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Comparative effects of treatment with diminazene aceturate and isometamidium chloride on kidney function of *Trypanosoma brucei* infected dogs

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Abstract

Trypanosome infections are commonly associated with kidney dysfunction. This study evaluated the effects of treatment with two commonly used trypanocides [diminazene aceturate (DA) and isometamidium chloride (IMC)] on kidney function of dogs experimentally infected with *Trypanosoma brucei*. Twenty dogs were used for the study. The dogs were randomly assigned to four groups (Groups 1, 2, 3 and 4) with five dogs per group as follows: Group 1 – Infected and treated with 7.0 mg/kg DA; Group 2 – Uninfected Untreated Normal Control; Group 3 – Infected Untreated Control; Group 4 – Infected and treated with 0.5 mg/kg IMC. One million *T. brucei* trypanosomes were used as the infection dose, and treatment with the trypanocides was done on Day 7 post-infection. Parasitaemia was checked daily until infection established and blood samples for assay of serum creatinine and urea (markers of kidney function) were collected weekly for the nine weeks of the experiment. Results showed that treatments with DA and IMC were able to eliminate the parasites from the blood stream of the treated dogs. 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), respectively. The infection led to significantly ($p < 0.05$) higher mean serum urea levels by day 21 PI, and significantly higher mean serum creatinine levels by days 14 and 21 PI, when compared with the normal control (Group 2). However, by day 49 PI and beyond, only serum urea levels was significantly ($p < 0.05$) higher in the Group 4 (IMC- treated) when compared to the normal control. These findings suggest that treatment with DA stabilized serum levels of creatinine and urea, and thus kidney function in *Trypanosoma brucei* infected dogs, more than treatment with isometamidium chloride.

Keywords: *Trypanosoma brucei* infection; Dogs; Kidney function; Diminazene aceturate; Isometamidium chloride.

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Introduction

The kidneys are a major part of the urinary system and are vital organs of the body involved in diverse range of functions including homeostatic, regulatory and excretory activities as well as hormone production (Barajas *et al*. 1992). The kidney and other parts of the urinary system eliminate waste products that are created when food is transformed to energy. The kidney also maintains the correct balance of water and electrolytes (salts) within the body and produces hormones (erythropoietin and renin), which are important in maintaining healthy blood pressure, producing blood cells and reabsorbing salts correctly. It equally processes Vitamin D. (Sherry, 2022). Clinically, kidney function is evaluated by measurement of blood/serum levels of metabolic products such as creatinine and urea (Braun *et al*., 2003; Kim *et al*., 2020; Salazar, 2014)

Trypanosomosis is an important protozoan disease of animals and man, which constitute a major constraint to livestock production in many areas of Africa, Asia and South America (Gutierrez *et al*., 2006). It is probably the only disease which has profoundly affected the settlement and economic development of major parts of the African continent (Wint and Rogers, 2000). Trypanosomosis is caused by several species of trypanosomes, including *Trypanosoma congolense, T. brucei, T. vivax, T. evansi, T. equiperdum, etc.* (Gutierrez *et al*, 2006). The most pathogenic trypanosome species responsible for the disease in domestic livestock are *T. vivax, T. congolense and T. brucei* in cattle, sheep and goats, and *T. simiae* in pigs. (Nantulya, 1999). In Latin America, dogs are important reservoirs of *T. cruzi* (Gurtler, 2007). Dogs are also commonly infected with *T. rangelli*, a human parasite in Latin America that is endemic for this species (OIE, 2008). Other trypanosome species such as *T. brucei brucei and T. congolense* also infect dogs and are thus of veterinary and economic importance (Kaggwa *et al*., 1984).

The therapeutic use of trypanocides for the treatment of and protection of domestic animals from trypanosomosis has been the mainstay of animal trypanosomosis control since early 1950s (Davey, 1957). Treatment of trypanosomosis using drugs had all along been done with homidium chloride and bromide, diminazene aceturate and isometamidium chloride (ILRAD, 1990), and recently quinapyrine sulphate has been reintroduced because of the need to especially combat camel trypanosomosis (Anene *et al*., 2001).

Diminazene aceturate is an aromatic diamidine derived from surfen (Eze *et al*., 2015). The molecule is marketed as the diaceturate salt and consists of two amidinophyl moieties linked by a triazene bridge: p,p- diamidiazoaminobenzene diaceturate tetrahydrate; N-1,3-diamidinophenyltriazene diaceturate tetrahydrate. In aqueous solution the compound is stable for 2-3 days (Olukunle *et al*., 2008). Diminazene binds to trypanosomal kinetoplast DNA (Newton, 1972). Diminazene inhibits synthesis of RNA primers, resulting in accumulation of replicating intermediates, thereby inhibiting k-DNA replication. (Brack and Delain, 1974). Shapiro and Englund (1990) have shown that diminazene specifically inhibits mitochondria type II topoisomerase in viable trypanosomes. Thus inhibition of DNA replication may also occur via this intercalation. Diminazene aceturate have shown efficacy when used to treat canine trypanosomosis at the dose 3.5 mg/kg in *T. brucei and in T. congolense* infection, and 7 mg/kg in *T. brucei brucei* and *T. evansi;* it is used more as a therapeutic drug (Aquino and Thomaz, 2007).

Isometamidium chloride has been marketed since 1961 as a prophylactic and therapeutic drug. Isometamidium differs from homidium by an additional moiety of amidinophenyl-azoamine (Anene *et al*., 2001), which in fact is part of the diminazene molecule.

Isometamidium can thus be seen as a 'hybrid molecule' which exhibits some of the properties of homidium and that of diminazene. Isometamidium is active against *T. congolense* and *T. vivax*, *T. brucei* and *T. evansi* infection in donkeys, horses, and camels (Zangs *et al*., 1991). In the dose range recommended for prophylactic purposes (0.5 – 1.0 mg/kg b.w), isometamidium chloride (IMC) has been used successfully to maintain the productivity of Zebu cattle exposed to tsetse challenge in both village and ranch management systems in East Africa (Moloo *et al*., 1987). However, considerable variation in prophylactic activity has been shown to vary in cattle from 2 – 22 weeks (Peregrine *et al*., 1991). Such variation in prophylactic activity appears to be independent of both the level of trypanosome challenge and the presence or absence of infection at the time of treatment (Peregrine *et al*., 1988).

Trypanosoma brucei is the predominant trypanosome species that infect dogs (Nwoha, 2013), and are known to be tissue invading parasites that cause pathologies in internal organs such as brain, heart, kidneys, liver etc. (Akpa *et al*., 2008). The kidneys of *T. brucei* infected cats have been reported to show severe widespread glomerular and tubular degeneration and necrosis with casts within the Bowman's capsule and collecting tubules (Nfon *et al*., 2000). Glomerulonephritis due to deposition of immune complexes, which ultimately compromise normal kidney function has also been associated with trypanosomosis (Nfon *et al*., 2000). Though African animal trypanosomosis caused by *T. brucei, T. congolense* and *T. evansi* are mostly characterized by elevated liver enzymes in serum, and high levels of blood urea nitrogen (BUN) and serum creatinine (Cr) and bilirubin concentrations (Aquinos *et al*., 2002; Nwoha *et al*., 2013), these parameters have not been extensively studied in Nigerian breed of dogs experimentally infected by *T. brucei*. There is therefore the need to have a clearer picture of the effect of *T. brucei* infection on the kidney function parameters (BUN, and Cr) of dogs and the consequent effects of treatment with the commonly used chemotherapeutic agents (diminazene aceturate and isometamidium chloride) on the kidney function of *T. brucei* infected dogs. The aim of this study was therefore to evaluate the effects of treatment with diminazene aceturate and isometamidium chloride on kidney function of dogs experimentally infected with *Trypanosoma brucei*.

Materials and Methods

Experimental Animals: Twenty Nigerian breed of dogs aged between 3 and 4 months were used for the study. They were purchased from Orie Orba market in Nsukka, Enugu State, Nigeria. They were housed in a fly-proof kennel at the Animal House of Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka. They were acclimatized for four weeks during which they were dewormed and de-ticked with ivermectin (Ivomec, Merck Sharp & Dohme, Holland) at the dose of 0.2 mg/kg subcutaneously. They were fed twice daily with food procured for the study and supplemented with one prepared by the Animal House Attendants. Water was provided *ad libitum* throughout the duration of the experiment. The dogs were screened and confirmed negative for trypanosomes by buffy coat method (Murray *et al*., 1977) before commencement of the experiment. They were also vaccinated with anti-rabies vaccine and DHLPP vaccine (Forte Dodge Company, USA).

Experimental design: The 20 dogs used for the study were randomly assigned to four groups (Groups 1, 2, 3 and 4) of five dogs per group. Fifteen of the dogs (those in Groups 1, 3 and 4) were infected by intra-peritoneal administration of 1×10^6 *Trypanosoma brucei*, while five dogs (those in Group 2) were

uninfected. The infective dose was estimated based on the method of Herbert and Lumsden (1976). The *Trypanosoma brucei* used for the study was obtained from the Nigeria Institute of Trypanosomiasis Research (NITR) Vom, Plateau State Nigeria. The parasites were maintained in albino rats before infection of the experimental animals.

Treatment with the trypanocides commenced 7 days post infection (PI). Diminazene aceturate (DA) [Veriben Ceva Sante Animals, Cedex France], reconstituted by dissolving 1.05 g of the drug per sachet in 12.5 ml of sterile water, was administered to the Group 1 dogs at the dose of 7.0 mg/kg. Isometamidium chloride (IMC) [Trypanidium-Samirin, Merial, Lyon France], reconstituted by dissolving one gramme sachet in 50 ml of sterile water, was administered to Group 4 dogs at the dose of 0.5 mg/kg. The groups and their specific treatments were thus as follows: Group 1: Infected and treated with diminazene aceturate (DA) at 7 mg/kg; Group 2: Uninfected, untreated (Normal Control); Group 3: Infected untreated (Negative Control); Group 4: Infected and treated with isometamidium chloride (IMC) at 0.5 mg/kg.

Blood samples for assay of serum creatinine and urea were collected from the dogs at day zero (baseline values), and subsequently at weekly intervals. The level of trypanosome parasitaemia was evaluated on daily basis.

Methods: The level of parasitaemia was estimated by the rapid matching method as described by Herbert and Lumsden (1976). Four millilitre of whole blood was collected from each dog each time of blood sampling for urea and creatinine assay. The blood was dispensed into clean labeled test tubes and allowed to stand for 45 minutes to clot. The test tubes were later centrifuged at 3000 rpm for 10 minutes to separate the sera from the clotted blood. The clear serum was used to assay for the urea and creatinine concentrations. Serum urea and creatinine concentrations were determined by colorimetric methods (Thomas, 1998), using a commercially available urea and creatinine test kits obtained from Dialab, Weiner Neudorf, Austria.

Handling of the experimental animals during the study: This study protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria Nsukka (Approval Reference No.: FVM-UNN-IACUC-2019-11/31). The dogs were fed and cared for well, and were also humanely handled all through the study.

Statistical Analysis: Data on serum levels of urea and creatinine were subjected to one way analysis of variance, and variant means were separated using Duncan's multiple range test. Significance was accepted at probability level less than 0.05. Summary results were presented as means with standard deviation.

Results

Clinical manifestations: 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 dogs in all the infected groups (Groups 1, 3 and 4), as from day 7 post infection when the treatment of infected groups (Groups 1, and 4) commenced. This persisted in the infected untreated group (Group 3) until the dogs died before the termination of the experiment. These clinical signs gradually disappeared in the treated groups (Groups 1 and 4) following treatment with the trypanocides. Dogs in the treated groups never showed signs of central nervous system abnormality. Depression and anorexia were observed again in two dogs in Group 1 (DA treated) following a relapse infection.

Parasitaemia: *Trypanosoma brucei* were found in the blood smear of the infected dogs

(Groups 1, 3 and 4) by day 6 – 7 post-infection (PI) [Table 1]. Treatment of dogs in Groups 1 and 4 commenced by day 7 PI when all the infected dogs became parasitaemic. The treated dogs (Groups 1 and 4) became aparasitaemic by day 14 PI (Table 1). The parasitaemia was progressive in the infected untreated group (Group 3). By day 28 PI, four dogs in Group 3 died and by day 56 PI all the dogs in Group 3 were dead (Table 1). On day 35 PI, relapse infection occurred in two out of the five dogs in Group 1 (DA treated), and by day 63 PI one dog in Group 1 (DA-treated) died (Table 1). Relapse of infection also occurred in one dog in Group 4 (IMC-treated) by day 56 PI, but no mortality was recorded in this group (Table 1).

Serum Urea: The serum urea levels of the Group 3 dogs (Infected untreated) was significantly ($p < 0.05$) higher than those of Groups 1 and 2 on Day 21 PI (Table 2). From day 49 PI to the end of the experiment, serum urea levels was significantly higher ($p < 0.05$) in Group 4 (IMC-treated) when compared with Group 2 dogs (Uninfected untreated Normal Control) [Table 2].

Serum Creatinine: Table 3 shows the mean serum creatinine levels of the various dog groups. Infection with trypanosomes led to significantly ($p < 0.05$) higher serum levels of creatinine in all the infected groups by Day 14 PI, with the significantly higher serum creatinine levels persisting in the Group 3 dogs up to Day 21 (Table 3). After day 21, no significant variations were recorded in the serum creatinine levels among the groups (Table 3).

Table 1. Parasitaemia in *Trypanosoma brucei* infected dog groups treated with either 7.0 mg/kg Diminazene aceturate (DA) or 0.5 mg/kg Isometamidium chloride (IMC).

Time (Days post infection)	Parasitaemia			
	Group 1 (Infected and treated with 7.0 mg/kg DA	Group 2 (Uninfected control)	Group 3 (Infected untreated control)	Group 4 (Infected and treated with 0.5 mg/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

[A = Aparasitaemia; M = Mortality; P = Parasitaemia; R = Relapse]

Numerator = Number of dogs either aparasitaemic, parasitaemic, relapsed or dead as indicated.

Denominator = Number of dogs in the group.

Table 2. The serum urea levels (mg/dl) of *Trypanosoma brucei* infected dog groups treated with either 7.0 mg/kg diminazene aceturate (DA) or 0.5 mg/kg isometamidium chloride (IMC).

Time (days post infection)	Mean serum urea levels (mg/dl) with standard deviation in bracket			
	Group 1 (Infected and treated with DA)	**Group 2 Uninfected, untreated**	**Group 3 Infected, untreated**	**Group 4 (Infected and 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)

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

Table 3: The mean serum creatinine (mg/dl) of *Trypanosoma. brucei* infected dog groups treated with either 7.0 mg/kg diminazene aceturate (DA) or 0.5 mg/kg isometamidium chloride (IMC).

Time (Days post-infection)	Mean creatinine (mg/kg) with standard deviation in bracket			
	Group 1 (Infected and treated with DA)	**Group 2 Uninfected, untreated**	**Group 3 Infected, untreated**	**Group 4 (Infected and treated with IMC)**
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)	0.68[b] (0.02)
21	0.55[ab] (0.09)	0.47[a] (0.15)	0.71[b] (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)

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

Discussion

The clinical signs reported for the dog groups infected with trypanosomes in this study, is consistent with earlier reports on canine trypanosomosis. The pyrexia which was observed following infection in this experiment is consistent with that reported by Stephen (1986). Pyrexia in trypanosomosis has been attributed to the metabolism of tryptophan to tryptophol by trypanosomes (Stephen, 1986). The accumulation of tryptophol in pharmacological doses in animals have been reported by Stephen (1986) to be responsible for rectal temperature changes or feverish condition seen in humoral antibody response to heterologous antigens.

The observed pale mucous membrane in the trypanosome infected dogs in this study, which indicates anaemia, is a consistent finding in animal trypanosomosis. This anaemia had been reported to be as a result of the combined effects of excessive red cell removal (erythrophagocytosis) by activities and proliferation of mononuclear phagocyte system (Anosa, 1988), haemolytic factors (Authie and Pobel, 1990), disorders of coagulation (Stephen, 1986), increased plasma volume and haemodilution (Stephen, 1986).

The administration of Diminazene aceturate (DA) and Isometamidium chloride (ISC) successfully reversed these clinical signs of trypanosomosis in the treated dogs, hence, the return to normal of rectal temperatures from day 14 PI. However, towards 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. Relapse on infection after treatment had earlier been reported by Nwoha and Anene (2011) in trypanosome infected dogs.

Previous reports by Amole, *et al*. (1982) showed variations in the pre-patent period following *T. brucei* infection, different from that recorded in the present study (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, the *T. brucei* infection in the infected dogs ran an acute course as opposed to chronic disease reportedly caused by *T. congolense, T. evansi, T. rangelli, T. cruzi, and T. caninum* (Amole, *et al*., 1982). The mortality in the infected untreated 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 levels given. DA is routinely used as a therapeutic agent since it is rapidly excreted in animals (Nwoha and Anene, 2011), whereas IMC has been used as a prophylactic drug at the dose range of 0.5 – 1.0 mg/kg especially to maintain productivity of dogs exposed to tsetse challenge (Moloo *et al*., 1987). In this study, relapse infection occurred at different times with the DA and IMC; day 35 PI 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. Moloo *et al*. (1987) 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 (days 14 or more post-infection) often leads to relapses as the parasite may have 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. Relapses recorded in this study are thought to be as a result of parasite drug resistance as treatment was instituted early in the infection (day 7 PI). This may explain the neurological signs recorded in

the infected untreated control towards the end of the experiment.

There was an infection-associated elevation of the serum levels of urea and creatinine which was very obvious in the infected untreated control in the present study. Serum creatinine and urea levels are kidney function markers and elevation of their concentration in blood are an indication of renal impairment or kidney dysfunction. The elevations in serum levels of creatinine and urea recorded in the infected untreated dog group in this study agrees with the reports of Aquino *et al.* (2002) and Nwoha *et al*. (2013) who recorded a similar result in African canine trypanosomosis caused by *T. brucei brucei, T. congolense*, and *T. evansi*. Similarly, Barr *et al*. (1991) 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 functional capacity as the levels of these substances that ought to be excreted and will therefore not have risen in the serum, are elevated. However, treatments with the trypanocides earlier stabilized blood levels of these kidney function markers, but later in the IMC treated group (Group 4), the serum urea levels were persistently significantly higher than those of those groups. It is thought that the significantly higher serum urea levels in the IMC treated group from day 49 to the end of the study could be as a result of the combined effect of the relapse infection and the longer time that isometamidium lasted in the blood stream, being a prophylactic drug as against a therapeutic drug that diminazene aceturate represents.

Conclusion: Based on the results of the study, it was concluded that treatment with diminazene aceturate was more effective than treatment with isometamidium chloride in stabilizing serum levels of creatinine and urea and thus kidney function in *Trypanosoma brucei* infected dogs.

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Conflict of interest

The authors declare no conflict of interest.

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