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# Clinical and hematological trends in *Ancylostoma caninum* infected puppies fed different levels of dietary protein

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## Abstract

Association between different dietary protein levels and susceptibility to *A. caninum* infestation in puppies was investigated for a period of ten weeks. In the investigation, thirteen puppies were divided into four groups: A, B, C and D; each with three puppies except group B which had four. Puppies in groups A and B were each infected orally with 500 *A. caninum* larvae obtained from naturally infected dog whereas puppies in groups C and D were not infected with *A. caninum.* Groups A and C were maintained on balanced commercial diet while groups B and D were maintained on unbalanced locally made diet for the whole period of the study. Body weight gain, egg per gram count, packed cell volume and hemoglobin content were monitored for a period of ten weeks. The results showed that infected with unbalanced diet puppies expressed more severe clinical signs and had statistically significant higher ($P<0.05$) mortalities and intestinal worm burden than the infected with balanced diet puppies. Mean worm burden was 352 ± 25.86 for the balanced diet fed group and 465 ± 11.57 for unbalanced diet fed group. The infected with balanced diet fed group had statistically significant higher ($P<0.05$) weight gain than the infected with unbalanced diet fed group. Mean weights were 8.27 ± 0.65 kg for balanced diet fed group and 3.30 ± 0.16 kg for unbalanced diet fed group. The infected with balanced diet fed group had statistically significant higher ($P<0.05$) PCV values than the infected with unbalanced diet fed group. Mean PCV were 25.34 ± 1.45% for the infected with balanced diet fed group and 15.73 ± 0.64% for the infected with unbalanced diet fed group. For hemoglobin content, the infected with balanced diet fed group had statistically significant higher ($P<0.05$) Hb values than the infected with unbalanced diet fed group. Mean Hb was 9.89 ± 0.43mg/dl for infected with balanced diet fed group and 8.07 ± 0.19mg/dl for infected with unbalanced diet fed group. For EPG counts, the infected with balanced diet fed group had lower EPG counts than the infected with unbalanced diet fed group, however, this result was not statistically significant ($P>0.05$). Means EPG was 8,050 ± 949 for infected with balanced diet fed group and 13,570 ± 2811 for infected with unbalanced diet fed group. From this study, it was concluded that undernourished puppies are more susceptible to *A. caninum* infection and developed more severe clinical signs than puppies in good nutritional plane. In addition to regular deworming, feeding of nutritionally balanced diet should be recommended to dog keepers as ways of controlling *A. caninum* in dogs in poor income countries where helminthiasis remains a problem.

**Keywords:** *A. caninum*, Dogs; dietary protein; haematology; clinical signs

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## Introduction

A repeated cross-sectional study on canine helminthes demonstrated a wide array of intestinal worms of which *Ancylostoma caninum* was the most prevalent worm among the local dogs kept by indigenous people of Tanzania (Makene et al., 1997; Kusiluka et al., 1999; Swai et al., 2012). The importance of these worms were further evidenced in a review of clinical cases at a veterinary clinic in Tanzania which showed that *Ancylostoma caninum* is the most leading endoparasite diagnosed (Muhairwa et al., 2008). High prevalence of helminthiasis is indication of poor management or concern for animals although substantive data for this is not available in Tanzania. Dog management in Tanzania is like that in other developing countries where dogs are frequently seen roaming in streets, indicating little care particularly in terms of feeding, housing, parasite control and preventive health care.

The attitude of different societies towards dogs is largely determined by culture and development (Van Heerden, 1989). Lack of knowledge, illiteracy and low income in most communities in developing countries are usually responsible for subnormal small animal care (Van Heerden, 1989). In such communities, dogs are usually not dipped and dewormed regularly, as a result ticks and fleas are encountered on dogs throughout the year and worm infestation is high (Van Heerden, 1989). Common worms encountered are *Ancylostoma* species, *Dipylidium caninum,* Toxocara species *Taenia hydatigena, Spirocerca lupi* and heartworms (Dada et al., 1979; Wanchira et al., 1993). The ideal dog management is well practiced in developed countries (Susan, 1997) and by limited affluent dog keepers in developing countries (Van Heerden, 1989).

For optimal health and welfare, dogs need good care which include food, shelter and routine care including grooming, preventive health care, parasite control and household hazards control for indoor dogs (Susan, 1997). Major preventive health care includes vaccination against diseases like canine distemper, parvovirus infection, canine hepatitis, canine leptospirosis, tracheobronchitis and rabies (Cleaveland et al., 2006) and control of endoparasites such as roundworms, whipworms, tapeworms and heartworms (Kopp et al., 2007) and ectoparasites which include fleas, ticks and mites (Susan, 1997).

Little information regarding the influence of diet on the susceptibility of those dogs to worm infestations is currently available. This study was therefore designed to investigate the effect of diet on the susceptibility of puppies to *A. caninum* infestation in Morogoro municipality. The objective of this study was to investigate the clinical and haematological trends in puppies following *A. caninum* infestation.

## Materials and Methods

### Study area

The study was conducted between March and August 2007 at Sokoine University of Agriculture in Morogoro Municipal. The Morogoro Municipality is located in the Coastal zone of Tanzania, 195 kilometers from Dar-es-Salaam, 600 meters above sea level, at latitudes 4.49° South and longitudes 37.40° East. It receives mean annual rain of about 800 mm to 1200 mm and temperatures ranging between 16°C and 30°C. It covers an area of about 260 square kilometers with a human population of 228,863 and it has about 5,000 dogs.

### Puppies

Thirteen puppies of mixed breed and of either sex, 3 to 5 months of age were purchased from dog keepers in Morogoro. Prior to the experiment, all puppies were dewormed by Ascaten-P® tablets which contain mebendazole, piperazine citrate and praziquantel. The puppies were dewormed at a dose rate of 22 mg/kg (mebendazole), 55 mg/kg (piperazine citrate) and 5 mg/kg (praziquantel). The treatment was repeated after 3 weeks to ensure that puppies are cleared from worm infestation. Clearance of infection was confirmed by faecal floatation technique as described Kusiluka et al. (1999).

### Source of *A. caninum* infective larvae

A naturally *A. caninum* infected dog, with egg per gram count (EPG) greater than 5,000 was identified and used as a source of infective larvae. EPG was confirmed by the use of McMaster Counting Technique as described by MAFF (1986).

### Preparation of faecal culture and isolation of *A. caninum* infective larvae

Faecal culture preparation, culturing, harvesting and storage of third stage larvae were conducted as described by Juergen and Prociv (2003). Briefly, 50 grams of faeces were mixed well with an equal volume of verminculate in a one litre plastic container. The mixture was moistened and incubated at 30°C and kept stirred daily. After five days the mixture was spread onto a 2 litre glass Petri dish, covered with a 5mm layer of washed coarse river sand and overlaid with two layers of dumpy surgical gauze. The top layer of the gauze into which infective third stage larvae were migrating was removed and replaced at 12 hours interval, rinsed in distilled water. Infective larvae were retrieved from the suspension by gravitational sedimentation and stored in buffer media (50 mM $NaHPO_4$. 22mM $KHPO_4$. 70mM NaCl) at 12°C and 6.8 PH to preserve their infectivity. The *A. caninum* infective larvae suspended in buffer media were serially diluted. The final volume of the larval suspension was adjusted so that 500 infective larvae were contained in 1.0 ml of the suspension (Victor et al., 1979).

**Infection of experimental animals**

Thirteen puppies were randomly distributed into four groups A, B, C and D which were housed in different compartments. Groups A, C and D consisted of 3 puppies each. Group B consisted of 4 puppies. Each group was identified with a neck collar colour and collar number. Group A and B puppies were infected orally with 500 *A. caninum* infective larvae through a pipette as described by Mittra et al. (1984). Group C and D were not infected with *A. caninum* infective larvae; they were positive and negative controls respectively. On day nineteen, thirteen puppies were screened for presence of *A. caninum* eggs in their faecal materials.

**Feeding of experimental animals**

Puppies in group A and C were kept on the commercial dog diet (SUPA DOG®) which contained 3,200 Metabolisable Energy/kg, 22.00% protein, 8% fats, 3.29% fibre, 1.21% calcium, 0.4% sodium, 0.5% chloride, 0.8% phosphorus, 1.35 lysine, 0.85 methionine, 2grams trimethylglycine, 150 grams iron, 400 mg manganese, 50 mg cooper, 350 mg zinc, 0.5 mg selenium, 50,000 IU vitamin A, 15,000 IU vitamin D, 75I U vitamin E, 5 mg vitamin K, 15 mg vitamin B1, 25 mg vitamin B2, 5 mg vitamin B6, and 0.05 mg vitamin B12 others include 0.5 mg biotin, 150 mg niacin, 100 mg chlorine, 80 mg pantothenic acid, 5 mg folic acid, 2.5 mg iodide, and 5 mg antioxidant. Puppies in group B and D were given unbalanced diet containing 35% corn starch, 35% dried grounded peas, 29% vegetable oils and 1% Sodium chloride as suggested by Forster and Cort (1931).

Water was given *ad libitum* to all puppies for the whole period of the study. During feeding, each puppy in each group was fed separately. Compartments were cleaned, dried and maintained free of fleas.

**Parameters measured**

Body weight measurement, faecal egg per gram count (EPG), packed cell volume (PCV) and Hemoglobin content (Hb) were taken after every 7 days for ten weeks.

**Determination of body weight and blood collection**

Body weight of each puppy was measured using a weighing scale. By using a 21 gauge needle, two ml blood from each puppy was collected and placed into a vacutainer containing Ethylene Damine Tetra Acetic acid (EDTA) for determination of packed cell volume (PCV) and Hemoglobin content (Hb).

**Determination of packed cell volume (PCV)**

Microhematocrit method was used to determine packed cell volume as described by Maxine (1986). A plane capillary tube 75.0 mm x1.0 mm was filled with blood from the vacutainer. The tube was filled up to 1cm from the end. Blood from outside of the tube was wiped while still wet. The vacant end of the tube was sealed with a plastic seal. The centre knurled bolt on the head of the high speed centrifuge was unscrewed and the cover plate removed. The capillary tubes were placed on the head in the slots with open ends towards the hub and sealed ends as close as possible to the rim of the head to prevent the tube from breaking while centrifuging. The cover was replaced securely and the centrifuge allowed running for five minutes at 12,500 revolutions per minute. Then the tubes were removed and by using a linear scale hematocrit reader the percent of PCV for each individual dog was read.

**Determination of haemoglobin content (Hb)**

Cyanmethemoglobin method was used to determine hemoglobin as described by Maxine (1986). About 5 ml of Drabkin's diluent solution was poured in a colorimeter cuvette. Using a sahli pipette 0.02 ml of blood was added to it and the pipette was rinsed at least three times with the diluent. The mixtures were thoroughly mixed and allowed to stand at least for 10 minutes before reading. The optical density (OD) was then read in the spectrophotometer at the 540 nm wavelength. The equivalent haemoglobin was read from the calibration curve. At the end of the study, puppies in group 'A' and the survivors of group 'B' were euthanized by injecting saturated magnesium sulphate solution intravenously as described by Brander and Pugh (1977) to recover the worms.

## Results

Mild diarrhea and rough hair coat were observed in all 3 puppies in three weeks after infection; however, these abnormalities disappeared by the fifth week. No mortality was observed in this group. Three weeks after infection, all four puppies had rough hair coat and watery diarrhoea. In the fourth week, the puppies had reduced appetite, bloody and tarry diarrhea and pale mucous membranes. Two of the four puppies were severely affected; they had potty bellies, ventral and conjunctiva edema. One puppy died suddenly at the end of the fourth week after infection. The other puppies survived up to the end of sixth week when another puppy died with the same clinical signs. The two remaining puppies had intermittent diarrhea, sometimes bloody and tarry, fluctuating appetite, body weakness and stunted growth.

At post-mortem, carcasses were emaciated, pale and edematous. Livers were ischemic with fat infiltration, they had hemorrhagic enteritis with swollen intestinal mucosa and *A. caninum* worms were found in the intestinal lumen and some were found attached into the intestinal mucosa. All worms were recovered, using a magnifying lens. All were counted and identified microscopically. On clinical examination all the three puppies had no any obvious abnormalities for the whole period of the study.

No puppies of group D developed specific clinical signs apart from progressive weight loss; and all puppies survived up to the end of the study.

Mean number of worms recovered from puppies infected with balanced diet was 352±25.86, which was statistically significant lower (P<0.05) than the infected with unbalanced diet group (mean = 465±11.57).

The infected with balanced diet group had a better body weight than the other groups (Fig. 1). During the study, this group had mean body weight gain ranging between 0.17 kgs in the second week and 1.42 kgs in the seventh week. No body weight gain in the ninth week was observed and the mean body weight gain was negative in the eighth week. Generally, in this group, body weight was increasing consistently. The infected with unbalanced diet group had mean body weight gain ranging between 0.08 kg in the fifth week and 0.375 kg in the first week (Fig. 2). No body weight gain was recorded in fourth, sixth and eighth weeks and the mean body weight gain was negative in the third, seventh and ninth weeks. Generally, this group had mean body weight decreasing consistently (Fig. 1).

The uninfected with balanced diet group had mean body weight gain ranging between 0.33 kg in the fourth week and 1.25 kg in the seventh week. The mean body weight gain was negative in the eighth week only (Fig. 2). Generally, this group had mean body weight increasing consistently (Fig. 1). The uninfected with unbalanced diet group had its mean body weight gain ranging between 0.17 kg in the fourth and sixth weeks and 1.25kg in the first week. There was no body weight gain in the last three weeks and the mean body weight gain was negative in the second, third and seventh weeks (Fig. 2).

Mean body weight gain of infected puppies on balanced diet was statisitically significantly higher (P<0.05) than that of infected puppies on unbalanced diet.

Initially the infected with balanced diet group had slightly high EPG counts than the infected with unbalanced diet group (Fig. 3). One week after introducing the diets the unbalanced diet group had its EPG counts rising higher than that of the balanced diet group to reach its highest value of 26,950 five weeks after infection. The EPG counts started to decline six weeks after infection to reach its lowest value of 3,200 counts eight weeks after infection, then it started to rise again nine weeks after infection (Fig. 3). The balanced diet group had EPG counts raised to 12,000 counts three weeks after infection, and then it declined gradually to reach its lowest EPG count of 3,867 in the tenth week (Fig. 3). At the end of the study period, the balanced diet group had EPG counts declining while the unbalanced diet group had EPG count rising.

Analysis of the effect of diet on egg per gram counts of puppies infected with *A. caninum* and fed different diets showed that with the mean 8,050 ± 949 for the balanced diet group and 13,570 ± 2811 for unbalanced diet group, the diets had statistically non significant (P>0.05) effect on egg per gram counts of the puppies in the two groups.

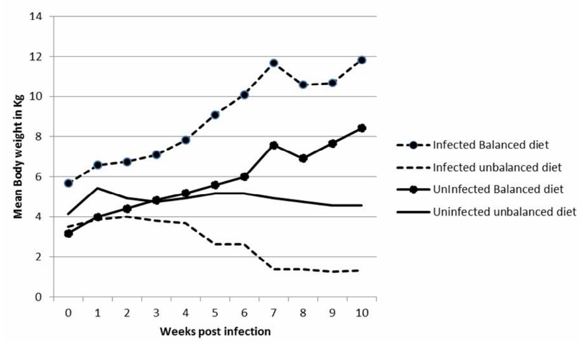


**Fig. 1: Mean body weights of puppies in different weeks**

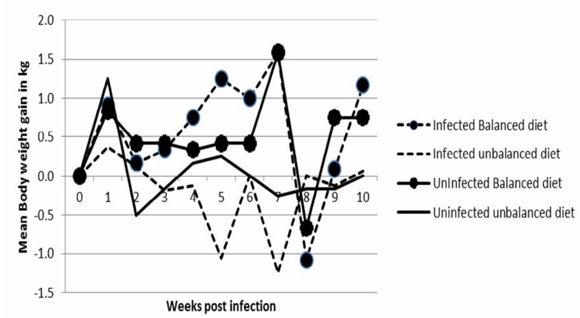


**Fig. 2: Mean body weight gain of puppies in different weeks**

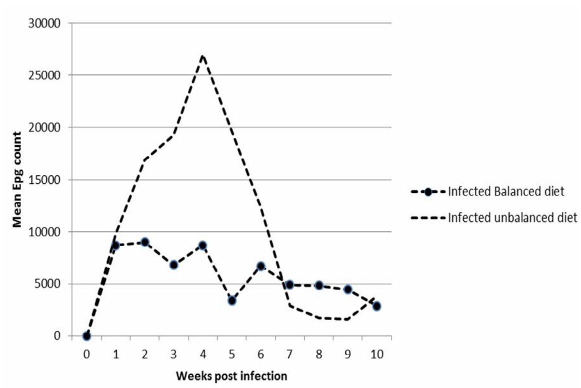


**Fig. 3: Mean egg per gram (EPG) counts in different weeks**

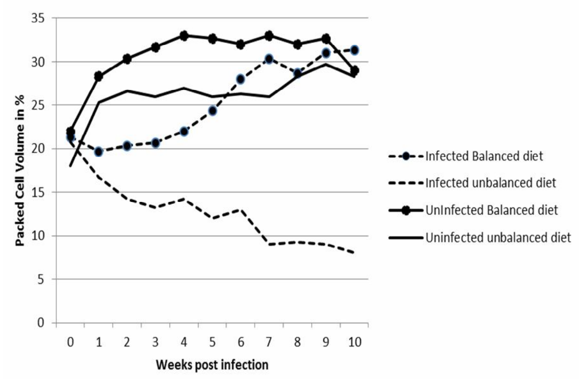


**Fig. 4: Mean PCV of puppies (percentages) in different weeks**

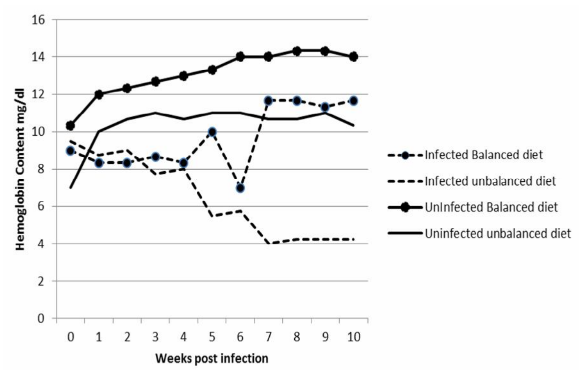


**Fig. 5: Mean haemoglobin (mg/dl) in different weeks**

At the start, mean PCV was 21.3, 20.75, 22 and 18% for the infected with balanced diet, infected with unbalanced diet, uninfected with balanced diet and uninfected with unbalanced diet respectively (Fig. 4). The mean PCV for the uninfected with balanced diet group increased with time and reached its highest value of 33% in the fourth and seventh weeks. This group had the highest mean PCV than the other groups at all times of the study period except in the tenth week. Uninfected with unbalanced diet group had mean PCV ranged between 18% at the start and 29.6% in the ninth week (Fig. 4). The infected with balanced diet group had mean PCV ranged between 19.6% and 31.3%. The lowest mean PCV was observed in the first week and the highest mean PCV was observed in the tenth week. The infected with unbalanced diet group, as for the infected with balanced diet group, initially had mean PCV declining to reach its lowest value of 13.25% in the third week after it started rising again to reach its highest reading of 18.5% in the ninth week but at the end of the study in the tenth week it started declining again.

The mean PCV were 30.58±0.98, 26.13±0.90, 25.34±1.45 and 16.64±0.66% for uninfected with balanced diet, uninfected with unbalanced diet, infected with balanced diet and infected with unbalanced diet group respectively. These values indicate a statistically significant ($P<0.05$) difference of the mean PCV among the experimental groups.

The initial mean Hb were 9, 9.5, 10.3 and 7 mg/dl for the infected with balanced diet group, infected with unbalanced diet group, uninfected with balanced diet group and uninfected with unbalanced diet group, respectively (Fig. 5). At the beginning, mean Hb for the uninfected with balanced diet group increased with time to reach its highest value of 14.3 mg/dl in the eighth and ninth weeks. This group had the highest mean Hb at all times of the study period. Uninfected with unbalanced diet group had its mean Hb ranged between 7mg/dl and 11mg/dl but there were some fluctuation. The mean Hb for the infected with balanced diet group started declining to reach its lowest value of 8.3mg/dl in the second, third and fifth weeks, then it reached its highest value of 11.6 mg/dl in the eighth and tenth week (Fig. 5). The infected with unbalanced diet group, as for the infected with balanced diet group, initially had mean Hb declining to reach its lowest value of 7.2 mg/dl in the fourth week after which it started rising again slowly to reach 8.5 mg/dl in the eighth, ninth and tenth weeks.

The mean Hb was 13.10±0.37, 10.36±0.35, 9.89±0.43 and 8.07±0.19 mg/dl for uninfected with balanced diet group, uninfected with unbalanced diet group, infected with balanced diet group and infected with unbalanced diet group respectively. These values indicated a statistically significant ($P<0.05$) difference in the mean Hb values among the experimental groups.

## Discussion

The results of the present study demonstrated that severity of clinical signs due to *A. caninum* infection is associated with the nutritional status of the animal. More severe clinical signs occurred in puppies fed with unbalanced diet than in puppies fed with balanced diet. The study has demonstrated that unbalanced diet in puppies with *A. caninum* infestation leads to significant increase of intestinal worm burden which in turn results in increased faecal egg per gram counts, more severe clinical and pathological manifestations and deaths of puppies than does the balanced diet. Furthermore, the results have shown that poor diet with *A. caninum* infection leads to significant depression of both PCV and Hb and a significant decrease in body weight gain in puppies. This is the first report of an association of *A. caninum* infestation and nutritional status of dogs in Tanzania.

In the present study, *A. caninum* infection in puppies fed unbalanced diet resulted in significant development of more severe clinical and pathological manifestations than those of infected with balanced diet group. These findings concur with Forster and Cort, (1931) observations which was explained to be a result of lowered resistance of the undernourished puppies in the unbalanced diet group compared to the resistance of the well fed puppies in the balanced diet group.

Worm burden was higher in the infected with unbalanced diet group than in the infected with balanced diet group. This finding is in line with those of Forster and Cort, (1931) who demonstrated that strong immunity in dogs fed balanced diet helps the dogs to expel some of the worms in faeces. These findings underline the importance of provision of balanced diet to puppies to lessen the *A. caninum* burden and reduce severity of clinical disease.

Comparison of growth rate between infected with balanced diet group and the infected with unbalanced diet group showed that irrespective of the infection, balanced diet caused higher body weight gain in the puppies than unbalanced diet. These findings were also reported by Forster and Cort, (1931) who found that with good nutrition, puppies became resistant and expelled some of the worms outside through faeces. Under reduced worm burden the puppies of this group were able to put weight. The present findings suggest that *A. caninum* infestation has less effect on body weight gain in balanced diet fed normal puppies. Thus in addition to regular deworming of dogs using appropriate anthelmintics, good nutrition should be advocated as one way of controlling canine helminthiasis.

Mean egg per gram count of the infected puppies was higher in the unbalanced diet group than in the balanced diet group. However, contrary to Forster and Cort (1931) observations, the difference was not statistically significant. This might be due to the short duration (ten weeks) used in the present study compared to twenty or more weeks used in the previous studies. Also according to Lillis (1967) worm sex ratio might have influenced the results such that numbers of eggs produced were not correct indicators of the degree of parasitism and infestations. Therefore, future studies should consider duration of the study and sex ratio of the infective larvae.

Mean PCV was highest in the uninfected with balanced diet group, followed by uninfected with unbalanced diet group then the infected with balanced diet group. The lowest mean PCV was found in the infected with unbalanced diet group. These results are in agreement with Ndiritu and Al-Sadi (1977) who showed that the uninfected groups had no worms which could cause blood loss through ingestion and bleeding from the attachment site. But the balanced diet group was fed good diet which presumably contributed to the formation of hematological constituents (Dale, 2006). Despite the infection, the infected with balanced diet group had PCV value greater than that of the infected with unbalanced diet group, this concurs with Forster and Cort, (1931) findings that with good diet the puppies got enough nutritional support to develop immunity which helped them to clear infection thus reducing the worm burden, blood uptake by the worms and intestinal hemorrhages, at the same time contributing to the formation of new blood constituents (Dale, 2006).

The infected with unbalanced diet group had the lowest PCV because of blood loss due to blood ingested by the worms and to the bleeding from the points of their attachment in the intestinal mucosa (Ndiritu and Al-Sadi, 1977). But since the puppies were fed poor diet, they were not capable of developing new blood constituents rapid enough to fill the gap (Dale, 2006). The explanations for the variation in haemoglobin content (Hb) in puppy groups in this study follow the same trend and carry the same reasons as described above for PCV.

Balanced diet fed puppies suffer less severe clinical *A. caninum* infestation and have greater body weight gain than puppies on unbalanced diet. Despite the fact that *A. caninum* infestation in puppies reduces red blood cell constituents (PCV and Hb), proper feeding of the puppies at early stages of infestation, reduces this effect significantly to allow puppy survival.

To reduce the effect of worm infestation and improve healthy status of dogs, regular deworming with appropriate anthelmintics is essential and it should be accompanied with proper nutrition. Education to dog owners on the basic dog management aspects which involve routine care of the dogs, preventive health care, internal and external parasite control and nutrition is required so as to improve the health and welfare of dogs in Morogoro.

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