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Research Article

Comparative Study on the Effect of *Trypanosoma Brucei* Infections on the Kidney Functionality and Chemotherapeutic Interventions in Dogs. A Case Study of Diminazene Aceturate and Isometamidium Chloride.

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Abstract

The kidney functionality of twenty growing Nigerian dogs (Mongrel) of both sexes weighing between 3.0-8.0 kg were used to assess the kidney function following infection with *Trypanosoma brucei* (*T. brucei*) and the comparative efficacy of diminazene aceturate (DA), and Isometamidium chloride (IMC)on the damage infection could cause if any by *T. brucei*. The purpose of the work was to find out which of the trypanocides (DA and IMC) can better reverse the kidney function parameters studied, following infection. The dogs were randomly assigned into four groups (1-4) with five dogs per group as follows: 1= infected and treated with DA (7.0mg/kg; 2= uninfected untreated; 3= infected and not treated with either of the trypanocides; 4= trypanosome infected and treated with IMC (0.5 mg/kg). Diminazene aceturate (DA) and isometamidium chloride (IMC) were able to eliminate the parasites from the blood stream. However, relapse of infection was observed in two of the dogs from group 1 and one dog in group 4 by days 35 and 56 post infection (PI), none of the treated dogs died following relapse of infection except one dog from group 1. The infection caused a significant increase ($p<0.05$) in the mean blood urea nitrogen (BUN) by 21 post infection (PI), whereas the infection caused a significant increase in the mean creatinine (Cr) by days 14 and 21 PI when compared with the normal control (group 2). However, by day 49 PI and beyond, BUN was significantly higher in group 4 (IMC- treated) when compared to the normal control, unlike group 1 (DA- treated) which was similar to the normal control. The findings suggested that DA-treated satisfactorily reversed BUN and Cr to normal; unlike IMC which could not reverse the BUN satisfactorily, but reversed Cr to normal value satisfactorily.

Keywords: Dogs; Diminazene Aceturate; Isometamidium Chloride; Kidney Function; *Trypanosoma Brucei*

Introduction

The kidneys perform functions in the body which includes excretory, homeostasis and hormone production. Trypanosoma brucei bucei is known to be the most occurring trypanosome in dogs [1]. This species has been incriminated in causing tissue and organ damages [2]. Immune-mediated glomerulonephritis which compromises the kidney functionality has been associated with trypanosomosis [3].

The activities of liver enzymes such as serum alkaline phosphatase, (AP), serum alanine amino transferase (ALT), aspartate aminotransferase (AST) is known toincrease following

infection with animal trypanosomosis [2]. Similarly blood urea nitrogen (BUN), Creatinine (Cr), and bilirubin concentration increases in serum following infection [1].

Therefore, there is the need to have a clearer picture of the effect of trypanosomosis caused by *T.brucei* on the kidney function parameters (BUN, and Cr) as the changes may cause some of the pathophysiological mechanisms in the development of the disease. This will go a long way in helping clinician in the management of the disease using diminazene aceturate (DA) or isometamidium chloride (IMC). Hence the need for this work.

Materials and Methods

Experimental Animals

Three to eight kilograms mongrel dogs (Nigerian breed of dogs) of about 3-4 months and weighing between 3-8 kg were used for this investigation. After being acclimatized for 4 weeks within which period they were dewormed and deticked using ivermectin (Ivomec, Merck sharp and dohmes; B.V. Hearlem, Holand) at the dose of 0.2mg/kg subcutaneously (s.c). They were fed once daily and water was provided Ad. Libitum till the end of the experiment; also they were confirmed negative for trypanosome by buffy coat method [4]. They were equally vaccinated against parvovirus, leptospirosis, canine distemper, canine hepatitis and canine panleucopaenia; also rabies diseases using DHLPP vaccine and antirabies vaccine (Forte Dodge Company USA) before commencement of the experiment. They were also vaccinated against rabies.

Design of the experiment

The experimental dogs were randomly assigned into 4 groups as follows

- **Group 1:** Infected with 1,000,000 Trypanosoma. brucei (T. brucei) and treated with diminazene aceturate (DA)- at the dose of 7mg/kg.
- **Group 2:** Uninfected, Untreated (negative control)
- **Group 3:** Infected with 1,000,000 T. brucei) Untreated (positive control).
- **Group 4:** Infected with 1,000,000 T. brucei, treated with isometamidium chloride (IMC)- at the dose of 0.5mg/kg.

The parasites were maintained in rats before infection of the experimental dogs intraperitoneally at 1,000,000 trypanosome/ml of saline diluted blood. The quantification of the parasites was done using the method of 7 Herbert and Lumsden (1976) [5].

Serum collection

Serum was harvested from whole blood. This was achieved by aspirating 4mls of whole blood into a clean container and allowing it to stand for some time to facilitate serum yield. The serum was centrifuged at 2000revolution per min for 10 minute to separate the sera from the blood cells. The serum was used to assess the concentration of urea and creatinine (4). milimeters of whole blood was put into clean tubes and allowed to stay for some time to enhance serum yield. The serum was decanted and centrifuged at 2000 rpm for 10 minutes to separate the sera from the cells. The clear serum was used to assay for the serum urea concentration and serum creatinine concentration.

Drug administration

Diminazene aceturate (Veriben Ceva SANTE ANIMALS, CEDEX France), reconstituted by dissolving 1.05g of the drug per sachet in 12.5 ml of sterile water and 7.0mg/kg was administered to the test dogs.

Isometamidium chloride (Trypanidium-Samirin merial, Lyon France; was reconstituted by dissolving 1g of the sachet in 50ml of sterile water and 0.5/kg was given to the dogs.

Estimation of Parasitaemia

- The parasitaemia level was estimated by the matching method as described by [5].
- Urea
- Serum urea concentrations was determined using a colorimetric method [6].
- Creatinine
- Serum creatinine concentrations was also determined using a colorimetric method [6].
- Handling of experimental animals during the study
- Manuscript complied with current animal welfare and ethical guidelines Of University of Nigeria Nsukka, Faculty of Veterinary Institutional Animal Care Committee. Reference No. FVM-UNN-IACUC-2019-1131.

Statistical Analysis

Data generated from urea and creatinine were presented as means with standard deviation. The data was subjected to analysis of Variance (ANOVA) and the means separated using Duncan's multiple range tests. Means were considered significant at $p < 0.05$.

Results

Parasitaemia/clinical manifestation

During the course of the infection, clinical signs of pyrexia, depression, dullness, pale mucous membrane, ocular discharges, corneal opacity, staggering and aggression were observed in the infected untreated (group 3) until the dogs died before the termination of the experiment. Also turbid urine, elevated urine PH, increase in the number of pus and epithelial cells, leading to increased specific gravity and turbidity of the urine were observed. These clinical signs in the treated groups (groups 1 and 4) gradually disappeared following treatment with the trypanocides. The treated groups never showed any sign of any central nervous sign. The signs of depression, and anorexia were observed again in three dogs in group 1 (DA treated) following a relapse infection.

The *Trypanosoma brucei* (*T.brucei*), infected dogs (groups1, 3, and 4) were positive for *T. brucei* by day 6-7 post infection (PI). Treatment for groups 1 and 4 commenced by day 7 PI when all the infected dogs became parasitaemic. The treaed dogs (groups 1 and 4) became aparasitaemic by day 14 PI. The parasitaemia was progressive in the infected untreated group (group 3). By day 28 PI, 4members of group 3 died and all died by day 56 PI. By day 35 PI, relapse infection occurred in two members of group1 dog (Diminazene aceturate- DA treated), and by day 63 PI one member of group 1 (DA-treated), died. Relapse of infection also occurred in one member of group 4 (Isometamidium chloride-IMC) by day 56 PI. No mortality was recorded in this group till the termination of the experiment (Table 1).

Blood urea nitrogen

The infection caused a significant increase ($p < 0.05$) in the mean blood urea nitrogen (BUN) by day 21 PI when compared with the normal control (Table 2), but the treated groups (groups 1 and 4) were similar to the normal control (group 2). From day 49 PI and beyond, BUN was significantly higher in group 4(IMC-treated) compared with the negative control unlike group1 (DA- treated which was similar to the negative control.

Blood Creatinine

Table 3 shows the mean creatinine (Cr) of the various groups. Infection did cause a significant ($p < 0.05$) increase in the mean Cr by days 14 and 21 PI when compared with the normal control, beyond this point, till the termination of the experiment, the treated groups (groups 1 and 4) became similar.

Time (Days post infection)	Parasitaemia of the various groups			
	Group 1 (Infected treated with 7.0mg/kg DA	Group 2 (Uninfected control)	Group 3 (Infected untreated control)	Group 4 (Infected treated with 0.5mg/kg IMC
0	A 5/5	A 5/5	A 5/5	A 5/5
1	A 5/5	A 5/5	A 5/5	A 5/5
2	A 5/5	A 5/5	A 5/5	A 5/5
3	A 5/5	A 5/5	A 5/5	A 5/5
4	A 5/5	A 5/5	A 5/5	A 5/5
5	A 5/5	A 5/5	A 5/5	A 5/5
6	P 3/5	A 5/5	P 4/5	P 4/5
7	P 5/5	A 5/5	P 4/5	P 4/5
14	A 5/5	A 5/5	P 4/5	A 5/5
21	A 5/5	A 5/5	P 4/5	A 5/5
28	A 5/5	A 5/5	M 4/5	A 5/5

35	R 2/5	A 5/5	M 4/5	A 5/5
42	R 2/5	A 5/5	M 4/5	A 5/5
49	R2/5	A 5/5	M 4/5	A 5/5
56	R 2/5	A 5/5	M 5/5	R 1/5
63	M 1/5	A 5/5	M 5/5	R I/5

Table 1: Shows Parasitaemia of *Trypanosoma brucei* infected dog groups treated with either 7.0mg/kg Dinazene aceturate or 0.5mg/kg Isometamidium chloride.

A= Aparasitaemia

M= Mortality

P= Parasitaemia

R= Relapse

Numerator= Number either aparasitaemic, parasitaemic, relapsed or dead as indicated. Denominator= Number in a group.

Time (days post infection)	Mean blood urea nitrogen (BUN)- (mg/dl) with standard error in bracket			
	Group 1 Infected treated with *Trypanosoma brucei* and treated with DA	Group 2 Uninfected, untreated (negative control)	Group 3 Infected, untreated (positive control)	Group 4 Infected treated with IMC
0	11.07^{a} (4.92)	8.65^{a} (4.06)	11.83^{a} (4.11)	10.58^{a} (4.06)
7	8.52^{a} (2.77)	10.66^{a} (3.36)	8.88^{a} (1.79)	7.47^{a} (1.61)
14	6.94^{a} 2.31	7.90^{a} (2.01)	15.79^{a} (9.42)	14.31^{a} (7.77)
21	7.44^{a} (2.02)	6.87^{a} (2.29)	15.72^{b} (5.32)	12.61^{ab} (6.39)
28	8.83^{a} (3.75)	8.50^{a} (3.26)	13.05	7.29^{a} (1.03)
35	9.08^{a} (2.18)	7.70^{a} (1.95)	8.07	9.94^{a} (4.84)
42	8.54^{a} (4.36)	5.63^{a} (2.79)	7.13	12.73^{a} (7.00)
49	7.25^{ab} (4.00)	4.34^{a} (2.12)	3.38	13.35^{b} (7.22)
56	7.28^{ab} (4.85)	4.81^{a} (2.12)	-	13.98^{b} (6.96)
63	7.63^{a} (5.28)	5.66^{a} (3.66)	-	15.45^{b} (6.14)

Table 2: The weekly blood urea nitrogen (BUN)- (mg/dl) of Trypanosoma brucei infected dog groups treated with either diminazene aceturate or iso metamidium chloride.

^{ab} Different superscripts in a row indicate significant difference between the group means ($p < 0.05$)

Reference value: 10-20

Time (days Post infection)	Mean creatinine (mg/kg) with standard error in bracket			
	Group 1 Infected treated with *Trypanosoma brucei* and treated with Diminazene aceturate	Group 2 Uninfected untreated (negative control)	Group 3 Infected untreated (positive control)	Group 4 Infected with *T.brucei* and treated with isometamidium chloride
0	0.45[a] (0.11)	0.45[a] (0.24)	0.42[a] (0.20)	0.52[a] (0.04)
7	0.43[a] (0.08)	0.43[a] (0.14)	0.42[a] (0.05)	0.42[a] (0.07)
14	0.65[b] (0.09)	0.46[a] (0.009)	0.63[b] (0.18)	0.48[b] (0.02)
21	0.55[ab] (0.09)	0.47[a] (0.15)	0.71 (0.20)	0.43[a] (0.19)
28	0.37[a] (0.08)	0.40[a] (0.15)	0.61	0.43[a] (0.12)
35	0.34[a] (0.07)	0.47[a] (0.15)	0.27	0.36[a] (0.05)
42	0.30[a] (0.10)	0.31[a] (0.18)	0.39	0.37[a] (0.26)
49	0.29[a] (0.15)	0.28[a] (0.013)	0.38	0.30[a] (0.20)
56	0.29[a] (0.17)	0.25[a] (.0.21)	-	0.23[a] (0.09)
63	0.27[a] (0.09)	0.33[a] (0.20)	-	0.29[a] (0.21)

Table 3: The weekly mean serum creatinine (mg/dl) of *Trypanosoma. brucei* infected dog groups treated with either diminazene aceturate or isometamidium chloride.

[ab] Different superscript in a row indicate significant difference between the group means ($p < 0.05$)

Reference value: 0.05-1.6.

Discussion

It was observed that anaemia that presented itself as pale mucous membranes is always a regular findings in trypanosomosis of animals. This agrees with the findings of previous workers [7-9]. and it was suggested to be due to the effect excessive removal of red cells by the activities of increases of mononuclear phagocyte system, hemolytic factors such as free fatty acids of 12-20 carbon atoms and rise in plasma volume.

Feverish condition observed in this work agrees with the findings of [9]. and he attributed it to the metabolism of tryptophan to tryptophenol by the infection isometamidium chloride (ISC) and diminazene aceturate (DA) administration successfully reversed these trends in the treated dogs, hence, the return to normal of rectal temperature from day 14 PI. However, toward the end of the experiment (days 56 and 63 PI), the pyrexia reoccurred among the treated dogs; this is obviously due to relapse infection that occurred in the treated groups. The parasite [10]. had a similar result.

It was observed that Infection caused no changes in the body weight of the infected dogs throughout the duration of the experiment. This observation shows that infection runs an acute course (6-7days PI), and may be responsible for the no significant changes in the body weight. Reduction in body weight had been reported in chronic case of canine trypanosomosis especially towards the terminal stage of the disease [10].

Previous workers [11]. observed variations in the prepatent period *T. brucei* as seen in this work (6-7 days). This may be due to the strain of the *T. brucei* stock used or due to inherent traits of the infected dogs. In this experiment, it was deduced that canine trypanosomosis caused by *T. brucei* ran an acute course as opposed to chronic disease caused by *T. congolense, evansi, T. rangelli T. cruzi, and T. caninum* [11]. The mortality in the infected control dogs within 28 days post infection may be due to the fact that increased parasitaemia might have overwhelmed the immune response thereby not allowing sufficient time for the dogs to

produce enough antibodies to fight the invading parasites. The trypanocides (DA and IMC) cleared the parasitaemia following treatment, an indication that they were efficacious in the treatment of *Trypanosoma brucei* in dogs at the dose level given. DA is used as a therapeutic agent since it is rapidly excreted [10]. in animals, whereas IMC has been used as a prophylactic drug at the dose range of 0.5-1.0mg/kg especially to maintain productivity of dogs exposed to tsetse challenge [12]. In this study, relapse infection occurred in different times with the DA and IMC (day 35PI and day 56 PI) respectively. Relapses could either be due to drug resistance or as a result of the parasites ability to cross the blood brain barrier and invade brain tissue [12] established that early treatment (3-4 days PI) often leads to permanent cure, and when relapses occur in such circumstance, it is due to drug resistance. On the other hand, delayed treatment (days14 or more Post infection) often leads to relapses as the parasite has entered the brain tissue from where it re-enters the vascular system when the effect of the drug in the blood stream would have waned; as the trypanocides molecule is too large to reach the brain tissue. Relapses in this study were as a result of parasite drug resistance as treatment was instituted early in the infection (day 7 PI). This may explain the neurological signs observed in the positive control towards the end of the experiment.

There is increases of creatinine and blood urea nitrogen, a kidney function marker, an indication of renal impairment or kidney functionality, following infection. This agrees with the works of [1,13]. who recorded a similar result in African canine trypanosomosis caused by *T. brucei brucei, T. congolense*, and *T. evansi*. Similarly, [14]. also recorded elevated creatinine and blood urea nitrogen in acute phase of chagas disease caused by *T. cruzi*. The rise in concentrations of serum creatinine and blood urea nitrogen suggests that the kidneys are losing their functionality as these substances ought to be excreted and will therefore not rise in the serum. However, treatments returned to normal these markers except blood urea nitrogen (BUN) in the IMC treated where we recorded significant ($p < 0.05$) increase in BUN when compared with the normal towards the end of the experiment. This could be because of the relapse infections that occurred within this period and the longer time that isometamidium lasted in the blood stream, being a prophylactic drug as against a therapeutic drug that diminazene aceturate represents. These findings-rise in creatinine and blood urea nitrogen following infection as an indication of renal impairment was corroborated by the work of Darling., *et al*. (2009) [15]. who recorded turbid urine as against normal urine colour in dogs which is pale, yellow and clear; also they recorded elevated urine P^H and an increase in the number of pus and epithelial cells which could lead to an increase in specific gravity and turbidity of the urine; in their work on canine urinalysis; these were also observed in this work. All these suggest serious renal function impairment [16,17].

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