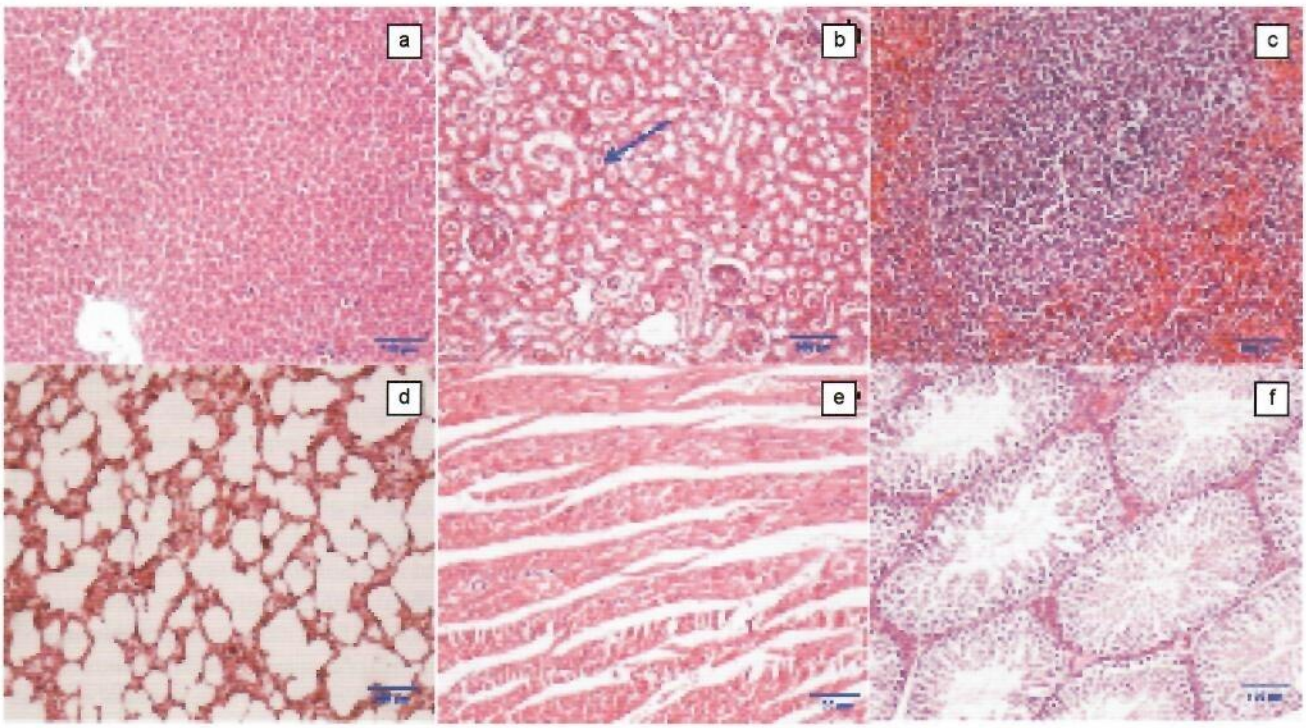

Fig. 5. Rat infected with leopard isolate of *T. evansi.* Liver showing normal architecture of hepatocytes (a), Kidney showing few cells and epithelial debris in the lumen of the tubules (arrow) with normal architecture of glomerulus and tubules (b), Spleen showing normal architecture of white pulp and red pulp (c), Lung showing normal architecture of alveoli and cells (d), Heart showing normal architecture of skeletal muscles (e), Testis showing seminiferous tubules containing the spermatozoa's in the centre of the tubules (f). Haematoxylin and Eosin stain, Scale bar=100 μm.

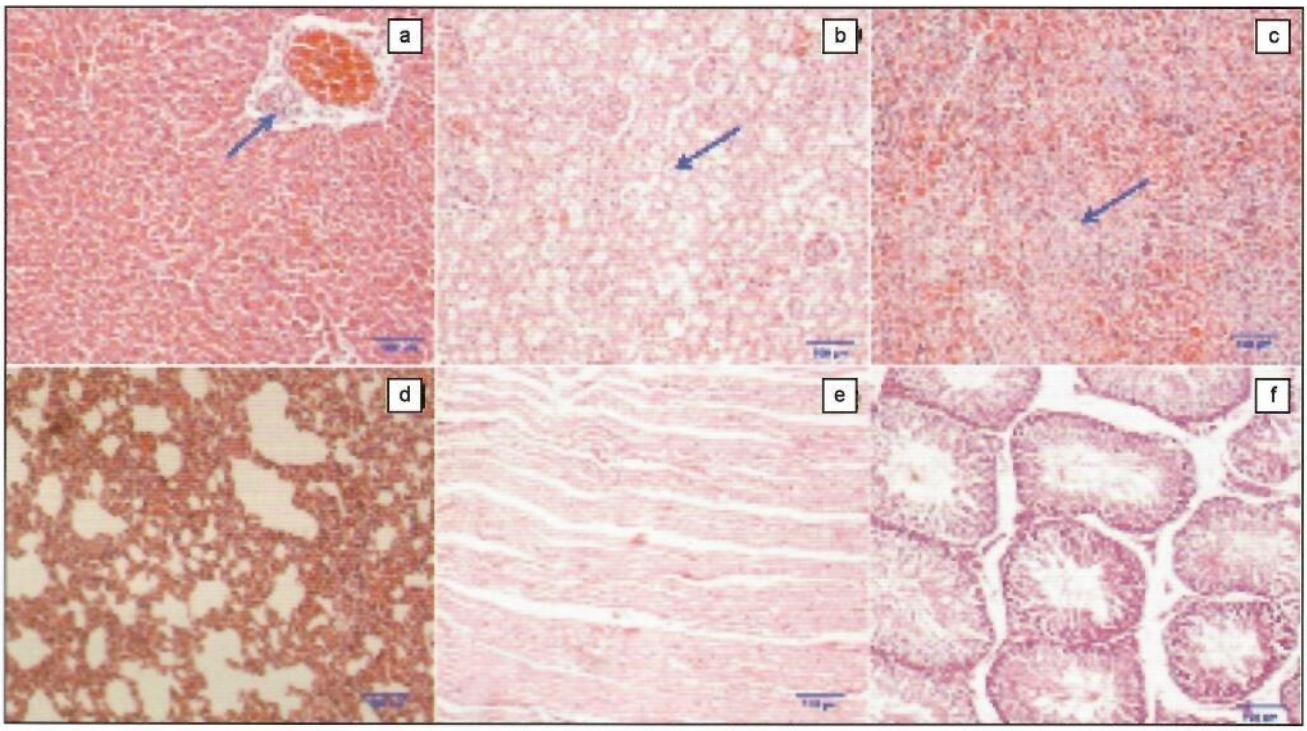

Fig. 6. Rat infected with lion isolate of *T. evansi*. Liver showing congestion of blood vessels with infiltration of mononuclear cells (arrow) and normal architecture of hepatocytes (a), Kidney showing cells and epithelial debris in the lumen of the tubules (arrow) with normal architecture of glomerulus (b), Spleen showing mild loss of lymphocytes (arrow), with white pulp and red pulp (c), Lung showing normal architecture of alveoli and bronchioles (d), Heart showing normal architecture of skeletal muscles (e), Testis showing seminiferous tubules containing the spermatozoa's in the centre of the tubules (f). Haematoxylin and Eosin stain, Scale bar=100 µm.

concurred with earlier studies (Biswas *et al.* 2001, Virmani *et al.* 2004, Bal *et al.* 2012, Sivajothi *et al.* 2014, Krishnamoorthy *et al.* 2016). The pathological changes observed in liver of rats infected with dog and lion isolates were of varying degrees as compared to buffalo isolate, but not much change in leopard isolate. Congestion, haemorrhages and fatty degeneration of hepatocytes may be due to hypoglycaemia, leading to starvation of the cells and anoxia due to anaemia in *T. evansi* infected animals as reported (Suryanarayana *et al.* 1986, Uche and Jones, 1992, Biswas *et al.* 2001). The kidney showed degeneration and atrophied glomerulus and secreting substance, tissue debris in the lumen of proximal and distal convoluted tubules in all the four isolates infected rats and corroborated with previous reports (Bal *et al.* 2012, Sivajothi *et al.* 2014, Krishnamoorthy *et al.* 2016). The changes in kidneys are mainly due to toxins produced by the parasite and accumulation of immune complexes which may impair the structure and function of the kidney as reported earlier (Morrison *et al.* 1981, Ngeranwa *et al.* 1993). In the present study, spleen showed haemorrhages, congestion, loss of lymphocytes, necrosis which progressed from D4 to D7 and more pronounced in buffalo isolate than in dog and lion isolates, no observable changes in leopard isolate. Haemorrhages, congestion, absence of germinal centers, hemosiderosis, increased follicular cells, focal necrosis and formation of giant cells due to aggregation of histiocytes in spleen were also developed during the progression of the disease (Biswas *et al.* 2001). The presence of *T. evansi* or their toxic metabolites might stimulate splenic cells, resulting in varying degrees of anaemic anoxia, which may induce splenic damage as observed in donkeys (*Equus asinus*) experimentally infected with *T. evansi* (Suryanarayana *et al.* 1986). However, the reduced proliferative activity followed by increase in the number of macrophages and multinucleated giant cells with severe disruption in the splenic architecture was observed in *T. brucei* infected dog (Morrison *et al.* 1981), but not observed in the present study. Lung revealed haemorrhages and infiltration of mononuclear cells and plasma cells in rats infected with buffalo and dog isolate of *T. evansi* on D7, but not in leopard and lion isolates. Congestion and haemorrhages of lungs were mainly due to inflammatory response to *T. evansi* parasite resulting in vasodilatation and exudation, concurred with previous studies (Bal *et al.* 2012, Biswas *et al.* 2001, 2010, Krishnamoorthy *et al.* 2016). However, Nagle *et al.* (1980) observed no changes in the lungs of *T. rhodesiense* infected rabbits and concurred with the leopard and lion isolates of *T. evansi* infected rats. Heart of rat showed congestion and streaming of trypanosomes in endocardium along with red blood cells in buffalo isolate and concurred with previous report (Biswas *et al.* 2001), but no observable changes in other three isolates of *T. evansi* infected rats. However, mild degenerative changes, interstitial edema along with presence of the trypanosomes in blood vessels of heart were reported in mice infected with cattle isolate of *T. evansi* (Bal *et al.* 2012). The testis showed interstitial edema in

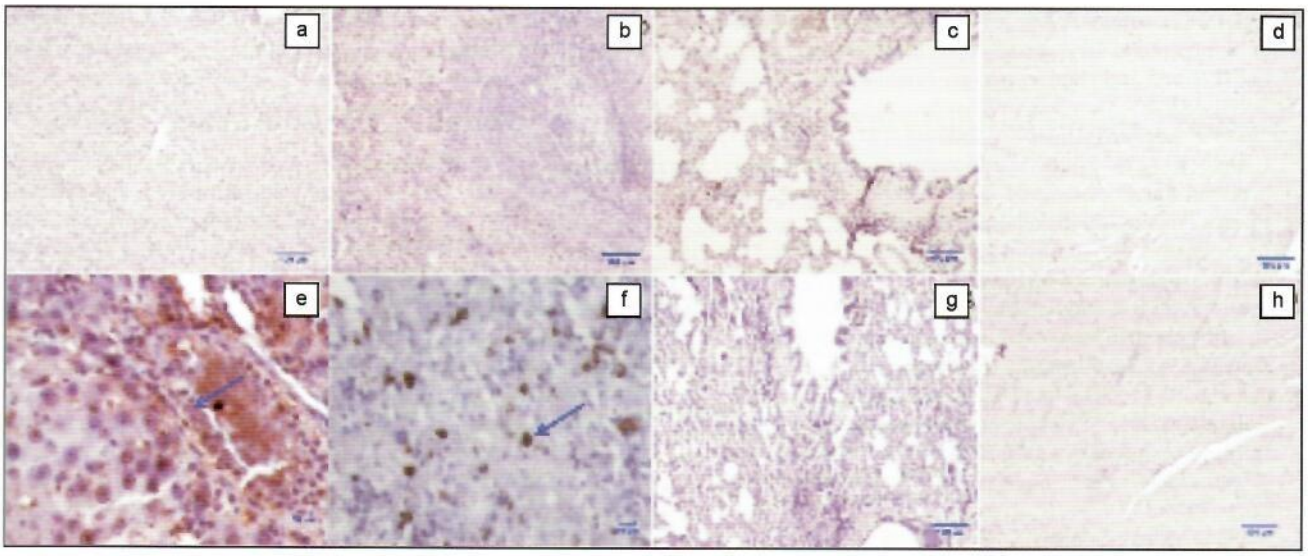

Fig. 7. Rat infected with different isolates of *T. evansi* on immunohistopathology showing negative for trypanosome antigens in control Liver (a), Spleen (b), Lung (c), Heart (d), the presence of Trypanosome antigens (arrow) in Liver blood vessels (e) and Spleen (f) of rats infected with buffalo isolate of *T. evansi* on D7 and negative in lung (g) and heart (h) of rats infected with different isolates of *T. evansi*. DAB chromogen and Haematoxylin counter stain, Scale bar=10 μm, 100 μm.

buffalo and dog isolates infected rats, but no changes in the leopard and lion isolate. The *T. evansi* utilizes oxygen and glucose for its growth and multiplication, which results in depletion of these metabolites leading to degenerative changes in the animals. Further, the consumption of oxygen by trypanosomes in the host for their multiplication leads to hypoxemic state, as a result of which animal tissues are deprived of oxygen and it results in degenerative changes in all the vital organs (Bal *et al.* 2012). The rats revealed the nonspecific histopathological changes in the organs in the present study because of the deprivation of oxygen and glucose to the tissues. Further, changes developed in the organs may be either due to cellular damage caused by toxicants released by the parasite, or due to immunological reaction (Bal *et al.* 2012).

The presence or absence of trypanosome antigens in various tissues by immunohistopathology are given in Table 1. The immunohistopathological changes in various organs of the control and experimentally infected with different isolates of *T. evansi* in rats are given in Fig. 7. The control liver, spleen, lung and heart revealed absence of trypanosome antigens in all rats on D1–D7 (Fig. 7a, b, c, d). Liver of the rat infected with buffalo isolate showed the presence of trypanosome antigens in the blood vessels on D7 (Fig. 7e). On D7, spleen revealed the presence of trypanosome antigens staining dark brown in colour in rat infected with buffalo isolate (Fig. 7f). This might be due to presence of trypanosomes in the large blood vessels of liver and due to destruction of trypanosomes along with red blood cells in spleen. The observations in the present study corroborated with previous study, in which the trypanosomes were demonstrated in the brain of hog deer (Tuntasuvan *et al.* 2000). However, no trypanosomes were present in the tissues of the other three isolates of *T. evansi* infected rats. The lung and heart of the rats infected with different isolate did not reveal the presence of trypanosome antigens during D1–D7 (Fig. 7 g, h).

Based on the clinical signs, trypanosome counts in the blood smear examination, serum glucose and BUN levels, histopathology and immunohistopathology studies, it can be concluded that there was variation between these four isolates (buffalo, dog, leopard, lion) of *T. evansi* on pathogenicity in rats. The *T. evansi* isolates from domestic animals (buffalo and dog isolates) are more pathogenic compared to wild animals (leopard and lion isolates). These four isolates may be arranged in the order of pathogenicity with ascending order as leopard, lion, dog and buffalo isolates based on the present study. Further studies are required to ascertain the host pathogen interaction of the rodents with different isolates of *T. evansi* in the molecular and sub-cellular levels.

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REFERENCES

Bal M S, Singla L D, Kumar H, Vasudev A, Gupta K and Juyal P D. 2012. Pathological studies on experimental *Trypansoma evansi* infection in Swiss albino mice. *Journal of Parasitic Diseases* **36**(2): 260–64.

Bancroft J D and Stevens A. 1996. *Theory and Practical of Histological Staining Techniques*. 6th ed. Churchill Livingstone, London. pp 247–260.

Biswas D, Choudhury A and Misra K K. 2001. Histopathology of *Trypanosoma* (Trypanozoon) *evansi* infection in bandicoot rat. I. Visceral organs. *Experimental Parasitology* **99**: 148–59.

Biswas D, Choudhury A and Misra K K. 2010. Histopathology of *Trypanosoma* (Trypanozoon) *evansi* infection in bandicoot rat. II. Brain and choroid plexus. *Proceedings of Zoological Society* **63**: 27–37.

Fatihu M Y, Adamu S, Umar I A, Ibrahim N D G, Eduvie L O and Esievo K A N. 2008. Studies on effects of lactose on experimental *Trypanosoma vivax* infection in Zebu cattle. 1. Plasma kinetics of intravenously administered lactose at onset of infection and pathology. *Onderstepoort Journal of Veterinary Research* **75**: 163–72.

Janeen D, Alan S R and Sandra A T. 1972. *Trypanosoma lewisi*: *In vitro* growth in mammalian cell culture media. *Experimental Parasitology* **31**: 225–31.

Krishnamoorthy P, Sengupta P P, Das S, Ligi M, Shome B R and Rahman H. 2016. Cytokine gene expression and pathology in mice experimentally infected with different isolates of *Trypanosoma evansi*. *Experimental Parasitology* **170**: 168–76.

Lanham S M and Godfrey D G. 1970. Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. *Experimental Parasitology* **28**: 521–34.

Lattig G J. 1964. A method for the measurement of blood urea nitrogen. *American Journal of Clinical Pathology* **41**: 565–67.

Morrison W I, Murray M, Sayer P D and Preston J M. 1981. The pathogenesis of experimentally induced *Trypanosoma brucei* infection in dog. II. Changes in the lymphoid organs. *American Journal of Pathology* **102**: 182–94.

Nagle R B, Dong S, Guillot J M, Mc Daniel K M and Lindsley H B. 1980. Pathology of experimental African trypanosomiasis in rabbits infected with *T. rhodesiense*. *American Journal of Tropical Medicine and Hygiene* **29**: 1187–95.

Narladkar B W. 2018. Projected economic losses due to vector and vector-borne parasitic diseases in livestock of India and its significance in implementing the concept of integrated practices for vector management. *Veterinary World* **11**(2): 151–60.

Ngeranwa J J, Gathumbi P K, Mutiga E R and Agumbah G J. 1993. Pathogenesis of *Trypanosoma* (*brucei*) *evansi* in small east African goats. *Research in Veterinary Science* **54**: 283–89.

Paim F C, Duarte M M F, Costa M M, Da Silva A S, Wolkmer P and Silva C B. 2011. Cytokines in rats experimentally infected with *Trypanosoma evansi*. *Experimental Parasitology* **128**: 365–70.

Sengupta P P, Balumahendiran M, Suryanarayana V V S, Raghavendra A G, Shome B R, Gajendragad M R and Prabhudas K. 2010. PCR-based diagnosis of surra-targeting VSG gene: experimental studies in small laboratory rodents and buffalo. *Veterinary Parasitology* **171**: 22–31.

Sengupta P P, Balumahendiran M, Balamurugan V, Rudramurthy G R and Prabhudas K. 2012. Expressed truncated N-terminal variable surface glycoprotein (VSG) of *Trypanosoma evansi* in *E. coli* exhibits immuno-reactivity. *Veterinary Parasitology* **187**: 1–8.

Sengupta P P, Rudramurthy G R, Ligi M, Roy M, Balamurugan V, Krishnamoorthy P, Nagalingam M, Singh L and Rahman H. 2014. Sero-diagnosis of surra exploiting recombinant VSG antigen based ELISA for surveillance. *Veterinary Parasitology* **205**: 490–98.

Singla L D, Juyal P D and Sandhu B S. 2001. Clinico-pathological response in *Trypanosoma evansi* infected and immuno-suppressed buffalo-calves. 18th International conference of WAAVP, WAAVP, Stresa, 26–30 August 2001.

Singla N, Parshad V R and Singla L D. 2003. Potential of *Trypanosoma evansi* as a biocide of rodent pests. (Eds) Singleton G R, Hinds L A, Krebs C J, Spratt D M. Rats, mice, and people: rodent biology and management. ACIAR, Canberra, pp 43–46.

Sivajothi S, Rayulu V C and Sujatha K. 2014. Study of histopathological changes in experimental *Trypanosoma evansi* infected rats. *Proceedings of Zoological Society* **68**(2): 112–15.

Snedecor G W and Cochran W G. 1980. *Statistical Methods*. 7th edn. Iowa State University Press, Ames, Iowa, USA. Pp 196–252.

Suryanarayana C, Gupta S L, Singh R P and Sadana J R. 1986. Pathological changes in donkeys (*Equus asinus*) experimentally infected with *T. evansi*. *Indian Veterinary Medicine Journal* **6**: 57–59.

Tuntasuvan D, Mimapan S, Sarataphan N, Trongwongsa L, Intraraksa R and Lucking A G. 2000. Detection of *Trypanosoma evansi* in brains of the naturally infected hog deer by streptavidine-biotin immunohistochemistry. *Veterinary Parasitology* **87**: 223–30.

Trinder P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry* **6**: 24–25.

Uche U E and Jones T W. 1992. Pathology of experimental *Trypanosoma evansi* infection in rabbits. *Journal of Comparative Pathology* **106**: 299–309.

Virmani N, Sengupta P P and Panisup A S. 2004. Pathomorphological changes in rats following infection with *Trypanosoma evansi* isolated from a heterologous host. *Indian Journal of Animal Research* **38**: 29–32.

Wolkmer P, Da Silva A S, Traesel C K, Paim F C, Cargnelutti J F, Pagnoncelli M, Picada M E, Monteiro S G and Lopes S T. 2009. Lipid peroxidation associated with anemia in rats experimentally infected with *Trypanosoma evansi*. *Veterinary Parasitology* **165**: 41–46.