



## Experimental *Ascaris suum* infection in Yankasa lambs: Parasitological and pathological observations

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### Abstract

The effects of experimental *Ascaris suum* infection in Yankasa lambs were investigated. Twenty four (24) Yankasa lambs aged 6-8 months were purchased and randomly divided into two groups (1 and 2). The lambs in group 1, consisting of 16 animals, were orally infected with 1500 infective *A. suum* eggs daily for seven consecutive days while those in group 2, consisting of 8 animals were maintained as non-infected/control group. All the experimental animals were closely monitored for 10 weeks, during which faecal samples were collected and analysed; and biochemical parameters of the blood samples were also evaluated. A total of seven animals (six from the infected and one from the control group) were humanely sacrificed on days 7, 14, 28 and 56 post-infection (p.i.) for larval/worm recovery, gross and histopathological examinations of organs. The values of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) from animals in the infected group increased significantly ( $P<0.05$ ) on day 14 p.i. The animals from the infected group were observed to have a significant increase in the mean values of creatinine and urea on day 28 p.i. All liver samples from the sacrificed infected animals showed varying degrees of diffuse whitish areas of necrosis. Similarly, histopathology revealed different levels of mononuclear cellular infiltration in the liver of the sacrificed infected animals. In two of the infected animals, the kidneys were congested. By comparison, the corresponding organs from animals in the control group were normal. Eight (8) *A. suum* larvae were recovered from the lungs of one infected animal sacrificed on day 28 post-infection. However, no egg was detected in the faeces of the lambs. It is concluded, based on the findings of this study that *A. suum* is infective to Yankasa lambs but is only slightly pathogenic to the lambs and did not develop to patency.

**Keywords:** *Ascaris suum*, Histopathology, Lambs, Larvae, Pathology

Received: 29-12-2016

Accepted: 11-04-2017

### Introduction

The large round worm of pigs, *Ascaris suum*, is reported to migrate in the tissues of a wide range of animals, including sheep (Vassilev, 1960; Fitzgerald, 1962; Johnson, 1963; McDonald & Chevis, 1965). Milk spot liver is a well-established terminology used to denote the whitish healing foci occurring in the liver stroma of pigs when *Ascaris suum* larvae are immobilized by the host's inflammatory reaction (Kelly, 1993). In abattoirs, the presence of milk spots in the livers of affected animals could lead to considerable losses due to offal condemnations (Barker *et al.*, 1993).

Condemnation of up to 70% of lamb livers from one farm in the (UK) was associated with *Ascaris suum* (Mitchell & Linklater, 1980).

Non-specific hosts usually come in contact with infective *Ascaris* eggs in joint enclosures or on pasture grounds manured with contaminated pig slurry (Borland *et al.*, 1980; Gunn, 1980; Mitchel & Linklater, 1980; Jepson & Hinton, 1986), or when pigs and sheep are grazed on the same pasture grounds (Thamsborg *et al.*, 1999). In Nigeria, pigs and sheep are mostly reared on extensive and semi-intensive systems of management (Ajala &

Osuhor, 2004; Holland, 2013). Additionally, *Ascaris suum* is a very fecund parasite; producing eggs that are resistant to environmental factors. Also, estimates of daily *Ascaris* female egg production are generally up to 200,000 eggs (Sinniah, 1982) even though the number of eggs a female produces decreases with worm load (Sinniah & Subramaniam, 2009). Thus, there are very high chances that unusual hosts such as sheep could become infected upon ingestion of pastures contaminated with infective *Ascaris suum* eggs. In view of this, it was considered worthwhile to evaluate the possible infectivity and pathogenicity of *Ascaris suum* in lambs.

## Materials and Methods

### *Experimental animals and management*

Twenty four (24) male Yankasa lambs, aged 6-8 months were purchased from a local market, and housed in fly and tick-proof pens of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. The lambs were acclimatized for two weeks, during which screening for internal and external parasites, treatment and prophylaxis were accomplished accordingly. The animals were fed twice a day with groundnut haulms, maize bran and *Digitaria* spp hay; while water and salt licks were provided *ad libitum*.

### *Experimental design*

The experimental animals were weighed, ear-tagged for proper identification and randomly divided into two groups (1 and 2). Group 1, the infected group, consisted of 16 animals while Group 2, the control/non-infected group, consisted of 8 animals. Animals in the groups were kept in separate pens for a period of twelve weeks.

### *Isolation of infective eggs*

Eggs of *Ascaris suum* were obtained from female worms collected from the intestines of pigs from slaughter slabs in Sabon Gari, Zaria.

The worms were collected in a beaker containing 50 ml of normal saline (0.9%), and transported to the Helminthology Laboratory of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. The uteri of the worms were dissected open using forceps into a petri dish and washed with 0.5 M KOH solution into a beaker as previously described (Fairbairn, 1961). The eggs were then agitated gently in the KOH solution for 30 minutes in order to dissolve the sticky albuminous layer. The suspension was then transferred into centrifuge tubes and spun at 349 relative centrifugal force (rcf) xg for 3 minutes, and the supernatant gently decanted, leaving about 0.5 ml which contained the eggs. The eggs were then washed two times with distilled water

and twice more with embryonating fluid (0.1 M sulphuric acid) according to the method described by Fairbairn (1961). The eggs collected were suspended in fresh embryonating fluid, transferred to Petri dishes and incubated for 30 days at 27°C (Dubinsky *et al.*, 2000), after which they were washed, and stored in distilled water at 4°C until needed.

### *Inoculation*

The solution containing the eggs was gently rocked to achieve an even distribution. Eggs in 0.1 ml of distilled water were counted under 10X objective of a light microscope. Each of the animals in group 1 was given 1500 infective eggs orally, each day for a week. The dose was administered using a 1 ml sterile-syringe and quickly followed with 20 ml of distilled water in order to ensure that the dose was wholly administered.

### *Faecal examination for eggs*

Beginning from day 7 post-infection until the end of the experiment, faecal samples were collected directly from the rectum of each infected animal, three times a week to determine if the infections had reached patency. Faecal flotation technique (MAFF, 1986) was carried out for egg detection.

### *Biochemical parameters*

Blood samples (3 ml each) from all animals were collected by jugular venipuncture into vacutainer tubes without anticoagulant and serum harvested from clotted blood. This was done on a weekly basis from day 0 to week 8 of the experiment that lasted for 12 weeks.

Blood urea and creatinine concentrations were determined using photometric procedures as described by Tietz (1986).

The total serum proteins were determined by the Biuret method. Serum albumin was determined by the use of Bromocresol green method (Weichselbaum, 1946) while the serum globulin fraction was determined as the difference between serum total protein and albumin fraction (Nnadi *et al.*, 2007).

Serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed according to the method described by Steven & Michael (2008).

### *Examination for lesions and histopathology*

On day 7 post-infection, two lambs from the infected group and one lamb from the control were sacrificed. On days 14, 28 and 56 post infection, one lamb each, from the infected group was sacrificed. The liver, heart, lung, kidneys and small intestine of each slaughtered lamb were examined for the presence of any gross lesions. Sections from these organs were fixed in 10% formalin solution, dehydrated in absolute alcohol,

cleared in xylene and embedded in paraffin for preparation of fine blocks in paraffin wax. Sections of 5µm thickness were cut and stained using the haematoxylin and eosin staining technique (Lillie, 1965). The sections were screened and examined for histopathology using a light microscope at 200X magnification.

**Larval recovery**

Tissues from the liver, lung and heart of the sacrificed infected lambs were finely cut with scissors, crushed and the Baermann's technique (Greenway & McCraw, 1970) was employed to recover the larvae. The finely chopped tissues were suspended in a double layer of gauze, submerged in saline in Baermann apparatus, and left overnight (Greenway & McCraw, 1970). Afterwards, the fluid was allowed to gravitate

from the stem of the funnel into centrifuge tubes and spun at 36 rcf xg for 5 minutes so as to induce the larvae to gravitate. The sediment was then checked for the presence of larvae using a stereomicroscope at 10X magnification.

**Data analysis**

Data collected were analyzed using Student's t-test (Graphpad Prism Software version 5.0). Significance of differences between group means was determined at  $P \leq 0.05$  and presented in charts.

**Results**

**Egg recovery and culture**

About 1.3 million eggs were recovered from the 5 female *A. suum* that were dissected, and after 30 days of Laboratory culture at 27°C; 70% of the eggs became infective, with each containing a fully developed larva (Plate 1).

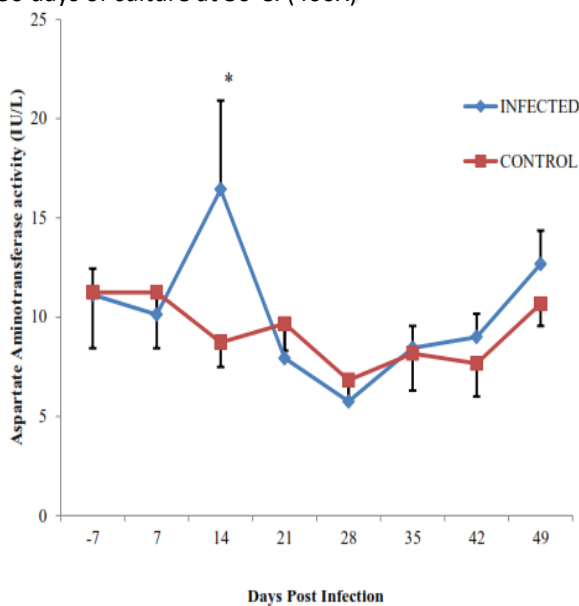
**Biochemical parameters**

The mean plasma activities of aspartate aminotransferase (AST) ( $16.44 \pm 4.47$  vs  $8.73 \pm 1.24$ ) and alanine aminotransferase (ALT) ( $8.00 \pm 2.18$  vs  $3.60 \pm 0.54$ ) were significantly higher ( $P < 0.05$ ) in the infected than in the control lambs on day 14 post-infection (Figures 1 and 2, respectively).

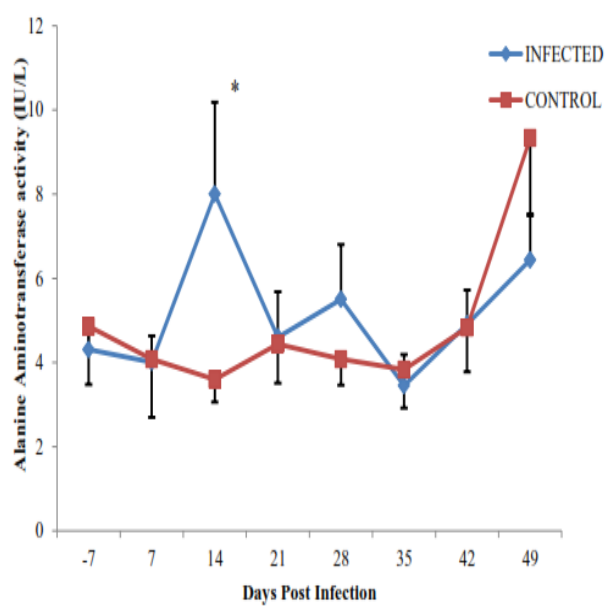
The mean concentrations of urea ( $7.85 \pm 1.23$  vs  $4.35 \pm 0.58$ ) and creatinine ( $86.50 \pm 11.99$  vs  $51.25 \pm 6.84$ ) of lambs in the infected group were significantly higher ( $P < 0.05$ ) on day 28 post-infection (Figures 3 and 4, respectively). However, there was no significant difference ( $P > 0.05$ ) in the activities of alkaline phosphatase (ALP) between



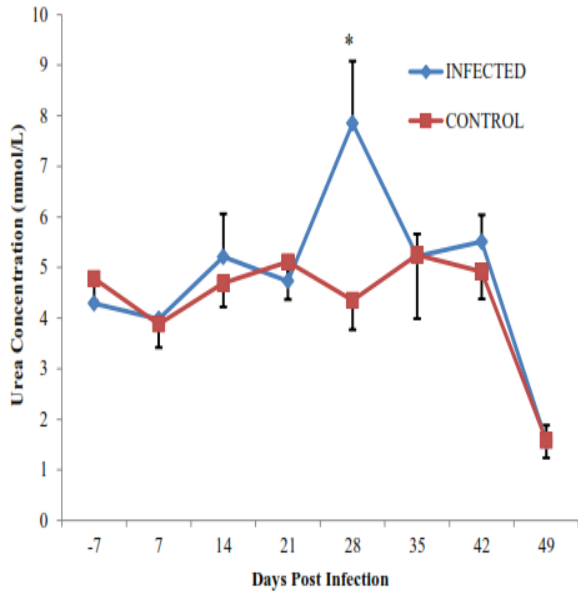
**Plate I:** Infective eggs of *Ascaris suum* (arrows) after 30 days of culture at 30°C. (400X)



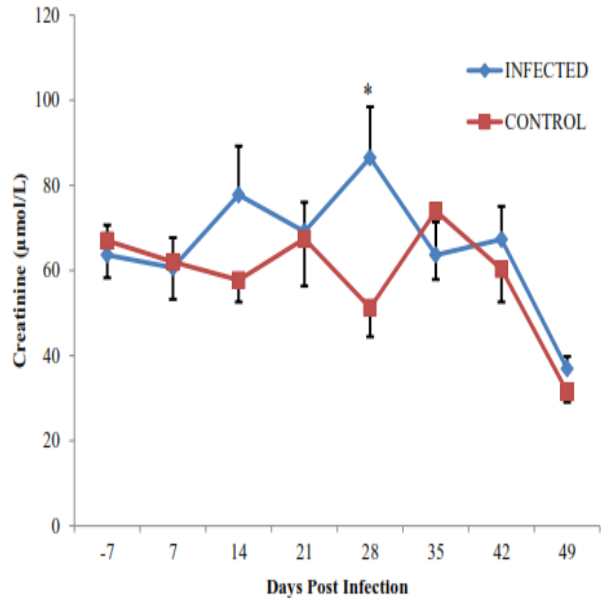
**Figure 1:** Serum AST activity (IU/L) of *Ascaris suum*-infected and control lambs (Mean ± SEM); \* = significantly different at  $p \leq 0.05$



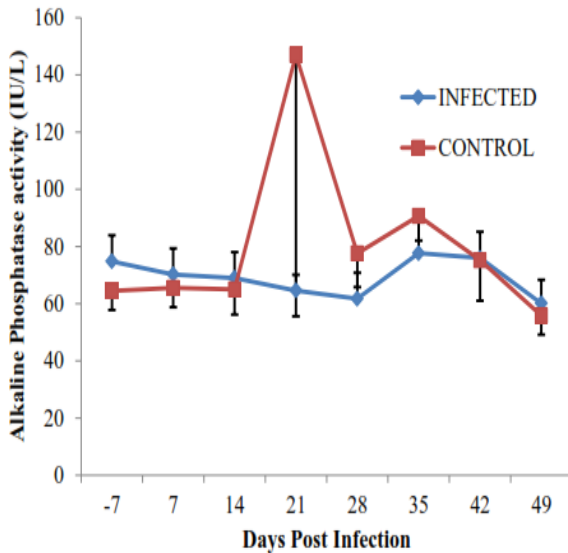
**Figure 2:** Serum ALT activity (IU/L) of *Ascaris suum*-infected and control lambs (Mean ± SEM); \* = significantly different at  $p \leq 0.05$



**Figure 3:** Serum urea concentration (mmol/L) of *Ascaris suum*-infected and control lambs (Mean  $\pm$  SEM); \* = significantly different at  $p \leq 0.05$



**Figure 4:** Serum creatinine concentration ( $\mu\text{mol/L}$ ) of *Ascaris suum*-infected and control lambs (Mean  $\pm$  SEM); \* = significantly different at  $p \leq 0.05$

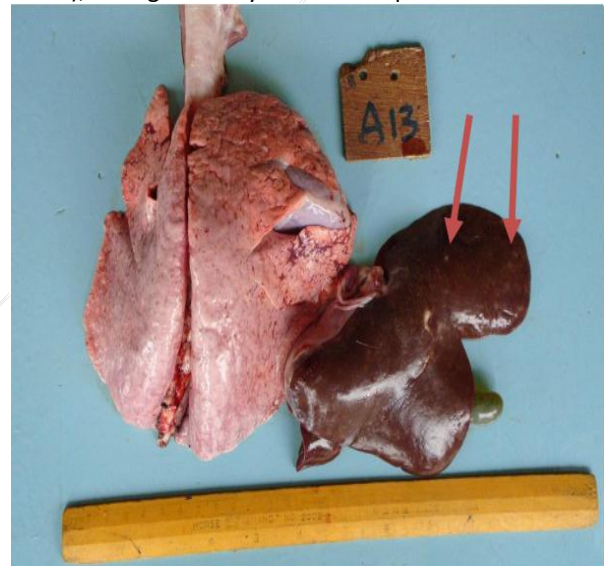


**Figure 5:** Mean ( $\pm$  SEM) ALP serum activity (IU/L) in the *A. suum*-infected and control lambs

the infected and the control groups (Figure 5). Similarly, there was no significant difference ( $P > 0.05$ ) in the concentrations of albumin.

#### Pathological findings

**Gross pathology:** Each of the two infected lambs that were sacrificed 7 days post-infection had tiny, white, pin-point lesions on the liver surface, although the lesions were more pronounced in one (Plate II) than the other (Plate III). In contrast, no lesions were observed on the liver surface of the non-infected animal sacrificed on the same day (Plate IV). Furthermore, no observable gross pathological findings were seen on the lungs, heart, kidneys and spleen of all sacrificed lambs.



**Plate II:** Tiny, white, pin-point necrotic foci (Arrows) on the liver of a lamb sacrificed on day 7 after oral infection with 10,500 infective *Ascaris suum* eggs

In animals sacrificed on day 14 post-infection, the foci became larger ( $1 \times 2$  mm) and more noticeable (Plate V). By days 28 and 56 post-infection the foci were almost inconspicuous.

**Histopathology:** On days 7 and 14 post-infection, areas of mononuclear cellular infiltration were observed in the liver parenchyma (Plate VI), while the kidney interlobular spaces were congested (Plate VII).

By days 28 and 56 p.i, the mononuclear cellular infiltration persisted in the liver, but to a lesser degree. It was also observed that the kidney of the animal sacrificed on day 56 p.i was congested (Plate VIII). No lesions were observed in the corresponding organs of the non-infected animals.





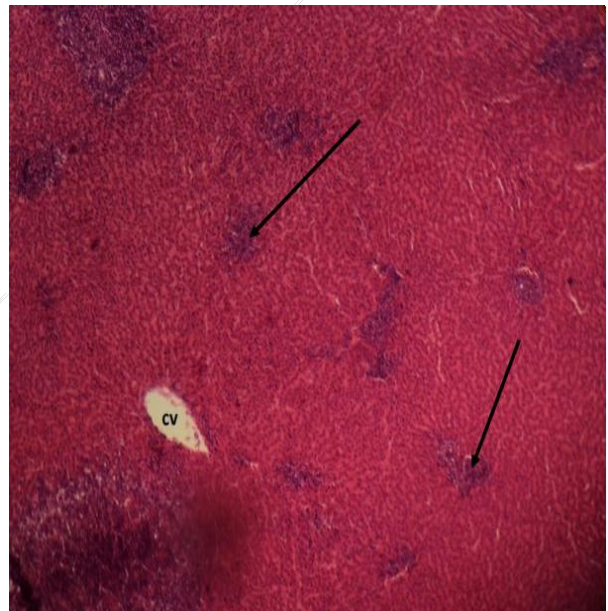
**Plate III:** Liver of an infected lamb sacrificed on day 7 post-infection with relatively less diffuse, tiny, faint, pin-point necrotic foci (arrows)



**Plate IV:** Liver of a non-infected lamb sacrificed on day 7 post-infection, showing no observable lesions



**Plate V:** Liver of a lamb slaughtered 14 days post-infection with 10,500 infective *Ascaris suum* eggs; showing larger, whitish necrotic areas (Arrows)



**Plate VI:** Photomicrograph of a section of the liver of *Ascaris suum*-infected lamb sacrificed on day 14 post-infection. Areas of mononuclear infiltration (arrows); CV - central vein (H & E; 200X)

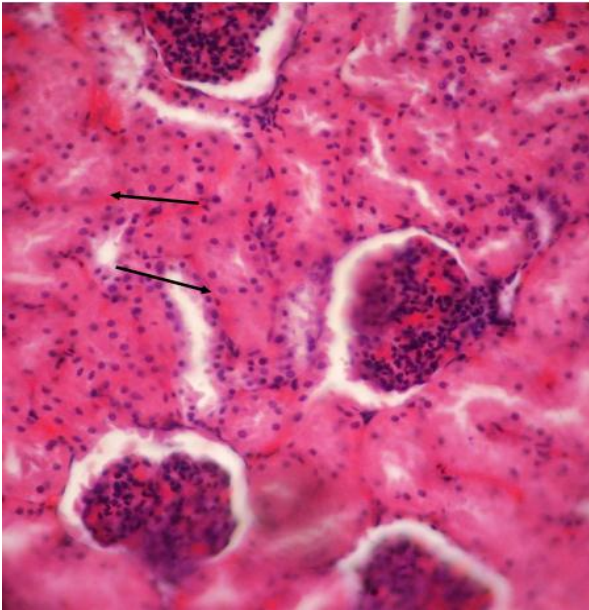
#### Larval recovery

Eight (8) larvae were recovered from the lung of an infected lamb that was sacrificed on day 28 post-infection. However, no larva or adult worm was detected upon examination of the contents of the gastro-intestinal tract. Larva was also not recovered from any of the infected lambs sacrificed after day 28. Similarly, no larva was detected in the lung or other organs of the non-infected animals throughout the experiment. The two ends of one of the larvae so recovered are shown in Plates IX and X.

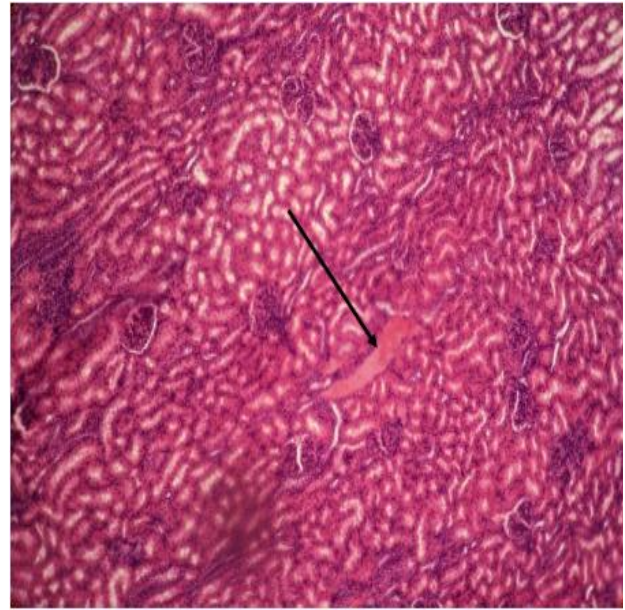
#### Discussion

The effects of experimental *Ascaris suum* (*A. suum*) infection in Yankasa lambs were investigated. The findings of pathological lesions and *A. suum* larvae recovered from the lungs, following infection of lambs with 10,500 infective eggs are a strong proof of the infectivity of *A. suum* infective eggs to the lambs. However, the infection did not reach patency, likely because Yankasa sheep is not the definitive host for the parasite.

The elevation in the values of AST and ALT on day 14 of infection in the infected group may be an indication of liver damage, caused by the migrating *A. suum* larvae, and consequent leakage of these



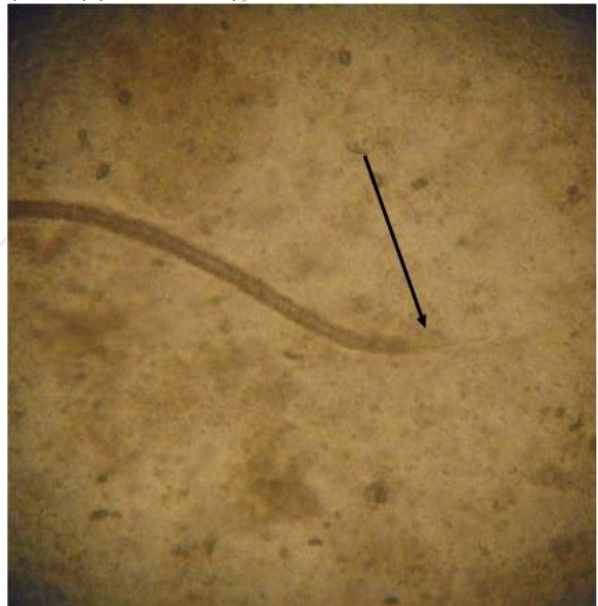
**Plate VII:** Photomicrograph of a section of the kidney of a lamb sacrificed 7 days post-infection with 10,500 infective *Ascaris suum* eggs. Congested intertubular spaces (arrows) (H & E; 400X)



**Plate VIII:** Photomicrograph of a section of the kidney of a lamb sacrificed 56 days post-infection with 10,500 infective *Ascaris suum* eggs. Congested blood vessel (arrow) (H & E; 200X)



**Plate IX:** Anterior end of larva recovered from the lungs of a lamb sacrificed on day 28 post-infection with 10,500 infective *Ascaris suum* eggs (arrow), showing blunt round head (400X)



**Plate X:** Tail end of larva recovered from the lungs of a lamb sacrificed on day 28 post-infection with 10,500 infective *Ascaris suum* eggs (arrow), showing a pointed tail tip (400X)

enzymes into the extracellular compartment and, subsequently, into the blood. The findings of increased ALT and AST activities observed in this study were contrary to the reports of Dubinsky *et al.* (2000), who observed that a long-term infection of lambs with the dose of 100 and 1000 eggs for 23 days did not influence the activities of AST and ALT. However, the higher infective dose (1500 x 7 eggs) used in this study may have been responsible for this difference.

The significant increase in the values of urea and creatinine concentrations in the infected group

on day 28 of infection may be suggestive of impairment of renal function, perhaps due to the congestion observed during histopathological examination. However, since larvae were not recovered in tissues, in addition to lack of observed gross lesions in the kidneys, it is difficult to attribute the congestion to the parasite migration.

The occurrence of whitish pin-point gross lesions, which were eventually identified by histopathology examination to be infiltrating mononuclear cells on the liver on day 7 post-infection, strongly suggests



that larval migration had occurred in this organ. Previous study (Brown *et al.*, 1984) had reported small hepatic necrotic foci infiltrated with eosinophils and lymphocytes in lambs infected with higher dose of infective eggs of *A. suum*. This study revealed that only sporadic lesions persisted from day 28 to day 56 post-infection, as was earlier reported by Roepstorff *et al.* (1997) and Dubinsky *et al.* (2000). This may be attributed to the progressive effacement of the lesions as the healing process proceeded.

In this study, larvae were detected in the lungs of the lamb that was sacrificed on day 28 post-infection, but not in the other animals that were sacrificed at the later stage of the infection. The low rate of larval recovery may be due to the recovery method that was employed or the number of animals sacrificed for larval recovery. Similar studies have reported high numbers of *A. suum* larvae from the lungs of experimentally infected ruminants (Fitzgerald, 1962; Morrow,

1968; McCraw, 1975), following administration of high single or repeated doses of the infective eggs. Dubinsky *et al.* (2000) also detected more larvae in the liver than in the lungs on day 7, but the larvae recovered from the lungs on day 21 were three times higher than those recovered from the liver. However, no larvae were recovered from the liver in this study. Likewise, no larvae were detected in the liver of infected lambs by Fitzgerald (1962) and Clark *et al.* (1989). This may be ascribed to differences in reaction of the liver tissue of pigs and sheep to migrating *A. suum* larvae as documented by Brown *et al.* (1984). In addition, different breeds of sheep may have different reactions to the migrating *A. suum* larvae. Overall, this study has shown that *Ascaris suum* is infective to Yankasa sheep, causing few pathological effects. This therefore calls for an improved management system that will curb the infection in pigs in order to avoid accidental infection of sheep and other unusual hosts.

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