



## Response of Nigerian local breed of dog to graded doses of *Ancylostoma caninum* infection

IK Idika\* & CO Nwosu

Department of Veterinary Parasitology & Entomology, University of Nigeria, Nsukka, Nigeria

\*Correspondence: Tel.: +234 8036464810; E-mail: idika.idika@unn.edu.ng

### Abstract

The experiment investigated the response of Nigerian local breed of dog to different doses of *Ancylostoma caninum* infection. Sixteen dogs aged 6 to 7 months and assigned to 4 groups (A – D) of 4 dogs each were used. Groups A, B and C were infected with 100, 200 and 400 *A. caninum* infective larvae ( $L_3$ ) while group D served as the uninfected control. Faecal egg count (FEC), red blood cell (RBC) counts, haemoglobin concentrations (HBC), packed cell volume (PCV) and body weight (BWT) were evaluated weekly from day 0 (D0) to D56 post-infection (Pi). The dogs were humanely sacrificed on D56 Pi to determine the adult worm count (WC). The mean FEC of dogs given 400  $L_3$  (group C) was significantly ( $P < 0.05$ ) higher than those given 100  $L_3$  (group A). There was no significant ( $P > 0.05$ ) difference between the mean FEC of groups B (200  $L_3$ ) and C dogs. The group C dogs had significantly higher ( $P \leq 0.05$ ) worm burden than those in groups B and A. There was a dose dependent reduction in RBC counts, HBC and PCV of the infected dogs which was most significant ( $P < 0.05$ ) in group C followed by group B. The control and group A dogs had a BWT gains of 0.75 and 0.15 kg, respectively at the end of the experiment when compared to their D0 BWT while groups B and C lost 1.70 and 3.30 Kg of their BWT by D56 respectively. The results of this study showed that while the FEC did not differ significantly among the infected dogs, other measures of parasite intensity, namely, WC, PCV, RBC, HBC and BWT differed in a dose dependent manner.

**Keywords:** *Ancylostoma caninum*, Dogs, infective dose, Nigeria, Response

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### Introduction

The Nigerian local breed of dog is the most predominant dog breed in Nigeria and is of a major socioeconomic importance to the people (Aiyedun & Olugasa, 2012). However, infection by gastrointestinal (GI) helminthes particularly *Ancylostoma caninum* has remained a major factor that impedes the successful rearing of dogs in most tropical and subtropical regions of the world, including Nigeria (Anene *et al.*, 1996; Overgaauw & Boersema, 1998; Zelon, 2003; Ramirez-Barrios *et al.*, 2004). Both kennel and free-roam dogs are frequently infected with these hookworms, but sometimes dogs could be infected without apparent evidence of the parasites' presence (Endrias *et al.*, 2010). Consequently, canine ancylostomosis could vary in severity from asymptomatic infection to rapidly fatal exsanguinations depending on the number of hookworm larvae available to infect the host along with host factors such as breed, age and acquired resistance (Bowman *et al.*, 2003).

Ancylostomosis in dog is usually diagnosed by identification and count of the worm eggs in the faeces (Soulsby, 1982) of the dog. Faecal ggg count (FEC) is also important in estimating the worm load and in assessing the efficacy of treatment. Therefore, clinicians rely on the FEC to make inference on the intensity of the infection. However, given that the severity of the disease varies with varying numbers of the infective dose in a particular breed, it should be desirable to determine reliable markers of intensity of the infection in a particular breed. Such information on the host-parasite interaction will help in designing control programmes that are tailored for the breed rather than the generic periodic or routine anthelmintic treatments that have always been the practice. Anthelmintic treatment should, as a matter of necessity, be given with careful considerations in order to reduce the selection pressure for anthelmintic resistance and maintain good refugia. Dogs require care tailored to their individual needs as certain factors may dictate

more intensive monitoring and/or treatment for some whilst others may require a less aggressive approach (ESCCAP, 2010).

Hence, this study was designed to investigate the response of the Nigerian local breed of dog to varying doses of *Ancylostoma caninum* infective larvae. It is hoped that information from the study will improve our understanding of the dynamics of the infection in this breed and thus aid in the clinical assessment and management of hookworm disease in the breed. It is also believed that the ability to predict the intensity of the infection will enhance the effectiveness of anthelmintics in the dogs given that absorption, storage, presentation and efficacy of anthelmintics are influenced by worm burden (Junquera, 2014).

## Materials and Methods

### Experimental animals

A total of 16 non-castrate male dogs aged 6 to 7 months old and purchased from local markets around Nsukka were used in this study. They were identified with neck tags and body markings and kept in groups of two dogs per 1m x 1m x 1m metal cage which stood 0.4m off the floor. A detachable tray was placed at the bottom of each cage to collect voided urine and faeces. The animals were fed twice daily (morning and evening) with home-made food and supplemented with commercial dog feed (PEDIGREE®). Water was served *ad libitum* in stainless bowls. The dogs were kept under close observation for 2 weeks, during which daily observations of their clinical conditions were made. The faeces and blood of the dogs were examined on the day of experimental infection to ensure that they were free from blood and gastrointestinal parasites.

### Isolation and preparation of the *A. caninum* inocula

The infective *A. caninum* larvae used in this study were harvested from faecal cultures prepared using faeces collected from a donor dog which was infected with *A. caninum* that was isolated from a naturally infected dog. Larval inocula were prepared by diluting larval suspensions with distilled water to obtain the required concentration/number of L<sub>3</sub> in 1 mL of suspension and the larvae administered orally using an improvised stomach tube.

### Study design

The dogs were assigned to four groups (A – D) of four dogs each on the basis of their body weights, after successful completion of the period of quarantine and health evaluation as earlier described. Each dog in groups A, B and C were experimentally infected orally with 100, 200 and

400 L<sub>3</sub> of *A. caninum* respectively while those in group D served as the uninfected control. Faecal samples from the dogs were examined daily from day (D) 12 post infection until patency was observed in all the infected groups. Thereafter, faecal egg counts (FEC), body weights (bwt) and haematological parameters were evaluated weekly from the day of infection (D0) to D56 post-infection (Pi). The animals were sacrificed on D56 Pi and adult worm count determined.

### Observation of clinical signs

Physical examinations with emphasis on the general appearance and mood, colour of the visible mucous membranes, state of hydration of the skin and nature of the haircoat were carried out daily on each dog. Observations were also made daily on the appetite, consistency and colour of the faeces of each dog.

### Faecal egg count

Faecal examination for *Ancylostoma* eggs was done using floatation technique as described by CAPC (2015). Following patency, egg counts were determined twice-weekly for individual dogs up to D56 of the experiment using the modified McMaster technique as described by CAPC (2015).

### Post mortem worm count

The dogs were humanely sacrificed by intravenous injection of magnesium sulphate (MgSO<sub>4</sub>) and the worm isolation and count determined as described by Sowemimo & Asaolu (2008b). The dogs were eviscerated with the stomach and intestine of each dog dissected out. Thereafter, the intestines were separated into the various segments; duodenum, jejunum, ileum, caecum and colon by ligation. Each of these segments was dissected out and placed into appropriately labeled containers before being cut open longitudinally. The contents of each segment were emptied into appropriately labeled containers by washing with a jet of tap water and gentle scrapping with a finger. All worms observed were picked out with a pair of forceps and washed in normal saline to remove adherent particles. Isolated worms were identified and counted. The percentage worm establishment was determined using the formular:  $\frac{Ad}{L_3} \times 100$  where Ad and L<sub>3</sub> represent the number of adult *A. caninum* recovered from a dog and the number of infective larvae administered to the dog, respectively.

### Determination of packed cell volume (PCV)

The PCV of each dog was determined by the microhaematocrit method on D0 and weekly thereafter till D56 Pi.

**Red blood cell (RBC) count**

The number of circulating red blood cells per microlitre of blood was determined for each dog on D0 and weekly thereafter till D56 using the haemocytometer method.

**Haemoglobin concentration (Hb)**

The Hb concentration (g/dL) of each dog was determined using the cyanomethaemoglobin method (Tietz, 1976) on D0 and weekly thereafter till D56.

**Body weight (Bwt)**

Each dog was weighed on D0 and weekly thereafter by placing them inside a pre-weighed basket placed on a weighing balance and the body weight recorded in kilogram.

**Statistical analysis**

Data generated were analyzed using SPSS 15 for windows. Parameters recorded on more than a single day (Body weights, FEC, PCV, RBC and HBC) were analyzed by the Repeated Measures ANOVA in general linear model (GLIM) (Crawley 1993) while those generated on a single day (weight gain/loss and worm burden) were analyzed by one way ANOVA. Variant mean were separated by the Duncans multiple range test and probabilities (P) of 0.05 or less were considered significant

**Results**

**Clinical signs**

The dogs in groups A (100 L<sub>3</sub>) and D (control) did not show any clinical signs suggestive of infection throughout the duration of the study. Mild diarrhea was however observed among dogs in groups B (200 L<sub>3</sub>) and C (400 L<sub>3</sub>) between days 18 and 20 post infection. Also pale mucous

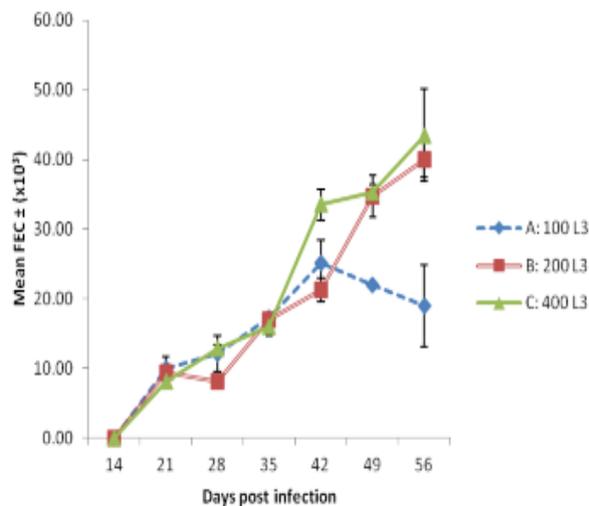
membrane was observed among dogs in group C from D49 post infection till the end of the experiment. Both the infected and uninfected dogs had good appetite and were generally alert and in apparent good health throughout the experiment.

**Faecal Egg Counts (FEC)**

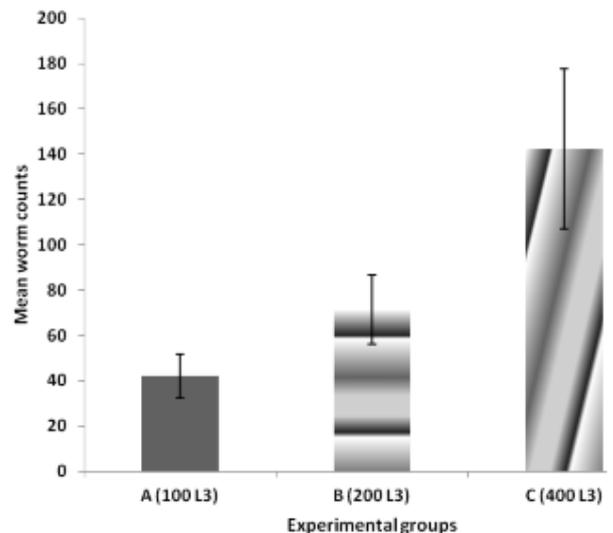
Figure 1 shows the mean FEC/gram of faeces of the dogs infected with the various doses of *Ancylostoma caninum* larvae. The pre-patent period of the worm as shown by the occurrence of hookworm eggs in the faeces was 14.00 ± 0.50 days (range: 14 - 15 days). There were no significant differences (P > 0.05) among the FECs of all the infected groups from patency up to D35 post-infection (Pi). Thereafter, the mean FEC recorded for group C (400 L<sub>3</sub>) became significantly (P < 0.05) higher than that recorded for group A (100 L<sub>3</sub>) up to the end of the experiment. Similarly, the mean FEC of dogs in group B was significantly (P < 0.05) higher than that of group A from D49 till the end of the experiment. Analysis of the FEC by repeated measure showed that the main effect of the doses of infection on the FEC was not significant (P > 0.05). There was a highly significant (P < 0.05) effect of time on the FEC as the values increased significantly with time.

**Post mortem worm count**

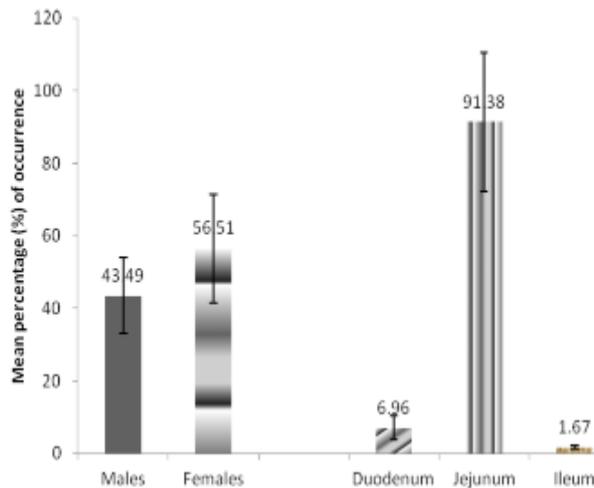
The mean worm burdens ± SEM of the dog are shown in Figure 2. Analysis by One-way ANOVA indicated that dogs given 400 L<sub>3</sub> (Group C) had significantly higher (P ≤ 0.05) worm burden (142.4 ± 35.4) than those given 200 L<sub>2</sub> (Group B: 71.5 ± 15.2) and 100 L<sub>3</sub> (group A: 42.0 ± 9.6). Consequently, the percentage worm establishments were 42, 35.8 and 35.6% for



**Figure 1:** Mean faecal egg count of Nigerian local breed of dogs infected with varying doses of *Ancylostoma caninum* infective larvae



**Figure 2:** Mean worm counts of Nigerian local breed of dogs infected with varying doses of *Ancylostoma caninum* infective larvae and sacrificed at D56 Pi



**Figure 3:** Sex of adult *Ancylostoma caninum* worms recovered from Nigerian local breed of dogs and their distribution along the digestive tracts

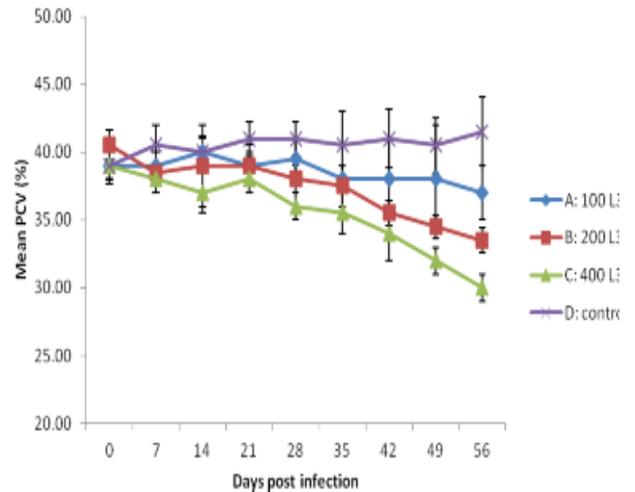
groups A, B and C respectively. Figure 3 shows the proportion of male and female worms recovered and their distribution in the gastrointestinal tracts (GIT). There was no significant ( $P > 0.05$ ) difference between the sexes of the worms recovered. Adult worms were recovered only from the small intestine but none from either the stomach or caecum of the infected dogs. On the basis of worm distribution in the small intestine, the majority of the worms resided in the Jejunum (91.4%) compared to either the duodenum (6.96%) or the ileum (1.67%).

#### Packed Cell Volume (PCV)

Figure 4 shows changes in the PCV of the control dogs and those infected with different doses of *Ancylostoma caninum* infective larvae. There were drops in the mean PCV of all the infected dog groups compared to the control. The drop in PCV was not significant ( $P > 0.05$ ) between the control and group A throughout the experiment compared to those of dogs in groups B and C that became significantly ( $P < 0.05$ ) lower than that of the control from D42 and 28 Pi respectively till the end of the experiment. The PCV of group C was also significantly ( $P < 0.05$ ) lower than those of groups A and B from D49 to the end of the study. Analysis by Rm ANOVA showed that the main effect of the infection on the PCV of the dogs was significant ( $P < 0.05$ ) in group C but not in groups A and B.

#### Red blood cell count

The mean red RBC counts ( $\times 10^6$  per  $\mu\text{l}$  of blood) of the dog groups are shown in Figure 5. There was no significant ( $P > 0.05$ ) difference between the mean RBC counts of the control and groups B and C up to D42 Pi. Thereafter, the mean RBC counts of groups B and C dogs became significantly ( $P < 0.05$ ) lower than that of the control up to the end of the



**Figure 4:** Mean packed cell volume (%) of Nigerian local breed of dogs infected with graded doses of *A. caninum* and their control

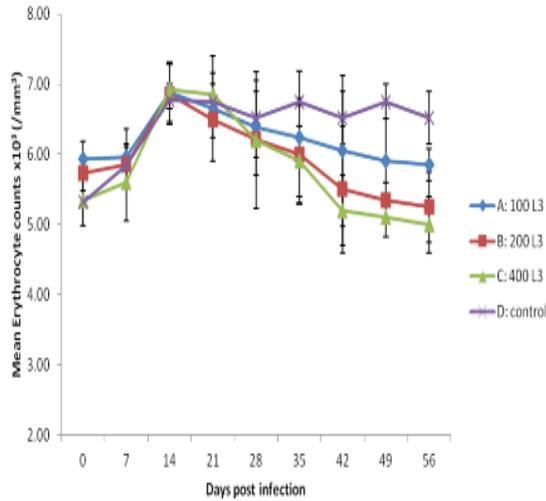
experiment. There was no significant ( $P > 0.05$ ) difference between the mean RBC counts of the control dogs and dogs in group A throughout the experiment. Analysis by rm ANOVA, fitting RBC counts as the dependent variable revealed that the infection had a significant ( $P < 0.05$ ) dose dependent effect on the RBC counts of the dogs with the effect being greatest among group C dogs which had the lowest RBC counts followed by groups B and A in that order.

#### Haemoglobin concentration (g/dL)

The result of the Hb concentration (HBC) as presented in Figure 6 showed that the mean HBC of the control dogs was not significantly different from those of the infected dogs up to D21 Pi for groups B and C and D42 for group A. Thereafter, the mean HBC of the control dogs became significantly ( $P < 0.05$ ) higher than those of the infected dogs till the end of the study on D56 Pi. The HBC of the dogs infected with 100  $L_3$  (group A) was significantly ( $P < 0.05$ ) higher than those of groups B and C from D28 till the end of the experiment. Analysis by Rm ANOVA, fitting Hb concentrations as the dependent variables showed that the infection had a significant ( $P < 0.05$ ) dose dependent effect on the Hb concentration of the dogs. The effect was greatest among Group C dogs which had the lowest Hb concentration followed respectively by groups B and A in that order. There was also a significant effect of time on the Hb concentrations of the dogs

#### Body weight (Kg)

Analyses of the body weight gained or lost by the dogs at the end of the experiment as shown in Figure 7 showed that the control dogs and those in group A gained 0.75 and 0.15 kg Bwt respectively at the end of the experiment while dogs in groups



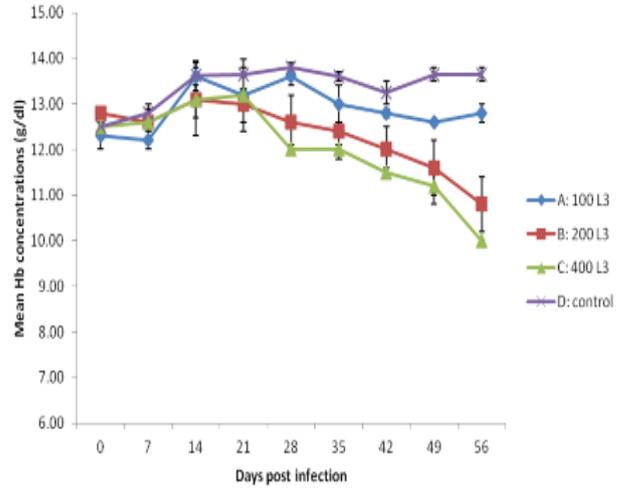
**Figure 5:** Mean red blood cell counts  $\times 10^6$  (per  $\mu$ l of blood) of Nigerian local breed of dogs infected with graded doses of *A. caninum* and their control

B and C lost 0.30 and 0.70 Kg of their Bwt respectively compared to their D0 Bwt.

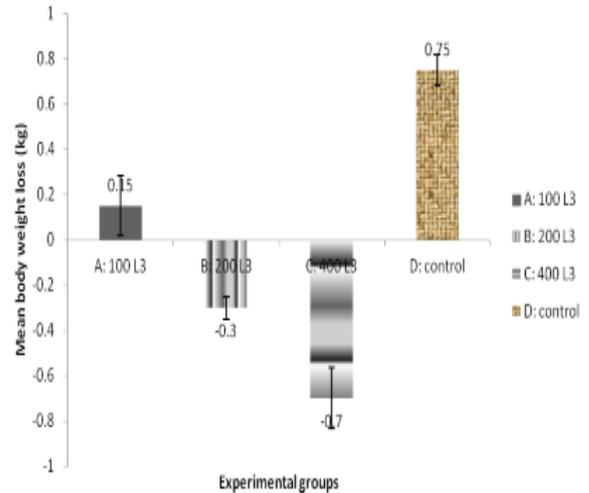
**Discussion**

Ancylostomosis has been increasingly recognized as an important clinical problem in dogs all over the world (Bowman *et al.*, 2003). The results obtained in this study showed that the doses of infective *A. caninum* larvae used did not produce overt clinical disease in the dogs as there was no noticeable clinical sign besides pale mucous membrane and transient diarrhoea that were observed in some of the infected dogs. However, Laboratory examination showed that the worms had effects on the hematological values and body weight of the dogs thus suggesting a subclinical course. Subclinical infections are frequently observed in most naturally acquired gastrointestinal nematode infections in Nigerian local dogs under field conditions (Anene *et al.*, 1996; Idika *et al.*, 2016).

A pre-patent period of 14-15 days as shown by the occurrence of hookworm eggs in the faeces of the infected dogs in this study is slightly shorter than the pre-patent period of 15-18 days reported by Soulsby (1982). In this study, there was no significant difference in the FEC recorded for groups B and C despite the fact that group C dogs received a higher infective dose of *A. caninum* larvae. However, the mean adult worm count of the dogs given 400 L<sub>3</sub> (Group C) was significantly higher than those given either 200 L<sub>3</sub> or 100 L<sub>3</sub>. This is in agreement with the works of Krupp (1961) and Sowemimo and Asaolu (2008a) who noted that the number of eggs laid by female *A. caninum* decreased with increase in worm load. The implication of this finding is that FEC may not be a good indicator trait for measuring the worm



**Figure 6:** Mean haemoglobin concentrations (g/dl) of Nigerian local breed of dogs infected with graded doses of *A. caninum* and their control



**Figure 7:** Mean body weight changes (kg) of Nigerian local breed of dogs infected with graded doses of *A. caninum* and their control

burden and intensity of ancylostomosis in Nigerian dogs and this could affect the clinical assessment of the disease.

Based on post mortem, the predilection site of the worm is basically the jejunum, although they were also found in the duodenum and ileum. This result is similar to the findings of Melo *et al.* (1977) who recovered 97.5% of *A. caninum* from the jejunum of 45 dogs examined for the nematode. It is also similar, in part, to previous reports that a greater proportion of the adult *A. caninum* was found in the jejunum than any other region of the small intestine (Sowemimo & Asaolu, 2008b). The results of this study however, contradict other reports that adult *A. caninum* were found mainly in the duodenum (Pinto, 1944; Soulsby, 1982), caecum

and colon (Krupp, 1961) and stomach (Melo *et al.*, 1977).

the blood loss in the intestine due to the feeding habits of the adult parasites (Lefkaditis, 2001; Soulsby, 1982). Similarly, anaemia was observed among the infected dogs as there were decreases in their erythrocyte values which were significant in dogs infected with 200 and 400 L<sub>3</sub> when compared to the uninfected dogs despite the fact that the dogs appeared apparently healthy. The fall in the haematological values was also directly proportional to the doses of the *A. caninum* given; further suggesting that the graded doses of *A. caninum* used in this study were able to produce a mild anemia in the dogs.

The effect of *A. caninum* was also evident on the body weights (BWT) of the infected dogs compared to the uninfected. The result suggests that while the control dogs gained weight at the end of the experiment, the infected groups, particularly groups B and C lost varying amounts of weight by D56. The weight loss was attributed to the effect of the parasites on nutrient absorption. Hookworm infection had been associated with a specific malabsorption syndrome that may be

Anemia is the major pathological effect of *Ancylostomosis* in dogs and this is related to correlated in severity with the parasitic load (Bowman *et al.*, 2003).

In conclusion, the results of this study have shown that the Nigerian local breed of dog is readily susceptible to *A. caninum* infection and that the doses of infection used could only produce mild clinical disease. The implication of such mild form of hookworm disease is that it may evade the attention of the owner to remain untreated with a resultant contamination of the environment with *A. caninum* eggs. The study further showed dose dependent effects of *A. caninum* on the measures of pathology in the host, namely, PCV, HBC and BWT whereas such correlation was not obtained between infecting doses and the FEC. This illustrates the limitations of relying on FEC alone in determining the severity of hookworm diseases, especially in secondary ancylostomosis which is usually the case under field conditions. It is therefore recommended that other ancillary Laboratory examinations such as the determination of the PCV and HBC be used to corroborate with the results of FEC.

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