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SERUM ASSESSMENT OF DOGS EXPERIMENTALLY INFECTED WITH SINGLE TRYPANOSOMA CONGOLENSE AND CONJUNCT TRYPANOSOMA CONGOLENSE AND ANCYLOSTOMA CANINUM INFECTIONS AND TREATMENT WITH DIMINAZENE ACETURATE AND MEBENDAZOLE

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ABSTRACTS

Aim: The health dangers associated with disease caused by different species of trypanosomes in dogs necessitated the serological assessment of dogs experimentally infected with single Trypanosoma congolense (T.congolense) and conjunct Trypanosoma congolense (T.congolense) and Ancylostoma caninum (A.caninum) infections and treatment with diminazene aceturate and mebendazole. Materials/ methods: Twelve mongrels of both sexes weighing between 4 to 8 kg were used in the study. They were randomly grouped into 3 to 4 members each. Group I was uninfected control, Group II was infected with T.congolense, Group III was infected with conjunct T.congolense/A.caninum. Post acclimatization, GPII was infected with 200 L_3 infective dose of A.caninum. Two weeks post infection 2.5 $x10^6$ doses of T.congolense was used to infect GPII and GPIII. **Result:** Results show a significant increase ($p<0.05$) in total protein, bilirubin and cholesterol level of GPII and GPIII. Transient increase in calcium ion was recorded only in GPII. Conversely a significant decrease ($p<0.05$) in albumin was recorded in both GPII and GPIII. **Conclusion:** The biochemical alterations were more severe in mixed infection of T.congolense/A.caninum compared to single T.congolense. Treatment improved altered serum analytes. Hence against this backdrop effort must be put in place to prevent its occurrence in dogs.

KEYWORDS: total protein, albumin, calcium ion, bilirubin, cholesterol, T.congolense, A. caninum.

INTRODUCTION

In Nigeria, African canine trypanosomosis is caused mainly by T.brucei brucei and T.congolense (Ezeokonkwo et al., 2010; Nwoha and Anene, 2011). Trypanosoma congolense infection causes varying disease ranging from subacute to acute disease conditions in dogs (Bengally et al., 2001; OIE, 2009). Similarly Ancylostoma or hookworm is a voracious blood sucking gastrointestinal helminth parasite of dogs (Dan, 1999). Ancylostoma caninum is an endemic parasite in our environment and as such animals are repeatedly infected and may require frequent treatments to sustain improvement in health of the population at risk (Quinnell et al., 2004; Saathoff et al., 2005). Both trypanosomosis and ancylostomosis are parasitic diseases which may occur in mixed infection in dogs especially in the south-eastern region of Nigeria (Nwoha, 2011). Serum biochemistry profile is one of the most informative laboratory tests in Veterinary Medicine that involves the analysis of the serum or plasma for clinical and metabolic changes in individual animals or herd and are used in diagnosis and disease monitoring (Mundim et al., 2007; Bukowski, 2012). Proper assessment of biochemistry profiles will reveal the clinical status of animal, the organ function, nutritional state and determine therapeutic prescription (Mundim et al., 2007; Bukowski, 2012). In clinical biochemistry, the use of wide range of properly chosen tests are preferred to a specific test in order to reveal the effect of a disease condition in different systems of the body through the process of pattern recognition. Some biochemical anomaly in trypanosomosis includes: hypoalbuminaemia and hypoproteinaemia in T.cruzi infection in dogs (Kjos et al., 2008) and in T. brucei infection in dogs (Aquino et al., 2002; Nwoha et al., 2013). There are other cases of hyperproteinaemia in T. brucei brucei infection in rabbits (Adenike and Adeyemi, 2009; Takeet and Fagbemi, 2009; AHPC, 2011). In T. evansi infection in carmel (Mahmood et al., 2014). Similarly, significant increases in bilirubin concentration have been recorded in T. brucei brucei infection in dogs (Omotainse et al., 1994; Nwoha et al., 2013); and in rabbits (Adenike and Adeyemi, 2009; Arowolo et al., 1988) as well as in T. congolense infection in dogs (Gow et al., 2007) and rabbits (Takeet and Fagbemi, 2009). There are records of elevation in cholesterol and high density lipids (HDL) in

experimental T.brucei infection in rabbits (Nakamura, 1998) and in T.congolense infection in rabbits (Takeet and Fagbemi, 2009). Conversely, a decrease in cholesterol and HDL was recorded in experimental T. brucei / T.congolense infection in goats (Biryomumansho and Katunguka-Rwakishaya, 2007).

AIM

It would therefore be useful to carry out serological assessment of dogs experimentally infected with single Trypanosoma congolense and conjunct Trypanosoma congolense and Ancylostoma caninum infections and treated with diminazene aceturate and mebendazole

MATERIALS/METHODOLOGY

Experimental Animals

Twelve mongrel breed of dogs of both sexes weighing between 4.0 and 8.0kg were used in this experiment. The dogs were 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 (Woo, 1970). 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 100mg twice daily for 3 days and also treated with sulfadimidine at the dose of 48mg/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.

Ethical approval

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. All 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.

Parasites and infections

Trypanosoma congolense

Kilifi strain of T. congolense obtained from the National Institute of Trypanosomosis and Oncocerciasis Research (NITOR), Nigeria was used. The parasite was a primary isolate from a cow in Kaduna. It was maintained in rats, and subsequently passage in a donor dog from where parasites were collected for infection of the experimental dogs.

Estimated 2.5×10^6 of T. congolense suspended in 1ml 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 Herbert and Lumsden (1976).

Ancylostoma caninum Infection

The concentration of larval suspension was estimated using an automatic pipette (Biotht Peoline®), according to the method of MAFF (1977).

Reconstitution of Diminazene aceturate

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

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 100mg twice daily for 3 consecutive days. Treatment was repeated 2 weeks later.

Experimental Design

Dogs were randomly divided into 3 groups of 4 members in each group. GROUP I was uninfected dogs (control), GROUP II was infected with Trypanosoma congolense, GROUP III was mixed infections of Trypanosoma congolense and Ancylostoma caninum.

Post acclimatization, Ancylostoma caninum infection was done on GPIII alone. Two weeks later Trypanosoma congolense infections were done on GPII and GPIII. Three weeks post trypanosome infection; GPII and GPIII were treated with diminazene aceturate. Mebendazole was used only on GPIII and a repeat treatment given 2 weeks later.

Serum Biochemistry Blood Collection and Preparation of Serum Samples.

Five milliliter of blood was collected through the cephalic vein of each of the experimental dogs and dispensed into dried appropriately labelled sterile test tubes with screw caps and kept slanted and allowed to clot. The blood samples were immediately transported to the Department of Veterinary Medicine Laboratory. The samples were left at room temperature for about 2 hours to yield sera and then centrifuged at 11000 revolutions per minute for 5 minutes and sera obtained were separated into clean labelled tubes and stored at -20^oC until analyzed for biochemical constituents.

Biochemical Analyst

The serum Total protein, Bilirubin, albumin, cholesterol, and calcium were determined using Randox Text Kits according to the manufacturer's prescriptions.

RESULTS

Total Protein

The mean± se of total protein was shown in (Table 1). Significant increases ($p<0.05$) were recorded in GPII and GPIII by week 7 and continued to week 9 in GPIII. By week 8, no significant difference ($p<0.05$) was recorded in GPII compared to the control (GPI).

Total Albumin

The mean± se of total albumin was shown in (Table 2). By week 3 post infection, there was a significant decrease ($p<0.05$) in albumin in GPIII which continued to week 7. Similarly a significant decrease ($p<0.05$) was recorded in GPII by week 4 up to week 7. The decrease recorded in GPIII was more compared to that in GPII. By week 8 to 12, no significant change ($p<0.05$) was observed in both GPII and GPIII compared to the control (GPI).

Total Bilirubin

The mean ±se of total bilirubin was shown in (Table 3). Significant increases were recorded in both GPII and GPIII only on week 6 post infection. Subsequently no significant difference ($p<0.05$) was observed between the groups (GPII and GPIII) compared to the control (GPI).

Cholesterol

The mean± se of cholesterol was shown in (Table 4). A significant increase ($p<0.05$) was observed in GPIII by week 3 post infection and continued to week 6. A significant increase ($p<0.05$) was equally observed in GPII by week 4 up to week 6. Subsequently no significant difference ($p<0.05$) was observed between GPII and GPIII compared to GPI.

Calcium ion

The mean± se of calcium was shown in (Table 5). A significant increase ($p<0.05$) was observed only in GPII by week 4 post infection, subsequently no significant difference ($p<0.05$) was recorded between GPII compared to the control (GPI).

Table: 1. Mean ± SE Total protein (mg/dl) of dogs with experimental single T.congolense and conjunct T.congolense / A. caninum infections and treated with diminazene aceturate and mebendazole

Experimental Period(Weeks)	GPI (control)	GPII (*Tc*)	GPIII (*Tc/Ac*)
0	6.4±0.90^{a}	5.3±0.40^{a}	5.5±0.60^{a}
1 ⇧	6.7±0.30^{a}	5.8±0.50^{a}	5.8±0.80^{a}
2	6.8±0.20^{a}	5.9±0.50^{a}	5.8±0.70^{a}
3 ⇳	6.9±0.50^{a}	5.7±0.20^{a}	5.7±0.20^{a}
4	6.1±0.20^{a}	5.8±0.40^{a}	5.8±0.30^{a}
5	6.0±0.30^{a}	5.1±0.50^{ab}	5.2±0.30^{ab}
6 * +	6.7±0.80^{a}	7.3±0.70^{b}	7.7±0.10^{b}
7	6.9±0.10^{ab}	6.0±0.50^{ab}	8.0±0.80^{b}
8 * +	6.5±0.20^{a}	5.4±0.30^{a}	8.8±0.70^{b}
9 *	6.8±0.60^{a}	6.0±0.20^{a}	8.9±0.40^{b}
10	6.5±0.50^{a}	6.2±0.30^{a}	---
11	6.6±0.20^{a}	6.1±0.30^{a}	---
12	6.2±0.20^{a}	6.0±0.30^{a}	---

Superscripts a b represent the homogeneity between the experimental groups at probability $p< 0.05$.
⇧ Infection with A. caninum
⇳ Infection with trypanosomes
+ Treatment with mebendazole
* Treatment with diminazene aceturate
Ac Ancylostoma caninum
Tc Trypanosoma congolense

Table: 2. Mean ± SE Total Albumin (mg/dl) of dogs with experimental single T.congolense and conjunct T.congolense / A. caninum infections and treated with diminazene aceturate and mebendazole.

Experimental Period(Weeks)	GPI (control)	GPII (*Tc*)	GPIII (*Tc/Ac*)
0	1.9±0.50^{a}	1.1±0.40^{a}	2.3±0.60^{a}
1 ⇧	2.6±0.20^{a}	2.3±0.30^{a}	2.1±0.10^{a}
2	2.7±0.20^{a}	2.4±0.20^{a}	2.1±0.40^{a}
3 ⇳	2.8±0.50^{a}	2.5±0.20^{a}	2.0±0.30^{b}
4	2.3±0.20^{a}	2.0±0.30^{ab}	1.3±0.10^{b}
5	2.6±0.20^{a}	1.8±0.30^{bc}	1.3±0.20^{c}
6 * +	2.0±0.50^{a}	1.3±0.20^{b}	1.4±0.20^{b}

7	2.4±0.20[a]	1.5±0.20[bc]	1.1±0.00[c]
8 * +	2.2±0.20[a]	2.0±0.20[a]	2.0±0.30[a]
9 *	2.5±0.10[a]	2.3±0.10[a]	2.4±0.20[a]
10	2.5±0.20[a]	2.4±0.30[a]	-----
11	2.4±0.10[a]	2.3±0.10[a]	-----
12	2.6±0.10[a]	1.9±0.10[ab]	-----

Superscripts a b c represents the homogeneity between the experimental groups at probability p< 0.05.
⇧ Infection with A. caninum
⇳ Infection with trypanosomes
\+ with mebendazole
* with diminazene aceturate
Ac Ancylostoma caninum
Tc Trypanosoma congolense

Table 3. Mean ± SE Total Bilirubin (mg/dl) of dogs with experimental single T.congolense and conjunct T.congolense / A. caninum infections and treated with diminazene aceturate and mebendazole.

Experimental Period(Weeks)	GPI (control)	GPIII *(Tc)*	GPIV *(Tc/Ac)*
0	0.3±0.10[a]	0.4±0.20[a]	0.4±0.10[a]
1	0.3±0.10[a]	0.2±0.10[a]	0.3±0.10[a]
2	0.2±0.10[a]	0.3±0.10[a]	0.2±0.10[a]
3 ⇳	0.3±0.10[a]	0.2±0.00[a]	0.3±0.00[a]
4	0.2±0.10[a]	0.2±0.00[a]	0.2±0.00[a]
5	0.2±0.10[a]	0.3±0.00[a]	0.2±0.10[a]
6 * +	0.2±0.10[a]	0.4±0.10[ab]	0.4±0.10[ab]
7	0.4±0.10[a]	0.4±0.10[a]	0.3±0.10[a]
8 * +	0.3±0.10[a]	0.3±0.00[a]	0.3±0.10[a]
9 *	0.2±0.10[a]	0.3±0.40[a]	0.4±0.40[a]
10	0.3±0.30[a]	0.3±0.10[a]	-----
11	0.2±0.10[a]	0.2±0.10[a]	-----
12	0.3±0.10[a]	0.3±0.00[a]	------

Superscripts a b represent the homogeneity between the experimental groups at probability P < 0.05.
⇧ Infection with A. caninum
⇳ Infection with trypanosomes
\+ Treatment with mebendazole
* Treatment with diminazene aceturate
Ac Ancylostoma caninum
Tc Trypanosoma congolense

Table 4. Mean ± SE Cholesterol level (mg/dl) of dogs with experimental single T.congolense and conjunct T.congolense / A. caninum infections and treated with diminazene aceturate and mebendazole.

Experimental Period(Weeks)	GPI (control)	GPII *(Tc)*	GPIII *(Tc/Ac)*
0	78.3±15.90[a]	163.0±58.70[a]	171.0±34.80[a]
1 ⇧	138.1 ±2.00[a]	120.2±0.20[a]	132.0 ±11.90[a]
2	128.0± 3.70[a]	123.8±0.20[a]	140.0±12.90[a]
3	121.0±10.80[a]	124.0±9.00[a]	141.0±14.20[b]
4 ⇳	121.0±3.00[a]	150.0±16.90[b]	145.0±15.90[b]
5	141.0±26.80[a]	186.0±12.20[b]	177.0±33.30[b]
6 * +	111.0±6.30[a]	164.0±10.10[b]	168.0±8.50[b]
7	130.0±10.80[a]	141.0±21.20[a]	110.0±18.70[a]
8 * +	127.0±6.90[a]	70.0±25.00[b]	120.0±16.70[a]
9 *	125.0±3.00[a]	130.0±23.00[a]	123.0±1.00[a]
10	130.0±2.90[a]	134.0±1.90[a]	----
11	124.0±2.00[a]	136.0±3.00[a]	----
12	114.0±23.30[a]	100.0±16.90[a]	---

Superscripts a b represent the homogeneity between the experimental groups at probability p<0.05.
⇧ Infection with A. caninum

⇳ Infection with trypanosomes
\+ Treatment with mebendazole
* Treatment with diminazene aceturate
Ac Ancylostoma caninum
Tc Trypanosoma congolense

Table: 5. Mean ± SE Calcium ion conc. (mg/dl) of dogs with experimental single T.congolense and conjunct T.congolense / A. caninum infections and treated with diminazene aceturate and mebendazole.

Experimental Period (Weeks)	GPI (Control)	GPII *(Tc)*	GPIII *(Tc/Ac)*
0	10.2±3.70[a]	9.0±0.60[a]	10.8±0.80[a]
1 ⇧	10.1±2.00[a]	10.1±2.00[a]	9.4±1.00[a]
2	9.2±1.00[a]	9.3±1.00[a]	9.0 ±1.00[a]
3 ⇳	10.4±0.10[a]	9.5±0.50[a]	8.1±0.30[a]
4	9.2±0.20[ab]	8.4±1.00[ab]	8.0±0.20[a]
5	8.0±1.10[a]	7.9±2.20[a]	7.1±3.00[a]
6 * +	7.8±1.00[a]	3.6±0.50[a]	5.3±3.00[a]
7	9.1±1.30[a]	11.2±1.00[a]	9.1±1.60[a]
8 * +	7.8±2.40[a]	5.7±1.50[a]	4.2±1.80[a]
9 *	8.3±5.70[a]	9.0±2.00[a]	9.7±6.00[a]
10	9.0±2.90[a]	9.3±2.80[a]	----
11	10.2±1.00[a]	9.8±3.90[a]	----
12	10.3±0.20[a]	7.0±1.40[a]	----

Superscripts a b represent the homogeneity between the experimental groups at probability $p<0.05$.
⇧ Infection with A. caninum
⇳ Infection with trypanosomes
\+ Treatment with mebendazole
* Treatment with diminazene aceturate
Ac Ancylostoma caninum
Tc Trypanosoma congolense

DISCUSSION

Hyperproteinaemia recorded in T.congolense infection may be associated with massive release of protein during infection. Protein influx occurs during inflammatory process in trypanosomosis as observed in T. brucei infection in dogs (Nwoha and Anene, 2016). This was enhanced in the conjunct infection of T. congolense/ A.caninum due to synergic effect of hookworm in the group. Similar observation was made in inflammatory conditions in humans (Cardon, 1943). This corroborates the works of Ohaeri and Eluwa (2011) and Taiwo et al. (2003) in brucei brucei infection in various species of animals. It also agrees with Orhue et al. (2005) in T. brucei infection in rabbits. It however opposes hypoproteinaemia recorded by Otesile et al. (1991) in T. brucei infection in boars and in T.congolense infection in cattle (Sadique et al., 2001). Ironically, there are cases of no significant effect on serum protein in trypanosomosis in animals (Anene et al., 2010; Biryomumaisho et al., 2003). Treatment of the infected groups improved the condition only in the single infected group but persisted in the conjunct group due to relapse of infection which was exaggerated in the group. Persistent hyperproteinaemia in the conjunct group could engender complications of oxygen deficit causing a reduction in oxygen carrying capacity of rbcs (Cardon, 1943). Such enhances cell aging and degenerative changes commonly observed in trypanosomosis in animals (Ekanem and Yusuf, 2008; Akanji et al., 2009).

The significant ($p<0.05$) decreases observed in total albumin in the T. congolense infections may be attributed to loss of albumin in urine as observed in Agu and Egbuji (2002). The decrease was more in the conjunct group compared to the single T.congolense due to additional loss of albumin through enteropathy from hookworm infection (Nwoha et al., 2013).

Transient increase in bilirubin in both the single and conjunct groups could be due to complications of liver obstruction. Treatment of the infected group improved the clinical conditions as observed in Rashid et al. (2008).

There was no significant difference ($p<0.05$) in cholesterol level of single and conjunct T.congolense infected groups. The observed increase could be due to liver malfunction arsing from the inflammatory effect of T. congolense and activities of hookworms in the liver. (Urquhart et al., 1998; Akpa et al., 2008). Liver impairment essentially affects production of triglyceride clearance enzymes thereby reduces lipid excretion from the system (Kary, 2008). This observation was in agreement with works of Nakamura (1998) in T.brucei infection in rabbits. In T. b. gambiense infection in Monkey (Abenga and Anosa 2006) and in T.congolense infection in rabbits (Takeet and Fagbemi 2008). Some other workers recorded a decrease in cholesterol level in experimental T. brucei /T.congolense infections in goats

(Biryomumaisho et al., 2003) and in T. brucei brucei infection in pigs (Adamu et al., 2009).

The transient increase in calcium ion concentration in the groups may not be regarded much in T.congolense infection in dogs. Conversely Osuna et al. (1990) observed a significant ($p<0.05$) increase in calcium ion in T. cruzi parasitized cells and attributed it to intracellular release of calcium deposit especially from the host mitochondria. The confounding factor in our findings may be attributed to species specificity. In conclusion conjunct infections of T. congolense/A.caninum produce more severe disease condition in dogs compared to single infection. Its therefore important to prevent such occurrence in dogs.

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