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Original Research Article

The Relapse of Single and Mixed Infections of *Trypanosoma congolense and Trypanosoma brucei* in Red Sokoto Bucks after Treatment with Isometamidium Chloride

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Abstract: Field reports by Veterinarians indicate that Isometamidium Chloride used in the treatment of trypanosomosis is not efficacious. This study was designed to determine the efficacy of 2% of this drug in the treatment of single and mixed infections of *T. Congoense* and *T. brucei* in Red Sokoto Bucks. Twenty four (24) Red Sokoto Bucks, 8 months - 1 year old were randomly selected and divided into four groups viz (I, II, III, and IV) of six each. These were used to investigate the relapse of infections of single and mixed trypanosome parasites. Group 1 served as un-infected control; Group II and III were inoculated with 2 ml of 1 x 10^6 *Trypanosoma brucei* and *T. congolense* respectively while Group IV were inoculated with 2 ml of 1x10^6 parasites each of *T. congolense* and *T. brucei* . Animals in the infected groups were treated on day 14 post patency with Isometamidium Chloride at the dose rate of 0.5 mg/kg IM. The Bucks were monitored pre-infection, post-infection and post treatment daily for rectal temperature, weekly body weight, PCV, WBC, total proteins and clinical signs. There was aparasitaemia 12hours after treatment. Relapse of the parasites were observed in all infected and treated group of Bucks two weeks post treatment. A buck died in Group II, three goats died in Group III while all goats died in Group IV. Isometamidium Chloride was observed to be efficacious against *T. brucei* and *T. congolense* 12 hours after treatment and this was maintained for two weeks when there was a relapse of the parasite. The need for further studies to determine the reason for the relapse is imperative and may necessitate a repeat of treatment two weeks later.

Keywords: Chemotherapy, *Trypanosoma brucei*, *Trypanosoma congolense*, Isometamidium chloride, Mixed infection, Red sokoto bucks.

INTRODUCTION

Trypanosomias is a complex, debilitating, Zoonotic protozoon disease of man and animal [1]. Natural infections with Trypanosoma congolense, T. vivax, T. brucei, and T. evansi have been described in goats. Trypanosomosis in goats produces acute, subacute, chronic, or subclinical forms, being T. vivax, T. congolense, and T. evansi, the most invasive trypanosomes for goats [2].

In Sub-saharan Africa, treatment and prophylaxis of trypanosomosis in cattle, sheep and goats is by the use of compounds like, Diaminazene (an aromatic diamidine), Homidium (a phenanthridine.), and Isometamidium (a phenanthridine aromatic amidine) [3]. Quinapyramine (a quinolone pyrimidine) is recommended for use against Trypanosomiasis only in camels and horses [4].

Natural mixed infections have been reported [5, 6] although it is not known whether the interaction of the trypanosome species in mixed infections would affect sensitivity of each species to the drug. Field experience, reports multiple specie relapse of parasites after single treatment with antitrypanosomal drugs especially Isometamidium Chloride.

Plasma trypanocidal levels of these drugs have been known to last between 1-6 months [7]. Isometamidium Chloride produces lethal effects in the trypanosomes by displacing magnesium ions and polyamines from ribosomes and also modifies cytoplasmic membranes and ribosomes [8], resulting in the inhibition of phospholipid synthesis, basic amino acid transport, and oxygen uptake in the Tri-carboxlic Acid Cycle of the trypanosome [9]. Isometamidium Chloride is usually administered to trypanosome infected animals by deep intramuscular (IM) injection

of 1% or 2% solution at the dose rate of 0.25- 0.5 mg/kg [10-12]. It is necessary to evaluate the efficacy of Isometamidium Chloride on single and mixed infection of trypanosome in Red Sokoto Bucks (RSB).

MATERIALS AND METHODS

Experimental Goats

Twenty-four Red Sokoto bucks aged 8 months to 1 year were procured from the open market of Kafur village in Katsina State. The animals were confined in arthropod-proof pens in the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The goats were fed on crop residues, wheat and guinea corn bran, *Andropogan gayanus* hay, groundnut cake, salt lick and potash. Water was provided *ad libitum*.

Screening for Parasites

The goats were screened for gastro-intestinal and haemo-parasites using floatation and sedimentation methods as described by Cole [13] and thin blood smear as described by Woo [14].

Trypanosoma Parasite

Trypanosoma congolense and *Trypanosoma brucei* used in this study were obtained from the National Institute for Trypanosomiasis and Onchocerciasis Research (NITOR), Vom, Nigeria.

Preparation of Inoculum

The parasites were initially multiplied in albino rats by inoculating each of the stabilates into albino rats and monitored for parasitaemia using the thin blood smear method and viewed under light microscope.

Two milliliters of infected blood containing about 2 x 10^4 trypanosomes were harvested from the albino rats via the corneal artery using capillary tubes and 1.0 ml each intravenously inoculated into two donor goats to further multiply the parasites and obtain sufficient inoculum for the experimental infection. The number of trypanosomes injected into the savannah brown goats was estimated as described by Herbert and Lumsden [15]. The two donor goats showed patent parasitaemia on days 7 and 13 for *T. brucei* and *T. congolense* respectively.

Animal identification and Grouping

The goats were tagged and grouped into 4 (I, II, III and IV) of 6 goats. Group I goats served as the uninfected untreated control Group; Groups II and III goats were intravenously inoculated with *T. brucei* and *T. congolense* ($1x10^6$ parasites each) respectively, while Group IV goats were inoculated with a mixed infection of *T. brucei* and T. *congolense* ($1x10^6$ parasites each). The number of trypanosomes per inoculum was estimated as described by Herbert and Lumsden [15].

Monitoring of Parasitemia and other physical parameters

Parasitemia was estimated daily using wet mounts of the jugular blood prior to patency of parasitaemia, while parasite clearance following treatment were determined daily for four weeks using the haematocrit centrifuge technique [14].

Rectal temperature readings of each animal in each group was taken using a digital thermometer two times daily at 8 a.m. and 6 p.m. and daily average temperatures were obtained from the data.

The goats were weighed on a weekly basis throughout the experimental period and mean weekly weights recorded.

Statistical analysis

The data generated were expressed as mean ± SEM. Analysis of variance (ANOVA) with Turkey‟s Mutiple comparison post-hoc tests using Graph Pad Prism version 4.0 for windows (from Graph Pad Software, San Diego, California, USA) was used to compare the level of significance between the test Groups. Values of $P< 0.05$ were considered significant.

RESULTS

The results of this Study are presented on Table 1 to Table 4 respectively

Table 1: Mean ±SEM of Temperature (^oC) of Red Sokoto Bucks infected with *Trypanosoma brucei, T. congolense* and mixed infection of *T. brucei* and *T. congolense* and treated with Isometamidium chloride.

Infection Status	Control	*T. brucei* Infected Goats	*T. congolense* Infected Goats	*T. brucei* + *T. congolense* Infected Goats
Pre-infection	37.74±0.09	37.81±0.19	37.50±0.16	37.50±0.16
Post-infection	36.51±0.13	39.59±0.14***	38.00±0.32***	40.88±0.18***
Post-therapy	36.81±0.29	37.50±0.16	37.91±0.31***	37.03±0.18
Relapse	36.42±0.38	37.05±0.04	38.58±0.32	38.70±0.62***

*** = P<0.001. There was a statistically significant difference (P < 0.001) in daily rectal temperature between infected and treated Groups

Table 2: Mean ±SEM of weight (kg) of Red Sokoto Bucks infected with *Trypanosoma brucei, T. congolense* and a mixed infection of *T. brucei* and *T. congolense* and treated with Isometamidium Chloride.

Weight (kg)	Control Goats	*T. brucei* Infected Goats	*T. congolense* Infected Goats	*T. brucei + T. congolense* Infected Goats
Pre-infection	12.40±0.68	11.00±0.40	10.20±0.45	11.02±0.55
Post-infection	13.65±1.09	8.38±0.63***	9.92±0.51	9.33±0.61
Post-therapy	12.10±1.11	8.792±0.68	9.75±1.03	7.42±0.81
Relapse	12.10±1.11	9.00±0.79	7.80±0.94***	6.23±0.82***

*** = P<0.001

Table 3: Mean ±SEM of haematological parameters of Red Sokoto Bucks Infected with *Trypanosoma brucei, T. congolense* and mixed infections of *T. brucei* and *T. congolense* and treated for four weeks with Isometamidium Chloride and evaluated.

Haematological parameters	Control	*T. brucei*	*T. congolense*	*T. congolense + T. brucei*
Packed Cell Volume (%)				
Pre-infection	25.40±1.11	24.00±3.04	24.33±3.53	23.92±2.00
Post-infection	26.33±2.01	20.00±1.48	20.75±1.41***	9.38±3.53***
Post-therapy	27.40±2.82	27.40±2.82	20.80±1.41***	0.71±1.23***
Relapse	29.33±2.91	25.67±1.86	19.70±0.84***	16.67±1.01***
Haemoglobin concentration (g/dl)				
Pre-infection	8.51±0.24	7.88±1.07	8.03±0.64	7.88±0.53
Post-infection	9.97±1.03	6.62±0.49***	6.66±0.21***	5.05±0.10***
Post-therapy	9.24±1.52	8.71±0.79***	6.88±0.12***	6.88±0.12***
Relapse	9.93±0.85	8.71±1.01	6.76±0.29***	4.21±0.25
White Blood Cell ($X10^3/\mu l$)				
Pre-infection	11.97±1.03	10.6±0.52	11.99±0.78	10.59±0.71
Post-infection	11.99±0.78	7.90±1.04***	7.07±0.73***	6.86±0.34***
Post-therapy	11.30±1.74	7.07±0.73***	6.78±0.22***	6.86±0.34***
Relapse	10.59±0.71	9.88±0.96	9.43±1.08	9.28±0.54
Total Protein (g/dl)				
Pre-infection	6.16±0.26	6.39±0.12	6.12±0.19	5.99±0.24
Post-infection	6.12±0.19	6.27±0.28***	5.00±0.05***	5.40±0.13***
Post-therapy	6.70±031	6.87±0.53***	5.90±0.24***	3.42±0.94***
Relapse	6.70±0.31	6.50±0.36	4.25±1.32	1.41±1.32

*** = P<0.001

Table 4: Mean ±SEM of differential leucocyte counts of Red Sokoto Bucks goats infected with *Trypanosoma brucei, T. congolense* a mixed infection of *T. brucei* and *T. congolense* and treated with isometamidium chloride.

Differential Leucocyte counts (X10/µl)	Control	*T. brucei*	*T. congolense*	*T. congolense + T. brucei*
Neutrophils				
Pre-infection	6.29±0.35	4.26±0.50	5.05±1.15	4.75±0.75
Post-infection	6.30±0.36	6.84±1.14**	3.03±0.57**	6.97±0.20**
Post-therapy	6.29±0.35	2.00±1.18	3.02±0.57**	1.42±0.12**
Relapse	6.30±0.36	4.55±0.63	3.60±0.69**	2.18±0.85**
Lymphocytes				
Pre-infection	6.39±0.81	6.10±0.18	6.75±1.03	6.48±0.75
Post-infection	6.43±0.90	5.31±0.65**	3.30±0.51**	3.22±0.36**
Post-therapy	6.39±0.81	6.83±1.14**	3.03±0.57**	3.22±0.20**
Relapse	6.40±0.70	5.39±0.67	5.96±0.99**	5.29±0.59
Monocytes				
Pre-infection	0.07±0.81	0.13±0.05	0.09±0.03	0.54±0.44
Post-infection	3.45±0.62	0.00±0.00**	0.15±0.06**	0.11±0.02**
Post-therapy	1.01±0.25	0.15±0.03**	0.15±0.03**	5.05±0.10**
Relapse	2.06±0.78	1.01±0.88	1.03±0.87	1.08±0.87
Basophils				
Pre-infection	0.12±0.12	0.18±0.18	0.00±0.00	0.02±0.02

Post-infection	0.02±0.02	0.10±0.02**	0.29±0.06**	0.17±0.03**
Post-therapy	0.02±0.02	0.02±0.02**	0.29±0.06**	3.82±0.80**
Relapse	0.77±0.77	0.77±0.77	0.77±0.77	4.13±0.22
Eosinophils				
Pre-infection	0.20±0.07	0.18±0.06	0.29±0.12	0.21±0.03
Post-infection	1.52±0.41	0.00±0.00**	0.15±0.06**	0.15±0.06
Post-therapy	1.86±0.64	0.21±0.03**	0.15±0.18	0.12±0.02**
Relapse	1.52±0.41	1.12±0.78	0.96±0.81	1.06±0.79
Band cells				
Pre-infection	0.64±0.39	0.09±0.05	0.06±0.25	0.07±0.02
Post-infection	0.60±0.40	0.02±0.02**	0.18±0.05**	0.16±0.04**
Post-therapy	0.69±0.36	0.08±0.02**	0.18±0.05	0.11±0.12
Relapse	0.67±0.33	0.12±0.03	0.35±0.09	0.26±0.07

** = P<0.001

DISCUSSION

The appearance of pyrexia in this study corresponds with the appearance of parasitaemia as reported by Anosa and Isoun [16, 17] and Adeiza [18] in Red Sokoto bucks. The reason for the relapse in this study was not readily available.

The values of body weight (kg) were increasingly decreasing throughout the experiment due to body wasting. The finding is similar to the work of Abenga *et al.,* [19] who reported muscle wasting in murines with mixed infections of *T. brucei* and *T. congolense*. The reason for muscle waste is reduction in feed and water intake which occurred within 4-6 days post infection. In this study, this speculation was also observed by [20, 21].

The observed fall in PCV and haemoglobin concentrations in all the infected bucks were highest in bucks with mixed infections ($P < 0.001$), this coinciding with the patency of the infection and with the fluctuating parasitaemia, and this agrees with previous work in sheep, goats, and Wister rats infected with *T. evansi* [22, 23], *T. congolense* and *T. brucei* infection in murine [19] suggesting that trypanosomes were responsible for the progressive development of anaemia [17, 24-36].

Pathologically, the mucous membrane and carcasses of these bucks were very pale, an indication of severe anaemia as observed in the subacute and relapsed phase of the disease. This finding were similar to those observed by [16, 17, 27-30]. The progressive decrease in PCV ($P<0.001$) observed in the relapse of infection as compared to that of pre infection stage suggests that the virulence of the parasite post infection is more severe than that of the initial infection.

Considering the decrease in WBC counts as observed post-infection in all infected bucks may be attributed to the immunosuppressive actions of trypanosome infection [31, 32]. The leucopenia in the *T. brucei*, *T. congolense* and the mixed infection group observed in this study is similar with findings in *T. congolense T. brucei* and mixed infections in murines [19]. Leucopenia due to lymphocytosis has been implicated in trypanosomosis and these conditions are usually as a result of wax and wear syndrome on the animal immune system caused by the ever changing variable surface glycoprotein of the infecting trypanosome [31].

Leucocytosis is a common finding in trypanosomosis in endemic areas where animals are exposed to the parasite and are not likely to suffer mortality from the infection. This is based on the fact that tolerant animals develop leucocytosis and non tolerant animals develop leucopenia [33]. leucopenia observed in bucks which had shown the relapsed *T. congolense* and mixed infections indicated immunosuppression in the goats leaving them with an impaired immune defensive mechanism hence the higher mortality observed in this goats two weeks post treatment. There was no marked leucopenia observed post therapy, perhaps the treatment was responsible for moderating the reduction. The leucopenia seen here is in agreement with the report of Anene *et `al.* [34] in dogs. Eosinophilia and monocytosis observed in this study contradicted the reported eosinopenia and normal monocytes levels in *T. congolense* infected cattle by Naylor, 1971a. Thus these findings are indications of good immune response by the infected goats to the infection.

The observed increase in serum total protein in all the infected and treated bucks is suggested to be caused by the following mechanism: increase release of specific enzyme and some intracellular protein secondary to parasite–induced cell membrane disruption as observed by Orhue *et al*. [35]; Esievo *et al.,* [36], Olaniyi *et al.,* [37]; and increased concentration of immunological response against the parasite [35, 38]. In the later part of the study, there was observed decrease in the total protein which could be

due to decreased abnormal serum concentration as also observed by other reports [17, 25, 36, 38]. This decrease in total protein could also explain the relapse of the parameter due to reduced immunological immunoglobulins.

The observed increase in serum urea levels in all the infected Groups is in agreement with the work of Isoun in [16] in *T. brucei* infected calves and which is said to be as a result of increase in protein catabolism. Igbokwe and Mohammed (1992) in *T. brucei* infected Red Sokoto bucks; [39] *T. congolense* infected cattle and *T. vivax* infected cattle (Fiennes, 1974; 1980), *T. brucei* infected rabbits (Jenjins *et al.,* 1974) and dogs [40]. This they explained may be due to increased red blood cell destruction or increased haemolytic crisis (erythrocliasis). This also may explain the observation in this study that the level of urea increased soon after the fall in PCV and Hb became prominent.

The observed serum glucose concentration significant decrease in all the infected groups both post infection and post therapy are silmilar with the findings of Raisinghani and Lodha (1980) in camel which is said to be due to excessive utilization of blood glucose by the parasites for their metabolism [17]. It was also observed in this study that the glucose level was greatly increased in group II and group IV post therapy and this could be interpreted as shown by Jatkar and Singh [41] that parasite count is inversely proportional to glucose concentration. Increased metabolic rate caused by fever and hepatocyte degeneration could also be a reason for hypoglycemia in trypanosomosis (Cadioli *et al*., 2006) as shown in the post infection phase of all the infected groups. In contrast, Hilali *et al*. [38] reported no significant changes in the mean serum glucose of buffalo calves infected with *T. evansi*.

The observation in this study of limb paralysis, lethargy, circling, paddling and staggering movement in the affected groups in the chronic cases agrees with the observation of [27] that trypanosomosis causes CNS derangement. The observed facial, limb and scrotal oedema in the infected goats may be due to hypoproteinaemia and hypoalbuminaemia as observed in the study.

It has been suggested that, in relapsed cases, the tissue invasive parasites enter privileged sites inaccessible to the drug, from where they break through into the peripheral circulation later, to re-establish an infection [42]. Jennings *et al. (*1977) established an inverse relationship between the duration of infection and the occurrence of relapse. The main host and parasite related factors in drug resistance in trypanosomosis have been documented by Leeflang [43], Williamson [44] and ILRAD (1990). The host-related factors of drug resistance in trypanosomosis include the concentration of the drug used, variation in drug metabolism between individuals and diminished activity of a drug in a host with suppressed immune system, poor distribution of the trypanocides in infected tissues inaccessible to the drug, The longer the duration of infection before treatment the greater the chances of relapse. The bucks became aparasitaemic with 100% clearance after treatment was instituted There was relapse of the parasite in all the 6 bucks (100%) the mixed infection group and 3 bucks (50%) in the *T. congolense* group, while *T. brucei* group showed least relapse of the parasite with one buck (10%). Therefore, the interaction of both Trypanosome species in the mixed infections in this study enhanced the relapse of *T. congolense* infection, Jatau (2008), reported similar observation. The reason for relapse is not yet clear. All reasons given above are possible observations. More definite explanations are needed.

CONCLUSION

This study has shown that Isometamidium Chloride was efficacious in the treatment of both multiple and single infections of *T. brucei* and *T. congolense* infections within 24 hours of treatment, also, the significant haematological changes observed may be used as a possible explanation of the relapse experience in this study. However, further studies are needed to determine a definite explanation for the relapse.

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