Clinco-pathological Responses to Experimentally Transmitted *Trypanosoma vivax* Infection in Sudanese Nubian Male Goats

Nadia M. Osman[1]; Fadl[2], M.; Zakia[1] A. Mohamed and A/Rahman[1], A. H.
(1) Centarl Veterinary Research Laboratories Centre, P.O. Box 8067 (Alamarat), Khartoum, Sudan. (2) Department of Parasitology, Faculty of Veterinary Medicine, Khartoum North, P. O. Box 32, University of Khartoum, Sudan.

ملخص البحث

أجريت هذه الدراسة لمعرفة قابلية الماعز النوبي للإصابة بطفيل المثقبية *Trypanosoma vivax* والتأثير المرضى للطفيل عليها. وذلك بحقن طفيل عن طريق الوريد عزل من بقرة مصابةً بمنطقة السوكى بولاية سنار، السودان. أظهرت التغييرات التشريحية العيانية شحوب الأغشية المخاطية، لون الدم الباهت، ضمور العضلات، نزف الكلى واحتقان الدماغ، كما ظهرت عدة تغيرات مجهرية في أنسجة بعض الأعضاء الداخلية أهمها:- إرتشاح الخلايا وحيدة النواة مع وجود فراغات في خلايا بركنجى في القلب، إحتقان نخر و خثره وعائية في الكبد، زيادة في سمك الفواصل النسيجية في الرئة، ترسيب صبغة الهيموسيدرين في الطحال، نزف في نخاع الكلي مع تنكس خلايا الأنابيب البولية، أيضا نزف وتنكرز في خلايا الغدة الكظرية، وذمة مع وجود خثره في بعض الأوعية الدموية في الغدد الليمفاوية، بينما أظهر الدماغ دباق و فراغات في المادة الرمادية مع تنكرز الخلايا العصبية و أظهرت الخصي، نخر و أورام حبيبيه متعددة. شملت دراسة المعالم في مصل الدم؛ زيادة معنوية في تركيز البروتين الكلى،الجلوبيولين، فعالية إنزيم الاسبارتيت امينو ترانفيريس واليوريا. اما تراكيز الالبيومين، الكرياتنين و أملاح الصوديوم و البوتاسيوم فقد ظلت طبيعيه. نخلص من هذه الدراسة الي أن ذكور الماعز النوبي ذو قابلية للإصابة بطفيل *T. Vivax* ,*الذي* يمكن ان ينقل ميكانيكيا بواسطة الحشرات *العاضة*.

Summary

The susceptibility of Nubian goats to *Trypanosoma vivax* (*T. vivax*) infection was studied using an isolate obtained from an infected cow at ElSuki District, Sennar State (tsetse flies free area), Sudan. Gross changes included pale mucuous membranes, watery blood, muscle atrophy, renal haemorrhages and congestion brain.

The most common histopathological changes were wide-spread infiltrations of mononuclear cell in several organs, vacuolation of Purkinje cells of the heart. The livers displayed congestion and areas of coagulative necrosis. Lungs showed thickening of the alveolar septa. Sections of spleen revealed deposition of haemosiderin pigment. Changes in kidneys included haemorrhages in the medullary rays and degeneration of tubular cells. Adrenal glands showed haemorrhages and degeneration of some cortical cells. There were oedema and thrombosis in some blood vessels in the lymph nodes.

The brain displayed gliosis, while testes showed necrosis and multiple granulomas. Serum biochemical alterations included significant increase ($P<0.05$) in aspartate aminotransferase (AST), total proteins, globulins and urea values.

No significant alterations ($P>0.05$) rarely were observed in serum albumin, creatinine, sodium (Na+) and potassium (K+) values. It can be concluded that Nubian male goats are highly susceptible to experimentally induced *T. vivax* infection that transmitted mechanically by biting flies.

Introduction

Pathological studies on trypanosomes infection in animals have been carried out by several investigators (Losos and IKede, 1972; Murray, 1974; Masake, 1980). Van Bogaert (1962) described perivascular mononuclear cells infiltration associated with degenerative changes in the brain of a goat experimentally infected with *Trypanosoma brucei gambiense*. Murray (1974) and Sayer *et al*. (1977) described significant myocardial changes associated with *T. brucei* infection in both dogs and mice.

Infections with *Trypanosoma vivax* in goats and *T. congolense* can result in the impairment of the microcirculation in the skeletal muscles and brain (Van den Ingh *et al*, 1976). Moreover, Brown *et al* (1990) have reported that infection with *T. brucei* and *T. vivax* leads to necrosis in many organs and even death of infected cattle due to extravascular accumulations of trypanosomes. Several changes were observed at necropsy in different organs of goats experimentally infected with *T. evansi;* mainly a combination of lymphadenopathy, splenomegaly, hepatomegaly, testicular enlargement, anaemia and consolidation of the anterior lobes of the lungs. Testicular changes, especially aspermia, were also reported (Dargantes *et al*, 2005). Adamu *et al.* (2007) have stated that *T. vivax* infection in bulls can cause severe testicular and epididymal damages.

Trypanosome infections were reported to cause several sero-biochemical changes in different animal species (Anosa, 1988). These included increase in serum transaminase activity, total serum proteins, gamma globulins, iron, chloride, bicarbonate, inorganic phosphate, creatinine, urea, and plasma fibrinogen. A decline in serum albumin, potassium, copper and magnesium were also reported (Gray, 1963; Clarkson *et al*, 1975; Anosa and Isoun, 1976; Ogunsanmi *et al*, 1994). On the other hand, Osaer *et al* (2000) reported a significant decline in total protein and albumin associated with a significant increase in plasma urea in ewes experimentally infected with *T. congolense.*

The present work was conducted to study the clinico-pathological responses of Sudanese male Nubian goats to mechanically transmitted *T. vivax* infection.

Materials and Methods

Trypanosoma vivax stock (Fig.1) was isolated from the blood of a confirmed naturally infected cow in El Suki district, Sennar State, Sudan. The infected blood was inoculated intravenously into a recipient goat in ELSuki and the goat was then transferred to the premises Central Veterinary Research Laboratories Centre at Khartoum. Blood was collected from the infected goat and preserved in liquid nitrogen (-179ºC) untill used.

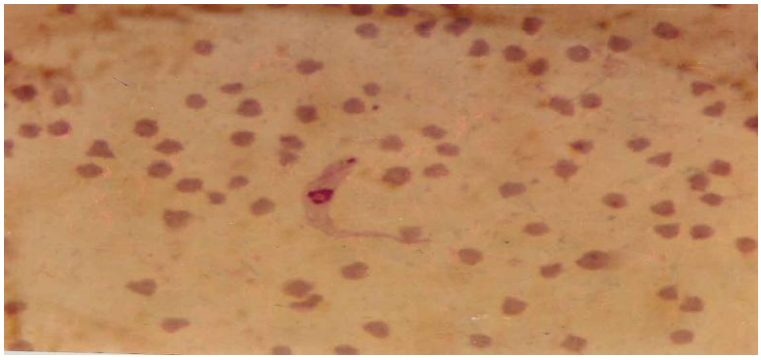

Fig. 1: ***Trypanosoma vivax* in a thin blood smear of an experimentally infected goat (Giemsa's stain x 100).**

Experimental animals:

Twenty Sudanese Nubian male goats' 6 to 8-month-old were purchased from Hillat Kuku Animals Market in Khartoum North and kept in fly-proof pens at the Central Veterinary Research Laboratories Centre, Soba, Khartoum-Sudan. An acclimatization

period of 30 days was allowed before the goats were screened for the presence of haemoparasites as well as internal and external parasites. They were drenched with albendazole (Vety Care pharmaceuticals Ltd., Pakistan), and received Oxytetracycline Hydrochloride, (Formaceutici Gellini S.P.A, Italy) and the anticoccidial Sulphadiamidine sodium, (Vetwic, Elnasr pharmaceutical chemicals Co, Egypt). Furthermore, they were also sprayed with the Asuntol acarcide (Coumaphos, Bayer, A.G, Germany). The animals were maintained on dry sorghum hay and water *ad libitum* with daily concentrate supplementation.

Fifteen goats were intravenously infected with one ml of infected blood (1×10^4 trypanosome/ml blood). The other five animals were kept as uninfected control group. The experimental animals were bled twice weekly into plain and heparinized vacutainers for 13 weeks, commencing one week before infection. Serum was separated and kept at -20^{o}C until analyzed.

Biochemical analysis:

Aspartate aminotransferase (AST) was determined according to Reitman and Frankel (1957). Total proteins and albumin were determined by the methods of Welchselbaum (1946) and Kertsman (1971), respectively; urea nitrogen (Evans, 1968), creatinine by (Hare, 1950 and Kostir and Sonka (1952), Sodium and potassium by Varley (1967).

Necropsy:

Necropsy was performed on all animals that died during the experiment on days 5, 27, 33, 35, 42, 50 post infection (PI) and on euthanized goats (After recumbency) (on days 66 and 85 PI). Specimens from brains, livers, hearts, spleens, lymph nodes, lungs, kidneys, adrenal glands and testicles fixed in 10% formalin. They were processed by conventional method, embedded in paraffin sectioned at 4-5µm and stained with haematoxylin and eosin (H&E).

Statistical analysis was performed using SPSS programme version 9.05. Significant levels were taken at $p \leq 0.05$.

Results

Clinical signs:

All infected Nubian male goats manifested lethargy, weakness, pale mucous membranes, rough coats, emaciation, recumbency followed by death of eight animals. All control animals were active and apparently healthy till the end of experimental period 13 week).

Gross Pathology:

Lesions included general emaciation, muscle atrophy, pale mucous membranes, gelatinous and haemorrhagic kidneys, hydropericardium, flabby hearts and congestion of brain blood vessels.

Histopathology:

Sections of the hearts of the infected animals demonstrated separation of myocardial muscle bundles, mononuclear cells infiltration (Fig.2) and vacuolation of Purkingi cells. The lungs showed thickening of the alveolar septa and interstitial tissue with proliferation of fibroblasts and infiltration of mononuclear cells (lymphoid cells, plasma cells and macrophages); alveolar oedema and emphysema were also seen (Fig. 3).

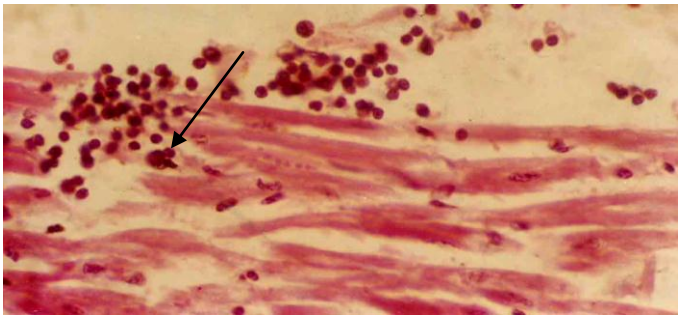

Fig. 2: Heart of a Nubian male goat experimentally infected with *T. vivax* showing separated muscle cells and infiltration of mononuclear cells. (H&E X 100).

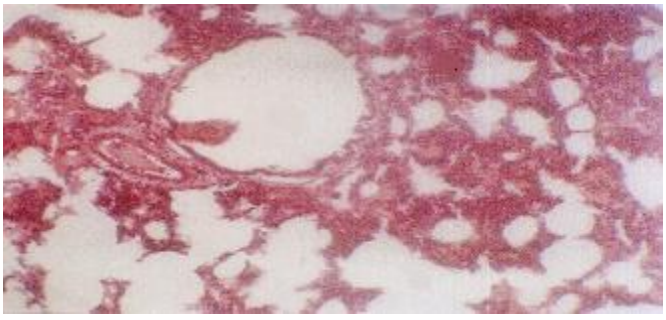

Fig. 3: Lung of a Nubian male goat experimentally infected with *T. vivax* showing a dilatation bronchioles. (H&E X 100.

In the liver, displayed hepatocellular swelling with collapsed or congested sinusoids, portal infiltration of lymphoid cells and a few polymorphonuclear cells were observed (Fig. 4); areas of coagulative necrosis associated with vascular thrombosis were occasionally seen. Spleen displayed deposition of haemosiderin pigment. Brain showed gliosis and congested blood vessels (Fig. 5). Some brain sections showed vacuolation of cerebral grey matter around degenerated contracted neurons. Changes in the kidneys included congestion in the medullary rays, degeneration and fragmentation of tubular epithelial cells. Other sections showed segmented glomerular tufts with presence of homogenous pink deposits. There was a depletion of cortical lymphoid follicles, oedema and thrombosis of blood vessels. Hemorrhages were present in the zona glomerulosa fasiculata with degenerative changes in the cortex. Testes showed degeneration and dissociation of seminiferous tubules cells, oedema, congestion and infiltration of mononuclear cells with proliferation of fibrous connective tissues. In certain areas the testicular tissue was replaced by multigranulomas. Focal interstitial mononuclear cell infiltration was seen in the epididymis (Fig. 6).

Biochemical changes:

Biochemical changes in serum (P<0.5) are shown in Figs. 7 and 8. A significant increase (P<0.5) was found in AST, total proteins, globulins and urea values in infected animals) when compared to uninfected controls. No significant changes were observed in other parameters.

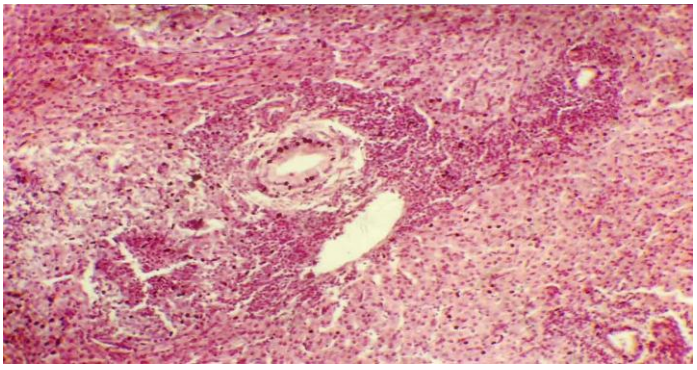

Fig. 4: Liver of a Nubian male goat experimentally infected with *T. vivax* showing intense infiltration of lymphoid cells in the portal tract. (H&E X 100).

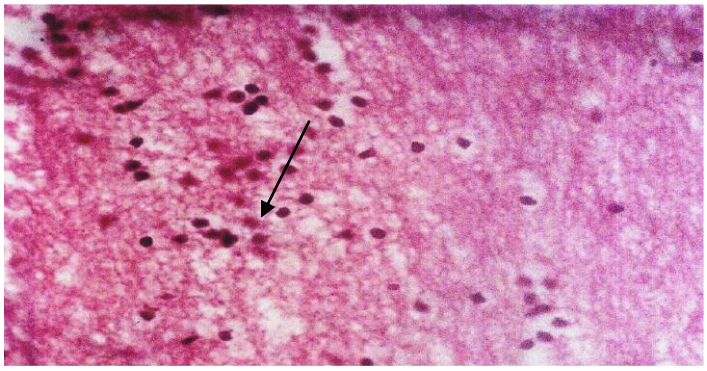

Fig. 5: Brain of a Nubian male goat experimentally infected with *T. vivax* showing gliosis (arrow) (H&E X 100).

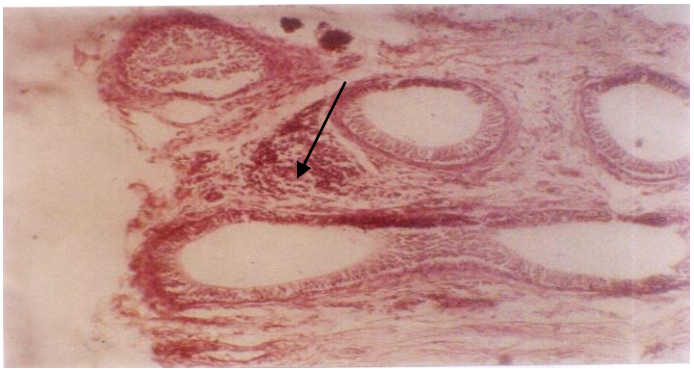

Fig. 6: Epididymis of a Nubian male goat experimentally infected with *T. vivax* showing focal infiltration of mononuclear cells (arrow) (H&E X 100).

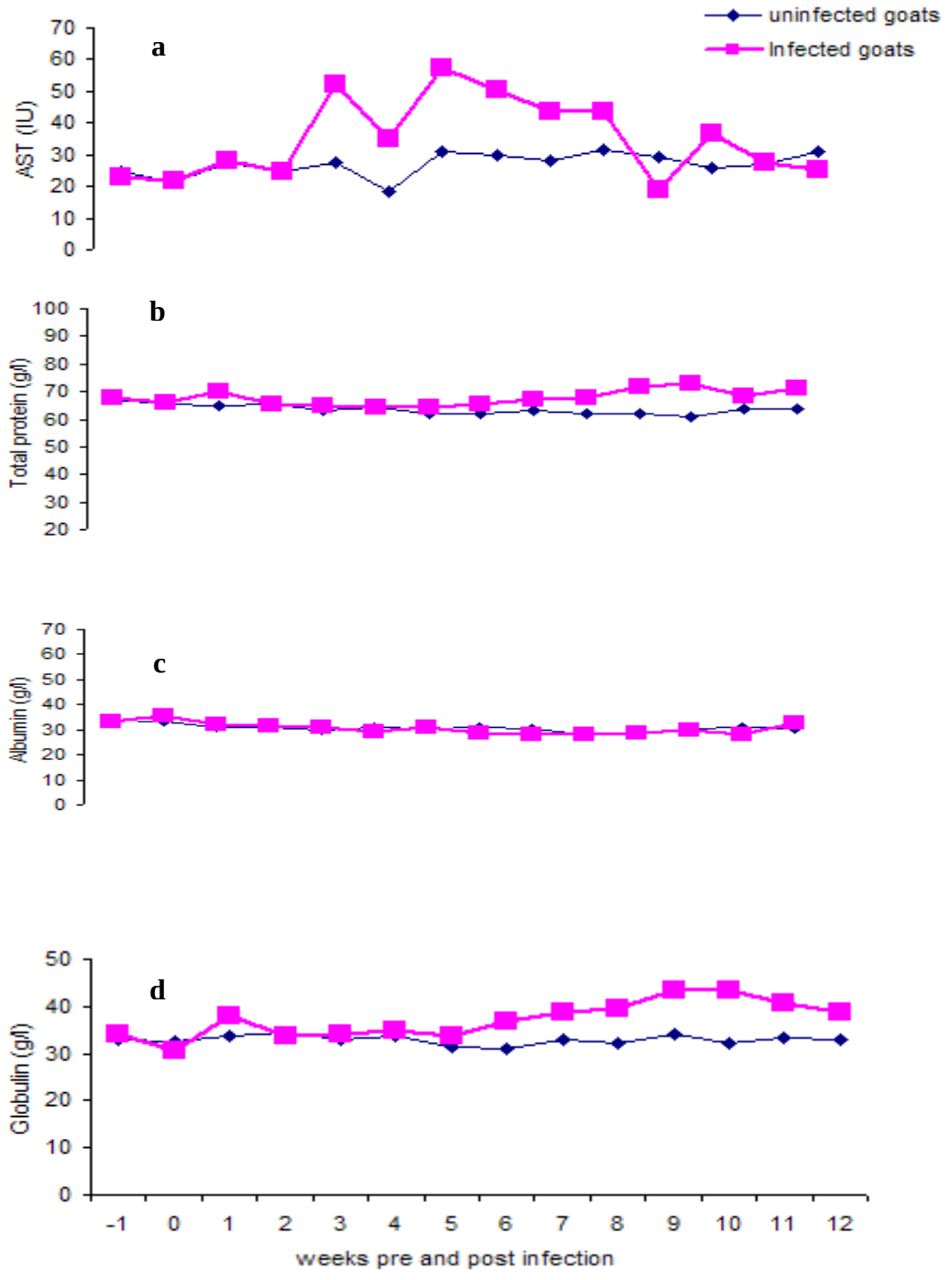

Fig. 7: Mean values of serum Aspartate aminotranspherase, Total proteins, Albumin and Globulin in Nubian male goats experimentally infected with *T. vivax* and their controls.

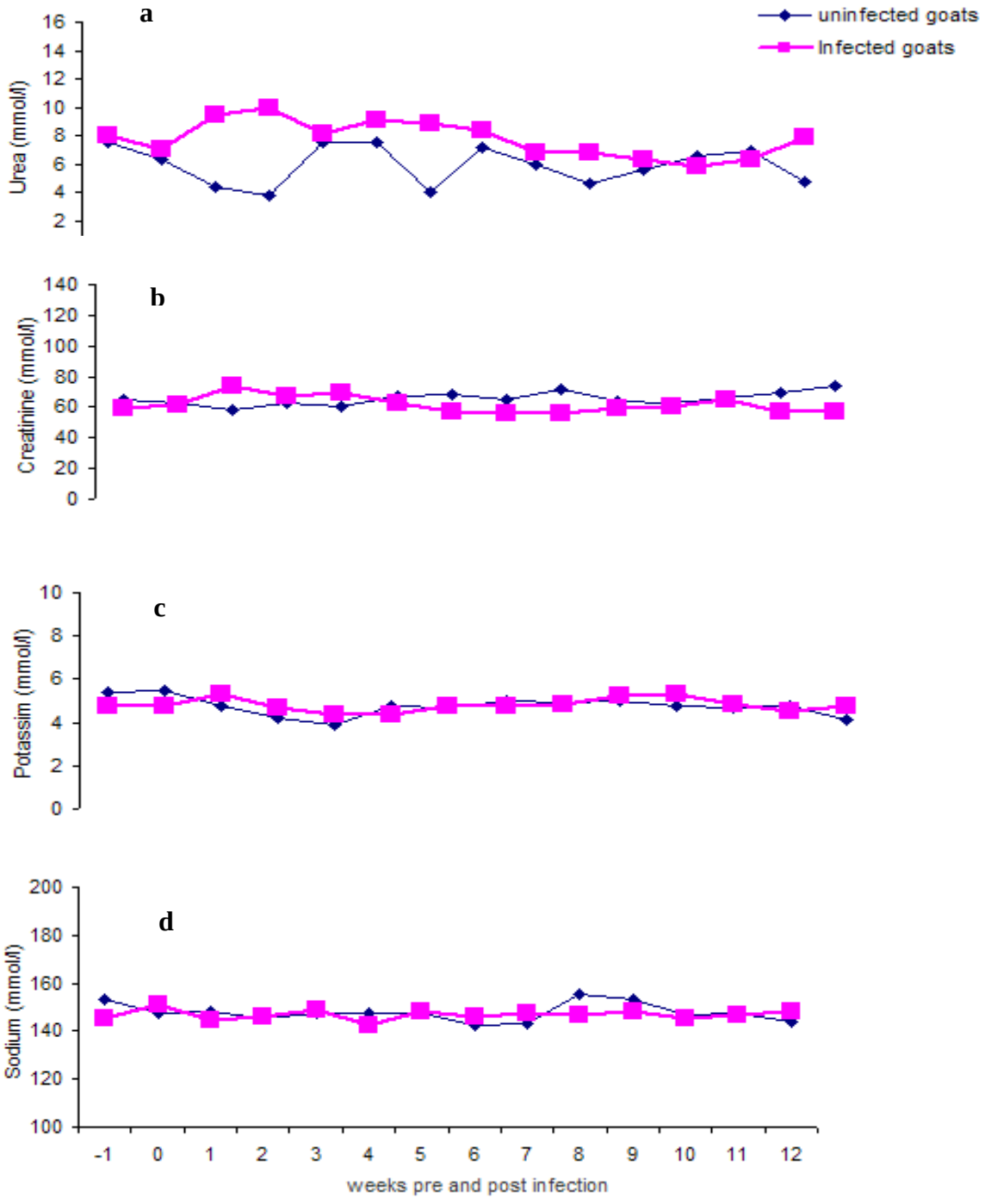

Fig. 8: Mean values of serum, urea, creatinine, potassium and sodium in Nubian male goats experimentally infected with *T. vivax* and their controls.

Discussion

In *T. vivax* infection the intensity of histopathological changes in different organs is always related to the state of chronicity in infected animals. *T. vivax* is always described as haematic organism that rarely invades tissues (Soulsby, 1982). However, our findings indicate that the parasite could invade tissues and this agrees with the findings of Losos (1986) who ascertained the invasive capacity of *T. vivax* to organs particularly the heart.

The results showed a significant increase in AST activity in infected animals. This agrees with the finding of Ogunsanmi *et al*. (1994) in *T. brucei* infection in West African goats (Egbe-Nwiyi *et al*. (2005) in *T. congolense* infection in experimental rats. The increase in AST activity might be partially due to hepatic damage caused by *T. vivax*. However, as AST enzyme is not a liver specific, the increase might also be associated with damages in other organs especially the heart and kidneys.

The present results show a significant increase in serum total proteins. Similar findings were reported by Anosa and Isoun (1976) in goats and Abenga and Anosa (2005) in experimentally infected velvet monkeys. This increase corresponded to an increase in globulins, while serum albumin showed no significant change. On the contrary, Otesile *et al*. (1991) reported hypoproteinaemia in *T. brucei* infected boars and Anosa and Isoun (1976) and Akingbemi *et al* (1995) reported hypoalbuminaemia and hyperglobulinaemia in *T. vivax* and *T. congolense* infected cattle and sheep, respectively. The hyperglobulinaemia observed after the first week of the infection might be due to the raised total proteins due to an immunological response towards the infection.

There was a significant increase in urea values in goats' infected and then *T. brucei* and *T. congolense*. This might be attributed to renal damage, as seen here, as a result of anaemia or accumulation of antigen antibody complexes in the kidneys and thus impairment of urea excretion. Although creatinine level showed no significant change in infected groups, a slight decrease in its concentration was shown to occur in infected goats, similar decrease was reported by Akingbemi *et al.* (1995) in sheep. However, Ogunsanmi *et al* (1994) reported an increase in creatinine levels in *T. brucei* infected sheep. The decrease in creatinine might be partly correlated to haemodilution (Akingbemi *et al.* 1995) or due to emaciation resulting from the decrease in muscle mass (Sukker *et al,* 1993).

In this study serum potassium and sodium concentrations did not show significant changes in *T. vivax* infected goats. This is in line with the findings of Otesile *et al.* (1991) in *T. brucei* infected boars. The ill-health and death of animals following trypanosome infections may be attributed mainly to the multiorgan failure and tissue damage associated with haemato and sero-biochemical changes (Urquhart, 1980; Anosa, 1988; Brown *et al.* 1990).

Most of the clinical signs and post mortem findings encountered in the current study were more or less similar to those previously reported by Losos and Ikede (1972) and Maikaje *et al.* (1991) in *T. vivax* infected sheep and by Saror (1980) in infected red Sokoto goats. In addition, the histopathological changes observed are in agreement with

the findings of Losos and Ikede (1972), Isoun (1975), Van den Ingh *et al.* (1976a), Saror (1980), Masake (1980), Olubayo and Mugera (1985), Gardiner *et al.*(1989) and Dargantes *et al*. (2005) in different host trypanosomes animal infections models. The Heart, liver and Lung changes observed here are similar to those reported by Van den Ingh *et al.* (1976) in *T. vivax* infected goats and cattle (Gradiner *et al*, 1989). Changes in heart muscles might be due to anaemia induced by *T. vivax* infection. Haemosiderosis in the spleen might be attributed to haemorrhages and haemolysis associated with *T. vivax* infection. Kidney changes might have been related to the anaemia or the accumulation of antigen antibody complexes. Changes Lymph nodes are similar to the findings of Dargantes *et al*. (2005) in *T. evansi* infected goats.

The present study has showed that *T. vivax* stocks circulating outside the tsetse belt in Sudan can maintain their virulence for a long time. The results also indicate that Nubian male goats (the most numerous goat eco-type present in the Sudan) are highly susceptible to *T. vivax* infection transmitted by intravenous injection of infected blood. In this context, it has been reported that, outside the tsetse fly belt, *T. vivax* infection can be maintained through mechanical transmission by biting flies (A/Rahman, 2005; Osorio *et al*. 2008), and may serve as a source of infection to other livestock. Further investigation on the susceptibility of Nubian goats to other Trypanosoma species, their role in the epizootiology of trypanosomiasis and the role of biting flies in the transmission of the parasite is highly warranted.

Acknowledgements

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References

A/Rahman, A.H. (2005). *Rev. Sci. Tech*., **24**(3): 965-972.

Abenga, J.N. and Anosa, V.O. (2005). *Afri. J. biotech***., 4:** 187-190**.**

Adamu, S.; Fatihu, M.Y.; Useh, N.M.; Mamman M.; Sekoni, V.O and Esievo K.A (2007). *Vet Parasitol*., **143**(1):29-34.

Akingbemi, B.T.; Mary, N, Madekurozwa. and Joshua, R.A. (1995). *Bull. Anim. Hth. Prod. Afri.*, ***XLIII*** (4): 269-275.

Anosa, V.O. (1988). *Rev. Elev. Med. Vet. Pays. Trop.*, **41** (2):151-164.

Anosa, V.O. and Isoun, T.T. (1976). *Trop. Anim. Hlth. Prod.*, **8**:14-19.

Brown, C.G.D.; Hunter, A.G. and Lukins, A.G. (1990). In *Handbooks on Animal Diseases in the Tropics*. 4th edn., M.M.H Sewell and D.W. Brocklesby (eds.). UK. Pp. 205-226.

Clarkson, M.J.; Penhale, W.J. and Mckenna, R.B. (1975). *J. Comp. Pathol.*, **85**:397-410.

Dargantes, A.P.**; Campbell, R.S.F.; Copeman, D. and Reid, SA. (2005)**. *J. Comp.Pathol*., **133**: 267-276.

Egbe-Nwiyi, T.N.; Igbokwe, I.O. and Onyeyili, P.A. (2005). *Afri. J. Bio. Res*.,8:197-201.

Evans, R.T (1968). *J Clin Pathol*. **21**(4):527-529.

Gardiner, P.R. (1989). *Adv. Parasitol.*, **28**: 229-317.

Gray, A.R. (1963). *Exp Prasitol.*, **14**:374-381.

Hare, R.S. (1950). *Pro. Soc. Exp. Biol. Med.*, **74**: 148.
Isoun, T.T. (1975). *Acta Trop.*, **32**(3):267-72.
Kendrick, R.K. (1968). *Vet. Bull.*, **38** (4): 191-197.
Kertsman, V.I. (1971). *Chemist. electract*., **74** :7.
Kostir, J.V. and Sonka, J.A. (1952). *Biochem. Biophys. Acta (Amst).*, **8**: 86-89.
Losos, G. J. (1986). *Infectious Tropical Diseases of Domesticated Animals*, 1st edn. Longman, USA, Pp.183-318.
Losos, G.J. and Ikede, B.O. (1972). *Vet. Pathol.*, **9**:1-71.
Maikaje, D.B; Sannusi, A; Kyewalabye, E.K. and Saror, D.I. (1991). *Vet. Parasitol.*, **38**(4): 267-274.
Masake, R. A. (1980). *Vet. Rec.*, **107**: 551-557.
Murray, M. (1974). The Pathology of African trypanosomiasis. In: C. Brent. and E. J. Holborow (eds.). *Progress in Immunology*, IIth, edn. North Holland Publishing Company, Amsterdam. The Netherlands. Pp. 181-192.
Ogunsanmi, A.O.; Akpavie, S.O. and Anosa, V.O. (1994). *Rev. Elev. Med. Vet. Pays. Trop.*, **47** (2): 195-200.
Olubayo, R.O. and Mugera, G.M. (1985). *Bull. Anim. Hlth. Prod. Afri*., **33:** 211-217.
Osaer, S.; Akinbamijo, O.O and Goossens, B. (2000). *Acta. Trop.*, **75**: 229-241.
Osorio, A.L.A.R., Madrugu, C.R., Desquesnes, M., Soares, C.O., Ribeiro, L.R.R and Goncalvesdacosta, S. C. (2008). *Mem.Inst.Oswaldo cruz*. **103**(1): 1-13.
Otesile, E. B; Fagbemi, B.O. and Adeyem, O. (1991). *Vet. Parasitol.*, **40**:207- **a** 5.
Reitman, S. and Frankel, S. (1957). *Am. J. Clin. Pathol.*, **28**: 51.
Saror, D. I. (1980). *Res. Vet. Sci*., **28**: 36-38.
Sayer, P.D.; Morrison,W.I.; Preston, J.M.; Hird, S.F.; Price, J.E. and Murray, M. (1977). *African trypanosomiasis in the dog.* ISCTRC.15th meeting, Banjul, the Gambia. OAU/STRC., **110**, 489-496.
Soulsby, E.J.L. (1982). *Helminths, Arthropods and Protozoa of domistacted animals,* **7th** edn**.** Bailliere Tindall, London, England**,** P.522**.**
Sukker, M.Y.; EL-Munshid, H.I. and Ardawi, M.S.M. (1993). *Concise Human Physiology,* 2nd edn. Black well scientific publications. London,UK. 440.p.
Urquhart, G. M. (1980). *Trans. R. Soc. Trop. Med. Hyg.*, **74**(6): 726-729.
Van Bogaert L. (1962): Experimental trypanosomal encephalitis: in Innes and Saunders, (editors) *Comparative Neuropathology*, New York Academic Press, USA, Pp. 465-481
Van den Ingh, T.S.; Zwart, D.; Schtoman, A.J.; Van Miert, A.S. and Veenendaal, **G. H. (1976).** *Res. Vet. Sci.*, **21** (30): 264-270.
Varley, H. (1967). *Practical Clinical Biochemistry*. 4thedn, Williams Heinemann ltd. and interscience books. London, 802. P.
Weichselbaum, T.E. (1946). *Am. J. Clin. Pathol.*, **16**:40-43.