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Clinical Effects of Mixed Infection of Trypanosomes and *Ancylostoma caninum* in Dogs and Treatment with Diminazene and Mebendazole (Nigeria)

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Authors' contributions

This work was carried out in collaboration between both authors. Author BMA designed the study and the protocol. Author RION wrote the first draft of the manuscript, managed the literature searches, and managed the experimental process and analyses of result. Both authors read and approved the final manuscript.

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ABSTRACT

The socio-economic importance of trypanosomosis and ancylostomosis in both humans and animal necessitated the investigation of the clinical signs of single and conjunct infection of both parasites in dogs. Sixteen dogs grouped into 4 of 4 members each were used in the study. GROUP I was uninfected dogs (control), GROUP II was infected with *Ancylostoma caninum* GROUP III was infected with *Trypanosoma brucei* (*T. brucei*), GROUP IV was mixed infections of *Trypanosoma brucei and Ancylostoma caninum* (*T. bruceilA. caninum*). Post acclimatization, *Ancylostoma caninum* infection was done on GPII and GPIV. Two weeks later *Trypanosoma brucei* infections was done on GPIII and superimposed on GPIV. Three weeks post trypanosome infection; GPIII and GPIV were treated with 7 mg/kg diminazene aceturate (Veribin®, CEVA Sante Animale- La Ballasteiére 33501 Libourne Cedex, France) x intramuscularly x once. Mebendazole

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(Vermin®, Janssen-Cilag Ltd 50 - 100 Holmers Farm Way, High Wycombe, Bucks, HP12 4EG UK) at 100 mg x *per os* twice daily for 3 days was used only on GPII and GPIV and a repeat treatment given 2 weeks later. Prepatent period of *T. brucei* infection was 5.00±1.30 days in single infection and 3.00±1.40 days in conjunct infection of *T. brucei* and *A. caninum.* Persistent parasitaemia resulted in repeated treatment with diminazene aceturate at 7 mg/kg and mebendazole at 100 mg twice daily for 3 days. The predominant signs revealed include; fluctuation in weight, lethargy, vomition, enlargement of popliteal lymphnodes, pyrexia, oedema of lower jaw and ocular discharges, enlarged abdomen, anaemia, cornea opacity and slight emaciation. The clinical signs were most severe in GPIV compared to GPIII. The egg per gram of faeces (EPG) in GPII was significantly higher than the mixed infection (GPIV). Treatment only slightly improved clinical manifestations.

In conclusion, most signs shown were consistent with trypanosomosis in dogs except abdominal enlargement which is a complication of *A. caninum*. Clinical signs therefore could serve as a diagnostic tool in the treatment of both conditions in dogs.

Keywords: Clinical signs; Trypanosoma brucei; Ancylostoma caninum; diminazene aceturate; mebendazole.

1. INTRODUCTION

Trypanosomosis is a wasting disease in which there is a slow progressive loss of condition characterized by progressive anaemia and weakness to the point of extreme emaciation, recumbency and death often sequel to heart failure [1]. It is one of the major diseases ravaging animals in Nigeria especially within Nsukka area in Enugu State. All species of trypanosomes, with the exception of some strains of *T. vivax* which produce a hyper acute and acute infection, are characterized by high parasitaemia, pyrexia, severe anaemia and haemorrhages on the mucosal and serosal surfaces [2]. It also produces some level of immunosuppression in infected animals [3]. The level of clinical manifestations is dependent on the severity of the disease in different species [1]. Similarly climatic condition such as cold weather plays a significant role in body physiology [4]. It significantly influences temperature changes in disease conditions. The relative humidity within Nsukka area during the months of July to September which coincides with period of rainy season could influence temperature variation in trypanosomosis. Ancylostomosis caused by *Ancylostoma caninum* is the most pathogenic of most gastrointestinal parasitic infections of dogs. It is an important cause of anaemia, and impairs the healthy wellbeing and productivity of infected dogs. Mixed infections of helminthosis and trypanosomosis are common in the field [5] and it would thus be useful to investigate the impact of such coinfection in dogs. On same note chemotherapy is the most widely used method of treatment and control of these infections [6,7] and treatment of infected animals result in clinical improvement [8]. In view of the effects of trypanosomosis and involvement of the host's immunity in optimizing medical therapies [9,10], it is necessary to examine clinical manifestations and response to treatment in single *T. brucei, A. caninum* and conjunct infection of both diseases.

2. MATERIALS AND METHODOLOGY

2.1 Experimental Animals

Sixteen mongrel breed of dogs of both sexes aged 5 to 6 months and weighing between 4.0 and 8.0 kg with average weight of 5 kg were used in this experiment. The dogs were purchased within Nsukka environ and acclimatized for 4 weeks before commencement of the experiment during which they were screened for blood parasites and confirmed negative by Giemsa-stain, thin blood smears and haematocrit buffy coat method [11]. They were dewormed with tablets of mebendazole (Vermin®, Janssen-Cilag Ltd 50 - 100 Holmers Farm Way, High Wycombe, Bucks, HP12 4EG UK) at the dose of 100 mg twice daily for 3 days and also treated with sulfadimidine at the dose of 48 mg/kg intramuscularly against systemic opportunistic bacterial infections. The experiment commenced a week later. The animals were kept in clean cages in a fly proof house and fed twice daily. Water was given *ad libitum*.

2.2 Care of Experiment Animals

The care of the animals was in conformity with the guideline for animals' experimentation of

Council for International Organization of Medical Sciences (CIOMS) for biomedical research involving animals. The dogs were humanely cared for and treated throughout the study. They were comfortably housed in properly ventilated pens in good hygienic condition and provided good and adequate feeding with clean portable drinking water. Authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.3 Parasites and Infections

2.3.1 *Trypanosoma brucei* isolate

Trypanosoma brucei used in the study was a local isolate obtained from a clinically infected dog from Nsukka area in Enugu State. The isolate was typed and confirmed in the department of Veterinary Parasitology and Entomology, University of Nigeria Nsukka. The parasites were maintained in rats and subsequently passage in a donor dog from where the experimental dogs were inoculated.

Estimated 2.5×10^6 of *T. brucei* suspended in 1 mL of normal saline was used to infect each experimental dog in the group. The quantity of parasites inoculated was estimated using the rapid matching method of [12].

2.3.2 *Ancylostoma caninum* infection

The concentration of larval suspension was estimated using an automatic pipette (Biotht Peoline®), according to the method of [13]. Small doses of 20 µL larval suspensions were placed as drops on a microscope slide and counted under ×40 objective of a light microscope (Ozympu®). Dogs were starved prior to infection for ease of establishment of infection. A dose of 200 infective L_3 suspended in 1mL of distilled water was delivered *per os* to each of the experimental dogs, using a 2 mL syringe without needle.

2.3.3 Reconstitution of diminazene aceturate

A 2.36 g Veribin® a brand of trypanocide containing 1.05 g of diaminazene aceturate was reconstituted with 15 mL of distilled water according to manufacturer's recommendation. The volume of diminazene acetutate administered to individual dogs in GPIII and GPIV, were calculated from their weight at the dose of 7 mg/kg via the intramuscular route.

2.3.4 Administration of mebendazole

Tablets of mebendazole (Vermin®, Janssen-Cilag Ltd 50 - 100 Holmers Farm Way, High Wycombe, Bucks, HP12 4EG UK) was given at the dose of 100 mg *per os* given twice daily for 3 days. Treatment was repeated 2 weeks later.

2.3.5 Experimental design

Dogs were randomly divided into 4 groups of 4 members in each group. GROUP I was uninfected dogs (control), GROUP II was infected with *Ancylostoma caninum,* GROUP III was infected with *Trypanosoma brucei*, and GROUP IV was mixed infections of *Trypanosoma brucei and Ancylostoma caninum.* Post acclimatization, *Ancylostoma caninum* infection was done on GPII and GPIV alone. Two weeks later *Trypanosoma brucei* infections was done on GPIII and superimposed on GPIV. Three weeks post trypanosome infection; GPIII and GPIV were treated with diminazene aceturate. Mebendazole was used only on GPII and GPIV and a repeat treatment given 2 weeks later.

Parasitaemia was determined using the wet mount method and the haematocrit buffy coat method [11]. The prepatent period of infection in the individual dogs was also determined.

2.3.6 Parameters monitored

The parasitaemia, clinical signs, temperature changes, egg per gram of faeces, were determined at daily intervals.

2.3.7 Evaluation of clinical signs

Clinical signs present were evaluated using the "score method" essentially as described in [14]. Briefly, range of variation in the lesions was divided into ordinal classes viz: Absent (0), Mild (+), Moderate (++) or Severe (+++). The result was first analyzed using paired sample T test and presented as mean percentage ± standard error. The level of significance was accepted at $p = 0.03$.

2.4 Statistical Analysis of Data

Data obtained on temperature and egg per gram of faeces (EPG) were presented as mean ±

standard error of mean (SEM). Statistical significance was analyzed using one way analysis of variance (ANOVA) and Duncan's multiple range test of SPSS version 16 soft ware package. The acceptance of level of Significance was at $P < 0.05$ [15].

3. RESULTS

The prepatent period of *T. brucei* infection was 5.00±1.30 days in single infection and 3.00±1.40 days in conjunct infection of *T. brucei* and *A. caninum.*

3.1 Parasitaemia

The results on parasitaemia are shown in (Table 1). Two out of 4 dogs in GPIV became positive on day 23 (3 days post infection). By day 24, the remaining two dogs became positive including two out of 4 dogs in GPIII. By day 25 of the experiment, all the *T. brucei* infected groups (GPIII and GPIV) had become patent with trypanosome infection. Treatment commenced on days 42 and 49. There was persistence of parasitaemia in a dog in GPIII and 3 dogs in GPIV (*T. brucei* and *T. brucei* + *A. caninum* infected groups respectively). By day 56, the number of relapse cases increased to 3 dogs in GPIII and all in GPIV. A repeat treatment same day cleared parasitaemia in both groups (GPIII and GPIV) except in a dog in GPIII. Further treatment on day 63 completely eliminated parasitaemia from both groups (GPIII and GPIV). By day 77, mortality was recorded in both GPIII and GPIV.

Fig. 1. Second degree emaciation (thin) in a dog with single *T. brucei/ A. caninum* infection at day 42 post infection

3.2 Feacal Egg Count

The results of faecal egg output were presented in (Table 2). The Prepatent period of *A. caninum* was established by 13 to 14 and 14 to 16 days, respectively in the conjunct *T. brucei /A. caninum* and single *A. caninum* groups. By day 21, the EPG in GPII was significantly ($p < 0.05$) higher compared to GPIV. This persisted up to day 42. Treatment on day 42 cleared EPG by day 49. There was recrudescence of EPG in both treated groups by day 56 and by day 63, a repeat treatment completely eliminated eggs in faeces (zero EPG) in both groups.

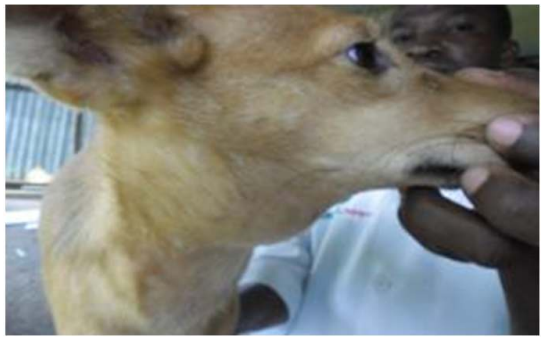

Fig. 2. Oedema of the lower jaw in a dog with single *T. brucei* infection at day 78 post infection

Fig. 3. Enlarged abdomen in a dog with conjunct *T. brucei/ A. caninum* infections at day 56 post infection

3.3 Clinical Manifestation

The clinical manifestations recorded in the infected dogs are shown in Table 3. Clinical signs of infection were only seen after 42days and 21 days respectively post infections of *A.caninum* and *Trypanosoma brucei* infection. The signs shown include; dark coloured foul smelling faeces, enlargement of popliteal lymphnodes, pyrexia, ocular discharges and

slight emaciation. These signs are mostly severe in GPIV compared to GPIII. By day 56, there was persistence of clinical signs despite institution of diminazene aceturate and mebendazole. Instead there were further manifestations of signs like swollen abdomen in GPIV, dullness in GPIII, pale mucous membrane in both GPIII and GPIV. By day 63, there was still persistence of most of the clinical signs including signs of passage of dark coloured foul smelling faeces in GPIII and GPIV. There was yet additional treatment with both diminazene aceturate and mebendazole in the groups. By day 65, there was moderate vomition in all the groups (GPII, GPIII and GPIV). There was also swelling of lower jaw in GPIII. By day 71, there was sudden boost in appetite in both GPIII and GPIV. By day 78, there was development of corneal opacity in GPIII, ocular discharges and emaciation in both GPIII and GPIV.

3.4 Change in Temperature

The result of change in temperature of the experimental groups was shown in Table 4. Following *A. caninum* infection, there was no significant ($p<0.05$) change in the temperature of the group. By day 28, there was a significant ($p<0.05$) increase in temperature in both GPIII and GPIV which persisted until day 49. By day 56 to day 84, there was no significant ($p<0.05$) change in temperature of both GPIII and GPIV compared to GPI.

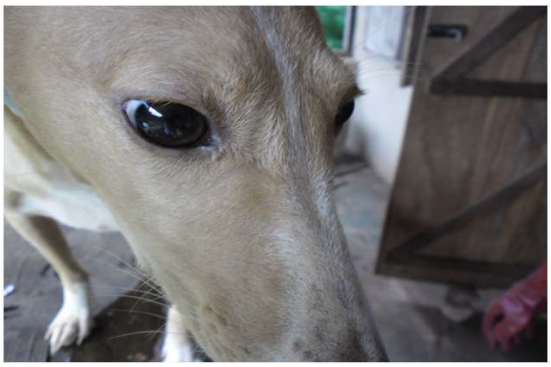

Fig. 4. Ocular discharges in a dog with conjunct *T. brucei /A. caninum* infection at day 56 post infection

Table 1. Parasitaemia of dogs with experimental single *T. brucei, A. caninum* and conjunct infections of *T. brucei* and *A. caninum* and treated with diminazene aceturate and mebendazole

Experimental period (Days)	GP I control	GP II *A. caninum*	GP III *T. brucei*	GP IV *T. brucei/ A. caninum*
0	0/4	0/4	0/4	0/4
1 ⇧	0/4	0/4	0/4	0/4
7	0/4	0/4	0/4	0/4
21 ⇳	0/4	0/4	0/4	0/4
22	0/4	0/4	0/4	0/4
23	0/4	0/4	0/4	2/4
24	0/4	0/4	2/4	4/4
25	0/4	0/4	4/4	4/4
26	0/4	0/4	4/4	4/4
27	0/4	0/4	4/4	4/4
28	0/4	0/4	4/4	4/4
35	0/4	0/4	4/4	4/4
42+*	0/4	0/4	4/4	4/4
49	0/4	0/4	1/4	3/4
56+*	0/4	0/4	3/4	4/4
63*	0/4	0/4	0/4	1/4
70	0/4	0/4	0/4	0/4
77	0/4	0/4	0/3	0/3
84	0/4	0/4	0/3	0/3

⇧ *Ancylostoma caninum infection* ⇳ *Trypanosoma brucei infection; Numerator- Number of aparasitaemic dogs; Denominator- Number of treated dogs; * Administration of diminazene aceturate ; + Administration of mebendazole*

Table 2. Mean egg per gram (EPG) ± se of dogs infected with experimental single *T. brucei*, *A. caninum* and conjunct infections of *T. brucei* and *A. caninum* and treated with diminazene aceturate and mebendazole

Experimental period (days)		GPI (control)	GP II *A. caninum*	GP IV *T. brucei* & *A. caninum*
0		ND	ND	ND
1	⇧	ND	ND	ND
7		ND	ND	ND
14		ND	1.050±110[b]	1.010±120[b]
21		ND	2.050±210[b]	1.600±147[b]
28		ND	2.850±247[b]	1. 025±225[c]
35		ND	3.075±229[b]	1. 250±222[c]
42	+	ND	3.525±206[b]	1.800±265[c]
49		ND	ND	ND
56	+	ND	1.350±529[b]	1.150±463[b]
63		ND	ND	ND
70		ND	ND	ND
77		ND	ND	ND
85		ND	ND	ND

Different superscripts (a,b) in a row indicate significant difference between the group means (p< 0.05).
⇧ *Ancylostoma caninum infection.* ⇳ *Trypanosoma brucei infection; + Treatment with mebendazole; ND=Non detected*

4. DISCUSSION

The period of disappearance of parasitaemia (Table 1) occurred as recorded by other researchers [16,8], thus confirms the potency of diminazene aceturate as a trypanocide. *Trypanosoma brucei* infection in dogs usually produces an acute infection of short prepatent period of 4-6 days [17,18]. Shorter prepatent period in the conjunct groups may be due to the effect of antigenic competition with *Ancylostoma* infection [19]. *Ancylostoma* parasites may have suppressed immune response to secondary trypanosome infection in GPIV thus enhancing early parasitaemia in the group compared to single infections where the immunity was higher. This agree with the reports of [20] and [21] who associated the prepatent period of species of trypanosome to immune status of the host. The delay in onset of clinical manifestation of ancylostomosis in GPII despite eggs in faeces (Table 3) may be due to acquired immunity from previous infection. Animals including humans that have suffered ancylostomosis and was duly treated often develop immunity against future infection. The immunity sustains and ameliorates the effect of ancylostomosis in the host. This contradicts the previous report of [22] who stated that clinical signs of ancylostomosis may appear long before passage of ova eggs in faeces in puppies infected inutero. The confounding factors in the present study may be the age difference and route of infection. The predominant signs in trypanosomes infected groups were (Table 3) consistent with previous records in trypanosomosis [8,23,24]. Emaciation in the groups was due to anorexia. Anorexia deprives the body of essential materials for synthesis of ATP, resulting in mobilization of body reserves.

The pustular dermatitis appeared in *T. brucei / A. caninum* group sequel to enhanced immunosuppression.

Cornea opacity observed in GPIII resolved following disappearance of trypanosomes from blood after repeated treatment. Cornea opacity resulted from infiltration of aqueous humour by inflammatory cells in response to trypanosome infection.

The oedema reported in this study is a common finding in canine trypanosomosis especially in the genus *Trypanozoon* [21,25,26]. Oedema of subcutaneous tissue in the trypanosome groups may be an immunologically mediated reaction manifested as increase vascular permeability and extravasation of fluid into extravascular spaces.

Table 3. Mean percentage (%) ± SE of comparative clinical manifestations in dogs infected with experimental single *T. brucei, A. caninum* and conjunct infections of *T. brucei* and *A. caninum* and treated with diminazene aceturate and mebendazole

Days	Clinical signs	GPI (control)	GPII *(Ac)*	GPIII (*Tb*)	GPIV (*Tb/Ac)*
1 ⇧	None	ND	ND	ND	ND
21	None			ND	ND
	Dark coloured foul- Smelling	ND	ND	ND	100.00± 0.00
42*+	feaces				
	Enlargement of popliteal lymphnodes	ND	ND	75.00± 0.25 [a]	100.00 ± 0.00 [b]
	Pyrexia	ND	ND	50.00±0.25 [a]	100.00± 0.00 [b]
	Ocular discharges	ND	ND	ND	ND
	Emaciation (second degree “thin”)	ND	ND	ND	50.00±0.29
56*	Emaciation (second degree “thin”)	ND	ND	ND	50.00±0.29
	Dullness	ND	ND	50.00±0.29	ND
	Enlargement of popliteal lymphnodes	ND	ND	100.00±0.00 [a]	100.00±0.00 [a]
	Swollen abdomen	ND	ND	ND	75.00±0.25
	Occular discharge	ND	ND	50.00±0.25 [a]	50.00±0.25 [a]
	Pale mucus membrane	ND	ND	100.00 ±0.00 [a]	100.00±0.00 [a]
	Pyrexia	ND	ND	50.00±0.29 [a]	100.00±0.00 [b]
	Passage of dark coloured foul- Smelling feaces	ND	ND	ND	100.00±0.00
63 * +	Dullness	ND	ND	75.00±0.25 [a]	25.00±0.25 [b]
	Passage of dark coloured foul- Smelling feaces	ND	ND	ND	100.00±0.00
	Enlargment of popliteal lymphnodes	ND	ND	100.00±0.00 [a]	100.00±0.00 [a]
	Enlargement of abdomen	ND	ND	ND	75.00±0.25
	Pyrexia	ND	ND	50.00±0.29 [a]	75.00±0.25 [b]
65	Vomition	ND	25.00 +	25.00±0.25 [a]	25.00±0.25 [a]
	Swelling of lower jaw	ND	ND	25.00±0.25	ND
71	Robust appetite	ND	ND	100.00±0.00 [a]	75.00±0.25 [b]
	Slight cornea congestion	ND	ND	ND	ND
	Cornea opacity	ND	ND	25.00±0.25	ND
	Enlargment of popliteal lymphnodes	ND	ND	50.00±0.29 [a]	75.00±0.25 [b]
	Pustular dermatitis	ND	ND	ND	25.00±0.25
78	Dissolution of Cornea opacity	ND	ND	25.00±0.25	ND
	Dullness	ND	ND	75.00±0.25 [a]	25.00±0.25 [b]
	Occular discharges	ND	ND	25.00±0.25 [a]	25.00±0.25 [a]
	Swelling of lower jaw	ND	ND	25.00±0.25	ND

Superscripts a b represents the homogeneity between the experimental groups at probability P<0.03
*A.c A. caninum infection; Tb- T. Brucei; Tb/Ac- T. brucei/A. caninum; ⇧A. caninum infection; Trypanosoma brucei; * Diminazene aceturate treatment; + Mebendazole treatment;*
Mean values within the range of 75.00 – 100.00 % = severe +++; Mean values within the range of 25.00 – 50.00% = moderate ++; ND =Non detected

The absence of pyrexia in GPII observed in this study is in agreement with previous works [22,24], and thus shows that ancylostomosis in dogs is not associated with pyrexia. The significant increase ($p< 0.05$) in temperature observed mostly in GPIV (Table 4) could be dependent on the enhanced level of released pyrogens in the severely stressed dogs. Trypanosomosis is often associated with an increase in body temperature above 39.0ºC [24]. Near absence of pyrexia in both GPIII and GPIV may be related to relatively high environmental

Table 4. Mean ± SE Temperature (°C) in dogs with experimental single *T. brucei*, *A. caninum* and conjunct with *A. caninum* infections and treated with diminazene aceturate and mebendazole

Experimental period (days)	GPI control	GPII (*A. c*)	GPII (*Tb*)	GPIV (*Tb*/*Ac*)
0	37.8±0.10[a]	37.8±0.20[a]	38.3±0.50[a]	37.9±0.10[a]
1 ⇧	37.6±0.30[a]	37.3±0.60[a]	37.6±0.30[a]	38.0±0.70[a]
7	38.3±0.20[a]	37.7±0.20[ab]	37.8±0.20[a]	37.8±0.10[a]
21 ⇳	37.9±0.50[a]	37.5±0.20[a]	38.0±0.80[a]	38.2±0.20[a]
28	37.8±0.70[a]	37.8±0.50[a]	38.5±0.79[ab]	39.4±0.70[b]
35	37.6±0.30[a]	37.5±0.20[a]	39.1±0.60[b]	39.0±0.20[b]
42 * +	38.2±0.50[a]	37.4±0.80[a]	38.6±0.60[ab]	39.7±0.40[b]
49	38.3±0.20[a]	38.2±0.40[ab]	38.3±0.90[ab]	39.3±1.00[b]
56 * +	38.0±0.40[a]	38.4±0.50[ab]	38.5±0.50[a]	38.2±0.70[a]
63*	38.0±0.60[a]	38.9±0.90[ab]	37.2±0.40[a]	38.0±0.50[a]
70	37.8±0.40[a]	37.8±0.50[a]	38.0±0.80[a]	38.0±0.80[a]
77	37.2±0.50[a]	38.3±0.70[a]	37.7±1.10[a]	38.0±1.10[a]
84	37.7±0.80[a]	38.0±0.50[a]	38.1±0.40[a]	38.4±0.30[a]

Superscripts a b c represents the homogeneity between the experimental groups at probability P≤ 0.05.
*+ Treatment with mebendazole; * Treatment with diminazene aceturate; ⇳ Infection with Trypanosoma brucei;*
⇧ Infection with A. caninum; Ac Ancylostoma caninum; Tb Trypanosoma brucei

humidity during the experimental period. Nevertheless, it could also be related to change in wave of parasitaemia seen in trypanosomosis reflecting periods of presence and absences of trypanosomes in the blood. The persistent pyrexia observed in GPIV despite treatments with diminazene aceturate was due to relapse of infection.

The prepatent period of *A. caninum* was shorter than previous report of 15-18 days in young dogs [20]. Low EPG observed in conjunct GPIV (Table 2) compared to single GPII could be the immunosuppressive effect of trypanosome on *Ancylostoma* thus enhancing the number of adult worms in the intestine. Earlier, [27] observed an inverse relationship between the number of adult worms and the number of eggs in the faeces. This contradicts the findings of previous workers [28,29] and [30] who observed significant increases in EPG in animals with concurrent infections with trypanosomosis and helminthosis. Also, [29] recorded high EPG in cattle with conjunct infections of *T. congolense* and haemonchosis compared with the single infections. The clinical signs observed were more and severe in the conjunct trypanosome / *A. caninum* group (GPIV) compared to the single *T. brucei* infection (GPIII) (Table 3). The presence of dark foul smelling faeces in the *Ancylostoma* groups indicates haemorrhages associated with injury to intestinal arterioles. Vomition in most members of *Ancylostoma* group could have been induced by the presence of *A. caninum* in the animals. Treatment with 7 mg/kg i/m of diminazene aceturate and mebendazole at 100 mg twice daily for 3 days slightly improved clinical conditions in the groups. This was observed in the enhanced appetite, dissolution of corneal opacity in single *T. brucei* infected group and disappearance of enlarged abdomen in conjunct *T. brucei / A. caninum* group. This supports the reports of clinical improvement in a dog treated with diminazene aceturate [21,6]. The reappearance of enlarged popliteal lymphnode in the conjunct *T. brucei / A. caninum* group, and oedema of the jaw in single *T. brucei* group maybe due to relapses in parasitaemia in the groups. Relapses of infection recorded at 2 week post treatment (Table 1) suggest resistance in the strains of trypanosomes used. Relapses in animals have been reported to often occur within 8-14 days post treatment as observed in *T. congolense* infection in diabetic rats [31] and in *T. congolense* infection in rabbits [32]. These corroborate findings in this study. In addition, relapse may be due to late administration of diminazene aceturate during the course of the disease as observed in *T. congolense* infection in goats [33]. Contrary to the results of this study [8] reported complete disappearance of *T. brucei* infection at single dose of diminazene at 7 mg/kg. Repeated doses were administered to enhance the therapeutic activity of diminazene aceturate and facilitated parasite clearance following relapse parasitaemia. Mebendazole effectively eliminated hookworm eggs in the feaces (Table 2) following treatment although there was

recrudescence of eggs in faeces 2 weeks post treatment. Mebendazole is one of the commonly used anthelmintic effective against *A. caninum* [34]. Subsequent shedding of eggs in the faeces of treated dogs may be due to migrating juvenile worms (L_3) that escaped the action of anthelminthic to re-establish infection in the intestine as matured egg producing adults. However, second dose of anthelminthic treatment administered 2 weeks after the first dose completely eliminated eggs from the faeces (Table 2) of the dogs.

5. CONCLUSION

In conclusion, the clinical signs manifested in the single *T. brucei* and conjunct infection with *A. caninum* were consistent. The severity of the disease conditions was more in the conjunct group compared to the single infection. Treatment of the diseases with diminazene aceturate and mebendazole caused slight improvement in the clinical condition due to restraint strain of *T. brucei* used in the study. Despite the challenge in relapses, manifested clinical signs could serve as first line tentative diagnostic tool in the management of both diseases in dogs.

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CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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