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Research Article
Responses of the Nigerian Local Breed of Dogs to Single Pulse, Trickle and Trickle Non-escalated Patterns of *Ancylostoma caninum* Infections

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Abstract
Background: Evidence abounds that Nigerian Local Breed of Dogs (NLBD) is highly susceptible to naturally acquired *Ancylostoma caninum* infection but there are very little or no information on their responses to experimental infection. Hence, the responses of the NLBD to varying patterns of *A. caninum* infection were investigated. **Methodology:** Sixteen NLBD aged 6-7 months and assigned into 3 infection groups (A-C) of 4 dogs each and 1 uninfected control group (D) were used for the study. Groups A, B and C dogs were each orally infected with 1000 L_3 of *A. caninum* by a single pulse, trickle non-escalated and trickle escalated infection patterns, respectively. Faecal Egg Counts (FEC), body weight (Bwt), Red Blood Cell (RBC) count, Packed Cell Volume (PCV), total serum protein (TP), serum albumin (ALB) levels and adult Worm Burden (WB) were evaluated. **Results:** The FEC of the dogs in group A was significantly (p<0.05) higher than those in groups B and C, whereas dogs in group C had significantly (p<0.05) higher WB than those of groups A and B. The pattern of infection had significant (p<0.05) effect on the PCV, RBC counts and body weight of the dogs, the effect being greatest on group A dogs which had significantly (p<0.05) lower mean values of these parameters than groups B and C. The mean TP and ALB levels of the dogs in group A were significantly (p<0.05) lower than those of groups B and C. **Conclusion:** The study showed that trickle infection led to higher WB but lesser FEC and pathological effects than the single pulse infection.

Key words: *Ancylostoma caninum*, infection patterns, response, local breed, FEC

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ancylostomosis is a widespread parasitic disease of dogs, particularly in the tropical and subtropical areas (Bethony *et al*., 2006; Bowman *et al*., 2010). The disease is caused mainly by *Ancylostoma caninum* and to a lesser extent, *A. braziliense*; the former being the more common and pathogenic of the two species. The disease is seen in dogs of all ages but especially severe in very young dogs. The disease may be acute and rapidly fatal in susceptible animals, although some dogs may develop a marked degree of resistance to the effects of the infection. Adult hookworms attach to the small intestine inducing blood loss from both direct consumption of blood by the parasite as well as 'Bleeding' around the attachment sites due to secretion of anticoagulants by the worms to promote feeding (Bowman *et al*., 2003; CAPC., 2015a). In nature, dogs are either infected by getting exposed to a large number of infective larvae at one single point in time which is referred to as a single pulse infection or by multiple regular infections called a trickle (repeated) infection regime. Experimental infections by the repeated infection regime are aimed at mimicking natural field conditions under which animals are continuously exposed to the infection (Idika *et al*., 2012).

In view of the possible variability in the response of some hosts to different patterns of infection with nematodes (Chiejina *et al*., 2009), it is therefore necessary to study the effects of these infection patterns on the intensity of ancylostomosis in Nigerian local dogs. Such information on the host-parasite relationship will help in designing a tailored control programme for the breed rather than the generic periodic or routine anthelmintic treatments that has always been the practice. Anthelmintic treatments should, as a matter of necessity, be given to dogs according to need in other to reduce the selection pressure for anthelmintic resistance and maintain good refugia. The European Scientific Counsel for Companion Animal Parasite's guideline noted that dogs require care tailored to their individual needs as certain factors may dictate more intensive monitoring and/or treatment, while others may suggest a less aggressive approach (ESCCAP., 2010). Hence, this study was designed to investigate the responses of the Nigerian local breed of dogs to experimental infection with *A. caninum* using varying patterns of infection.

MATERIALS AND METHODS

Experimental animals and their management: A total of 16 non-castrate male dogs aged 6-7 months old and purchased from local markets around Nsukka were used in this study. The dogs were housed in the Kennel Unit of the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka. On arrival at the animal house, the dogs were treated routinely for gastrointestinal and haemo and ecto-parasites. The dogs were identified with neck tags and body markings and kept in groups of two dogs per 1×1×1 m metal cage which stood 0.4 m off the floor. A detachable tray was placed at the bottom of each cage to collect voided urine and faeces.

Study design: The dogs were assigned to three infection groups (A-C) of 4 dogs each while the remaining 4 dogs were used as the uninfected control (Group D). Dogs in groups A, B and C were given a total of 1000 L_3 of *A. caninum* by a single pulse, trickle non-escalated and trickle escalated infection pattern, respectively as shown in Table 1. Faecal samples from the dogs were examined daily for *Ancylostoma* eggs from D12 post infection until patency and there after, weekly. Faecal Egg Counts (FEC), body weights and haematological parameters were evaluated from D0 upto 56 of the experiment. The animals were humanely sacrificed using intravenous injections of magnesium sulphate ($MgSO_4$) on D56 post infection to carry out post mortem worm count.

***Ancylostoma caninum* larvae and infection of the animals:** *Ancylostoma caninum* larvae used in this study were obtained from a donor dog which was infected with a local isolate of *Ancylostoma caninum.* Larval inocula were prepared by the dilution method to obtain the required concentration/number of L_3 in 1 mL of larval suspension and the larvae administered orally using an improvised stomach tube.

Observation of clinical signs: A physical examination of each dog with emphasis on the general appearance and mood, colour of the visible mucous membranes, state of hydration of the skin and the nature of their hair coat was carried out daily on each dog. Observations were also made daily on the general well being of the dog, appetite, consistency and colour of the faeces.

Faecal egg counts: Faecal examination for *Ancylostoma* eggs was done using the floatation technique as described by CAPC (2015b). Following patency, egg counts were determined

Table 1: Experimental protocol and the pattern of infection with *A. caninum* L_3

No. of animals (Groups)	D0	D7	D14	D21	D56
A	1000 L_3	0 L_3	0 L_3	0 L_3	Necropsy
B	250 L_3	250 L_3	250 L_3	250 L_3	Necropsy
C	100 L_3	200 L_3	300 L_3	400 L_3	Necropsy
D	0 L_3	0 L_3	0 L_3	0 L_3	Necropsy

twice-weekly for individual dogs up to D56 of the experiment using the modified McMaster technique (CAPC., 2015b).

Post mortem worm count: The dogs were humanely sacrificed by intravenous injection of magnesium sulphate ($MgSO_4$) and the worm isolation and count determined as described by Sowemimo and Asaolu (2008). The percentage worm establishment was determined using the formula:

$$\frac{\text{Ad}}{\text{L}_3}\times 100$$

where, Ad and L_3 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) and Red Blood Cell (RBC) counts: The PCV of each dog was determined by the microhaematocrit method and the RBC count per microlitre of blood by the haemocytometer method on D0 and weekly there after till D56 Pi.

Determination of total serum protein and albumin concentration: The total serum protein and albumin concentrations of the dogs (g dL^{-1}) were determined using a commercial kit (Randox Laboratories Ltd., UK) based on the Biuret test Bromocresol green methods (Burtis *et al*., 2005), respectively on D0 and biweekly thereafter till D56 Pi.

Body weight (Bwt): Each dog was weighed on day 0 and weekly there after 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 and RBC) were analyzed by the repeated measures ANOVA in the General Linear Model (GLM) while those generated in a single day (weight gain/loss and worm burden) were analyzed by one way ANOVA.

RESULTS

Clinical signs: Sporadic diarrhoea was observed among the infected dogs within the first 7 days of infection which was more severe and prolonged among the group A dogs, followed by those that were given trickle non-escalated infection (group B). Thereafter, transient constipation was noticed among the infected dogs between D14 and 24 Pi, which was also more severe and prolonged among group A dogs, followed by group B. Similarly, dogs in groups A and B had pale mucous membranes from D35 Pi. Generally, both the infected and uninfected dogs were generally alert and in apparent good health throughout the experiment.

Faecal Egg Counts (FEC): The mean pre-patent period as shown by the occurrence of hookworm eggs in faeces of the dogs was 14.00±0.50 days (range: 14-15 days). The faecal egg counts/gram of faeces (FEC) rose sharply in all the infected groups from D15 Pi to reach a peak on D35 in all groups (Fig. 1). Thereafter, the counts began to decline steadily till the end of the study on D56. There were no significant ($p>0.05$) differences in the FECs of all the infected groups from patency till D28 after which the FEC of dogs in group A became significantly ($p<0.05$) higher than those of either group B or C. A repeated measure (Rm) ANOVA analysis showed that the pattern of infection had significant ($p<0.05$) effect on the FEC of the dogs.

Worm count: Mean worm counts±SEM of the various groups of the dogs are shown in Fig. 2. A one-way ANOVA analysis indicated that dogs in group C had significantly ($p<0.05$) higher worm burden (451.5±17.5) than those of groups A (292.5±5.8) and B (301.5±34.2). Consequently, 29.3, 30.2 and 45.2% of the inoculums were, respectively recovered from groups A, B and C.

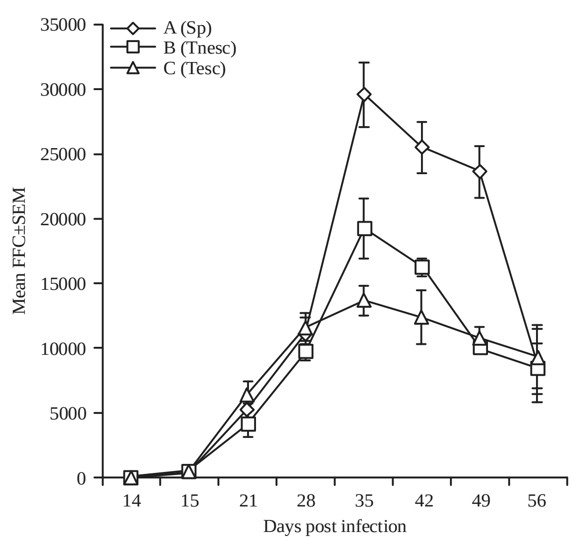

Fig. 1: Mean faecal egg counts/(per gram of faeces) ±SEM of Nigerian dogs infected with single pulse (Sp), trickle non-escalated (Tnesc) or trickle escalated (Tesc) doses of *Ancylostoma caninum* and the control

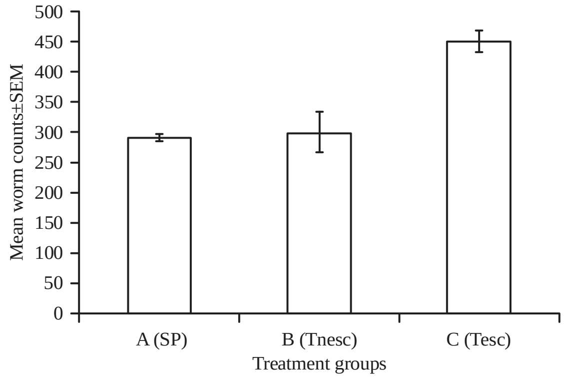

Fig. 2: Mean worm counts±SEM of Nigerian dogs infected with single pulse (sp), trickle non-escalated (Tnesc) or trickle escalated (Tesc) doses of *Ancylostoma caninum* and the control

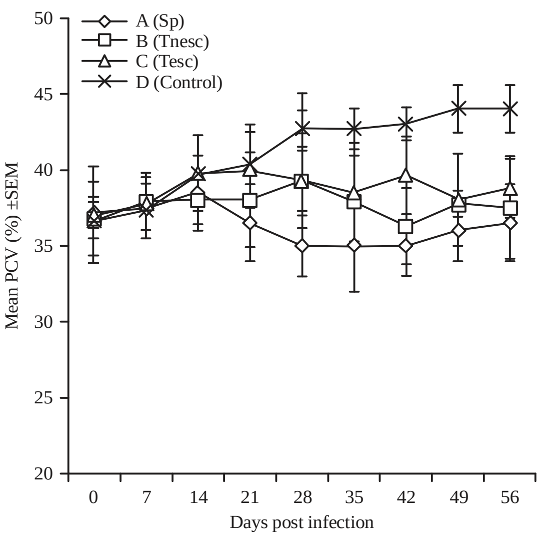

Fig. 3: Mean PCV (%) ±SEM of Nigerian dogs infected with single pulse (Sp), trickle non-escalated (Tnesc) or trickle escalated (Tesc) doses of *Ancylostoma caninum* and the control

Packed Cell Volume (PCV): Figure 3 shows the changes in the PCV of the control and infected groups. The PCV of all the groups were comparable between D0 and 7 Pi. Thereafter, the PCV of the control dogs became significantly ($p<0.05$) higher than those of groups A and B from D14 and group C from D28 till the end of the study on D56 Pi. Analysis showed that the pattern of infection with *A. caninum* had significant ($p<0.05$) effect on the PCV of the infected dogs. The decline in PCV was greatest among group A dogs followed by group B and C in that order.

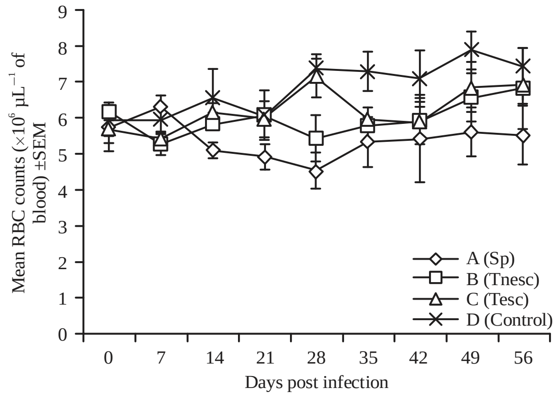

Fig. 4: Mean RBC counts ($\times 10^6$ μL^{-1} of blood) ±SEM of Nigerian dogs infected with single pulse (Sp), trickle non-escalated (Tnesc) or trickle escalated (Tesc) doses of *Ancylostoma caninum* and the control

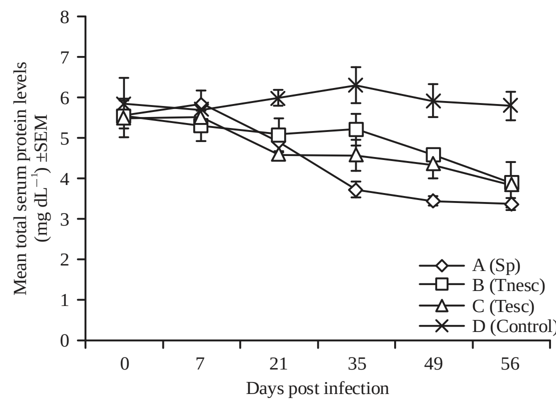

Fig. 5: Mean Total serum protein level (mg dL^{-1}) ±SEM of Nigerian dogs infected with single pulse (Sp), trickle non-escalated (Tnesc) or trickle escalated (Tesc) doses of *Ancylostoma caninum* and the control

Red Blood Cell (RBC) counts: The results of the RBC counts as illustrated in Fig. 4 showed that while the RBC count of the control showed a steady but gradual increase with time, those of the infected groups decreased on the other hand. The RBC counts of the infected and uninfected dogs were not significantly ($p<0.05$) different from each other except on D28 and 35. The infection as well as their pattern had significant ($p<0.05$) effect on the RBC counts of the dogs when groups (infection pattern) were fitted as the between subject factor, time as within subject factor and RBC counts as the dependent variables the effects being greatest on the group A dogs.

Total serum protein: The results of the total serum protein as indicated in Fig. 5 showed that the mean total serum protein level of all the groups were comparable between D0

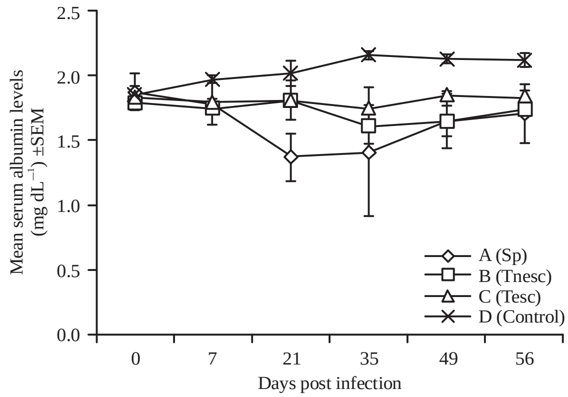

Fig. 6: Mean serum albumin level (mg dL^{-1}) $\pm$SEM of Nigerian dogs infected with single pulse (Sp), trickle non-escalated (Tnesc) or trickle escalated (Tesc) doses of *Ancylostoma caninum* and the control

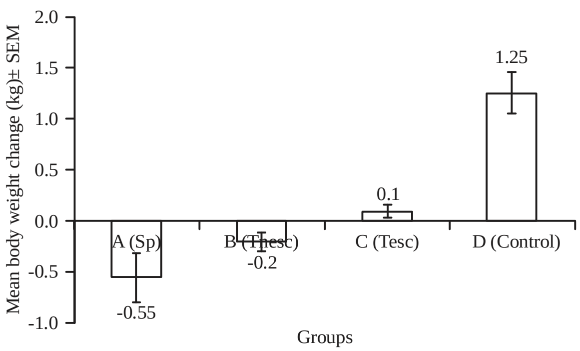

Fig. 7: Mean body weight changes (kg) $\pm$SEM of Nigerian dogs infected with single pulse (Sp), trickle non-escalated (Tnesc) or trickle escalated (Tesc) doses of *Ancylostoma caninum* and the control

and 7, following which those of the infected groups (A-C) decreased and became significantly ($p<0.05$) lower than that of the control group up to the end of the study. The analysis showed that the difference between the total serum protein levels of the two groups that received trickle infections (groups B and C) were not significant ($p>0.05$).

Serum albumin levels: The serum albumin levels of the experimental dogs are presented in Fig. 6. The result showed a gradual but fairly constant increase in the mean albumin levels in the uninfected group. The mean serum albumin levels of the infected groups remained relatively lower than that of the control but were comparable with one another throughout the duration of the experiment except on D21 when a significantly lower level was recorded for the dogs in group A compared to groups B and C as well as the control.

Body weight (Bwt): Figure 7 illustrates the mean body weight changes of the dogs. There was a gradual and fairly constant increase in the mean body weights of the uninfected dogs (Group D) throughout the duration of the experiment. The dogs in group D (control) and those given trickle escalated infection (group C) gained 1.25$\pm$0.6 and 0.10$\pm$0.06 kg, respectively in body weight at the end of the experiment (D56) when compared to their D0 body weight. On the other hand, the dogs given trickle non-escalated (group C) and single pulse (group A) infections lost 0.2$\pm$0.09 and 0.55$\pm$0.29 kg in their body weight.

DISCUSSION

The experiment was designed to observe the responses of Nigerian local breed of dogs infected with *A. caninum* using different patterns of infection. The results demonstrated clear variability among the dog groups in their responses to the varying patterns of infections used. The clinical observations made in this study showed that the infection was most severe in the dogs given single pulse infection (group A) followed by group B dogs which received a trickle non-escalated infection. The infection was characterized by transient diarrhoea within the 1st week of infection in most of the infected dogs which shortly gave way to constipation that lasted between 2 and 5 days in all the dogs in group A and two dogs in group B. No bloody faeces were observed except in one dog belonging to group A. There was also a slight depression of appetite in group A while dogs in groups B and C (given trickle infections) had a good appetite throughout the study. Ancylostomosis has been shown to be characterized by diarrhea, which is usually seen as early as the fourth day of infection when fourth stage larvae had reached the intestine and by the 8th day, fresh blood mixed with watery mucus may be seen in the faeces (Lefkaditis, 2001; Merck Veterinary Manual, 2014). It was assumed that the pattern of infection used was responsible for the severity of the infection seen in group A unlike the other groups (B and C) whose infections were almost symptomless besides slight weight loss.

The FEC results showed that following patency, FEC increased rapidly in all the infected dogs, peaked by D35 and had a continuous decline till the end of the experiment on D56. This is consistent with the observation of (Bowman *et al*., 2003) that maximum output of *A. caninum* eggs occurred after the 20th day of infection. In this study, the faecal egg counts and worm burdens were significantly influenced by the patterns of the infection used. Dogs with single pulse infection had significantly higher mean FEC than those given trickle infections. However, trickle infection pattern, particularly the escalated infection, resulted in the accumulation of higher worm burdens than the pulse infection such that dogs that were given trickle escalated infection had the highest mean worm count (451.5±17.5) followed by those given trickle non escalated (301.5±34.2) and pulse infection (292.5±5.8) in that order. Therefore, as the number of adult worms present in the dogs increased, their egg outputs decreased.

The pathogenesis of ancylostomosis is essentially that of an acute or chronic haemorrhagic anaemia (Bowman *et al*., 2003). Blood loss due to ancylostomosis starts about the eighth day post-infection, coinciding with the fourth moult and the development of the adult buccal capsule which enables it to grasp plugs of mucosa containing arterioles (Lefkaditis, 2001). Similarly, blood loss was evident in the present study as manifested by reductions in the PCV and RBC counts observed among the infected groups compared to the uninfected control. Analysis revealed that the infection as well as their pattern had significant effect on the above red cell parameters. That the effect was also greatest on dogs given single pulse infection (group A) was attributed to the fact that single pulse infection will likely produce an acute form of the disease, whereas, the immunizing effect of trickle infection will give rise to a more chronic disease. The severity of ancylostomosis has been shown to be related to the presence of acquired immunity (Bowman *et al*., 2010).

The results of the biochemical tests showed that the infection caused a significant reduction in the mean total protein and albumin levels of the infected dogs in relation to the control group which was most severe in the dogs that received single pulse infection. The loss in the serum proteins observed in this study was attributed to blood loss and malabsorption associated with *A. caninum* infection. Hookworm infection is associated with malabsorption syndrome that is correlated in severity with the parasitic load (Merck Veterinary Manual, 2014). Endogenous protein loss is a common feature of GI tract helminthosis which is in agreement with the results of the present study (Yu *et al*., 2000).

The assessment of the body weight changes in this study revealed that the pattern of infection had significant effect on the body weights of the dogs. This effect was more on the dogs that were given single pulse infection, followed by those given trickle non escalated (groups C) and trickle escalated (group B) infections in that order. Poor growth had been associated with hookworm infection (Bowman *et al*., 2010) just as was observed in the present study and it is believed to be as a result of the loss of blood and proteins associated with the infection. Most cases of ancylostomiasis do not result in severe anemia; however, persistent infections and re-infections do result in malnourishment (Bowman *et al*., 2010). Chronic protein loss during severe hookworm infection can result in hypoproteinemia and oedema (Hotez *et al*., 2004). Thus, changes in the protein metabolism of the GI tract may be pivotal to the pathogenesis of helminthosis.

CONCLUSION

In conclusion, this study has demonstrated that the patterns of *A. caninum* infections had a significant effect on the outcome of ancylostomosis in the Nigerian local dogs. The experiment has shown that the ancylostomosis is more severe in dogs which acquire the infection by a single pulse challenge while those that are exposed to a small multiple (escalated or non-escalated) doses of the infection are likely to tolerate and better manage the pathological effects of the infection. Also, at the level of the infective doses and study duration used in this study, it appeared that haematopoiesis and protein synthesis compensated sufficiently in the infected dogs such that they appeared normal and displayed only mild clinical and clinicopathologic signs of anemia. Consequently, the infective doses of up to 1000 L_3 as used in this study were capable of producing moderate pathologies and as such could be used as a working dose in experimental studies involving *A. caninum* in Nigerian local breed of dogs.

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