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The Daily Egg Production of *Ancylostoma caninum* and the Distribution of the Worm along the Digestive Tract of the Dog

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Abstract: The gastrointestinal tracts of 50 dogs aged 4-18 weeks were examined between January and October, 2005 for infection with hookworm, *Ancylostoma caninum*. The daily egg production of the worm using the formol-ether concentration technique was determined and also the position of the worm along the digestive tract was observed. The results showed that 27(54%) of the 50 dogs examined were infected and the daily average egg output/female *A. caninum* was estimated to be 2074.46 (range 821.51-25,160.00) with an average egg per gram of 624.59. The male/female worm ratio was estimated to be 1:1.4. The estimation of worm load based on egg count is useful in determining the intensity of infection in an animal. The distribution of the worm was such that 47.7% occur in the jejunum, 31.7% in the duodenum, 14.3% in ileum and 6.2% in the colon. There was no worm observed in the stomach and the caecum.

Key words: Egg production, fecundity, *Ancylostoma caninum*, dog, egg output

INTRODUCTION

It is difficult to accurately predict the number of parasitic worms present in the intestine of an infected host without direct post-mortem examination or treatment of the host with anthelmintics and subsequent collection of faeces containing purged parasites. The presence of mature female worms can be detected indirectly from examination of faecal material for parasite eggs, but this method does not necessarily quantify numbers of parasites present, in view of the effect of density dependence on worm fecundity (Keymer and Slater, 1987; Richards and Lewis, 2001).

Egg count is important in estimating the worm load and in assessing the efficacy of treatment. Routine egg counts can be performed using the formol-ether sedimentation technique (Allen and Ridley, 1970; Knight *et al.*, 1976) or the modified Kato-katz technique (Forrester and Scott, 1990; Ebrahim *et al.*, 1997). Several workers have made attempt to relate egg counts to the number of worms present in an infected animal. In some earlier studies, it was reported that the measurements of egg production per gram of faecal material (epg) are more reliable indicators of worm burden than measurements of eggs per day (epd) (Coadwell and Ward, 1982; Keymer and Hiorns, 1986; Seivwright *et al.*, 2004).

In a comprehensive review of the biology of *Ascaris lumbricoides* it was reported that a female *A. lumbricoides* lays 200,000 eggs/day (Crompton, 2001). Sinniah (1982) investigated the daily egg production of *A. lumbricoides* and reported that the daily average egg output/female worm was estimated to be 238,722 with an average egg per gram (epg) of 3540.

This study was conducted to determine the daily egg output/female *A. caninum*, the sex ratio of the worm and the position of the worm along the digestive tract of the dog.

MATERIALS AND METHODS

Area of Study

This study was conducted in the Department of Zoology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. Ile-Ife, a peri-urban community is located within latitude of 07°26' N - 07° 33' N and longitudes 004° 30'E-004 35'E. The climate of the area is typical with a characteristic dry season of about 6 months (April-September) (Akinbuwa and Adeniyi, 1996). The mean annual rainfall ranges between 1000 and 1250 mm, the mean annual relative humidity from 75 to 100% and the mean annual temperature is about 30°C (Ndifon and Ukoli, 1989). The detail description of the area of study is in my earlier report by Sowemimo (2007a).

Parasitological Procedure

A total of 50 dogs (25 males and 25 females) aged 4-18 weeks old were used for this study. Faeces passed for a period of 24 h was collected from each of the dogs and examined for hookworm eggs, *Ancylostoma caninum*. The faeces collected from each dog was weighed and then preserved in 10% aqueous formaldehyde solution in clean universal bottles labelled with the dog identification number (ID). The preserved faecal samples were processed and examined for *A. caninum* eggs by formol-ether concentration technique (Allen and Ridley, 1970). This involved passing a subsample of each faecal specimen through double-ply gauze of mesh size 0.25 mm² to remove rough materials and washing with distilled water, as necessary. The filtrate was collected in pre-weighed centrifuge tubes and was then centrifuged at 2500 rpm for 2 min. The supernatant was decanted and the tube allowed to drain for 5 min. The tube was weighed again to determine the weight of the faecal matter. The sediment in each tube was re-suspended in 3 mL of distilled water and an equal volume of diethylether was added and the bottle shaken thoroughly for a minute. The suspension was centrifuged again for another 3 min at 2000 rpm. Four layer profiles were observed with the three top layer decanted and the egg sediment left inside the tube. The egg sediment was mixed and a drop was transferred onto a clean glass slide, covered with coverslip and then examined for *A. caninum* eggs. The total number of eggs in each drop was estimated. The total number of drop of the egg sediment was determined and then the egg concentration per gram of faeces calculated. The total number of eggs in the whole faeces passed in 24 h for each dog was estimated.

Each dog was then sacrificed, the stomach and the intestine were removed. The intestine was measured longitudinally from the pyloric sphincter to the anus. Each intestine was then cut opened longitudinally in order to determine the number of adult worm of *A. caninum* present and their position in the intestine. Similarly, the stomach was slit opened and the content expressed in a Petri-dish containing saline. Adult worms of *A. caninum* observed were picked with forceps and washed in saline to remove adherent particles. Isolated worms were counted, identified and the sex of each helminth noted. All the information collected for each dog was recorded in a data sheet coded with the dog's reference number and the date of examination. The number of female *A. caninum* worms recovered from each dog was noted and from the estimated number of eggs in the faeces passed in 24 h, the daily egg output per female worm was calculated.

RESULTS

Sex Ratio

Ancylostoma caninum worms were recovered from 27 (54%) of the 50 dogs examined in this study. A total of 306 adults of *A. caninum* comprising 128 males and 178 females of the worm were recovered. Based on the number of adult worms recovered, the sex ratio of male: female was 1:1.4. Faecal egg output and fecundity in female *A. caninum* worms.

Weight of faeces collected in 24 h and the associated egg counts for each of the 27 infected dogs and the number of male and female worms recovered from the intestines of the infected dogs are shown in Table 1. From the table, the overall estimated average daily egg output per female *A. caninum* was 2074.46, while the egg output ranged from 821.51-25,160.0. The correlation of the egg production per female worm with the number of female adult worms showed that as the worm burden increased, the number of eggs produced per female worm decreased (Fig. 1).

Table 1: The numbers of *Ancylostoma caninum* worms recovered from the intestine of 27 dogs and the average egg output/female/day

Dog S/N	Weight of 24 h stool sample	EPG	Total egg in faeces	No. of worms collected		No. of eggs laid/female/24 h
				Female	Male	
1	22.6	118.0	2666.8	1.0	1.0	2666.80
2	37.4	368.0	13763.2	3.0	2.0	4587.73
3	29.2	162.0	4730.4	5.0	5.0	946.08
4	24.4	1126.0	27474.4	30.0	36.0	915.81
5	19.2	1390.0	26688.0	14.0	6.0	1906.29
6	22.3	263.0	5864.9	2.0	3.0	2932.45
7	15.1	2125.0	32087.5	29.0	18.0	1106.47
8	14.0	470.0	6580.0	6.0	2.0	1096.67
9	24.7	151.0	3729.7	2.0	4.0	1864.85
10	32.5	261.0	8482.5	5.0	2.0	1696.50
11	15.1	980.0	14798.0	5.0	6.0	2959.60
12	19.3	979.0	18894.7	23.0	16.0	821.51
13	66.4	338.0	22443.2	11.0	5.0	2040.29
14	18.5	419.0	7751.5	3.0	3.0	2583.83
15	27.8	156.0	4336.8	2.0	2.0	2168.40
16	29.3	120.0	3516.0	1.0	2.0	3516.00
17	10.5	382.0	4011.0	1.0	1.0	4011.00
18	9.7	656.0	6363.2	1.0	2.0	6363.20
19	32.5	750.0	24375.0	5.0	1.0	4875.00
20	33.0	950.0	31350.0	3.0	2.0	10450.00
21	13.3	517.0	6876.1	2.0	2.0	3438.10
22	16.9	633.0	10697.7	4.0	1.0	2674.40
23	25.9	397.0	10282.3	2.0	1.0	5141.20
24	18.5	1360.0	25160.0	1.0	1.0	25160.00
25	33.8	430.0	14534.0	5.0	1.0	2906.80
26	15.4	704.0	10841.6	5.0	2.0	2168.32
27	31.8	659.0	20956.2	7.0	1.0	2993.74
Total	659.1	16864.0	369254.7	178.0	128.0	
Average	24.4	624.6	13676.1	6.6	4.7	2074.46

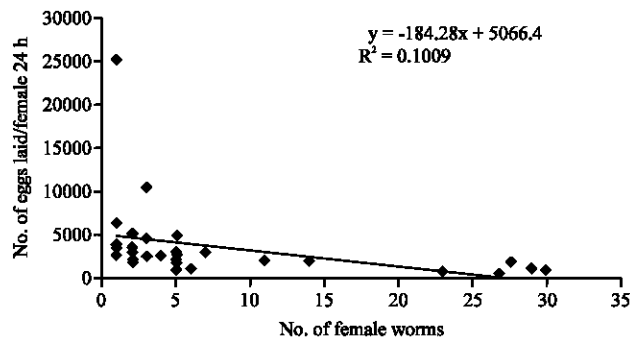


Fig. 1: Mean egg output/female *Ancylostoma caninum*/day in relation to female worm burden studied in 27 dogs

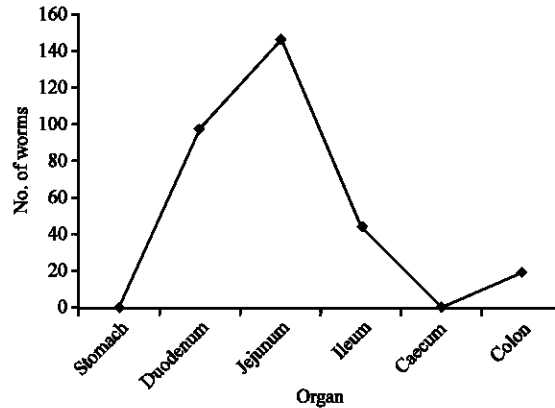


Fig. 2: No. of *Ancylostoma caninum* worm recovered and its distribution along the digestive tract of dogs

Distribution of *A. caninum* along the Intestine of the Host

The examination of the intestine of dogs revealed that majority of *A. caninum* recovered resided in the jejunum with 47.7% of the worms occupying this region. The worms were also recovered from the duodenum with 31.7% occupying this region while 14.3% were found in the ileum. The density of the worm decreased gradually towards the posterior end of the intestine with 6.2% of the worms residing in the colon (Fig. 2). There was no *A. caninum* worm recovered from the stomach and the caecum.

DISCUSSION

This study has revealed that on average a female *A. caninum* lays 2074.46 eggs daily (range 821.51-25,160.00) (Sowemimo, 2007b). The number of eggs laid by a female worm had been reported to vary from day to day. Yazima and Machida (1958) reported that egg production by *Ancylostoma caninum* in experimental dogs decreased with an increase in worm load. This is in agreement with the result of the present study where the number of eggs laid by female *A. caninum* decreased with increase in worm load. The position of the worm in the small intestine and competition for location offering optimum conditions for the parasite, have been suggested as factors which influence the number of eggs each female lays daily (Sinniah, 1982). On the other hand, Krupp (1961) suggested that the decrease in egg output with increase worm load may be due to the competition for food in view of the fact that the concentration of worms in a particular region could not be correlated with an increase or decrease in the rate of egg production.

Studies conducted by various investigators have shown that estimation of the number of egg/g of faeces (egg) is not an indication of the true worm burden (Sinniah, 1982; Richards and Lewis, 2001). They stated that it only reflects the number of adult females and not the total worm load. In addition the reliability of egg count have been reported to depend on several factors such as the amount of stool passed, the concentration of eggs within a sample of stool, the daily egg output/worm, the worm load, the age of the worm and the method use for estimating the egg (Sinniah, 1982).

The results of this study also showed that *A. caninum* was observed in every region of the intestine starting from the duodenum up to the colon. However, *A. caninum* was observed to be highly concentrated in the jejunum, where 47.7% of the worms were recovered (Sowemimo, 2007b). This result is similar to the findings of Mello *et al.* (1977) where it was reported that 97.5% of *A. caninum* were recovered from jejunum of 45 dogs examined for nematodes. This result is also similar to the

findings of Krupp (1961) who reported that a greater proportion of the adult *A. caninum* was found in the jejunum than any other region. The colon recorded the least percentage of the worm recovered in this study which is in agreement with the findings of Mello *et al.* (1977). The results of this study contradicts the findings of Pinto (1944) and Soulsby (1965) where they reported that *A. caninum* was found mainly in the duodenum. This result also differ from the findings of Krupp (1961) who reported that *A. caninum* worms were found in the caecum, whereas none were observed in the caecum in this study. This result also differ from the findings of Mello *et al.* (1977) where they reported that 0.1% of *A. caninum* worms were recorded from the stomach, whereas in this study no worm was observed in the stomach.

This study has shown clearly that faecal egg counts can still provide useful and reliable ways of estimating the egg production of the hookworm, *Ancylostoma caninum* despite some limitations. It also confirms the reports of previous studies, that the most preferred site of the worm, *A. caninum* in the intestine of dogs is the jejunum.

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