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Pathological Observations in *Trypanosoma evansi* Infected Yankasa Sheep

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ABSTRACT

The pathology of *Trypanosoma evansi* infection in Yankasa sheep was studied using an isolate obtained from the blood of an infected camel slaughtered at Kano Abattoir, Kano State, Nigeria. Pathological changes affecting various organs were noted including pale and anemic carcasses and serious atrophy of adipose tissue. The major gross lesions observed were lymphadenitis, lymphadenopathy, and splenomegaly.

Histopathological changes in the infected Yankasa sheep included fatty degeneration involving hepatocytes, erythrophagocytosis, and haemosiderosis associated with focal areas of hepatic necrosis in chronic cases. Glomerular necrosis, and that of proximal and distal collecting tubules, mononuclear cell infiltration, and congestion were observed in the kidneys of the *T. evansi* infected Yankasa sheep. Other lesions associated with the infection include emphysema, pulmonary congestion, and diffuse mononuclear cell infiltration into the lung parenchyma (pneumonia). There were no significant findings in the organs of the control sheep.

The results of this study have indicated the susceptibility of the Yankasa sheep to the *T. evansi* isolate. The full genetically characterization of the isolate was, however, not carried out.

INTRODUCTION

Pathological changes due to trypanosomosis in ruminants have been a subject of intensive research (Edwards et al. 1956; Losos and Ikede 1972; Ikede 1979; Saror 1980; Sekoni 1992). However, most of the published reports were on *T. vivax, T. brucei* and *T. congolense* (Ikede and Losos 1972; Saror 1980; Sekoni 1992), with little attention to pathological changes occurring in *T. evansi* infections in ruminants. Some of the few reported pathological changes in the organs of *T. evansi* infected rabbits and goats included degenerative changes in the spleen and lymph nodes, which interfered with the immune response of the host (Uche and Jones 1992). Other lesions occurred in the kidneys and heart. Similarly, lymphatic tissue hyperplasia, muscular atrophy, necrotic foci in the liver, kidneys, spleen and lungs, and bronchopneumonia have been reported in goats infected with *T. evansi* (Ngeranwa et al. 1993). The observed histopathological lesions included necrosis of hepatocytes, myocardium, and kidneys with mononuclear cellular infiltration. Splenic lymphoid hyperplasia and that of lymph nodes have been reported in infected goats (Ngeranwa et al. 1993).

The present study was carried out to investigate the pathological changes in Yankasa sheep following infection with the *T. evansi* isolate.

MATERIALS AND METHODS

Animals and experimental infection:

Fourteen intact male and female Yankasa sheep, aged between 9 and 13 months, were purchased locally and conditioned for a period of 9 weeks prior to the start of the experiment. The sheep were dewormed against helminth infection using oral Nemafax® at the rate of 7.5 mg/kg body weight. They were also sprayed with a solution of Supona® (Shell, London) against ectoparasites. They were subsequently kept in a tick-and fly proofed pen and maintained on hay, concentrates (cotton seed mixed with grain offal's), and salt-licks. Water was supplied ad libitum. At the end of 9 weeks of conditioning, they were randomly divided into two groups, and monitored for another 2 weeks for the purpose of obtaining baseline pre-infection data. they were also screened and certified negative for *T. evansi* and other haemoparasites prior to inoculation with the isolate. Six sheep (2 males and 4 females; Group 1) constituted the non-infected control group, while the remaining 8 (5 males and 3 females; Group 2) formed the experimentally infected group. Number tags were used to identify the sheep.

The *T. evansi* isolate used in the study was obtained from the blood of a naturally infected camel slaughtered at Kano abattoir. Samples of the infected blood were obtained in EDTA, transported immediately to the laboratory, and examined using the wet film method. Two milliliters of blood, positive for *T.*

evansi, was inoculated intravenously into a donor sheep in order to harvest a large number of the isolate for subsequent experimental infection of the Yankasa sheep.

When the donor sheep developed parasitemia of 3+ (three "pluses"; 20 trypanosomes per ×40 field) for 2 consecutive days, it was bled via the jugular vein and the infected blood was collected in EDTA and subsequently diluted using phosphate buffered saline glucose prior to inoculating the Yankasa sheep. Each of the infected sheep received approximately 2.0×10^6 parasites as estimated using the improved Neubauer haemocytometer method (Petana 1963). The inoculated sheep were allowed to go through the full course of the infection without treatment.

The sheep were daily observed for any abnormal behavior. All sheep *in-extremis* were humanely killed and immediately followed by postmortem examination.

Gross and histophatological evaluations:

Post-mortem examinations were conducted on two control sheep and those that were killed *in-extremis* as a result of the infection. Gross pathological examinations of various organs were carried out on the carcasses.

Cerebrospinal fluid, liver, and bone marrow impression and brain squash smears were prepared, stained with Giemsa, and microscopically examined under ×40 objective.

Sections of the liver, bone marrow, lymph nodes, kidneys, heart and lungs were obtained, fixed in 10% buffered neutral formaline, and processed manually using Technicon tissue processor. The tissues were sectioned at 5μ and stained using H&E. Samples obtained from the two control Yankasa sheep were similarly processed.

RESULTS

The carcasses of the infected sheep were anemic as indicated by paleness of the tissue and the mucous membranes, especially in those sheep that had chronic infection. Generally, splenomegaly and lymphadenopathy occurred in all the *T. evansi*-infected Yankasa sheep.

The carcasses of sheep P3046 and P3041 had petechial hemorrhages on the abomasal mucosa. However, sheep P3035 had congested lung with some evidence of parenchymal inflammation (pneumonia), hepatomegaly, catarrhal enteritis, enlarged adrenal glands, and oedematous lymphadenitis. Sheep P3036, P3037, and P3038 had relative lymphadenitis and splenomegaly.

Table 1. Course of infection in Yankasa sheep infected with *Trypanosoma evansi* and period when they were in-extremis.

Identification Number	Period in-extremis (Days post inoculation)	Course of infection
P3046	4	Acute
P3038	5	Acute
P3041	7	Acute
P3035	14	Acute
P3037	43	Chronic
P3036	59	Chronic

Histopathological findings:

Considerable histopathological changes occurred in all the tissues and organs of the infected Yankasa sheep. However, such changes were not observed in the tissues of the two control sheep.

a) Spleen

Histopathologically, the spleen of the infected Yankasa sheep showed dense lymphocyte proliferation at the germinal centers which extended into the red pulp. Haemosiderosis and haemosiderin-laden macrophage occurred in the spleen of such infected sheep. These features were more prominent in sheep P3036 which was *in-extremis* on day 59 post infection (Fig. 1). Similar findings were observed in the lymph nodes of the infected Yankasa sheep.

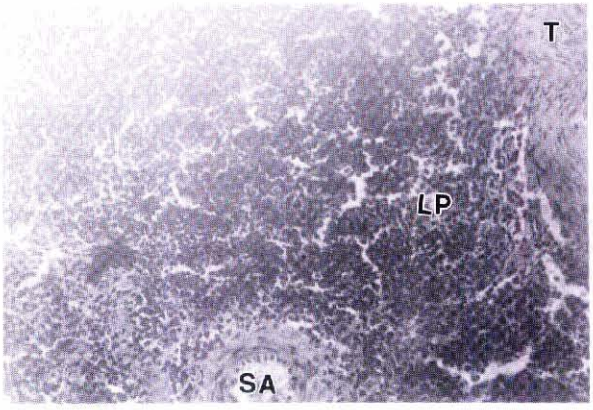

Figure 1. Photomicrograph of the spleen of *Trypanosoma evansi* infected Yankasa sheep (P3036) showing dense lymphocyte proliferation (LP) involving the germinal centers and the red pulp. Note splenic artery (SA) and trabaculae (T). (H&E, ×400)

b) Liver

The liver of the infected Yankasa sheep showed centrilobular and other focal areas of necrosis. Hepatic erythrophagia, haemosiderosis, and haemosiderin-laden macrophages occurred in sheep P3041, P3038, and P3035 (Fig. 2).

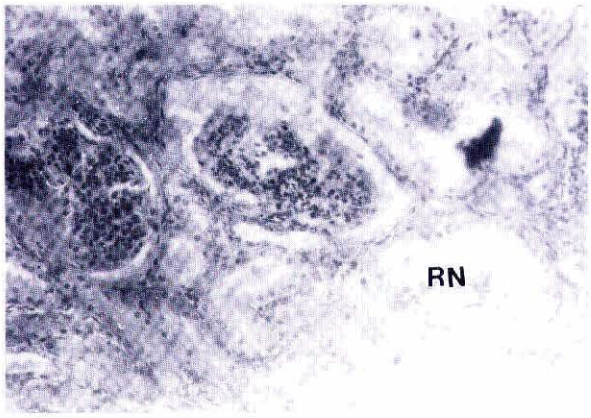

Figure 2. Photomicrograph of the liver of *Trypanosoma evansi* infected Yankasa sheep (P3036) showing centrilobular hepatocyte necrosis (CN). Note central vein (CV). (H&E, ×400)

c) Kidney

Non-suppurative glomerulonephritis involving the proximal and distal collecting tubules with a few mononuclear cell infiltration and congestion were observed in the kidneys of the infected Yankasa sheep (Fig. 3).

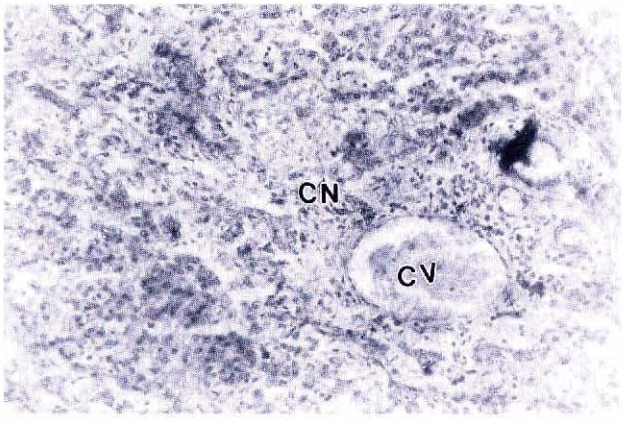

Figure 3. Photomicrograph of the kidney of *Trypanosoma evansi* infected Yankasa sheep (P3038) showing non-suppurative renal tubular necrosis (RN). (H&E, ×400)

d) Lungs

The lungs of the infected sheep showed congestion, emphysema, and diffuse mononuclear cell infiltration and pulmonary oedema in sheep P3041 and P3035 (Fig. 4). There were no significant changes in the lungs of sheep P3038.

e) Other findings

There were no significant histopathological findings in the intestine, testes, ovaries, epididymis, and heart of sheep P3046, P3038, P3041, P3035, P3037, and P3036. Similarly, no significant changes were observed in the spleen, liver, kidney and heart of the control sheep.

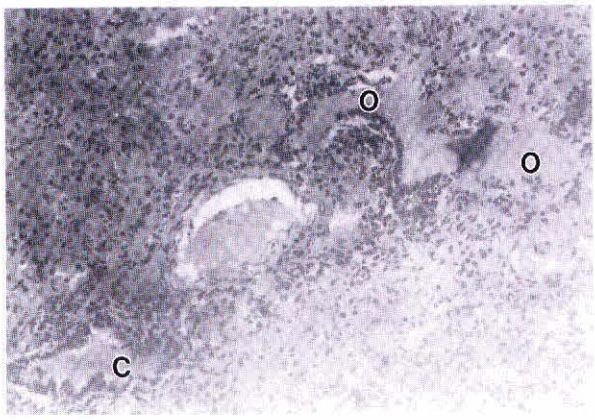

Figure 4. Photomicrograph of the lung of *Trypanosoma evansi* infected Yankasa sheep (P3041) showing mononuclear cell (MC) infiltration and pulmonary oedema (O). (H&E, ×400)

DISCUSSION

The results of the present study have clearly shown that the major histopathological features of infection in Yankasa sheep with the *T. evansi* isolate include lymphocytic proliferation in germinal centers of the spleen and lymph nodes, haemosiderosis, and haemosiderin-laden macrophages. These changes are indicative of extensive mononuclear cellular reaction due to the effect of trypanosomes on the organs of the *T. evansi* infected Yankasa sheep as was reported in *T. brucei* infected rats (Nwaorgu et al. 1981) and *T. evansi* infected rabbits (Uche and Jones 1992).

The pathological changes observed in the infected Yankasa sheep, particularly lymphadenitis, splenomegaly, and lymphoid hyperplasia, have been previously reported in *T. evansi* infected Madras red sheep (Saseendranath et al. 1995). These changes were similar to those reported in acute (Losos and Ikede 1972) and chronic (Van den Ingh et al. 1976) *T. vivax* infection in cattle, sheep and goats. The necrotic foci in the liver, kidney, and the pneumonic lesions seen in the two Yankasa sheep with chronic infection agree with the findings of Ngeranwa et al. (1993) in *T. evansi* infected goats.

The occurrence of haemosiderin-laden macrophage in the kidneys and lungs of trypanosome infected sheep are attributed to hemorrhages as a result of haemolysis involving the expanded mononuclear phagocytic system as reported in *T. brucei* infected donkeys (Ikede et al. 1977) and *T. vivax* infected goats (Saror 1980). This may also be the case in the present study.

Severe anemia was observed following the infection of Yankasa sheep in the present study. This was demonstrated by pale carcasses, watery blood, and in another aspect of the study, low erythrocyte counts, decreased hemoglobin concentrations, and low packed cell volumes.

The histopathological changes following infection of Yankasa sheep with the

T. evansi isolate, especially as observed in the chronic stage under the current study have demonstrated the susceptibility of this breed of sheep for the isolate.

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