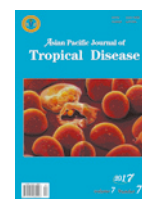


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Comparative mild, moderate and severe effects of Trypanosomiasis and Ancylostomiasis on the Haemogram of Local dog breeds

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ABSTRACT

Objective: To evaluate the haematological parameters and assess the comparatively mild, moderate and severe effects of trypanosomiasis and ancylostomiasis on local dog breeds.**Methods:** Diagnosis of trypanosomiasis was based on clinical signs and presence of *Trypanosoma congolense* in wet mount of infected animals. Diagnosis of ancylostomiasis was based on clinical signs and presence of *Ancylostoma caninum* eggs in fecal samples. Fecal samples were examined using modified McMaster technique. Haematological parameters assessed were packed cell volume (PCV), haemoglobin concentration (HbC), total leucocyte counts and differential leucocyte counts.**Results:** The severity of anaemia produced was graded into mild, moderate and severe. The infected dogs with mild trypanosomiasis and ancylostomiasis had slightly elevated temperatures. Other symptoms observed included dullness, depression, muscular weakness and pale mucous membranes. The infected dogs with mild ancylostomiasis also showed anorexia. The mean PCV and HbC values for mild infections with *T. congolense* and *A. caninum* were significantly ($P < 0.05$) lower than those of normal dogs. The mean total WBC counts for both mild trypanosomiasis and ancylostomiasis were within the normal range when compared with the values for normal dogs. In all the cases of trypanosomiasis and ancylostomiasis, the total differential counts of monocytes were significantly ($P < 0.05$) lower than the normal values. There were obvious clinical signs such as pyrexia, anaemia, enlarged lymph nodes, subcutaneous oedema and occasional ophthalmitis for most of the dogs with severe trypanosomiasis. The mean PCV and HbC values of severe trypanosomiasis and ancylostomiasis were significantly lower than the normal values and also lower than all other cases of the disease condition. Anaemia was associated with both disease conditions but more severe in acute cases of trypanosomiasis.**Conclusions:** This study shows that dogs in this area are at risk of trypanosome infection all the year round, although the incidence of infection is the highest during the rainy season. Understanding on the causes of the changes in the composition of the blood in mixed infections of trypanosomiasis and ancylostomiasis is necessary.

1. Introduction

Prioritization of zoonotic diseases is very important as it facilitates maximum optimization of resources, greater understanding of zoonotic diseases and implementation of policies promoting collaboration between multi-sectoral disciplines and sectors[1].

Trypanosomiasis, a zoonosis, is a haemoprotozoan disease of domestic and wild animals caused by *Trypanosoma*, the extra-erythrocytic haemoprotozoan parasite transmitted by *Glossina* spp. and infected meat[2,3]. Canine trypanosomiasis is a devastating disease leading to anaemia, pyrexia, pale mucous membrane, corneal opacity, infertility, abortions and death if not treated[4-6]. *Trypanosoma congolense* (*T. congolense*), *T. brucei* and *T. evansi* cause the disease in dogs. Dogs could also be infected with *T. gambiense*, *T. rangeli*, and *T. rhodesiense* which are human infective[7-9], but in Nigeria, trypanosomiasis in dogs due to *T. congolense* occurs more commonly[10].

Internal parasitism exerts unpleasant and deleterious effects, and

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it also poses serious health problems in dogs resulting in generalized ill-health, lowered resistance to infectious diseases and retarded growth[11].

Dog hookworm also called *Ancylostoma caninum* (*A. caninum*) due to its high prevalence and zoonotic importance has gained much attention in veterinary practice as well as public health research[12]. It is a very common nematode in tropical and subtropical countries[13].

The larvae of *A. caninum* are associated with creeping eruption in man which is generally referred as cutaneous larva migrans[14]. *A. caninum* is responsible for the induction of eosinophilic enteritis and unexplained abdominal pain with peripheral eosinophilia in man[15]. They are blood sucking nematodes, and in fact 740 million people in developing countries have been reported to be suffering from this disease[15]. Clinical manifestations of this disease include anaemia, body weakness, diarrhoea, dehydration, melena, oedema, alopecia, lichenification and death[13,16,17]. Dogs suffering from ancylostomiasis and other helminth infections generally pose a public health hazard as they act as reservoirs and a source of transmission of zoonotic gastrointestinal helminths to humans and other animals[18].

Haematological profiles have been extensively used in veterinary medicine for evaluation of clinical status, nutritional balance, nutrient and vitamin deficit conditions, monitoring of drug treatment and prognostics in individual animal as well as human[19]. Haematological studies are useful for the diagnosis of both human and animal diseases as well as investigation of the extent of damage to blood[20-22]. Haematological profile evaluations are important in assessing the health status of dogs infected with GI helminths[23]. Therefore, evaluation of haematological parameters was performed in this study to assess the comparatively mild, moderate and severe effects of trypanosomiasis and ancylostomiasis on local dog breeds.

2. Materials and methods

2.1. Study subjects, design and sampling

The study subjects involved local breed dogs that were presented at the University of Nigeria Nsukka, Veterinary Teaching Hospital. Haematological parameters of parasitized and non-parasitized dogs were compared for statistical difference and also with published reference values[24,25].

The sample size was determined by convenient sampling based on the number of blood samples that were concurrently collected and matched with fecal samples from the local dogs presented at the University of Nigeria Nsukka, Veterinary Teaching Hospital.

2.2. Laboratory analysis

Diagnosis of trypanosomiasis was based on clinical signs and presence of *T. congolense* in wet mount of infected animals as described previously[9]. Diagnosis of ancylostomiasis was based on clinical signs and presence of *A. caninum* eggs in fecal samples. Fecal samples were examined using modified McMaster technique

following standard procedures as described previously[16]. The severity of anaemia produced was graded into mild, moderate and severe using laid out guideline[26].

2.3. Haematological studies

Blood samples (5 mL) were simultaneously aseptically collected with the saphenous and cephalic venepuncture into well-labelled ethylene diamine tetra acetic acid (EDTA) tubes for the cases of trypanosomiasis and the ones collected from cases of ancylostomiasis were matched with the collected fecal samples. Blood samples were examined immediately or stored at 4 to 8 °C and examined within 24 h for the determination of different haematological parameters.

PCV was measured using the microhematocrit method as previously described[27]. HbC was determined using the cyanmethaemoglobin technique as described by Brar *et al.*[25]. The total red blood cell count was determined using a hemocytometer with an improved Neubauer counting chamber as described by Baker and Silverton[28], and the total and differential white blood cell counts (WBC) were determined using standard procedures as described by Schalm *et al.*[29].

2.4. Ethical considerations

The ethical guidelines governing the use and conduct of experiments using live animals were strictly observed and the experimental protocols were approved by the University of Nigeria, Nsukka Senate Committee on Medical and Research Ethics.

2.5. Statistical analysis

The normal values of haematological parameters (Table 1) for healthy dogs[24,25] were statistically compared with the mean values of those obtained from confirmed cases of infection with *T. congolense* and *A. caninum* using student *t*-test, analysed using ANOVA. All results were expressed as mean \pm SD and values of *P* < 0.05 were considered significant.

Table 1

Normal values and ranges of haematological parameters of local breeds of dogs.

Parameters	Normal	Ranges
PCV (%)	45	30–55
WBC ($\times 10^3/\text{mm}^3$)	12	5.6–16.6
HbC (g/dL)	15	12–16
Neutrophil (%)	69	60–70
Lymphocytes (%)	20	15–38
Monocytes (%)	6	3–8
Eosinophils (%)	0.5	0–2

3. Results

Tables 2–7 show values (mean \pm SD) for mild, moderate and severe infections with *T. congolense* and *A. caninum*, respectively.

The dogs with mild infection with *T. congolense* had slightly elevated temperatures. Other symptoms observed included dullness,

Table 2

Mild effects of trypanosomiasis on the mean monthly haemogram values of local breeds of dogs.

Month	PCV (%)	WBC ($\times 10^3/\text{mm}^3$)	HbC (g/dl)	Neutrophil (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Jan	25.00	12.20	9.40	58.00	17.10	7.10	3.10
Feb	23.50	9.60	8.00	58.90	31.00	1.00	8.00
Mar	24.10	10.70	10.70	59.00	16.10	3.00	7.00
May	25.70	11.00	9.20	59.00	19.00	2.00	3.00
Jun	24.00	16.00	9.60	57.00	21.00	7.00	6.00
Jul	25.00	11.00	10.00	61.00	17.00	3.00	4.00
Sept	27.00	9.70	9.80	67.00	15.10	2.00	3.00
Oct	25.50	11.90	11.10	61.00	17.00	1.00	4.00
Nov	37.00	11.20	13.20	54.00	17.00	1.00	11.00
Dec	37.00	9.60	11.00	64.00	16.00	2.00	4.00
Mean	27.38	10.30	9.20	59.70	17.63	2.90	5.31
SD	19.20	5.69	6.32	11.38	6.32	8.20	12.87

Table 3

Mild effects of ancylostomiasis on the mean monthly haemogram values of local breeds of dogs.

Month	PCV (%)	WBC ($\times 10^3/\text{mm}^3$)	HbC (g/dl)	Neutrophil (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Jan	33.00	18.90	10.60	58.00	39.00	0.00	6.00
Feb	24.00	7.20	11.40	56.00	11.00	0.00	5.00
Mar	30.00	14.60	8.40	89.00	67.00	0.00	0.00
May	20.00	5.80	7.00	30.00	57.00	0.00	3.00
Jun	23.00	5.70	6.00	49.00	60.00	0.00	2.00
Jul	27.00	3.30	6.30	37.00	16.00	1.00	2.00
Sept	25.00	11.10	9.00	79.00	32.00	0.00	5.00
Oct	28.00	9.90	10.50	67.00	46.00	1.00	0.00
Nov	20.00	8.70	7.00	52.00	36.00	0.00	2.00
Dec	25.00	18.20	9.80	63.00	35.00	0.00	2.00
Mean	25.50	10.34	8.60	57.00	39.90	0.20	2.70
SD	11.38	12.60	7.26	73.00	39.50	9.48	5.70

Table 4

Moderate effects of trypanosomiasis on the mean monthly haemogram values of local breeds of dogs.

Month	PCV (%)	WBC ($\times 10^3/\text{mm}^3$)	HbC (g/dl)	Neutrophil (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Jan	16.50	15.70	6.10	64.00	14.00	0.00	4.00
Feb	19.30	18.50	6.60	81.00	16.10	1.00	0.00
Mar	19.20	17.40	5.80	75.00	25.10	0.00	4.00
May	27.10	14.60	5.60	53.00	20.00	0.00	6.00
Jun	30.10	21.80	7.10	49.00	24.00	0.00	5.00
Jul	31.00	29.90	10.80	61.00	18.50	0.00	4.00
Sept	26.00	24.70	8.60	80.00	8.00	0.00	0.00
Oct	24.00	17.00	7.10	82.00	19.00	0.00	2.00
Nov	26.00	25.70	6.80	58.00	25.00	0.00	3.00
Dec	18.00	20.60	6.70	68.00	20.00	0.00	0.00
Mean	23.72	20.59	7.25	63.10	20.38	0.10	2.80
SD	18.90	8.90	5.70	55.97	17.60	9.48	5.69

Table 5

Moderate effects of ancylostomiasis on the mean monthly haemogram values of local breeds of dogs.

Month	PCV (%)	WBC ($\times 10^3/\text{mm}^3$)	HbC (g/dl)	Neutrophil (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Jan	60.00	24.10	16.60	79.00	19.00	0.00	2.00
Feb	18.00	10.00	4.70	38.00	28.00	0.00	3.00
Mar	37.00	9.60	5.60	64.00	16.00	0.00	4.00
May	37.00	10.00	5.10	54.00	27.00	0.00	9.00
Jun	16.00	6.20	7.30	58.00	16.00	0.00	4.00
Jul	37.00	14.30	7.00	54.00	20.00	0.00	1.00
Sept	50.00	20.40	8.00	89.00	20.00	0.00	1.00
Oct	43.00	23.20	4.40	79.00	22.00	0.00	6.00
Nov	24.00	66.00	4.20	51.00	21.00	0.00	6.00
Dec	44.00	15.60	6.30	69.00	15.00	0.00	1.20
Mean	36.60	13.49	6.92	59.70	20.40	0.00	5.10
SD	33.00	20.87	16.40	11.38	11.54	0.00	8.54

Table 6

Severe effects of trypanosomiasis on the mean monthly haemogram values of local breeds of dogs.

Month	PCV (%)	WBC ($\times 10^3/\text{mm}^3$)	HbC (g/dl)	Neutrophil (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Jan	13.00	24.7	4.60	50.00	40.00	0.0	0.0
Feb	19.00	25.2	5.00	45.00	43.00	0.0	2.0
Mar	22.00	14.7	4.30	89.00	25.00	0.0	9.0
May	21.00	13.9	6.00	87.00	20.00	0.0	4.0
Jun	16.50	16.4	5.30	44.00	30.00	0.0	3.0
Jul	19.00	16.8	4.90	89.00	30.00	0.0	6.0
Sept	18.50	18.3	4.10	59.00	41.00	0.0	4.0
Oct	17.50	24.3	5.00	58.00	40.00	0.0	4.0
Nov	22.00	7.0	4.80	49.00	17.00	0.0	4.0
Dec	12.00	17.9	5.70	56.00	20.00	1.0	0.0
Mean	18.50	17.8	4.97	52.60	14.60	0.1	3.6
SD	9.49	4.11	4.74	17.39	12.86	0.0	5.6

Table 7

Severe effects of ancylostomiasis on the mean monthly haemogram values of local breeds of dogs.

Month	PCV (%)	WBC ($\times 10^3/\text{mm}^3$)	HbC (g/dl)	Neutrophil (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Jan	11.50	27.20	5.00	58.0	20.00	1.0	4.0
Feb	16.50	14.50	4.00	66.0	20.00	0.0	1.0
Mar	22.00	19.20	3.10	67.0	16.00	0.0	4.0
May	16.00	4.60	4.10	51.0	22.00	1.0	3.0
Jun	16.00	14.60	3.10	62.0	17.00	1.0	4.0
Jul	25.00	4.90	3.80	34.0	31.00	0.0	7.0
Sept	21.00	9.70	3.70	64.0	15.00	1.0	11.0
Oct	18.00	23.30	3.50	72.0	18.00	1.0	10.0
Nov	26.00	31.10	3.50	65.0	21.00	2.0	9.0
Dec	16.50	11.00	3.30	61.0	20.00	1.0	1.0
Mean	18.88	14.25	3.61	60.0	18.52	0.8	5.4
SD	10.10	8.90	3.20	27.8	15.80	8.9	9.5

depression, muscular weakness and pale mucous membranes. The mean PCV and HbC values for mild infection with *T. congolense* were significantly ($P < 0.05$) lower than the normal values (Table 1). The mean PCV and HbC values for mild infection with *A. caninum* (Table 3) were significantly ($P < 0.05$) lower than the normal values. Most dogs in this group recorded marked weakness, anorexia and slightly pale mucous membrane. However, values of mean PCV and HbC in mild infection with *A. caninum* were lower than those in dogs with mild *T. congolense* infection. The mean total WBC counts for both mild infections with *T. congolense* and *A. caninum* (Tables 2 and 3) were within normal range when compared with the normal values (Table 1).

Some of the dogs with mild and moderate infections with *A. caninum* had mean differential neutrophil and lymphocyte counts below the lower limit of the normal range. There was slightly significant difference ($P < 0.05$). For the mild infection with *A. caninum* in Table 3, the lymphocyte count is significantly higher ($P < 0.05$) compared with that in all cases of trypanosomiasis and ancylostomiasis (Table 2, 4–7), and the total differential count of monocytes was significantly ($P < 0.05$) lower than that of the normal values in Table 1.

In moderate infection with *T. congolense* as shown in Table 4, the mean PCV and HbC were significantly ($P < 0.05$) lower than normal values. The same finding is presented when compared the normal values (Table 1) with those in severe infection with *T. congolense* shown in Table 6. The effect of the moderate *T. congolense* infection on the mean PCV and HbC was shown in Table 4. In these cases,

higher rectal temperatures and higher parasitaemia were recorded with more obvious clinical signs than the moderate *A. caninum* infection (Table 5), compared with the normal values and this increase was not significant ($P > 0.05$). Leukopenia in moderate ancylostomiasis was significantly less ($P < 0.05$) when compared with the normal dogs (Table 5). Although most of the dogs with moderate *A. caninum* infection in Table 5 showed little or no clinical signs compared to their counterparts with moderate *T. congolense* infection (Table 4), their mean HbC value was significantly ($P < 0.05$) lower than that of dogs with *T. congolense* infection.

The mean differential neutrophil and lymphocyte counts for moderate *T. congolense* infection and severe *A. caninum* infection (Tables 4 and 7) were within normal range and there was no significant difference between these two groups, but the eosinophil counts were above normal range, indicating eosinophilia.

There were obvious clinical signs such as pyrexia, anaemia, pale mucous membrane, enlarged lymph nodes, subcutaneous oedema and occasional ophthalmitis for most of the dogs with severe trypanosomiasis. The clinical signs observed included pale mucous membrane, diarrhoea, weakness and emaciation. The mean PCV and HbC of severe cases of trypanosomiasis and ancylostomiasis are shown in Tables 6 and 7 respectively, which were significantly lower than that of the normal values and also lower than all other cases of the disease condition. However the effect of trypanosomiasis on the PCV was more severe than that in severe ancylostomiasis (Tables 6 and 7). The difference however was not significant ($P > 0.05$). The values of HbC for severe trypanosomiasis shown in Table 6 was

higher than that of severe ancylostomiasis (Table 7), although there was no significant difference ($P > 0.05$).

The mean total WBC counts in Tables 6 and 7 were significantly ($P < 0.05$) higher than the normal value, but in the severe cases of ancylostomiasis (Table 7) it was significantly ($P < 0.05$) lower than that in severe cases of trypanosomiasis (Table 6). There was a corresponding decrease in the total differential neutrophil and lymphocyte counts in severe cases of trypanosomiasis and ancylostomiasis. Lymphocytosis was significantly ($P < 0.05$) greater in severe cases of ancylostomiasis than that observed in severe cases of trypanosomiasis. Also the eosinophil count observed in severe cases of ancylostomiasis and trypanosomiasis in the present study (Tables 6 and 7) was significantly higher ($P < 0.05$) when compared with that of normal local dogs (Table 1), although that of severe cases of ancylostomiasis was significantly ($P < 0.05$) higher than that of severe cases of trypanosomiasis.

It is interesting to note that the mean total WBC counts in severe ancylostomiasis and trypanosomiasis were significantly ($P < 0.05$) higher than those in all other cases of the disease except moderate trypanosomiasis.

From the general overview, the mean values of PCV and HbC in all cases of the infections were lower than the normal value and the mean total WBC counts and the total mean differential WBC counts were increasing and decreasing in the ratio of 3:1 for neutrophils and lymphocytes respectively.

4. Discussion

Trypanosomiasis is a widespread disease of animals in Nigeria and a continual major cause of mortality in companion animals especially dogs[30-32]. Trypanosomiasis causes great haematological alterations in the infected animals[33]. Anaemia is a cardinal feature of trypanosomiasis, in which red blood cells are removed from the circulation by the mononuclear phagocytic system[5]. In the present study, the incidence of trypanosomiasis is associated with lower PCV and HbC values for the mild, moderate and severe cases. This agrees with the findings of other published researches[2,6]. Low PCV value observed in this present study may be a result of acute haemolysis due to growing infection[34].

There is also corresponding decrease in the HbC values in almost all cases of canine trypanosomiasis, except for very few dogs with high HbC value which was not statistically significant ($P < 0.05$); the decrease in HbC values agrees with the reports by Guedes *et al.*[35]. The WBC counts of the infected dogs increased relative to the normal value in some cases; this agrees with the finding of other reports, which observed similar findings in goats suffering from trypanosomiasis[36], in dogs infected with *Trypanosoma brucei brucei*[37], and in rats infected with *Trypanosoma brucei brucei*[38]. The other researchers also reported leucocytosis in canine trypanosomiasis[35,39], but in the report by Adeyemi *et al.*[38], leucocytosis was preceded by either a slight depression or no change in mean leucocyte counts. This result did not agree with the reports of leucopenia[31,40,41] in natural cases of canine trypanosomiasis.

The low lymphocyte counts seen in this paper agree with the findings of Kaggwa *et al.*[39]. The relative lymphocytopenia in the cases of moderate to severe anaemia and related clinical signs may be suggestive of canine trypanosomiasis; this does not agree with the findings of the published reports[41,42], which reported lymphocytosis in a case of canine trypanosomiasis. The neutrophil counts varied as the disease developed. The changes in neutrophil counts in the cases of canine trypanosomiasis were in two phases. There was an initial rise which occurred in the acute phase of the disease and decrease as the infection subsided; the neutropenia observed in this present study agrees with findings of Nongo *et al.*[41]. In the mild case of canine trypanosomiasis, there was a marked decrease in monocyte counts, which agrees with the findings of other reports[43,44]. The elevated mean eosinophil counts in cases of trypanosomiasis agrees with the reports of eosinophilia in natural cases of canine trypanosomiasis[31].

Dogs are frequently infected by one or more species of endoparasites, which can lead to secondary infections that cause high morbidity and death[45]. *A. caninum* parasitizes the small intestine in felids and domestic and wild canids leading to anaemia and intestinal lesions[46,47].

Anaemia observed in the cases of canine trypanosomiasis in this present study is almost always associated with canine ancylostomiasis. Anaemia was observed in the dogs diagnosed to have ancylostomiasis, which is consistent and in agreement with previous studies reporting anaemia as an important consequence of the pathological process in *A. caninum* infections[48-50]. There were no elevated PCV or HbC values as described by Miller[51]. The very low PCV and HbC values observed in this present study agree with findings of previous reports[45,52]. These findings can be attributed to poor nutritional status, age of the dogs infected by *A. caninum*, the sex and stage of the disease. The findings could also be attributed to blood loss due to blood ingested by the worms and to the bleeding from the points of their attachment in the intestinal mucosa[53]. However, in a few cases of the dogs even in moderate and severe infection, the PCV and the corresponding HbC values were high. This interesting finding could be attributed to differences in immune status of these animals, dehydration due to diarrhoea and vomiting, therefore leading to haemoconcentration[54].

In the cases of canine ancylostomiasis, eosinophilia was common and this agrees with the findings of other studies[45,55]. Eosinophilia appeared to be a non-differential finding for the two conditions studied in this present study. This alteration (eosinophilia) may be related to the inflammatory response that is generated during the migratory phases which occur before the parasite reaches sexual maturity[45].

Lymphocytosis observed in this present study in severe cases of ancylostomiasis is in agreement with the findings of Dracz *et al.*[45]. Lymphocytosis is characteristic of the inflammatory processes like trypanosomiasis, in which there is increased lymphopoiesis in response to the antigenic and cytokine stimuli that trigger the host response against the infection[56].

The fall in circulating white blood cells in general cells in general especially the neutrophils, is connected with their role in the defence

of the host against infection, some of these cells being destroyed in the process^[57]. The relative lymphocytosis observed in this study may be attributed to the role of lymphocytes in antibody production and their corresponding activity in the general body immunological response.

The findings in this study may not reflect the true incidence of trypanosomiasis, as the study subjects selected were just clinic population. Only few cases of the infected dogs had PCV within normal ranges. This could be due to the fact that the dogs are trypanotolerant. This study shows that dogs in this area are at risk of trypanosome infection all the year round, although the incidence of the infection is highest during the rainy season. On this basis, it is suggested that more attention should be paid to controlling this disease by veterinarian and animal care givers in this zone during the rains when the incidence is very high. However understanding of the causes of the changes in the composition of the blood in these studied parasitic infections is necessary.

Conflict of interest statement

We declare that we have no conflict of interest.

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