Serum Biochemical and Liver Enzymes Changes in Dogs with Single and Conjoint Experimental Infections of *Trypanosoma brucei brucei* and *Ancylostoma caninum*

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Abstract: The serum biochemical changes in dogs with experimental single and conjoint infections of *Trypanosoma brucei* and *Ancylostoma caninum* were studied. Four groups (1-4) of five dogs each were used for the study. Groups 1-3 were infected, respectively with 120 L₃ of *Ancylostoma caninum*, 1.5×10^6 mL⁻¹ of *Trypanosoma brucei brucei* (federe strain) only and a combination of *Ancylostoma caninum* and *Trypanosoma brucei brucei* while Group 1 served as uninfected control. The results show that the infections produced significant changes in the conjoint infection than the single infections. There was apparently shorter pre-patent period of *T. brucei brucei* (4-6 days) in the conjoint infection as against 6-9 days recorded in the single *T. brucei brucei* infection. There was also a shorter pre-patent period of 13 days as against 19 days in the single *Ancylostoma caninum*. There was significant high ($p < 0.05$) Blood Urea Nitrogen (BUN) in the infected Groups compared to the control. Also, the total bilirubin and creatinine values of the infected groups were significantly higher ($p < 0.05$) than the control. However, the creatinine levels of Group 3 were unaffected. The serum activities of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) in all the infected groups were significantly higher ($p < 0.05$) while the serum Alkaline Phosphatase (ALP) activity was significantly lower ($p < 0.05$) than the control. It was concluded that conjoint infection of dogs with *Trypanosoma brucei brucei* and *Ancylostoma caninum* which resulted in marked biochemical alterations can be ancillary to diagnosis and useful in prognosis during such natural infections.

Key words: *Trypanosoma brucei brucei*, *Ancylostoma caninum*, single and conjoint infections, serum biochemistry, dogs

INTRODUCTION

Trypanosomosis is a major disease of animals in sub-Saharan Africa (WHO, 2007). The disease is characterised by anaemia and suppression of the host's immune responses (Anosa, 1988; Anene *et al.*, 1989). This leads to suppression of immune response to core vaccines or infections (Rurangirwa *et al.*, 1983) and render animals susceptible to opportunistic infections (Nantulya *et al.*, 1982). Gastrointestinal nematode infections are recognized as major causes of impaired productivity in livestock and domestic animals in the tropics (Chiejina, 1986). The infections are mostly sub-clinical probably due to host acquired or innate resistance (Chiejina, 1987; Fakae, 1990). Fakae and Chiejina (1993) noted that in natural animal populations in sub-Saharan Africa, gastrointestinal helminths causes anemia and the most common and pathogenic for dogs is *Ancylostoma caninum* (Soulsby, 1982; Anene *et al.*, 1996).

Under natural conditions, trypanosomosis and helminthosis often occur in mixed infections (Goossens *et al.*, 1997). The phenomenon of immunosuppression in trypanosomosis may lead to increase in worm burden in such mixed infections with gastrointestinal parasites (Dwinger *et al.*, 1994; Goossens *et al.*, 1997). Consequently, the evaluation of haematological and biochemical parameters in animals are useful adjuncts in the clinical assessments of animal patients and the changes in serum biochemical indices are indicative of ill-health (Coles, 1986). This research was therefore designed to determine the serum biochemical changes that occur in dogs experimentally infected singly and conjunctly with *T. brucei brucei* and *A. caninum*.

MATERIALS AND METHODS

Twenty local dogs of both sexes, weighing between 2.7 and 4.2 kg were purchased from the local market and

used for the study. They were acclimatized for 3 weeks in a netted kennel during which they were investigated for gastrointestinal parasites, blood parasites and ectoparasites and treated accordingly. They were randomly divided into 4 groups of 5 members each. GPI (uninfected control), GPII (*Ancylostoma caninum* infected dogs) GPIII (*Trypanosoma brucei brucei* infected dogs) GPIV (Conjunct infections with *Trypanosoma brucei brucei* and *Ancylostoma caninum*).

Parasites and infections

Trypanosomes/infection: The *T. brucei* used was a Federe strain obtained from the National Institute of Trypanosomiasis Research (NITR) Vom, Plateau State, Nigeria. The parasites were cryopreserved in liquid nitrogen from where donor rats were initially infected. The parasites were thus maintained by serial passage in mice in the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka. Each experimental animal was infected intraperitoneally with 1.5×10^6 trypanosomes suspended in 1 mL of normal saline. The parasites were estimated using the Rapid Matching Method as described by Herbert and Lumsden.

Ancylostoma caninum culture/infection: Faecal samples obtained from dogs at the Nsukka local markets were screened for presence of hookworm eggs. Positive samples were then cultured to obtain infective L₃ and confirmed in the Department of Veterinary Parasitology and Entomology, University of Nigeria Nsukka. Faeces positive for hookworm eggs were mixed thoroughly washed and passed through a sieve with constant stirring using a spatula.

The faecal suspension was then centrifuged at 3,000 g for 5 min using a bench centrifuge (Techmel and Techmel, Texas, USA). The supernatant was poured off and the sediment mixed uniformly and lightly spread onto moist filter study (Whatman®, England) on petri dishes kept at room temperature (25-30°C) and moistened daily to ensure optimum conditions. After 7 days, jets of water from a wash bottle were sprayed on the filter paper and the infective larvae L₃ were allowed to swim out into the pool of water. The larval suspensions were collected and stored at 4°C until used. Each dog was then infected orally with 120 infective L₃ suspended in 1 mL of distilled water, using a 2 mL syringe without needle.

Serum collection: Exactly 3 mL of blood was withdrawn from the cephalic veins of each experimental dog. The blood was delivered into sterile universal bottles with screw caps. It was transported slanted in a cooler containing ice pack to the laboratory within 2 h. The sera

were left at room temperature for at least another 2 h to clot and separate. They were then decanted into clean test tubes and centrifuged at 12000 g for 5 min. Sera obtained were stored in clean labelled bottles at -20°C until analyzed. The serum total protein, total bilirubin, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), urea, creatinine and albumin were determined using Randox® Text kits according to the manufacturer's prescriptions.

Statistical analysis: Data generated were analyzed statistically using SPSS 9.0 Software package by the application of Analysis of Variance (ANOVA). Duncan's multiply range tests were used to separate the means (Scenedor and Cocharm, 1973). Probability values less or equal to 0.05 ($p < 0.05$) were considered significant.

RESULTS AND DISCUSSION

Total protein: The mean serum total protein was significantly lower ($p < 0.05$) in all the infected groups than the control from week 2 till the end of the experiment (Table 1). It is noteworthy that the decrease in the total protein was particularly marked in Group 3 and 4 especially at week 2, 3 and 5 post infections. There was a significant low ($p < 0.05$) serum albumin level in all the infected groups than the control from week 3-5 post infection (Table 2). Almost similar to what was observed in the total protein; the decrease in serum albumin was marked in Group 3 and 4.

Table 1: Mean total protein $\pm$ SE (mg/100 mL) of dogs infected with *T. brucei* or *A. caninum* alone and conjunct *T. brucei/A. caninum*

Experimental period (week)	Groups			
	I <i>A. caninum</i>	II Control	III <i>A. caninum</i>	IV <i>T. brucei</i> and <i>A. caninum</i>
0	5.30 $\pm$ 0.10	5.23 $\pm$ 0.12	5.30 $\pm$ 0.10	5.73 $\pm$ 0.54
1	8.72 $\pm$ 0.55	7.68 $\pm$ 0.80	8.30 $\pm$ 0.42	9.37 $\pm$ 0.20
2	8.63 $\pm$ 0.63 ^a	7.12 $\pm$ 0.92 ^b	1.32 $\pm$ 0.20 ^c	2.03 $\pm$ 0.12 ^c
3	8.18 $\pm$ 0.49 ^a	7.42 $\pm$ 0.60 ^a	5.52 $\pm$ 0.55 ^b	4.50 $\pm$ 0.43 ^b
4	8.38 $\pm$ 0.79 ^a	5.36 $\pm$ 0.35 ^b	5.40 $\pm$ 0.40 ^b	4.78 $\pm$ 0.62 ^b
5	8.1 $\pm$ 0.400 ^a	7.24 $\pm$ 0.56 ^{ab}	5.03 $\pm$ 0.77 ^b	4.80 $\pm$ 1.80 ^b

Table 2: Mean albumin $\pm$ SE (g/100 mL) of dogs infected with *T. brucei* or *A. caninum* and conjunct *T. brucei/A. caninum*

Experimental period (week)	Groups			
	I <i>A. caninum</i>	II Control	III <i>A. caninum</i>	IV <i>T. brucei</i> and <i>A. caninum</i>
0	2.50 $\pm$ 0.280	2.50 $\pm$ 0.040	2.50 $\pm$ 0.04	2.52 $\pm$ 0.050
1	3.34 $\pm$ 0.070 ^a	2.84 $\pm$ 0.140 ^{ab}	2.78 $\pm$ 0.12 ^b	2.94 $\pm$ 0.280 ^{ab}
2	3.53 $\pm$ 0.670	4.56 $\pm$ 0.670	2.34 $\pm$ 0.38	2.93 $\pm$ 0.800
3	4.40 $\pm$ 0.340 ^a	2.0 $\pm$ 0.1700 ^c	0.90 $\pm$ 0.32 ^b	1.03 $\pm$ 0.400 ^b
4	4.43 $\pm$ 0.070 ^a	1.90 $\pm$ 0.120 ^b	1.50 $\pm$ 0.17 ^b	1.43 $\pm$ 0.250 ^b
5	4.40 $\pm$ 0.070 ^a	3.10 $\pm$ 0.380 ^c	1.00 $\pm$ 0.31 ^b	0.65 $\pm$ 0.050 ^b

Different superscripts (a-c) in a row indicate significant difference between the group means ($p < 0.05$)

There was an initial significant high ($p<0.05$) serum urea level in all the infected groups than the control group at 1 week post infection (pi) followed by a decrease from weeks 3-5 of the experiment (Table 3). This decrease was lower in Group 2 than in both the conjunct infection (Group 4) and the single *T. b. brucei* infection (Group 3) although, it was not significant ($p<0.05$). There was a significant high ($p<0.05$) serum creatinine levels in Group 3 and 4 than the control at week 2 (Table 4). Though the serum creatinine level decreased over time in the course of the experiment, the levels in Group 3 and 4 were significantly higher ($p<0.05$) than the control. There was a significant high ($p<0.05$) total serum bilirubin level in Group 4 at week 2 pi than the control (Table 5). This increase continued till the end of week 5. There was however, no significant difference ($p<0.05$) between Group 2 and 3 when compares to the control all through the experiment (Table 5). The levels of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were significantly higher ($p<0.05$) than the control

(Table 6 and 7). Whereas the serum levels of ALT and AST in Group 2 were significantly lower ($p<0.05$) than Group 3 and 4. However, their values were significantly higher than the control all through the experiment (Table 6 and 7). Then, serum level of Alkaline Phosphatase (ALP) was significantly lower ($p<0.05$) in all the infected groups than the control (Table 8). Overall, the ALP level of all the infected groups decreased as the experiment progressed. Even so, there was no significant difference ($p<0.05$) between the infected groups (Table 8).

The decreases in the total serum protein especially in Group 3 and 4 (Table 1) were probably as a result of decreased hepatic biosynthesis. Decreases in the total serum protein have been reported in *Trypanosome brucei* infected pigs and goats (Otesile *et al.*, 1991; Witola and Lovelace, 1997) as well as in rats (Taiwo *et al.*, 2003; Orhue *et al.*, 2005). The hypoproteinaemia observed in all the infected groups may be as a result of progressive loss of albumin in urine from the *T. brucei brucei* infected dogs. Also, some degenerative changes in the liver and

Table 3: Mean urea $\pm$ SE (mg/100 mL) of dogs infected with *T. brucei* or *A. caninum* alone and conjunct *T. brucei/A. caninum*

Experimental period (week)	Groups			
	I Control	II <i>A. caninum</i>	III <i>T. brucei</i>	IV <i>T. brucei</i> and <i>A. caninum</i>
0	11.33 $\pm$ 0.67	11.25 $\pm$ 1.250	11.00 $\pm$ 0.770	11.33 $\pm$ 1.33
1	23.00 $\pm$ 3.10 ^a	48.40 $\pm$ 10.78 ^b	69.75 $\pm$ 7.080 ^b	73.67 $\pm$ 7.88 ^b
2	23.67 $\pm$ 0.33	19.40 $\pm$ 1.960	24.20 $\pm$ 6.350	16.00 $\pm$ 1.47
3	22.00 $\pm$ 1.53 ^a	12.68 $\pm$ 2.740 ^b	11.50 $\pm$ 1.930 ^b	12.00 $\pm$ 2.12 ^b
4	23.00 $\pm$ 0.00 ^a	7.50 $\pm$ 2.470 ^b	10.00 $\pm$ 4.620 ^b	11.33 $\pm$ 2.19 ^{ab}
5	22.33 $\pm$ 3.28 ^{ab}	7.33 $\pm$ 1.860 ^a	9.67 $\pm$ 5.040 ^b	10.50 $\pm$ 1.50 ^c

Table 4: Mean creatinine $\pm$ SE (mg/100 mL) of dogs infected with *T. brucei* or *A. caninum* alone and conjunct *T. brucei/A. caninum*

Experimental period (week)	Groups			
	I Control	II <i>A. caninum</i>	III <i>T. brucei</i>	IV <i>T. brucei</i> and <i>A. caninum</i>
0	0.53 $\pm$ 0.13	0.54 $\pm$ 0.090	0.53 $\pm$ 0.07	0.53 $\pm$ 0.13
1	0.50 $\pm$ 0.00	0.87 $\pm$ 0.990	0.32 $\pm$ 0.27	0.33 $\pm$ 0.23
2	0.56 $\pm$ 0.07 ^a	2.80 $\pm$ 1.670 ^{ab}	9.53 $\pm$ 4.47 ^{bc}	14.00 $\pm$ 0.00 ^c
3	0.50 $\pm$ 0.60	1.60 $\pm$ 2.080	0.90 $\pm$ 0.58	2.70 $\pm$ 3.29
4	0.53 $\pm$ 0.13 ^a	1.06 $\pm$ 0.330 ^{ac}	0.93 $\pm$ 0.19 ^c	2.70 $\pm$ 1.90 ^c
5	0.50 $\pm$ 0.10 ^a	0.88 $\pm$ 0.350 ^a	0.70 $\pm$ 0.00 ^a	1.65 $\pm$ 0.35 ^b

Table 5: Mean total bilirubin $\pm$ SE (mg/100 mL) of dogs infected with *T. brucei* or *A. caninum* and conjunct *T. brucei/A. caninum*

Experimental period (week)	Groups			
	I Control	II <i>A. caninum</i>	III <i>T. brucei</i>	IV <i>T. brucei</i> and <i>A. caninum</i>
0	0.17 $\pm$ 0.00	0.17 $\pm$ 0.07	0.17 $\pm$ 0.07	0.17 $\pm$ 0.07
1	0.17 $\pm$ 0.00 ^a	0.26 $\pm$ 0.05 ^b	0.30 $\pm$ 0.04 ^c	0.18 $\pm$ 0.03 ^{ab}
2	0.18 $\pm$ 0.03 ^a	0.35 $\pm$ 0.16 ^{ab}	0.29 $\pm$ 0.29 ^{ab}	1.05 $\pm$ 0.33 ^b
3	0.15 $\pm$ 0.05 ^a	0.50 $\pm$ 0.24 ^{ab}	0.27 $\pm$ 0.03 ^a	1.13 $\pm$ 0.31 ^b
4	0.13 $\pm$ 0.00 ^a	0.73 $\pm$ 0.27 ^a	0.47 $\pm$ 0.09 ^a	1.73 $\pm$ 0.39 ^b
5	0.13 $\pm$ 0.03 ^a	0.60 $\pm$ 0.24 ^a	0.47 $\pm$ 0.09 ^a	2.15 $\pm$ 0.70 ^b

Different superscripts (a-c) in a row indicate significant difference between the group means ($p<0.05$)

Table 6: Mean alanine aminotransferase mean $\pm$ SE (iu L⁻¹) of dogs infected with *T. brucei* or *A. caninum* alone and conjunct *T. brucei/A. caninum*

Experimental period (week)	Groups			
	Uninfected/control	<i>A. caninum</i>	<i>T. brucei</i>	<i>T. brucei</i> and <i>A. caninum</i>
0	17.25 $\pm$ 1.00	17.33 $\pm$ 2.190	17.33 $\pm$ 1.760	17.33 $\pm$ 2.190
1	14.67 $\pm$ 0.88 ^a	29.25 $\pm$ 4.390 ^{ab}	53.00 $\pm$ 17.24 ^{ab}	61.25 $\pm$ 15.53 ^b
2	15.33 $\pm$ 1.67 ^a	50.25 $\pm$ 13.41 ^{ab}	65.00 $\pm$ 13.76 ^b	69.33 $\pm$ 21.09 ^b
3	15.33 $\pm$ 2.00	54.00 $\pm$ 24.99	79.33 $\pm$ 12.25	87.33 $\pm$ 32.71
4	14.67 $\pm$ 1.30	52.75 $\pm$ 10.43	86.75 $\pm$ 3.840	82.67 $\pm$ 15.21
5	15.33 $\pm$ 2.30 ^a	55.00 $\pm$ 1.410 ^a	92.33 $\pm$ 17.46 ^b	93.50 $\pm$ 9.500 ^b

Table 7: Mean aspartate aminotransferase $\pm$ SE (iu L⁻¹) of dogs infected with *T. brucei* or *A. caninum* alone and conjunct *T. brucei/A. caninum*

Experimental period (week)	Groups			
	I Control	II <i>A. caninum</i>	III <i>T. brucei</i>	IV <i>T. brucei</i> and <i>A. caninum</i>
0	20.60 $\pm$ 0.24	21.40 $\pm$ 1.660	21.40 $\pm$ 1.660	20.60 $\pm$ 2.110
1	21.00 $\pm$ 3.21 ^a	34.75 $\pm$ 5.170 ^{ab}	55.00 $\pm$ 14.39 ^{ab}	72.00 $\pm$ 25.24 ^b
2	20.67 $\pm$ 0.67 ^a	41.6 $\pm$ 7.2400 ^{ab}	63.25 $\pm$ 10.36 ^{bc}	73.25 $\pm$ 11.35 ^c
3	20.67 $\pm$ 4.10	42.67 $\pm$ 30.44	106.00 $\pm$ 50.52	138.75 $\pm$ 51.81
4	21.00 $\pm$ 0.00 ^a	45.67 $\pm$ 13.92 ^{ab}	103.67 $\pm$ 13.64 ^b	139.00 $\pm$ 15.87 ^{ab}
5	21.67 $\pm$ 2.96 ^{ab}	40.25 $\pm$ 3.920 ^a	105.00 $\pm$ 4.580 ^{ab}	135.00 $\pm$ 3.00 ^b

Table 8: Mean alkaline phosphatase $\pm$ SE (iu L⁻¹) of dogs infected with *T. brucei* or *A. caninum* and conjunct *T. brucei/A. caninum*

Experimental period (week)	Groups			
	Uninfected/control	<i>A. caninum</i>	<i>T. brucei</i>	<i>T. brucei</i> and <i>A. caninum</i>
0	76.67 $\pm$ 13.86	76.67 $\pm$ 15.21	76.33 $\pm$ 27.69	76.33 $\pm$ 24.25
1	74.67 $\pm$ 12.02	51.00 $\pm$ 12.93	41.25 $\pm$ 9.660	58.50 $\pm$ 8.810
2	75.00 $\pm$ 8.540 ^a	19.60 $\pm$ 3.430 ^b	14.00 $\pm$ 0.000 ^b	14.00 $\pm$ 0.000 ^b
3	74.67 $\pm$ 16.67	17.50 $\pm$ 3.500	27.67 $\pm$ 13.67	14.00 $\pm$ 0.000
4	76.50 $\pm$ 9.580	23.00 $\pm$ 9.000	9.67 $\pm$ 9.6700	23.33 $\pm$ 4.670
5	75.67 $\pm$ 8.090 ^a	23.00 $\pm$ 9.000 ^{ab}	9.33 $\pm$ 9.3300 ^b	21.00 $\pm$ 7.000 ^{ab}

Different superscript (a-c) in a row indicate significant difference between the group means ($p<0.05$)

intestines may have predisposed the dogs, especially those in Group 2 to osmotic imbalances as a result of the activities of the worm. This leads to protein losing enteropathies from the loss of nutrients required for the synthesis of albumin. Decrease in total serum protein has been previously reported to be caused by intestinal malabsorption of proteins (Gregor and John, 1976). Hypoalbuminaemia (Table 2) observed in all the infected groups were pronounced in Group 3 and 4 and this was attributed in *T. brucei brucei* infected rats (Agu and Egbuji, 2002) to the progressive loss of albumin in the urine. The severe decrease in Group 4 was due to the combined effects of the infections in the dogs.

The initial decrease and subsequent increase in the Blood Urea Nitrogen (BUN) of the infected groups agrees with the reports of Kwem *et al.* (2000) where these fluctuations were attributed to early organ damage following the peak parasitaemia in the 1 week of infection. Similar observations have been reported in *T. brucei gambiense* infected monkeys (Jerry and Victor, 2007) and filariasis in dogs (Hashem and Badawy, 2008). These observations were attributed to increases in metabolic acidosis as well as intravascular haemolysis. The subsequent progressive significant ($p < 0.05$) decrease in BUN could be associated with the failure of the liver to synthesize protein. The changes observed in the total bilirubin were that of hyperbilirubinaemia (Table 4) in Group 4. This is consistent with the findings of Kwem *et al.* (2000) and Jerry and Victor (2007) in *T. brucei gambiense* infected monkeys and was attributed to hepatic damage and consequent inability of the liver to conjugate bilirubin in circulation. In this study, the Group 4 dogs may have been particularly affected because of the liver impairment as a result of the combined effect of the activities of the young larvae of *A. caninum* and sequestered trypanosomes in the liver. The elevated serum creatinine levels in Group 3 and 4 following infection (Table 5) may be associated with impaired kidney function. It may also be as a result of sequestration of the trypanosomes in the muscle tissues of the heart leading to damage to the cardiac smooth muscles and release of creatine kinase with attendant increase in the circulating creatinine (David and Micheal, 2003). The increased activities of ALT and AST, especially in Group 3 and 4 may be due to the effects of trypanosomes in the tissues. Also, the parasites may release these enzymes as metabolites into the blood circulation as their homogenates and suspensions have reported to show activities of these enzymes (Stephen and Gray, 1960). The high levels of AST and ALT observed in Group 4 and 3 show that the higher activity in Group 4 was more as a consequence of

Trypanosoma brucei brucei infection. More so the migrating larvae of *A. caninum* may cause damage to host cells in the wake of their migration leading to mobilization of inflammatory cells along their tracts causing fibrosis and necrosis with the release of these enzymes and their increased activities.

The decreased ALP activity in Groups 2-4 following infection could be attributed to early hepatic damage. Kwem *et al.* (2000) observed progressive liver fibrosis in *Trypanosoma vivax* infected cattle which led to abnormal decrease in ALP as the disease progressed. Akpa *et al.* (2008) recorded a significant increase in the three liver enzymes (AST, ALT and ALP) and attributed it to sequestration of *T. brucei brucei* in several organs including the liver causing damage to the cells. The findings from this study however did not quite agree with this report.

CONCLUSION

It was clear from the study that significant alterations in biochemical parameters occurred in dogs infected singly or conjunctly with *T. brucei brucei* and *A. caninum*. These changes were particularly more marked in case of conjunct infections, probably engendered by the stress of concurrent infection and the immunosuppressive effects of trypanosomiasis in dogs. These changes were non-specific and therefore may be of limited use in the diagnosis of conjunct infections. They may however be useful ancillary evidence and the degree of changes may provide prognostic information.

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