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Full Length Research Paper

Serum biochemical and liver enzymes changes in dogs with single and conjunct experimental infections of *Trypanosoma brucei* and *Ancylostoma caninum*

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The serum biochemical changes that occur in dogs with single and conjunct experimental infections of *Trypanosoma brucei* and *Ancylostoma caninum* were studied. Four groups (GPI, GPII, GPIII and GPIV) of five dogs each were used for this study. GPI was the uninfected control while GPII, GPIII and GPIV were infected with *A. caninum*, *T. brucei* and conjunct *A. caninum/T. brucei,* respectively. Results show that the disease was more severe in the conjunct infection than in the single infections. This was apparent from the shorter prepatent period of *T. brucei* infection (four to six days) in GPIV (conjunct) when compared with six to nine days in GPIII (*T. brucei* alone). Infection with *A. caninum* also showed a shorter patency period of 13 days in GPIV when compared with 19 days in GPII (*A. caninum* alone). Significant decrease (P < 0.05) in total protein occurred in all the infected groups due to hypoalbumineamia. There was a transient rise followed by a sustained decline in the blood urea nitrogen (BUN) concentration in all the infected groups. Total bilirubin and creatinine recorded a significant increase (P< 0.05) in the infected groups, except in GPII where the creatinine level was unaffected. The liver enzymes: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) showed significant increase in the infected groups, while alkaline phosphatase (ALP) showed a significant decrease (P < 0.05). These biochemical changes were in all cases more profound in the conjunct infection, and could thus be ancillary to diagnosis and useful in prognosis during natural infections.

Key words: *Trpanosoma brucei, Ancylostoma caninum*, total protein, albumin, creatinine, blood urea nitrogen, bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT).

INTRODUCTION

Trypanosomosis is still a major disease of animals in sub-Saharan Africa (WHO, 2007). Anaemia is a predominant symptom and a reliable indicator for the severity of trypanosome infection (Anosa, 1988). Another striking feature of African trypanosomes is their capacity to cause immunosuppression in the affected hosts (Anene et al., 1989; Chiejina et al., 2003). The most important consequence of trypanosome suppression in animals is the down-regulation of host immune responses to vaccines used to control important diseases (Scott et al., 1977; Rurangirwa et al., 1983). It also renders animals more sensitive to secondary infections (Nantulya et al., 1982). Importantly, response to parasitic infections may also be suppressed. This is an aspect that has received limited attention in gastrointestinal (GI) nematodes of animals where concurrent infections lead to more severe worm infections (Griffin et al., 1981; Kaufmann et al., 1992; Dwinger et al., 1994; Goosens et al., 1997). Gastrointestinal nematodes are recognized as a major

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cause of impaired productivity in livestock and domestic animals in the tropics (Chiejina, 1986; Fabyi, 1987). Infections are mostly sub-clinical probably due to acquired or innate resistance (Chiejina, 1987; Fakae, 1990). Fakae and Chiejina (1993) also noted that in natural animal population in sub-Saharan Africa, gastrointestinal helminths causes anemia and of these, *Haemonchus contortus* is the most common and most pathogenic for ruminants (Preston and Allonby, 1979; Fritsche et al., 1993) and *Ancylostoma caninum* is the most pathogenic for dogs (Soulsby, 1982; Anene et al., 1996).

Goossens et al. (1997) confirmed that under natural conditions, trypanosomosis and helminthosis often occur in mixed infections. Hence, concurrent African trypanosome and gastrointestinal helminth infections are prevalent in sub-Saharan Africa where they are endemic. Evaluation of haematological and biochemical parameters in animals are useful adjuncts in the clinical assessments of animal patients, whereby changes in serum biochemical indices are indicative of ill-health (Coles, 1986; Bush, 1991).

This work is therefore designed with the objectives to determine the serum biochemical changes that occur in dogs experimentally infected with *Trypanosoma brucei* and *Ancylostoma caninum* singly and conjunctly.

MATERIALS AND METHODS

20 local dogs of both sexes, weighing between 2.7 and 4.2 kg were purchased from the local market and used. They were acclimatized for three weeks in a netted kennel during which they were investigated for gastrointestinal parasites, trypanosomosis and ectoparasitism and thus treated accordingly. They were randomly divided into four groups with five dogs in each group; GPI (uninfected control), GPII (*A. caninum* infected dogs) GPIII (*T. brucei* infected dogs), GPIV (conjunct infections with *T. bruce* and *A. caninum*).

Parasites and infections

Trypanosomes

The *T. brucei* used was a "Federe" strain obtained from the National Institute of Trypanosomosis Research (NITR) Vom, Plateau State. The parasites were cryopreserved in liquid nitrogen from where donor rats were initially infected. The parasite was thus maintained by serial passage in mice in the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka.

Trypanosome infections

Estimated 1.5×10^6 typanosomes suspended in 1 ml of normal saline were used to infect each experimental dog, and infection was done via the intraperitoneal route (i.p.). The quantity of parasite was estimated using the rapid matching method of Herbert and Lumsden (1976).

Ancylostoma caninum

Faeces were collected from dogs gotten from the local market around Nsukka. Positive samples were thus cultured in the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka.

Feacal culture

Homogenous faeces from the *A. caninum* positive samples were first washed with water and passed through a sieve after mashing with a spatula. The suspension was centrifuged at 3,000 rpm for 5 min using a bench centrifuge (Techmel and Techmel, Texas, USA). The supernatant was poured off and the sediment was mixed uniformly and lightly spread onto moist filter paper (Whatman®, England) on Petri dishes. The Petri dishes were kept at room temperature (25 to 30°C) and moistened daily to ensure optimum conditions. The cultures were harvested after one week by spraying jets of water from a wash bottle. The larval suspensions were extracted by using a 10 ml syringe. The suspensions of infective larvae were stored in the refrigerator in test tubes pending use.

A. caninum infection

The concentration of larval suspension was estimated using an automatic pipette (Biotht Peoline®); small doses of 20 µl larval suspensions were placed as drops on a microscope slide and counted under 4× objective of a light microscope (Ozympu®). Estimated infective doses were contained in volumes of approximately 1000 µl. Infection was per os using a 2 ml syringe without needle. Animals were not fed prior to infection so as to establish infection. A dose of 120 infective L3 suspended in 1 ml of distilled water was delivered per os per dog.

Serum collection

Exactly 3 ml of blood was withdrawn from the cephalic veins of the 20 experimental dogs. It was delivered into sterile universal bottles with screw caps. It was transported slanted in a cooler containing ice pack to the Department of Veterinary Medicine Laboratory within 2 h. It was left at room temperature for another 2 h to clot and separate. The sera were decanted into clean test tubes and centrifuged at 12000 *g* for 5 min. Each serum obtained was stored in clean labeled bottles at -20°C until analyzed.

Serum biochemistry

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. The experiment was carried out in duration of five weeks.

Statistical analysis

The means and standard errors of the parameters were calculated. The differences in the means were analyzed statistically using SPSS 9.0 soft ware package by the application of analysis of variance (ANOVA) and Duncan's multiple range tests (Scenedor and Cochran, 1973). Probability values less or equal to 0.05 ($p < 0.05$) were considered significant.

RESULTS

Total protein

The total protein (TP) (mean ± SE) is shown in Table 1.

Table 1. Mean total protein ± SE (mg/100 ml) of dogs infected with *T. brucei* or *A. caninum* alone and conjunctly with *T. brucei/A. caninum.*

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

Different superscripts (a, b and c) in a row indicate significant different between the group means (p< 0.05).

Table 2. Mean albumin ± SE (g/100 ml) of dogs infected with *T. brucei* or *A. caninum* and conjunctly with *T. brucei/A. caninum.*

Experimental period (week)	Group			
	I (Control)	II (*A. caninum*)	III (*T. brucei*)	IV (*T. brucei* and *A. caninum*)
0	2.50±0.28	2.50±0.04	2.50±0.04	2.52±0.05
1	3.34±0.07^{a}	2.84±0.14^{ab}	2.78±0.12^{b}	2.94±0.28^{ab}
2	3.53±0.67	4.56±0.67	2.34±0.38	2.93±0.80
3	4.40±0.34^{a}	2.0±0.17^{c}	0.90±0.32^{b}	1.03±0.40^{b}
4	4.43±0.07^{a}	1.90±0.12^{b}	1.50±0.17^{b}	1.43±0.25^{b}
5	4.40±0.07^{a}	3.10±0.38^{c}	1.00±0.31^{b}	0.65±0.05^{b}

Different superscripts (a, b and c) in a row indicate significant difference between the group means (P < 0.05).

Table 3. Mean urea ± SE (mg/100 ml) of dogs infected with *T. brucei* or *A. caninum* alone and conjunctly with *T. brucei/A. caninum.*

Experimental period (week)	Group			
	I (Control)	II (*A. caninum*)	III (*T. brucei*)	IV (*T. brucei* and *A. caninum*)
0	11.33±0.67	11.25±1.25	11.00±0.77	11.33±1.33
1	23.00±3.10^{a}	48.40±10.78^{b}	69.75±7.08^{b}	73.67±7.88^{b}
2	23.67±0.33	19.40±1.96	24.20±6.35	16.00±1.47
3	22.00±1.53^{a}	12.68±2.74^{b}	11.50±1.93^{b}	12.00±2.12^{b}
4	23.00±0.00^{a}	7.50±2.47^{b}	10.00±4.62^{ab}	11.33±2.19^{ab}
5	22.33±3.28^{ab}	7.33±1.86^{a}	9.67±5.04^{b}	10.50±1.50^{c}

Different superscripts (a, b and c) in a row indicate significant difference between the group means (P < 0.05).

There was a significant decrease (P < 0.05) from the 2nd week pi. in the three infected groups when compared with GPI. Here, decrease observed in GPIII was higher when compared to that in GPII while that in GPIV was highest.

Albumin

The albumin (mean ± SE) is shown in Table 2. There was a significant decrease (P < 0.05) in the three infected groups as compared to GPI. Also, similar to what was observed in TP, decrease observed in GPIII was higher as compared to that of GPII while that in GPIV was highest.

Urea

The urea (mean ± SE) is shown in Table 3. The result show an initial significant increase (P < 0.05) in the three

Table 4. Mean creatinine ± SE (mg/100 ml) of dogs infected with *T. brucei* or *A. caninum* alone and conjunctly with *T. brucei/A. caninum*.

Experimental period (week)	Group			
	I (Control)	II (*A. caninum*)	III (*T. brucei*)	IV (*T. brucei* and *A. caninum*)
0	0.53±0.13	0.54±0.09	0.53±0.07	0.53±0.13
1	0.50±0.00	0.87±0.99	0.32±0.27	0.33±0.23
2	0.56±0.07^{a}	2.80±1.67^{ab}	9.53±4.47^{bc}	14.00±0.00^{c}
3	0.50±0.60	1.60±2.08	0.90±0.58	2.70±3.29
4	0.53±0.13^{a}	1.06±0.33^{ac}	0.93±0.19^{ac}	2.70±1.90^{c}
5	0.50±0.10^{a}	0.88±0.35^{a}	0.70±0.00^{a}	1.65±0.35^{b}

Different superscript (a, b and c) in a row indicate significant difference between the group means ($P < 0.05$).

Table 5. Mean total bilirubin ± SE (mg/100 ml) of dogs infected with *T. brucei* or *A. caninum* and conjunctly with *T. brucei/A. caninum.*

Experimental period (week)	Group			
	I (Control)	II (*A. caninum*)	III (*T. brucei*)	IV (*T. brucei* and *A. caninum*)
0	0.17±0.00	0.17±0.07	0.17±0.07	0.17±0.07
1	0.17±00^{a}	0.26±0.05^{b}	0.30±0.04^{c}	0.18±0.03^{ab}
2	0.18±0.03^{a}	0.35±0.16^{ab}	0.29±0.29^{ab}	1.05±0.33^{b}
3	0.15±0.05^{a}	0.50±0.24^{ab}	0.27±0.03^{a}	1.13±0.31^{b}
4	0.13±0.00^{a}	0.73±0.27^{a}	0.47±0.09^{a}	1.73±0.39^{b}
5	0.13±0.03^{a}	0.60±0.24^{a}	0.47±0.09^{a}	2.15±0.7^{b}

Different superscripts (a, b and c) in a row indicate significant difference between the group means ($P < 0.05$).

infected groups at the 1st week pi. when compared with GPI. By the 2nd week pi., there was a significant decrease ($P < 0.05$) throughout the period of the experiment. The decrease observed in GPII was higher when compared when that in GPIII while that in GPIV was highest.

Creatinine

The creatinine (mean ± SE) is shown in Table 4. There was a significant increase ($P < 0.05$) by the two weeks pi. in GPIII and GPIV as compared to GPI. at three weeks pi., there was a decrease in the infected groups though when compared to GPI there was an increase. Increase in GPIV when compared with GPIII was high. There was no significant difference ($P>0.05$) between GPI and GPII.

Total bilirubin

The total bilirubin (mean ± SE) of dogs is shown in Table 5. There was a significant increase ($P < 0.05$) in GPIV at two weeks pi. till the end of the experiment. There was no significant difference ($P > 0.05$) between GPI, GPII and GPIII.

Liver enzymes

Alanine aminotransferase (ALT)

The ALT (mean ± SE) is shown in Table 6. There was a significant increase ($P < 0.05$) in GPIII and GPIV by the 1st week pi. to the end of the experiment. Increase in GPIV as compared to GPIV was high. There was no significant difference ($p> 0.05$) between GPI and GPII.

Aspartate aminotransferase (AST)

The AST (mean ± SE) is shown in Table 7. There was a
The AST (mean ± SE) is shown in Table 7. There was a significant increase ($P < 0.05$) in the GPIII and GPIV 1st week pi. The increase in GPIV as compared to GPIII was higher. There was no significant difference ($P > 0.05$) between GPI and GPII.

Table 6. Mean alanine aminotransferase mean ± SE (iu/l) of dogs infected with *T. brucei* or *A. caninum* alone and conjunctly with *T. brucei/A. caninum.*

Experimental period (week)	Group			
	I (Control)	II (*A. caninum*)	III (*T. brucei*)	IV (*T. brucei* and *A. caninum*)
0	17.25±1.0	17.33±2.19	17.33±1.76	17.33±2.19
1	14.67±0.88^{a}	29.25±4.39^{ab}	53.00±17.24^{ab}	61.25±15.53^{b}
2	15.33±1.67^{a}	50.25±13.41^{ab}	65.00±13.76^{b}	69.33±21.09^{b}
3	15.33±2.0	54.00±24.99	79.33±12.25	87.33±32.71
4	14.67±1.3	52.75±10.43	86.75±3.84	82.67±15.21
5	15.33±2.3^{a}	55.00±1.41^{a}	92.33±17.46^{b}	93. 50±9.50^{b}

Different superscripts (a, b and c) in a row indicate significant difference between the groups means (P < 0.05).

Table 7. Mean aspartate aminotransferase ± SE (iu/l) of dogs infected with *T. brucei* or *A. caninum* alone and conjunct *T. brucei/A. caninum.*

Experimental period (week)	Group			
	I (Control)	II (*A. caninum*)	III (*T. brucei*)	IV (*T. brucei* and *A. caninum*)
0	20.60±0.24	21.40±1.66	21.40±1.66	20.60±2.11
1	21.00±3.21^{a}	34.75±5.17^{ab}	55.00±14.39^{ab}	72.00±25.24^{b}
2	20.67±0.67^{a}	41.6±7.24^{ab}	63.25±10.36^{bc}	73.25±11.35^{c}
3	20.67±4.10	42.67±30.44	106.00±50.52	138.75±51.81
4	21.00±0.00^{a}	45.67±13.92^{ab}	103.67±13.64^{b}	139.00±15.87^{ab}
5	21.67±2.96^{ab}	40.25±3.92^{a}	105.00±4.58^{ab}	135.00±3.00^{b}

Different superscripts (a, b and c) in a row indicate significant difference between the group means (p< 0.05).

Table 8. Mean alkaline phosphatase ± SE (iu/l) of dogs infected with *T. brucei* or *A*. *caninum* and conjunctly with *T. brucei/A. caninum*.

Experimental period (week)	Group			
	I (Control)	II (*A. caninum*)	III (*T. brucei*)	IV (*T. brucei* and *A. caninum*)
0	76.67±13.86	76.67±15.21	76.33±27.69	76.33±24.25
1	74.67±12.02	51.00±12.93	41.25±9.66	58.50±8.81
2	75.00±8.54^{a}	19.60±3.43^{b}	14.00±0.00^{b}	14.00±0.00^{b}
3	74.67±16.67	17.50±3.50	27.67±13.67	14.00±0.00
4	76.50±9.58	23.00±9.00	9.67±9.67	23.33±4.67
5	75.67±8.09^{a}	23.00±9.00^{ab}	9.33±9.333^{b}	21.00±7.00^{ab}

Different superscript (a, b and c) in a row indicate significant difference between the group means (P < 0.05).

Alkaline phosphatase (ALP)

The ALP (mean ± SE) is shown in Table 8. There was significant decrease (P < 0.05) in the three infected groups when compared with the control (GPI) throughout the period of the work.

DISCUSSION

The hypoproteinaemia recorded in the infected groups is in agreement with the findings of other workers in trypanosomosis (Otesile et al., 1991; Witola and Lovelace, 1997; Taiwo et al., 2003; Orhue et al., 2005; Bisalla et al., 2007) and helminthosis (Ettinger and Feldman, 2005). It was essentially due to hypoalbumimeamia since low serum albumin was recorded in the three infected groups. The decrease in the total serum protein especially in the trypanosome infected groups (III and IV) was probably as a result of decreased hepatic biosynthesis and progressive loss of

albumin in urine (Agu and Egbuji, 2002). Also, some degenerative changes in the intestines of *Ancylostoma caninum* infected dogs may have led to protein loosing enteropathies and intestinal malabsorption of proteins (Gregor and John, 1976).

The initial increase and subsequent decrease in the blood urea nitrogen (BUN) of all the infected groups agrees with the reports by Kwem et al. (2000) where such fluctuations were attributed to early organ and tissue damage following peak parasitaemia in the first week of infection. Similar observations have been reported in *T. brucei gambiense* infected monkeys (Jerry and Victor, 2007) and filariasis in dogs (Mohammed and Ahmed, 2008). The subsequent progressive significant ($P < 0.05$) decrease in BUN could be associated with decrease in liver synthesis of urea (Ettinger and Feldmasn, 2005).

The changes observed in total bilirubin were that of hyperbilirubinaemia (Table 4) in trypanosome infected dogs. 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 conjunct group may have been particularly affected because of the combined effect of the activities of the young larvae of *A. canninum* and trypanosomes in liver tissues.

The elevated serum creatinine levels in groups III and IV 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 muscles and release of creatine kinase with attendant increase in the circulating creatinine (Daniel and Michael, 2003).

The increased activities of ALT and AST in trypanosome infected groups agrees with the reports of other workers (Kwem et al., 2000; Akpa et al., 2008; Ezeokonkwo, 2009) and may be due to the effects of trypanosomes in tissues including the liver (Justine and Oluwatosin, 2005). Also, the parasites may release these enzymes as metabolites into the blood circulation. Homogenates and suspensions of trypanosomes have been reported to show activity for these enzymes (Stephen and Gray, 1960). The higher activities of AST and ALT observed in the conjunct group were more as a consequence of *Trypanosoma brucei brucei* than *Ancylostoma caninum* infection since significantly higher value also occurred in single *Typanosoma* unlike *Ancylostoma* group. Nevertheless, migrating larvae of *A. caninum* are reported to cause damage to host cells in the wake of their migration leading to mobilisation of inflammatory cells along their tracts causing fibrosis and necrosis with the release of these enzymes and an increased activity (Soulsby, 1986; Urquhart, 1998).

The significant progressive decrease in ALP activity in all infected groups which was evident from the second week following infection could not be adequately explained. However, Kwem *et al*. (2000) made a similar observation of progressive abnormal decrease in ALP in association with liver fibrosis in *Trypanosoma vivax* infected cattle, and it was attributed to early hepatic damage. These finding contradicted the results of Akpa *et al*. (2008) who reported a significant increase in the enzymes and thus may be subjected to further investigation.

Conclusion

It was clear from this study that significant alterations in biochemical parameters have occurred in dogs infected singly or concurrently with *T. 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 trypanosomosis in the 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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