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Research Article

Experimental *Ascaris Suum* Infection in Yankasa Lambs: Clinical Responses

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ABSTRACT

The aim of this study was to investigate the responses of Yankasa lambs to *Ascaris suum* infection. Twenty-four (24) male Yankasa lambs aged 6-8 months were purchased and randomly divided into two groups (1 and 2). The lambs in group 1 consisted of 16 animals, and they were orally infected with 1500 infective *A. suum* eggs daily for seven consecutive days. In group 2, 8 animals were maintained as non-infected/control group. All experimental animals were closely monitored for 10 weeks. PCV, WBC, differential leucocytes count and other haematological parameters were evaluated. Student's t- test was used to test for differences between groups. Clinical signs observed in the infected animals were cough and dyspnoea. Significant differences ($P<0.05$) between the mean respiratory and pulse rates of the infected animals (28.03 and 83.78 beats/min) and those of the control animals (23.84 and 81.08 beats/min) were observed on day 14 post- infection. Non- significant ($P>0.05$) higher eosinophil counts were observed in animals from infected group than in animals from control group on days 7, 28 and 35 post- infection. There were significant differences in the counts of white blood cells, neutrophils, lymphocytes and monocytes at various weeks of the experiment between the animals from the infected group and those from the control group. However, the infection did not have any influence on body weight changes, Packed Cell Volume (PCV), serum total proteins, albumins, globulins and haemoglobin concentration. It is concluded that based on the findings of this study, *Ascaris suum*, although a common roundworm of pigs, is also found to cause clinical symptoms in Yankasa lambs but is only slightly pathogenic to the lambs. Therefore, an improved management system that will curb the infection in pigs so as to avoid accidental infection of sheep and other unusual hosts is recommended.

Key words: *Ascaris suum*, Lambs, Experimental infection, Clinical responses

INTRODUCTION

Ascaris suum, the large round worm of pigs, is reported to migrate in the tissues of a wide range of animals, including sheep (Fitzgerald, 1962; Johnson, 1963; McDonald and Chevis, 1965; Vassilev, 1960). 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 and Linklaler, 1980; Gibson and Lanning, 1981), or when pigs and sheep are grazed on the same pasture grounds (Thansborg *et a1*., 1999).

A. suum can cause significant clinical manifestations and reduce carcass quality in cattle and sheep. However, in areas of industrialized farming systems, the clinical impact of *A. suum* may be limited since most farms are specialized for a single type of livestock, and pig slurry is seldom applied on ruminant grazing areas. In contrast, in more extensive livestock production systems with mixed species or in areas where livestock are roaming freely, as is the case in many developing countries such as Nigeria, the impact of *A. suum* in abnormal hosts might be higher, although this may not have been documented (Celia, 2012).

In Nigeria, pigs and sheep are mostly reared on extensive and semi-intensive systems of management (Ajala and Osuhor, 2004; Celia, 2012). Additionally, *A. 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 and Subramaniam, 2009). Thus, there are very high chances that unusual hosts such as sheep could become infected upon ingestion of pastures contaminated with infective *A. suum* eggs. In view of this, it was considered worthwhile to evaluate the possible infectivity of *A. suum* and clinical manifestations of *A. suum* infection in lambs.

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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 (12) weeks.

Isolation of infective eggs

Eggs of *A. 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 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.

Clinical observations

Daily physical examination was carried out. Temperature changes, respiratory and pulse rates were evaluated. Also, body weight changes were monitored weekly.

Haematological Examination

1. Blood samples (5 ml each) from all animals were collected by jugular venipuncture into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA), as anticoagulant. This was done on a weekly basis from day 0 to the end of the experiment that lasted for 12 weeks.

- Packed Cell Volume (PCV) was determined by the microhaematocrit method (Benjamin, 1978).
- Total White Blood Cells (WBC) were determined by using Neubauer haemocytometer.
- Differential leukocyte counts of blood smears were determined by the Battlement Method (Kelly, 1974).

2. Serum was harvested from clotted blood.

- The total serum proteins were determined by the Bieuret 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).

Data analysis

The collected data were expressed as Means ±SEM and presented as Charts. The data were analyzed using Graphpad Prism Software version 5.0. Student's t- test was used to test for differences between groups. Significance of differences between group means was determined at $P \leq 0.05$.

RESULTS

Egg recovery and culture

A total of about 1.3 million eggs were recovered from the 5 female *A. suum* that were dissected, and after 30 days of culturing at 27˚C; most of the eggs (70%) became infective, with each egg containing a fully developed larva (Fig. 1).

Clinical signs

Cough and dyspnoea were noticed in the infected lambs, from day 7 after the initial infective dose until about 11 days after the last infective dose. No clinical signs were seen in lambs of the control group throughout the period of experiment.

Vital parameters

The mean (±SEM) temperatures as well as respiratory and pulse rates of the infected and the control groups for the 10-week experimental period are presented in Figures 2 to 4. The difference in the mean respiratory rates of the infected and the control groups was significant on day 14 of infection (Fig. 2). The mean respiratory rate of lambs in the infected group was significantly higher ($P<0.05$) than that of lambs in the control group (28.03±0.31 vs 23.84±0.28).

On the other hand, the mean temperatures of the infected group did not differ significantly ($P>0.05$) from those of the control group (Fig. 3). However, the difference in the mean pulse rates in the infected and the control groups (83.78±0.21 vs 81.08±0.98) (Fig. 4) was significant ($P<0.05$) on day 14 of infection.

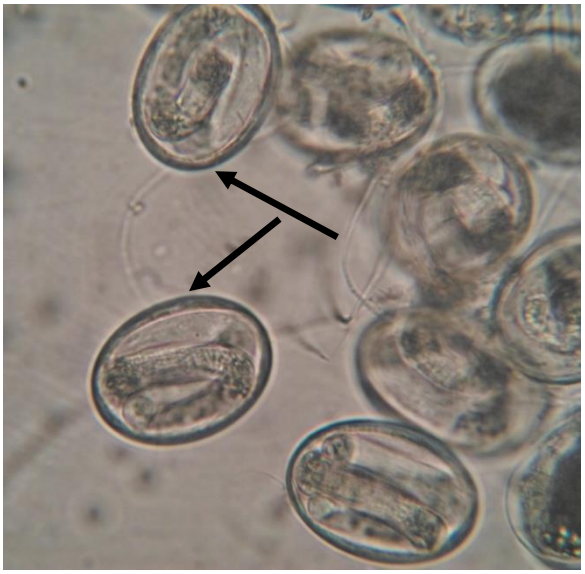

Fig. 1: Infective eggs of *A. suum* (arrows) after 30 days of culture at 30˚C. (× 400).

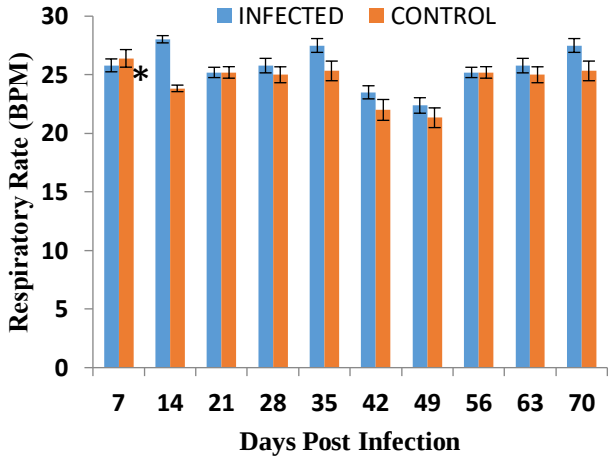

Fig. 2: Mean (± SEM) respiratory rates (cycles/min) in the *A. suum*- infected and control lambs. *significantly different at P<0.05.

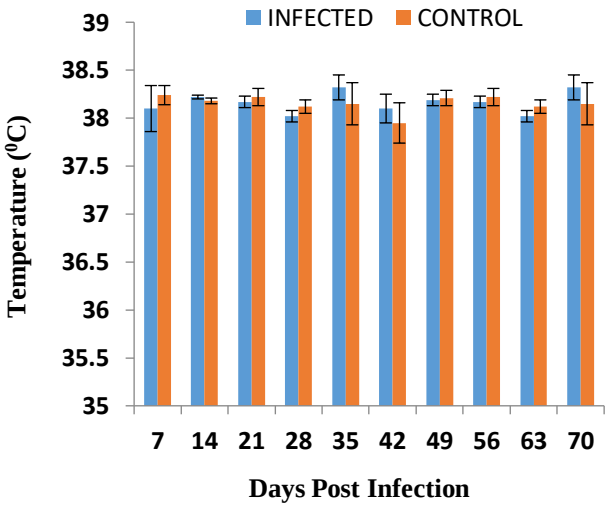

Fig. 3: Mean (± SEM) temperatures in the *A. suum*-infected and control lambs.

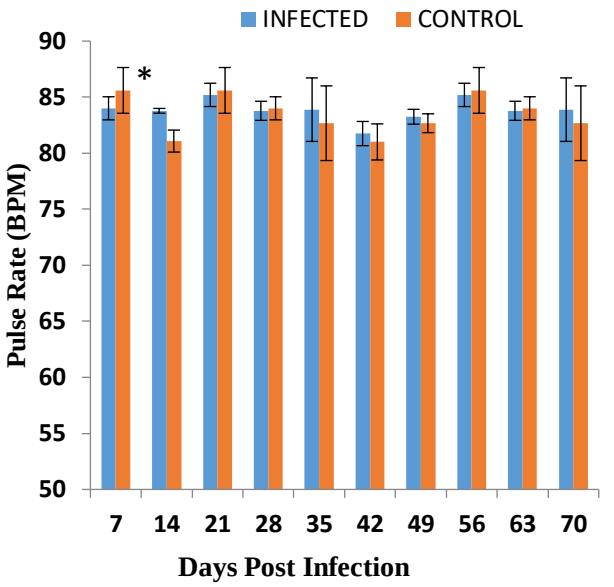

Fig. 4: Mean (± SEM) pulse rates in the *A. suum*-infected and control lambs. *significantly different at P<0.05.

Haematological findings

The mean (±SEM) PCV, Hb and TP are presented in Tables 2 to 4, respectively. The mean (±SEM) WBC, eosinophils, neutrophils, lymphocytes and monocytes are presented in Figs. 5 to 9, respectively.

In general, the mean PCV, haemoglobin and total protein concentrations in the infected group did not differ significantly (P>0.05) from those in the control group (Tables 2 to 4). Mean WBC was significantly higher (P<0.05) on days 7 and 35 of infection in the infected than in the control group (Fig. 5). The mean WBC of lambs in the infected group were significantly higher on day 7 (P=0.023) and day 35 (P=0.047) than those of the control group [(6.8±0.37 vs 6.00±0.17) and (5.91±0.12 vs 5.57±0.26)] respectively. The mean eosinophil counts in the infected group were higher on days 7, 28 and 35 of infection than in the control group though, the differences were not statistically significant (P>0.05) (Fig. 6).

Similarly, the mean neutrophil, lymphocyte and monocyte counts in the infected group were significantly (P<0.05) higher than those of the control group on days 56, 49 and 42 of infection respectively (Fig. 7-9).

Live weight changes

The mean (±SEM) body weights of Yankasa lambs in the infected and control groups during the experimental period are presented on Table 1. The mean body weights of the lambs in the infected group were consistently lower than those of the control lambs but the differences were not significant (P>0.05).

The effects of experimental *A. suum* infection in Yankasa lambs were investigated. The clinical responses observed following a trickle 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.

Table 1: Mean (± SEM) body weights of the *A. suum*-infected and control Yankasa lambs

Week	Infected	Control	P-values	Significance
1	18.20±0.92	18.40±0.72	0.420	NS
2	17.12±0.88	17.89±0.49	0.359	NS
3	16.40±0.75	17.50±0.70	0.417	NS
4	16.20±0.74	16.32±0.38	0.281	NS
5	15.67±0.99	16.13±0.39	0.510	NS
6	15.20±0.58	16.24±0.61	0.418	NS
7	15.60±0.68	16.91±0.58	0.424	NS
8	15.86±0.68	16.98±0.58	0.334	NS
9	15.92±0.39	17.00±0.16	0.442	NS
10	16.00±0.71	17.18±0.56	0.216	NS

NS- Not significant.

Table 2: Mean (± SEM) PCV (%) in the *A.* suum-infected and control lambs.

Day	Infected	Control	P-value	Significance
7	33.00±1.66	34.71±2.71	0.578	NS
14	31.90±1.52	33.83±3.86	0.592	NS
21	36.90±1.97	35.67±1.50	0.676	NS
28	40.11±6.51	38.50±3.02	0.291	NS
35	38.91±2.18	37.50±2.79	0.701	NS
42	41.00±3.11	41.4±3.67	0.932	NS
49	30.30 ±1.30	33.67±2.69	0.225	NS
56	30.75±1.66	34.67±3.23	0.268	NS

NS- Not significant.

Table 3: Mean (± SEM) haemoglobin concentration (g/dL) in the *A. suum*-infected and control lambs.

Day	Infected	Control	P-value	Significance
7	10.97±0.55	11.53±2.71	0.587	NS
14	10.55±0.52	11.20±1.28	0.592	NS
21	12.24±0.66	11.70±0.42	0.523	NS
28	13.36±2.71	14.97±0.98	0.514	NS
35	12.36±8.19	13.42±0.93	0.540	NS
42	13.71±1.02	13.74±1.22	0.983	NS
49	10.51±0.73	11.18±0.89	0.574	NS
56	10.22±0.55	11.53±1.07	0.465	NS

NS- Not significant.

Table 4: Mean (± SEM) total protein concentrations (g/dL) in the *A. suum*-infected and control lambs.

Day	Infected	Control	P-value	Significance
7	5.00 ±0. 32	5.31±0.46	0.587	NS
14	5.07±0.49	5.67±0.20	0.421	NS
21	7.13±1.34	5.57±0.88	0.436	NS
28	4.18 ±0.62	5.68±0.62	0.147	NS
35	5.45±0.64	4.07±0.49	0.166	NS
42	6.98±0.88	6.78±1.14	0.893	NS
49	5.94±0.51	4.33±0.77	0.574	NS
56	5.84±0.21	5.75±0.26	0.265	NS

NS- Not significant.

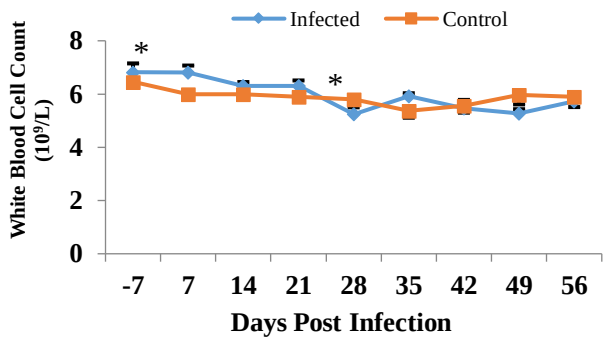

Fig. 5: Mean (± SEM) WBC count (10^9/L) in the *A. suum*-infected and control lambs * = significantly different at P<0.05.

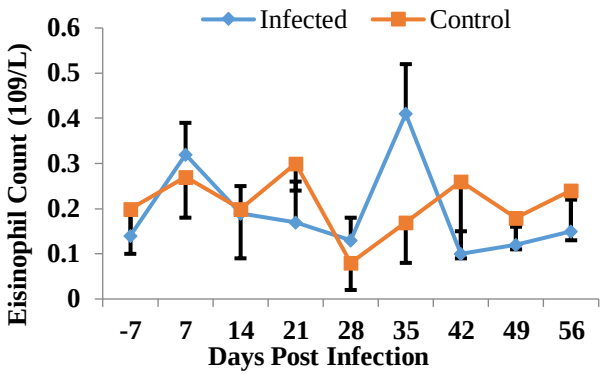

Fig. 6: Mean (± SEM) Eosinophil count (10^9/L) in the *A. suum*-infected and control lambs.

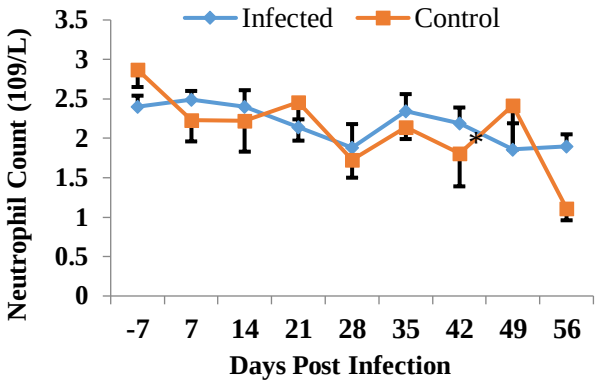

Fig. 7: Mean (± SEM) neutrophil count (10^9/L) in the *A. suum*-infected and control lambs. * = significantly different at P<0.05

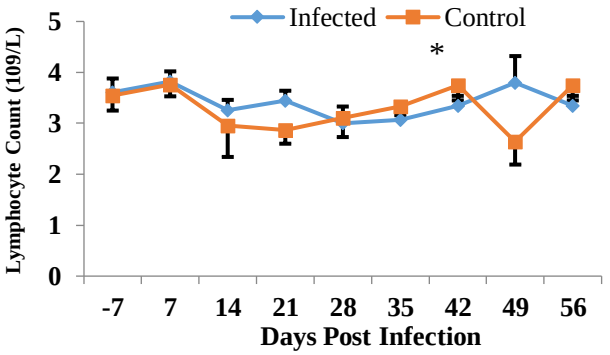

Fig. 8: Mean (± SEM) lymphocyte count (10^9/L) in the *A. suum*-infected and control lambs. * = significantly different at P<0.05

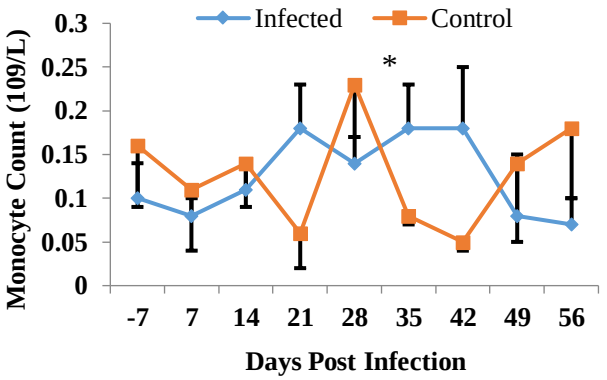

Fig. 9: Mean (± SEM) monocyte count (10^9/L) in the *A. suum*-infected and control lambs.* = significantly different at P<0.05.

DISCUSSION

The cough and dyspnoea observed in the infected animals were in agreement with the findings in the report of Krupicer *et al*. (1999) in which a prolonged infection of lambs with low doses of *A. suum* eggs resulted in a mild increase in the breath rate, which was accompanied with cough from day 6 of infection. Upon a single infection of lambs with large doses of *A. suum* eggs, Fitzgerald (1962) reported increased temperature and dyspnoe beginning from day 2 until day 8 post-infection. Brown *et aI*. (1984) observed similar symptoms on day 5 post-infection but the lambs were without clinical signs on day 15 post-infection.

Similarly, the infection in the present study did not appear to have significant effect on body weights. The significant difference in the respiratory and pulse rates on day 14 of infection could be attributed to the migration of the *A. suum* larvae in the lungs, which could have caused some damage to alveolar tissues thereby interfering with normal gaseous exchange. Evidence of migration in the lungs was reported in our earlier publication, whereby eight (8) *A. suum* larvae were recovered from the lungs of one infected animal sacrificed on day 28 post-infection (Isah *et al*., 2017).

The higher eosinophil count recorded in the infected than in the control group on days 7, 28 and 35 of infection may be an indication of increased mobilization of these cells from the bone marrow into the circulating blood. Since eosinophillia is a hallmark of parasitic infection, it is likely that the increase in circulating eosinophils was an attempt by the host to kill the larvae. This is because degranulation of eosinophils has been reported to kill parasite larvae through the A.D.C.C. (Antibody-dependent cell-mediated cytotoxicity) (Butterworth, 1984). Previous report (Krupicer *et al*., 1999) had indicated similar finding of eosinophillia following an experimental infection of lambs with low doses of *A. suum* eggs. The decrease observed at some points during the course of the experiment in the infected group could be merely relative. This is because high neutrophil count could cause relative decrease in eosinophil count (Latimer, 2011).

The significant increase in the WBC count on days 7 and 35 of infection in the infected group might be reflective of inflammatory process that might have been triggered by the migrating *A.* suum larvae in the liver and possibly other organs. Thus, the significantly higher neutrophil count recorded in the infected, compared to the control group on day 56 of infection, could be an indication of possible injury caused by the migrating larvae to the liver and perhaps, other organs.

Similarly, the significant increase in the lymphocyte count in the infected lambs might be an indication of attempt to develop specific immunity against the parasite (Latimer, 2011). Likewise, it may be inferred that the increase in monocyte count, particularly on day 42 of infection, was a normal body response to clear itself of cell debris that may have accumulated as a result of damage to tissues by migrating *A. suum* larvae.

The non-significant changes observed in the values of PCV, haemoglobin and total proteins, as well as the levels of albumins and globulins might be an indication that the parasite was not very pathogenic to this host species.

Therefore, this study has shown that *A. suum* is infective to sheep, causing few clinical signs.

Conclusions

This study concludes that *A. suum* is infective to Yankasa lambs but the infection did not reach patency. Clinical signs of cough and dyspnoea were noticed in the lambs but were less expressive.

Recommendations

Farmers should ensure that lambs are protected from grazing in such places where they may ingest pig slurry. Public enlightenment campaign on the dangers of such should be carried out. Efforts aimed at controlling the infection in pigs should be intensified, which in turn will help in preventing its occurrence in lambs and other accidental hosts.

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