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Pig trypanosomosis : comparative anaemia and histopathology of lymphoid organs

OMEKE (B.C.O.), UGWU (D.O.). La trypanosomose porcine : anémie et histologie comparées des organes lymphoïdes. *Revue Élev. Méd. vét. Pays trop.*, 1991, **44** (3) : 267-272

Après avoir infecté expérimentalement des porcs par des espèces pathogènes de *Trypanosoma brucei brucei* et de *Trypanosoma congolense* (le premier se montrant plus virulent que le second), les auteurs ont étudié un syndrome d'anémie accompagné des symptômes cliniques caractéristiques, les modifications hématologiques et d'histopathologie des organes lymphoïdes. Les périodes moyennes d'incubation ont été de 4 à 7 jours et on a observé des niveaux, généralisés mais variables, de la parasitémie et de la pyrexie sans mortalité. Les autres symptômes ont été l'hyperémie, des hémorragies pétéchiales conduisant à des plaques ou à des lésions, de l'innapétence, de la déshydratation avec ou sans ascites. La trypanosomose a abaissé significativement (P < 0,01) la valeur de l'hématocrite (PCV), la concentration en hémoglobine et les globules rouges. Elle a par contre accru la numération différentielle des globules blancs des animaux infectés. Les trypanosomes étaient localisés dans les tissus lymphatiques qu'ils ont détruits et dont les lésions essentielles étaient des hémorragies, la prolifération des infiltrations par les mononucléaires, la déformation des follicules et la nécrose ou la fibrose des tissus. Les auteurs mettent bien en évidence les effets significatifs résultant de l'immunosuppression, l'érythrophagocytose et par voie de conséquence des infections secondaires qui sont le lot de la production porcine dans les zones de trypanosomoses endémiques. *Mots clés* : Porc - Trypanosomose - Anémie - Histopathologie - Organe lymphoïde - Nigeria.

INTRODUCTION

Recent findings on African trypanosomosis (1, 11) confirm that trypanosomes other than *Trypanosoma simiae* are pathogenic to pigs. Similar observations have been made here at the Veterinary Teaching Hospital of the University of Nigeria, Nsukka.

Anaemia is a consistent finding (2) and tends to adversely limit livestock production (10, 14). It is suggested that consequent destruction of lymphoid organs predispose infected animals to severe anaemia. Besides normal functions of lymphoid organs including body defence mechanisms and erythropoiesis (4, 15), are impaired. To what extent this is true for pigs is subject to proper investigations.

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*. Pfizers'Nig. Ltd.

**. Merck, Sharpe, and Dohme. Ag. Vet.

In view of significance being placed on this disease (1, 11) in the recent times, the present study was carried out to comparatively evaluate haematological parameters and histopathology of certain lymphoid organs (lymph nodes, spleen, and liver) of pigs experimentally infected with pathogenic strains of *Trypanosoma brucei brucei* and *T. congolense*. Findings are related to pig productivity in endemic areas.

MATERIALS AND METHODS

Eighteen crossbred pigs aged between 11 and 13 months were purchased from a tsetse-free area of Northern Nigeria and used in the study between November 1989 and August 1990.

On arrival at Nsukka, they were quarantined in a fly-proof house for 6 weeks. During this period they were screened for haemoparasites, dewormed with Piperazine Adepate[R*] and given Amprolium[R**] in the feed. They were maintained on a concentrate diet (16 % crude protein) at a rate of 1.4 to 1.6 kg/pig/day.

At the end of acclimatisation, the pigs were randomly divided into three equal groups (A, B and C) of 6 each, and kept in three separate pens. Pigs in group A were infected with strain Y58/98 of *T. brucei* while those in group B were infected with strain Y58/35 of *T. congolense*. Both pathogenic trypanosomes were obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, where they were characterized. Pigs in group C were left as uninfected controls.

To obtain trypanosome strains for the pig infection, a sample was first injected into a group of mice at NITR. They were brought live to Nsukka. At peak parasitaemia, mice were bled using disodium ethylene diamine tetraacetic acid (EDTA) as anticoagulant. Each trypanosome suspension was diluted to a known concentration using a phosphate buffered saline (PBS) solution. About 1.8×10^6 trypanosomes of a particular strain was injected intraperitoneally to each pig in the respective groups.

Pigs were monitored daily for clinical symptoms and for levels of parasitaemia according to methods described by ASHMANN and SEED (3). Haematological value including packed cell colume (PCV), haemoglobin concentration (Hb), red cell corpuscules (RBC), and white cell cor-

puscules (WBC) were determined on a regular basis and according to the methods of KELLY (12) and COLES (5). All pigs were weighed at the start of the experiment and at slaughter.

Upon slaughter, aspirates were taken from the lymph node, spleen and liver and injected intraperitoneally into groups of healthy mice. Tail blood of these mice was microscopically examined each day for at least 10 days for the presence of trypanosomes. Part of each lymphoid organ was rinsed twice in PBS and cut into two, so that impression smears were made from the freshly cut surfaces. Smears were stained with Grunwald-Giemsa and examined under the microscope for presence of trypanosomes. The other part was fixed in Zenker's formol solution for 24 h, washed in running water for 24 h, processed through isopropanol chloroform series, and embedded in paraffin wax. Sections were cut at 5µ, mounted on clean slides and stained with Erhlich's haematoxylin and eosin for microscopic studies.

RESULTS

Mean incubation periods of 4 and 7 days, maximum (fluctuating levels of parasitaemia of 8 and 23 trypanosoes per high power microcopic field (HPF), and onset of clinical symptoms after 6 and 11 days post-infection were recorded for *T. b. brucei* and *T. congolense* infected pigs respectively (table I). None of the pigs consequently died, although infected groups gained less body weight than the controls which appeared healthy.

***TABLE I** Incubation period, levels of parasitemia, and body weights of pigs experimentally infected with* **Trypanosoma brucei brucei** *(A) and* **Trypanosoma congolense** *(B).*

Variables	Animal groups		
	A	B	C (controls)
Number of pigs used	6	6	6
Incubation period (days) (average)	3-7 (4)	6-11 (7)	— —
*Levels of parasitemia (average)	3-16 (8)	8-33 (23)	— —
Mean body weights (kg) at start of experience	56.3 ± 1.7[a]	54.2 ± 0.9[a]	56.3 ± 0.9[a]
at slaughter	59.8 ± 3.6[a]	59.6 ± 1.4[a]	75.0 ± 1.3[b]
Mean weight gain	3.5 ± 2.4[a]	5.4 ± 2.0[a]	18.7 ± 1.6[b]

* Parasitemia fluctuated, including a parasitemia in some individual pigs.
[ab] : $P < 0.05$.

Clinical symptoms were similar for both groups of infected pigs, except that they appeared more severe for those in group A. Symptoms included fluctuating pyrexia (38.4 - 41.4 °C), hyperaemia and parasitaemia ; loss of appetite, petechiations of the ears, genitals and ventral abdomen and dullness. Later on, dehydration ascites and scrotal plaques and/or lesions were observed.

Figure 1 shows that both trypanosomes significantly ($P < 0.01$) and progressively lowered the haematological values, except for the WBC differentials that rather tended to increase (table II). Values for control pigs were

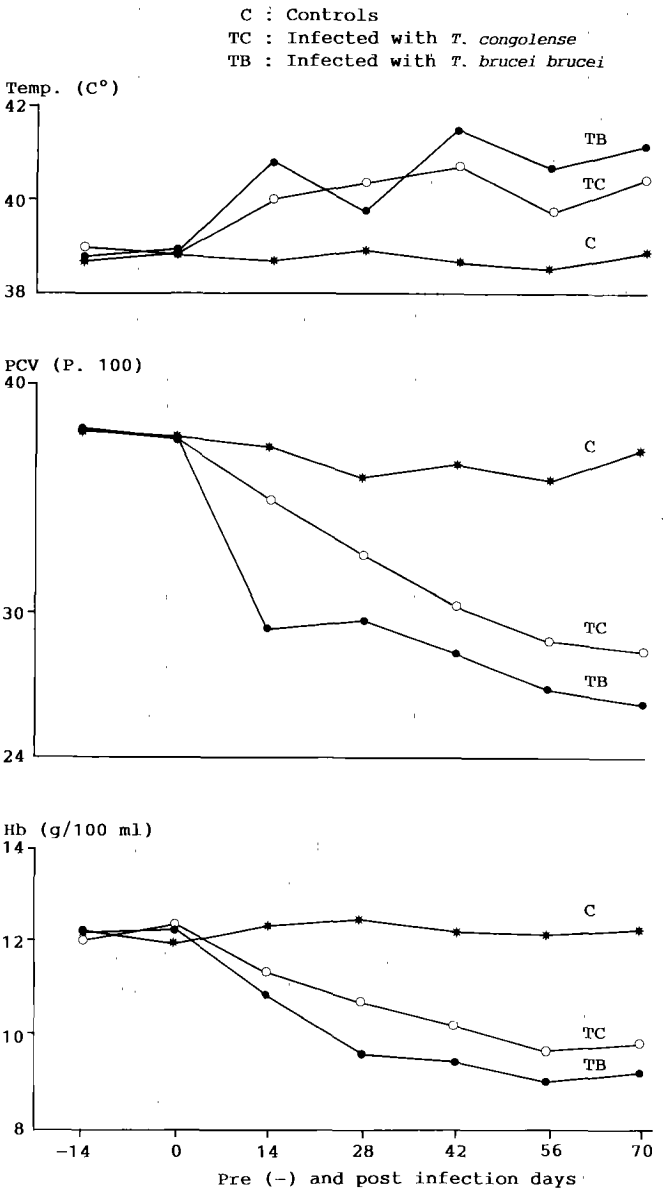

Fig. 1 : Mean body temperature, packed cell volume and haemoglobin concentration of pigs infected with trypanosome.

TABLE II *Mean [a]RBC and [b]WBC (differentials) of pigs experimentally infected with* **Trypanosoma brucei brucei** *and* **T. congolense.**

Treatment group	Blood cell types	Post infection weeks					
		- 2	0	2	4	6	8
A	RBC (× 10^6/mm^3)	6.7	6.8	4.7	4.3	4.4	4.1
	WBC (× 10^3/mm^3)	7.3	7.0	10.0	13.1	16.3	15.1
	% neutrophiles	40.3	31.8	28.3	19.9	19.7	21.7
	% lymphocytes	51.6	52.0	70.0	73.0	71.0	72.3
	% monocytes	4.5	3.8	3.0	5.3	6.3	4.7
	% eosinophiles	1.5	0.8	2.3	0.6	1.0	0.3
	% basophiles	—	—	—	—	—	—
	Presence of trypanosomes	—	+ve (4)*	+ve (3)	+ve (1)	+ve (3)	+ve (2)
B	RBC	6.2	6.8	5.5	5.1	5.1	4.8
	WBC	6.4	8.5	8.3	10.7	16.7	18.5
	% neutrophiles	41.5	42.0	29.3	25.0	30.1	28.3
	% lymphocytes	52.2	52.7	64.7	67.0	62.6	77.3
	% monocytes	5.0	5.3	5.0	6.0	6.3	5.7
	% eosinophiles	1.2	0.8	0.6	0.6	1.0	0.3
	% basophiles	0.3	—	—	—	0.1	—
	Presence of trypanosomes	—	—	+ve (6)	+ve (4)	+ve (5)	+ve (5)
C	RBC	6.1	6.2	5.9	5.8	5.0	5.9
	WBC	7.4	7.0	6.8	6.9	6.8	6.8
	% neutrophiles	36.3	37.4	36.3	39.6	38.0	38.0
	% lymphocytes	54.0	54.6	56.0	57.0	55.0	56.4
	% monocytes	6.7	5.6	4.6	3.0	4.2	4.0
	% eosinophiles	2.4	1.4	1.1	2.4	2.8	2.7
	% basophiles	0.6	—	—	—	—	—
	Presence of trypanosomes	—	—	—	—	—	—

[a]RBC : red blood corpuscles ; [b]WBC : white blood corpuscles.
* : number of pigs positive with trypanosomes.

TABLE III *Localisation of* **Trypanosoma brucei brucei** *(A), and* **T. congolense** *(B) in lymphoid organs of infected pigs.*

Pig		Post-infection slaughter week	Organ smears						Mean incubation in mice (days)
Group	Identity		L.N.	Spleen	Liver	L.N.	Spleen	Liver	
A	Tb_1	2	++	+	+	P	P	P	4.2
	Tb_2	4	++	++	+	P	P	P	4.6
	Tb_3	6	+++	++	+	P	P	P	5.1
	Tb_4	8	+++	++	++	P	P	P	5.1
	Tb_5	9	++	++	+	P	—	—	6.2
	Tb_6	10	+++	++	—	P	P	P	5.0
B	Tc_1	2	—	—	—	—	—	—	9.6
	Tc_2	4	+	+	—	P	P	—	7.2
	Tc_3	6	++	+	+	P	P	P	6.9
	Tc_4	8	+	—	—	P	P	—	10.2
	Tc_5	9	—	—	—	—	—	—	9.6
	Tc_6	10	+	+	+	P	P	—	9.0

+ : level of parasitemia ; P : positive (i.e. trypanosomes isolated in mice).

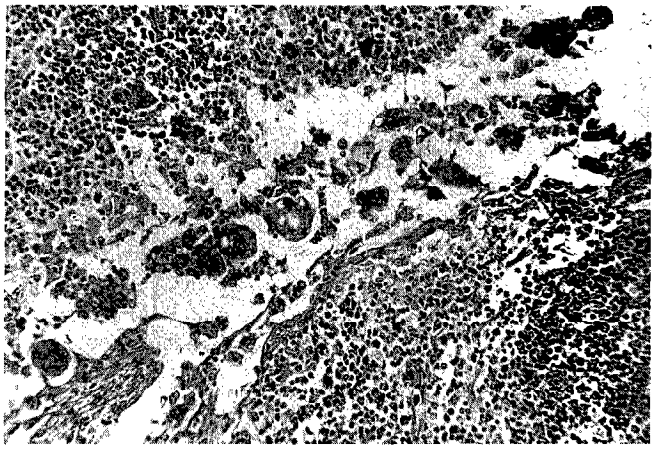

Photo 1 : Pig lymph node showing infiltration of monocyte, and Russel's bodies.

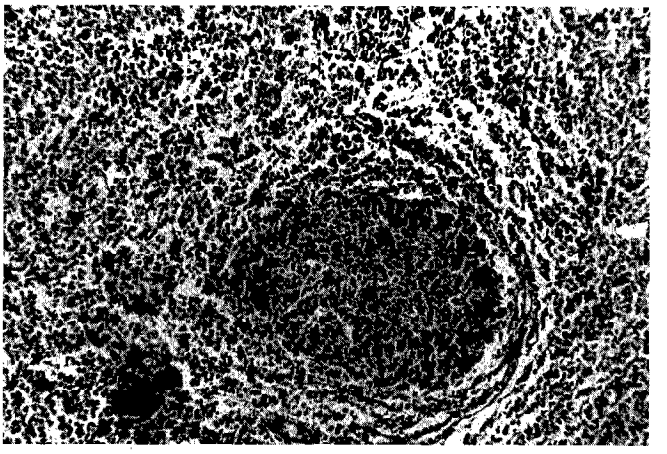

Photo 2 : Pig lymph node : lymphocyte repopulation due to T.B. brucei *infection (H & E x 80).*

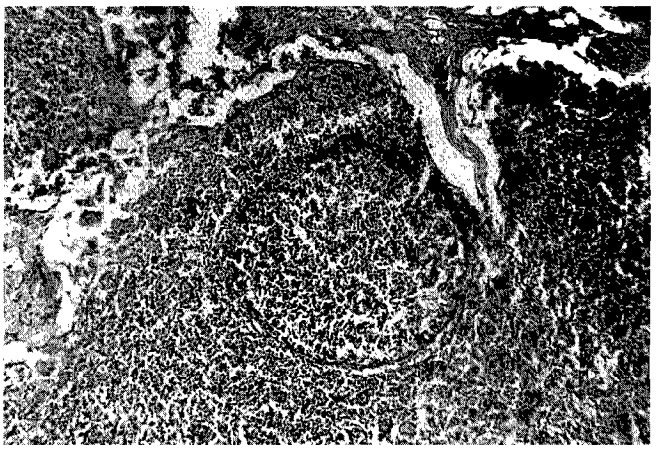

Photo 3 : Reactive nodules, areas of tissue necrosis and/or fibrosis, and lymphoid depletion due to T. congolense *infection.*

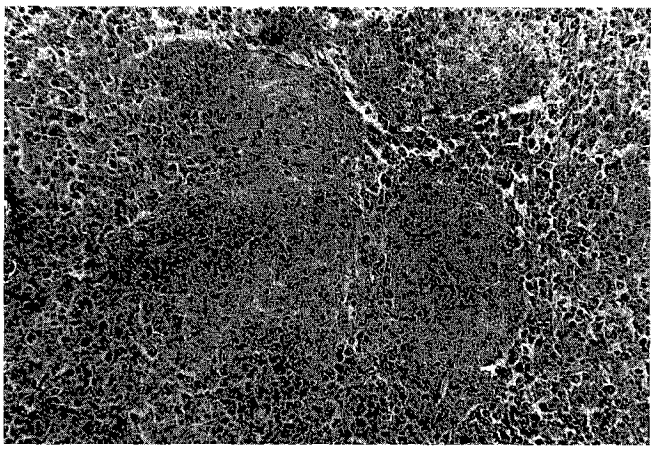

Photo 4 : Pig spleen, showing depletion of lymphoid tissue and hyperplasia of reticular cells around sheaths of arterioles (H & E x 32) due to T. congolense *infection.*

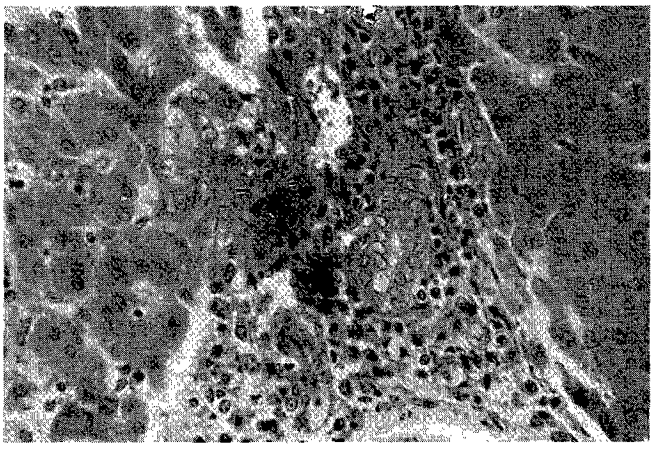

Photo 5 : Pig liver, showing mononuclear cells, germ cell degeneration, and enlargement of interlobular spaces (H & E x 160) due to T. b. brucei *infection.*

normal. Trypanosomes were found abundantly in the lymphoid organs of the pigs (table III), particularly in those infected with *T. b. brucei.*

Plates 1-5 show that the trypanosomes destroyed the tissue structure of the lymphoid organs. General lesions included massive haemorrhage ; perivascular accumulation of mononuclear cells, and Russel bodies ; proliferation and distorsion of primary and secondary follicles, tissue necrosis and/or fibrosis.

DISCUSSION

The results indicate the adverse effects of certain species of trypanosomes on the lymphoid functions of pigs. Both pathogens used were properly classified at NITR and

cannot be mistaken for *T. simiae* (13) usually assumed to be the only strain pathogenic to pigs. AGU and BAJEH (1) reported that the fatal outbreak of trypanosomosis in pigs at Mkar Gboko in the Benue State of Nigeria was due to *T. b. brucei*. These authors further infected pigs experimentally and confirmed this adverse effect. In the same way, KAGERUKA (11) noted the *T. congolense* was more prevalent and destructive to pigs than other trypanosomes in Zaire. In the present work, both strains of trypanosomes were confirmed to be pathogenic to pigs.

Although both trypanosomes failed to cause mortality in the adult pigs, similarity in clinical symptoms, changes in haematological values of infected pigs were noteworthy. Anaemia was evident in both infections. However, according to the results, *T. b. brucei* appeared to be more virulent than the *T. congolense* strains. This may be due to the preferential localisation of *T. b. brucei* in tissues as opposed to *T. congolense* that localises more in the blood (8, 17). This agrees with observations made by LOSOS and IKEDE (14) that fever is more significant with *T. brucei* while anaemia is more prominent with *T. congolense* infection. In both infections, there was destruction of lymphoid tissues leading to impairment of their functions. Pig production is adversely affected. IGBOKWE (7) similarly observed dyserythropoises due to erythroid injury and depressed erythroprotein and haemoglobin synthesis in infected animals.

The mechanisms leading to trypanosomosis anaemia are basically the same for all animals. However, it remains unclear which factors determine its pathogenicity. According to ANOSA (2), the resultant immunological complexes, and the activation of the complement and mononuclear phagocytosis system are mainly responsible for this disease. Several research workers (6, 16, 18) consider that extra-and intravascular haemolysis, haemodilution and inhibition of erythropoesis are major factors to trypanosomosis anaemia. IKEDE *et al.* (9) associated the mechanisms of anaemia with types of trypanosomes, because of their variations in strain antigenicity.

It is clear that trypanosomosis is characterized by the destruction of lymphoid tissues the effects of which include immunosuppression, erythrophagocytosis (7) and hence a poor animal performance, especially as they are prone to secondary intercurrent diseases (13, 14, 15).

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OMEKE (B.C.O.), UGWU (D.O.). Pig trypanosomosis : comparative anaemia and histopathology of lymphoid organs. *Revue Élev. Méd. vét. Pays trop.*, 1991, **44** (3) : 267-272

Anaemia with characteristic clinical symptoms, haematological changes and histopathology of lymphoid organs, was observed following experimental infection of pig with pathogenic species of *Trypanosomosa brucei brucei* and *Trypanosoma congolense*, the former being more virulent than the latter. Mean incubation periods were 4 and 7 days, and generalized fluctuating levels of parasitaemia and pyrexia without mortality were observed. Other symptoms included hyperaemia, petechial haemorrhages leading to plaques or lesions, inappetence, dehydration and/or ascites. Trypanosomosis significantly ($P < 0.01$) lowered the packed cell volume (PCV), haemoglobin concentration and red blood cells, but elevated white blood count (differentials) of infected pigs. Trypanosomes were localised in and destroyed the lymphoid tissues, the major lesions of which included haemorrhages, mononuclear infiltration prolification and distorsion of follicles, and tissue necrosis or fibrosis. Significant effects of resultant immunosuppression, erythrophagocytosis and hence secondary infections to pig production in trypanosome endemic areas are highlighted. *Key words* : Pig - Trypanosmosis - Anaemia - Histopathology - Lymphoid organs - Nigeria.

OMEKE (B.C.O.), UGWU (D.O.). Tripanosomosis del cerdo : comparación de la anemia y de la histopatología de los órganos linfoideos. *Revue Élev. Méd. vét. Pays trop.*, 1991, **44** (3) : 267-272

Los autores infectaron experimentalmente cerdos con especies patógenas de *Trypanosoma brucei brucei* y *Trypanosoma congolense* (siendo el primer más virulento que el segundo). Estudiaron un síndrome de anemia con síntomas clínicos característicos, modificaciones hematológicas e histopatología de los órganos linfoideos. El periodo medio de incubación fue de 4 a 7 días y se observaron niveles, generalizados pero variables, de la parasitemia y de la pirexia sin mortalidad. Otros síntomas fueron congestión, hemorragia causando placas o lesiones, inapetencia, deshidratación con o sin ascitis. La tripanosomosis reduzco significativamente ($P < 0,01$) el valor del hematocrita (PCV), la concentración de hemoglobina y los eritrocitos. En cambio, aumentó el recuento diferencial de los leucocitos de los animales infectados. Los tripanosomos se situaban en los tejidos linfáticos que destruyeron y cuyas lesions esenciales eran hemorragias, la proliferación de las infiltraciones por los mononucleares, la deformación de los folículos y la necrosis o la fibrosis de los tejidos. Los autores evidencian bien los efectos significativos resultando de la inmunosupresión, la eritrofagocitosis y en consecuencia infecciones segundarias que ocurren generalmente en las crías de cerdo en las zonas de tripanosomosis endemicas. *Palabras claves* : Cerdo - Tripanosomosis - Anemia - Histopatología - Organo linfoideo - Nigeria.

REFERENCES

1. AGU (W.E.), BAJEH (Z.T.). An outbreak of fatal *Trypanosoma brucei brucei* infection of pigs in Benue State of Nigeria. *Trop. Vet.*, 1986, **4** : 25-28.
2. ANOSA (V.O.). Haematological and biochemical changes in human and animal trypanosomiasis. Part II. *Revue Élev. Méd. vét. Pays trop.*, 1988, **41** (3) : 277-281.
3. ASHMANN (P.U.), SEED (J.R.). The effects of photoperiod and *T. brucei gambiense* infection on the reproductive organs of male *Microtus montanus*. *J. reprod. Fert.*, 1974, **40** : 51-60.
4. CALVAO-CASTRO (B.), HOCHMAN (A.), LAMBERT (P.N.). The role of host-immune response in the development of tissue lesions associated with African trypanosomiasis : in mice. *Clin. expl. Immunol.*, 1978, **33** : 12-18.
5. COLES (K.H.). Veterinary clinical pathology. 3rd ed. Philadelphia, Saunders Co, 1980. Pp. 113-114.
6. FIENNES (R.N.T.W.). Haematological study in trypanosomiasis of cattle. *Vet. Rec.*, 1954, **66** : 423-434.
7. IGBOKWE (I.O.). Dyserythropoiesis in animal trypanosomiasis. *Revue Élev. Méd. vét. Pays trop.*, 1989, **43** (3) : 423-429.
8. IKEDE (B.O.), LOSOS (G.J.). Pathogenesis of *Trypanosoma brucei* infection in sheep. I. Clinical signs. II. Cerebro-spinal fluid changes. III. Hypophyseal and other endocrine lesions. *J. Comp. Path.*, 1975, **85 (1) :** 23-31 ; 33-36 ; 37-44.
9. IKEDE (B.O.), MARGAREL (L.), TERRY (R.J.). Anaemia in trypanosomiasis : Mechanism of eryhtrocyte destruction in mice infected with *Trypanosoma congolense* or *Trypanosoma brucei*. *Acta. trop.*, 1977, **34** : 53-60.
10. ILEMOBADE (A.A.), BALOGUN (T.F.). Pig trypanosomiasis : effects of infection on feed intake, live weight gain, and carcase traits. *Trop. Anim. Hlth Prod.*, 1981, **83** : 128-136.
11. KAGERUKA (P.). Trypanosomose dans les élevages porcins du bas Zaïre. *Revue Élev. Méd. vét. Pays trop.*, 1987, **40** (1) : 49-53.
12. KELLY (W.R.). Veterinary clinical diagnosis. 2nd ed. London, Baillière Tindall, 1977. Pp. 261-296.
13. KILLICK-KENDRICK (R.), GODFREY (D.G.). A close association between *Glossina tachinoides* and domestic pigs near Nsukka, Eastern Nigeria. *Ann. Trop. Med. Parasitol.*, 1963, **57** (2) : 225-231.
14. LOSOS (G.J.), IKEDE (B.O.). Review of the pathology of diseases in domestic and laboratory animals caused by *T. congolense, T. vivax, T. brucei, T. rhodesiense* and *T. gambiense*. *Vet. Path.*, 1972, **9** : 1-71.
15. MUSOKE (A.J.), NANTULYA (V.M.), BARBET (A.F.), KIRONDE (F.), McGUIRE (T.C.). Bovine immune response in the development African trypanosomiasis : specific antibodies in variable surface glycoproteins of *T. brucei*. *Parasitol. Immunol.*, 1981, **3** : 97-106.
16. OMAMEGBE (J.O.), ORAJAKA (L.J.E.), EMEHELU (C.O.). The incidence and clinical forms of naturally occurring canine trypanosomiasis in the veterinary Clinical in Anambra State of Nigeria. *Bull. Anim. Hlth Prod. Afr.*, 1984, **32** (1) : 23-29.
17. SSENYONGA (G.S.Z.), ADAM (K.M.G.). The number and morphology of trypanosomes in the blood and lymph of rats infected with *Trypanosoma brucei* and *Trypanosoma congolense*. *Parasitology*, 1975, **70** : 255-261.
18. SULIMAN (N.B.), FELDMAN (B.F.). Pathogenesis and etiology of anaemia in trypanosomiasis with special reference to *Trypanosoma brucei* ans *Trypanosoma evansi* (review). *Protozool. Abstr.*, 1989, **13** (2) : 37-45.