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Results of Novidium® (Homidium Chloride) Chemotherapy on Clinical Manifestation of *Trypanosoma vivax* Infected Pregnant Yankasa and West African Dwarf [Wad] Ewes

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Abstract: The results of chemotherapy with the Trypanocidal drug Novidium(R), on the clinical manifestation of *Trypanosoma vivax* infected pregnant Yankasa (YK) and pregnant West African Dwarf (WAD) ewes was investigated. Three groups of pregnant YK and pregnant WAD ewes, comprising of 6 YK and 6WAD per group, were randomLy assigned to first, second and third trimesters of pregnancy and infected with *T. vivax* at each trimester. A fourth group comprising of 3 uninfected pregnant YK and 3 uninfected pregnant WAD ewes were the controls for the study. Two weeks post infection the infected ewes in each trimester period were divided into two equal groups of three ewes each. One group was treated with Novidium® (Homidium chloride), at 1.0 mg kg^{-1} body weight, the other group remained untreated. Blood samples from the treated ewes were negative for trypanosomes within 4 days post-chemotherapy. As the study progressed, clinical signs associated with trypanosomosis, such as anaemia indicated by low packed cell volume (PCV), loss in body weights, pyrexia and decline in total plasma protein (TP) values observed in the infected ewes, disappeared gradually following chemotherapy. Treatment had significant ($p \leq 0.05$) positive effects on PCV values of the treated YK and WAD ewes in the third trimester, TP values of treated YK in the first and second trimester, body weight of treated YK ewes in the second trimester, TP values of treated WAD ewes in the first, second and third trimester and body weights of treated WAD ewes in the second and third trimesters. The trimester of pregnancy and breed of ewe influenced the results of Novidium® chemotherapy on observed clinical parameters.

Key words: Trypanosomosis, chemotherapy, trimester of pregnancy, breed effects

INTRODUCTION

African trypanosomosis caused by tsetse-transmitted trypanosomes *Trypanosoma vivax*, *T. congolense* and *T. brucei,* is one of the important diseases of livestock in Africa. One of the most common characteristic features of the infection is the development of anaemia[1] and loss in body weights[2]. Trimester of pregnancy has been reported to influenced the severity of the clinical manifestation of Trypanosomosis in pregnant Zebu cattle [4]. Bawa *et al.*,[5] described anaemia, abortions, foetal mummification and loss in body weights in *T.vivax* infected second and third trimester pregnant YK and WAD ewes. The trimester of pregnancy and breed of ewe influenced the severity of the infection in the ewes. The infection was more severe in the third trimester and most severe in the pregnant YK than in the pregnant WAD ewes.

Detailed work prior to this investigation on the results of chemotherapy with trypanocidal drugs on clinical manifestation of *T.vivax* infected pregnant ewes in Nigeria appears to be lacking. Previous studies in other species have described the effects of trypanosomosis on male reproduction and the effects of chemotherapy on observed genital lesions[6] and deteriorated semen characteristics[5,6].

The objective of this study was to investigate the results of Novidium® (Homidium chloride) chemotherapy intervention on the clinical manifestation of *T. vivax* infected pregnant YK and WAD ewes, the predominant breeds of sheep in Nigeria.

MATERIALS AND METHODS

Animals: Fourty two healthy uniparous pregnant ewes, made up of 21 YK and 21 WAD sheep, were selected from a group of YK and WAD ewes that have earlier been synchronized by placing intravaginal progestagen sponges [Veramix®, Upjohn Ltd, West Sussex-England], containing 60.0 mg of Medroxyprogesterone, for 13 days. Forty eight hours after sponge withdrawal, the ewes were bred naturally with fertile rams of the same breed. Pregnancy was confirmed by radioimmunoassay (RIA)

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technique for progesterone (P_4) levels determination [7] and absence of oestrus 21 days after natural breeding by the rams.

The ewes were sprayed with an organophosphorus acaricide Rhodiacide [Rhone-Poulene Agrochimie, Germany]. The acaride was used at a dilution of 1.0 mL to 1.0 liter of water to spray the ewes. The ewes were dewormed with Flukazole® [Channelle, Ireland England], containing 1.5% w/v Levamisole and 0.6% w/v Niclofolan as active ingredients. The drug was administered orally at a dose of 7.5 mg kg^{-1} body weight. The ewes were also treated twice against common haemoparasites with long acting Terramycin [Pfizer Ikeja Lagos Nigeria], containing 216.0 mg of Oxytetracycline dihydrate per mL as active ingredient. The drug was administered intramuscularly at 20.0 mg kg^{-1} body weight. The ewes were checked for absence of trypanosomes infection using the haematocrit-centrifuge technique (HCT)[7].

Thereafter, 18 pregnant YK and 18 pregnant WAD ewes were selected from the ewes that have earlier been confirmed pregnant by RIA technique and non return to oestrus 21 days post natural breeding by the rams. These were randomLy assigned into three groups made up of 6 YK and 6 WAD ewes in each group, for first, second and third trimester of pregnancy studies. First, second and third trimesters are 0-50, 50-100 and 100-150 days of pregnancy respectively. Three pregnant YK and 3 pregnant WAD ewes served as controls for the study.

Infection: Stabilates of *T. vivax* (Stabilate.150) were obtained from the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria and used to infect donor goats. Trypanosomes were transferred from the goat into the experimental pregnant YK and WAD ewes at each trimester of pregnancy. The strain of *T.vivax* used in this study, was first isolated from a bovine at Kaya village in Zaria, Kaduna state, Nigeria. It was cloned and passaged twice in goats before it was cryo-preserved in 5% DMSO and stored in liquid nitrogen as stabilate 150. Prior to use, the stabilate was inoculated into mice and then transferred into a donor goat that subsequently developed parasitaemia.

Each ewe in the first, second and third trimester of pregnancy was inoculated via jugular vein with 2.0 mL of blood from the donor goat, containing approximately 2 x 10^6 trypanosomes. The ewes in the first, second and third trimester of pregnancy were infected at 23, 52 and 102 days of pregnancy respectively. The control ewes were left un-infected.

Parameters monitored pre- and post infection were rectal temperatures, packed cell volume (PCV) values, total plasma protein (TP) values according to Coles[10]. Blood samples taken in test tubes containing 10% Ethylene diaminetetracetate as anti coagulant (EDTA) were examined for parasitaemia by the haematocrit centrifuge technique (HCT) as described by [7] and thin blood smear examination. These parameters were estimated twice weekly. The body weights of the ewes were determined weekly using a top loading scale and recorded in kilograms (Kg). Percentage change in body weights post infection were calculated and recorded for each of the ewes.

The infection was described in the ewes as mild, when there were no obvious signs of anemia as shown by paleness of the mucous membranes and decrease in PCV values post infection to values not below 16.0%. No emaciation or abortions in the ewes. Severe, when there were obvious signs of anemia manifested by pale mucus membranes, severe decrease in PCV values post infection to values as low as 10.0%, with severe loss of body weight and emaciation, abortion and /or death of ewes. Very severe, whereby many ewes aborted before death or died without aborting, few days after the onset of parasitaemia and pyrexia.

Treatment: Two weeks post- infection the pregnant YK and WAD ewes in each trimester group were divided into two equal groups consisting of 3 pregnant YK and 3 pregnant WAD ewes each.. One group was treated with Novidium® intramuscularly, at 1.0 mg kg^{-1} body weight. (Novidium[R] is a brand of Homidium Chloride manufactured by May and Baker Ltd, Dagenham, England), the other group remained untreated. From each of the trimester groups, 3 YK and 3 WAD ewes were treated, while 3 YK and three WAD ewes remained untreated. The uninfected YK and WAD control ewes were not treated.

Statistical analysis: The SAS proc GLM procedure [8].was used to analysed the data collected from the various treatment groups of YK and WAD ewes.

RESULTS

Clinical signs: All the ewes were clinically healthy prior to the experimental infection. They were negative for blood parasites and had normal rectal temperatures. The haematological parameters, PCV and TP were within normal range. Trypanosomes were identified in the blood of the experimentally infected YK and WAD ewes within 3-4 and 3-9 days post infection, respectively.

All the YK and WAD infected ewes developed clinical trypanosomosis. The infection was mild (not

Table 1: Results of Novidium chemotherapy Parasitaemia levels of pregnant Yankasa and WAD ewes infected with T. vivax in their first, second and third trimesters of pregnancy

			Trimester		
Parameter	Breed	Treatment	1	2	3
Parastaemia levels (Mean ± SEM)	Yankasa	Non infected control	0.0±0.27^{a}	0.0±0.27^{a}	0.0±0.24^{a}
		Infected not treated	1.3±0.27^{b}	1.8±0.27^{b}	1.5±0.24^{b}
		Infected treated	0.6±0.27^{c}	0.9±0.27^{c}	0.7±0.24^{c}
	WAD	Non infected control	0.0±0.27^{a}	0.0±0.27^{a}	0.0±0.24^{a}
		Infected not treated	1.0±0.27^{b}	1.6.0±0.27^{b}	1.6±0.24^{b}
		Infected treated	0.6±0.27^{c}	0.7±0.27^{c}	0.3±0.24^{c}

Figures in the same row with different superscripts are significantly ($p \leq 0.05$) different

Table 2: Results of Novidium chemotherapy rectal temperatures of pregnant Yankasa and WAD ewes infected with T. vivax in their first, second and third trimesters of pregnancy.

			Trimester		
Parameter	Breed	Treatment	1	2	3
Rectal Temperature 0C (Mean ± SEM)	Yankasa	Non infected control	38.8±0.14^{a}	38.7±0.12^{a}	38.6±0.1^{a}
		Infected not treated	39.2±0.14^{b}	39.1±0.12^{b}	39.2±0.1^{b}
		Infected treated	39.0±0.14^{b}	38.8±0.12^{a}	38.8±0.1^{a}
	WAD	Non infected control	38.9±0.14^{a}	38.8±0.12^{a}	38.7±0.1^{a}
		Infected not treated	39.3±0.14^{b}	39.2±0.12^{b}	39.0±0.1^{b}
		Infected treated	39.1±0.14^{b}	38.9±0.12^{a}	38.7±0.1^{a}

Figures in the same row with different superscripts are significantly (0.05) different

Table 3: Results of Novidium chemotherapy on Packed cell volume values of pregnant Yankasa and WAD ewes infected with T. vivax in their first, second and third trimesters of pregnancy.

			Trimester		
Parameter	Breed	Treatment	1	2	3
% Packed cell values (Mean ± SEM)	Yankasa	Non infected control	29.1±0.7^{a}	30.2±1.0^{a}	30.4±1.0^{a}
		Infected not treated	22.8±0.7^{b}	22.4±1.0^{b}	22.6±1.0^{b}
		Infected treated	23.5±0.7^{b}	24.1±1.0^{b}	25.1±1.0^{c}
	WAD	Non infected control	29.6±0.7^{a}	31.8±1.0^{a}	31.5±1.0^{a}
		Infected not treated	23.6±0.7^{b}	24.2±1.0^{b}	22.3±1.0^{b}
		Infected treated	24.1±0.7^{b}	24.7±1.0^{b}	28.1±1.0^{d}

Figures in the same row with different superscripts are significantly ($p \leq 0.05$) different

Table 4: Results of Novidium chemotherapy on plasma protein values of pregnant Yankasa and WAD ewes infected with T. vivax in their first, second and third trimesters of pregnancy.

			Trimester		
Parameter	Specie	Treatment	1	2	3
Plasma Proteins g/dL (Mean ± SEM)	Yankasa	Non infected control	7.1±0.12^{a}	7.3±0.14^{a}	7.1±0.11^{a}
		Infected not treated	6.5±0.12^{b}	6.9±0.14^{b}	6.8±0.11^{b}
		Infected treated	7.0±0.12^{a}	7.4±0.14^{a}	6.9±0.11^{b}
	WAD	Non infected control	7.2±0.12^{a}	7.1±0.14^{a}	7.0±0.11^{a}
		Infected not treated	6.4±0.12^{b}	6.8±0.14^{b}	6.7±0.11^{b}
		Infected treated	7.2±0.12^{a}	7.9±0.14^{c}	7.1±0.11^{a}

Figures in the same row with different superscripts are significantly ($p \leq 0.05$) different

Table 5. Results of Novidium chemotherapy on % body weights changes of pregnant Yankasa and WAD ewes infected with T. vivax in their first, second and third trimesters of pregnancy.

			Trimester		
Parameter	Breed	Treatment	1	2	3
% Body weight changes(Mean ± SEM)	Yankasa	Non infected control	2.0±0.11^{a}	2.4±1.7^{a}	1.2±2.2^{a}
		Infected not treated	-2.2±0.11^{b}	-3.2±1.7^{b}	-9.3±2.2^{b}
		Infected treated	-0.64±0.11^{b}	1.5±1.7^{a}	-7.3±2.2^{b}
	WAD	Non infected control	3.86±0.11^{a}	0.4±1.7^{a}	3.7±2.2^{a}
		Infected not treated	4.1±0.11^{a}	-0.01±1.7^{b}	-5.2±2.2^{b}
		Infected treated	1.9±0.11^{c}	2.3±1.7^{a}	4.7±2.2^{a}

Figures in the same row with different superscripts are significantly ($p \leq 0.05$) different

severe), severe and very severe in the YK infected in their first, second and third trimester of pregnancy respectively. It was mild, severe and variable in the WAD ewes infected in their first, second and third trimester of pregnancy respectively. The trimester of pregnancy and breed of ewe influenced the severity of the infection.

Following Novidium(R) chemotherapy, trypanosomes were cleared from peripheral blood circulation of the infected-treated YK and WAD ewes within four days post treatment. Relapsed infections were treated again at the same dose. Following treatment, clinical signs and pyrexia earlier observed in the infected YK and WAD ewes subsided in the treated YK and WAD ewes. Consequently, resolution in PCV, TP and body weights were observed in the treated YK and WAD ewes, Tables 1 to 5 are summaries of the statistical analysis of the clinical data of the ewes.

Parasitaemia: All the infected ewes in the three trimesters of pregnancy developed high parasitaemia that fluctuated until death or treatment of the ewes, with the exception of one infected-untreated WAD ewe in the first trimester that self cured from the infection at 76 day post infection. No significant differences ($P \geq 0.05$) in parasitaemic levels were observed between the infected-untreated ewes of the two breeds (Table 1). The parasitaemic levels of the treated YK and WAD ewes were significantly ($p \leq 0.05$), lower than those of the untreated YK and WAD ewes (Table 1).

Rectal temperatures: All the YK and WAD infected ewes developed pyrexia. The onset of high pyrexia coincided with the first wave of high parasitaemia. Thereafter the ewes developed fluctuating pyrexia until treatment or termination of study at the end of each trimester period The rectal temperature of the infected-untreated ewes were significantly ($p \leq 0.05$) higher than those of the noninfected control ewes. No significant ($p \geq 0.05$) difference was observed between the rectal temperatures of the infected-untreated and the infected-treated YK and WAD ewes, in the first trimester, however in the second and third trimesters, the rectal temperatures of the infected-untreated and infected-treated ewes were significantly ($p \leq 0.05$) different (Table 2).

Packed cell values: Decline in PCV values were observed in all the infected YK and WAD ewes in the first, second and third trimesters of pregnancy. The PCV values of un-infected control ewes were significantly ($p \leq 0.05$) higher than those of the infected-untreated ewes (Table 3).

Following chemotherapy, increase in PCV values were observed in the infected- treated ewes in all the three trimesters of pregnancy. However, no significant ($p \geq 0.05$) difference was observed between the PCV values of the infected-untreated and the infected-treated YK and WAD ewes in the first and second trimesters of pregnancy. Significant ($p \leq 0.05$) difference was however observed in the third trimester between the infected-untreated and the infected-treated YK and WAD ewes (Table 3) In all the three trimesters of pregnancy the PCV values of the infected-treated YK and WAD ewes were significantly ($p \leq 0.05$) lower than those of the uninfected control YK and WAD ewes (Table 3). This showed that 49 days post chemotherapy with Novidium® did not completely ameliorate the effects of Trypanosomosis on PCV values in the treated ewes.

Total Plasma proteins: Decline in TP values were observed in all the infected ewes in the three trimesters of pregnancy and were ameliorated following chemotherapy with the exceptions of the TP values of the infected - treated YK ewes in the third trimesters of pregnancy (Table 4).

Percent change in body weights: Significant ($p \leq 0.05$) differences in the decline in body weights following infection were observed between the YK and WAD ewes in the first trimester (Table 5). The infected-untreated WAD gain weight compared to the loss of weight observed for the infected-untreated YK. The body weights of the infected-untreated and infected-treated WAD ewes were also significantly ($p \leq 0.05$) higher than those of the infected untreated and infected-treated YK ewes (Table 5).

In the second trimester, there was no breed difference ($p \geq 0.05$) in weight loss. The results of chemotherapy on weight loss, was also similar in both breeds and highly significant ($p \leq 0.0001$) Consequently, the infected-treated YK and WAD ewes had significantly ($p \leq 0.05$) higher body weights than the infected-untreated ewes (Table 5).

In the third trimester there was breed difference in severity of infection and weight loss between the two breeds of ewes. Consequently there was breed difference on the results of chemotherapy on the body weights of infected-treated YK and WAD ewes, The body weights of infected-treated WAD ewes were significantly higher than those of the infected-treated YK ewes (Table 5).

DISCUSSION

The results of the study showed that pregnant YK and WAD ewes are susceptible to T. vivax infection, the trimester of pregnancy and breed of ewe influenced the severity of the infection. All the infected ewes developed clinical Trypanosmosis with high and fluctuating

parasitaemia and pyrexia, weight loss and anaemia. These findings concur with earlier reports[1,2]. The observed weight loss and anaemia were however not completely ameliorated in the infected ewes by 49 days post chemotherapy with the trypanocide Novidium®. This observation agrees with previous reports [9].

The severity of infection and breed of ewe influenced the results of chemotherapy on body weights of treated pregnant YK and WAD ewes. In the first when the infection was mild in both the infected Yankasa and WAD ewes, the breed of ewe had significant positive effects ($p \leq 0.0001$) on body weights. The infected-untreated and infected treated WAD ewes both showed positive estimates for percent change in body weights compared to the negative estimates observed in the YK ewes (Table 1). In the third trimester when the infection was very severe in the YK and variable in the WAD ewes, the PCV, TP and body weights of the infected-treated WAD ewes were significantly ($p \leq 0.05$) higher than those of the infected-treated YK ewes (Table 3-5) The severity of the infection in the third trimester ewes influenced the results of chemotherapy on these parameters.. This finding concurs with previous reports[4,5].

It was concluded from this study that the breed of ewe, trimester of pregnancy and severity of infection influenced the results of Novidium(R) chemotherapy in pregnant YK and WAD ewes. Chemotherapy was generally more beneficial in the infected-treated pregnant WAD ewes than in the infected-treated pregnant YK ewes.

It is suggested that the beneficial effects of Novidium® chemotherapy in pregnant WAD ewes could be further investigated, if they could translate to successful gestation, lambing and resumption of normal reproduction in the infected-treated WAD ewes.

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