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Prevalence of Diminazene Aceturate Resistant Trypanosomiasis in Wad Sheep in Plateau State, Nigeria

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Abstract:- This study aimed to determine the effects of Diminazene, Levamisole, and Vitamin A on treating trypanosomiasis in West African Dwarf (WAD) sheep experimentally infected with Trypanosoma brucei. Twenty-four (24) adult WAD sheep of mixed sexes were acclimatized for two months and treated with Albendazole, Ivermectin, Tick and Flea Powder, and long-acting Oxytetracycline. The animals were confirmed negative for trypanosomiasis and brucellosis. They were then infected with Trypanosoma brucei and treated with Diminazene Aceturate, Levamisole, and Vitamin A two weeks after the infection. Parasitemia was established on the 4th day and was evident in all the infected animals six days post-infection. Reduced Total White Blood Cell count of treated animals did not affect clinical signs, clearance of parasites from the bloodstream, or antibody titers to Brucella abortus. In addition, the study also monitored rectal temperature and body weight changes. The data showed that using immunomodulators to treat West African Dwarf sheep infected with Trypanosoma brucei significantly increased the erythrocyte indices (PCV, Hb, and RBCs). Reduced Total White Blood Cell count of treated animals did not affect clinical signs, the clearance of parasites from the bloodstream, or antibody titers to brucella abortus antigen. High titres of Brucella abortus antibody recorded in all vaccinated animals up to the last week of the study indicate that trypanosomiasis did not suppress antibody production in these animals. A mortality rate of 25% was recorded in groups two, three, and five during the second week and 33% in group five during the third week. Death first occurred as early as two weeks post-infection in groups two, three, and five. The study concluded that Diminazene Aceturate, vitamin A, Levamisole, or a combination of Diminazene Aceturate, vitamin A, and Levamisole should be used to treat ovine trypanosomiasis.

Keywords:- *Diminazane Aceturate; Trypanosomiasis; Immunomodulators; West African Dwarf Sheep; Levamisole;Vitamin A.*

I. INTRODUCTION

African animal trypanosomiasis (AAT) is caused by a group of protozoan haemoflagellate parasites of the genus Trypanosoma. They produce persistent infection in the blood and induce profound immunosuppression[1]. Trypanosoma transmits the disease complex (T.) congolense, T. vivax, or T. brucei. Diminazene aceturate and Isometamidium chloride are the most commonly used therapeutic and prophylactic agents for treatment in Nigeria[1]. The Plateau state was previously considered free of AAT; the altitude is assumed too high to permit tsetse colonization, rendering it free of the tsetse vector and the trypanosomes they transmit. Consequently, the Plateau has been used in various predictive models to set the current limits for areas habitable for tsetse and to predict future limits[2]. However, a closer examination shows that tsetse flies and AAT have been present on the Plateau since the 1980s[2]. Reports from single village surveys and surveys undertaken in Local Government Areas have shown that these assumptions are no longer valid[2]. Trypanosoma brucei, T. congolense, and T. vivax cause disease in livestock (cattle, goats, sheep, and pigs)[3]

II. MATERIALS AND METHODS

A. Materials

The study bought twenty-four (24) adult West African Dwarf (WAD) sheep of mixed sexes from four markets (Mangu, Bukuru, Shendam, and Rikkos) spread across the state. The sheep were acclimatized for two months and treated with Albendazole (Tuyil Pharm Ind. Ltd., Nig), Ivermectin, Tick and Flea Powder (Propets Product & Serv., Nig), and long-acting Oxytetracycline (TETROXY LA®, Bimeda, Holland). The animals were screened and confirmed negative for Trypanosomiasis and Brucellosis.

- *Parasites*

The T. brucei isolate used in the study was a local isolate obtained from the National Veterinary Research Institute (NVRI) Vom Plateau State. The parasites were maintained in albino rats for research purposes until use in the experiment.

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➢ *Antigens/Drugs/Physiological Solutions/Chemicals*

The research used the following antigens, drugs, physiological solutions, and chemicals:

- Brucella abortus vaccine
- Brucella antigen
- Physiological saline; is prepared by dissolving 0.9 gms of sodium chloride (NaCl) in 100ml of distilled water.
- Diminazene Aceturate (Trypazen® by Pantex Holland) at a dose of 7.0mg/kg, intramuscularly (I/M). The Trypazen injectable solution was obtained by dissolving the 2.36g content of a sachet in 15 ml sterile water.
- Levamisole HCL (Levaject 100®, Farvet, Holland), intramuscularly at 5mg/kg.
- Vitamin A (Aquasol-A® by US Vitamins Ltd) at a dose of 50,000 IU/animal intramuscularly

B. Methods

The sheep were assigned into six treatment groups of four animals per group as follows:

- Group One: Uninfected and Untreated (Negative control).
- Group Two: WAD sheep Infected with Trypanosoma brucei and treated with diminazene aceturate; two weeks post-infection (Positive Control).
- Group Three: Infected with Trypanosoma brucei and treated with diminazene aceturate and Levamisole two weeks post-infection.
- Group Four: Infected with Trypanosoma brucei and treated with diminazene aceturate and Vitamin A two weeks after infection.
- Group Five: Infected with Trypanosoma brucei and treated with diminazene aceturate and Vitamin A three weeks after infection.
- Group Six: Infected with Trypanosoma brucei and treated with diminazene aceturate, Levamisole, and Vitamin A, two weeks after infection.

All the sheep were inoculated with Brucella abortus strain 19 antigen ten (10) days after infection.

➢ *Trypanosome Infection of Sheep*

Each sheep to be infected received 2ml of infected rat blood in physiological saline containing one million trypanosomes approximately. The organisms were inoculated intravenously into the sheep via the jugular.

➢ *Clinical Parameters*

Body weight was determined weekly using a weighing balance, and the values were recorded in kilograms. Rectal temperature[4] was obtained weekly, and the values were recorded in degree Celsius. Respiratory rate was monitored weekly and recorded in cycles per minute. Pulse rate was also monitored weekly and recorded in beats per minute. The study took respiratory rates in the morning hours.

➢ *Trypanosome count/estimation of parasitemia*

Estimating parasitemia/trypanosome counts using the rapid matching technique was performed [5]. A dilution (1:10) of the infected rat blood in Edetate Calcium Disodium (EDTA) was produced with physiological saline. The trypanosomes were counted per field of vision using an x5 eyepiece and x40 objective lens. The parasitemia per field of vision is compared with the chart and the number of organisms readout [5]

➢ *Diagnosis of Infection*

The researcher examined blood samples from the infected sheep daily until parasitemia occurred. Examination of the blood samples continued weekly until the end of the experiment. The wet blood film and microhematocrit buffy-coat methods were used.

➢ *Wet Blood Film Examination*

The wet blood film method isolated the parasites[6]. The trypanosomes in the blood film were visible under a high-power x40 objective lens. [6].

➢ *Micro-Hematocrit Buffy Coat Microscopy (MBC)*

A capillary tube was 3/4 filled with a blood sample and centrifuged at 2000G for 5mins (i.e., as in the measurement of PCV). The capillary tube was cut about 1mm below (to include the uppermost layer of RBC) and 3mm above (to have some plasma) the buffy coat layer. The content was expressed onto a slide and examined under a microscope with an x40 objective lens[7].

➢ *Brucella abortus Vaccine Innoculation*

Fifteen milliliters of distilled water dilute the *Brucella abortus* vaccine purchased from NVRI, Vom. Each sheep was then inoculated with 0.5ml of the solution subcutaneously.

➢ *Hematology*

A blood sample was taken from a sheep's jugular vein and mixed with an anticoagulant. The mixture was then gently rocked to mix the blood with the anticoagulant evenly. The number of RBCs in the fluid was measured using the micro-hematocrit method[6]. The tube containing EDTA-infused blood was thoroughly mixed, sealed, and centrifuged for five minutes at 10,000G using a micro-hematocrit centrifuge reader [5] and read as a percentage.

A Photo-colorimeter was used to determine the hemoglobin concentration. The Neubauer Counting Chamber method was used to examine for Total Red and White Blood Cell Count.

The study collected serology blood samples in containers without adding anticoagulants. The blood was left at room temperature to clot and then centrifuged at 3000G for 15 minutes. The serum was dispensed into a clean bottle and stored in a freezer at -20°C until analyzed.

➢ *Brucella abortus Antibody Assay/Serum Agglutination Test*

The serum agglutination test (SAT) and the British method were used[8]. Serial dilutions (doubling dilution) of serum in four test tubes produced titers of 1: 10 to 1: 80 IU/ µl from the first to the fourth test tube, respectively. 800µL of phenol saline was put into the first tubes of each row. 200µl of serum samples were added to the first tube of the appropriate row. They were well mixed at the end of which they were incubated at 37 ºC overnight. The reading of the result was done by observing the tubes against a dull black background with light coming from behind them[8].

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C. Statistical Analyses of Data

The results (except for the *Brucella abortus* assay) were analyzed statistically with the Statistical Package for the Social Sciences (SPSS) software package using one-way Analysis of Variance (ANOVA). The results are in tables and charts as Mean ± standard error (SE.)

The variant means were separated using Duncan's multiple-range tests[9]. The level of significance was $P<0.05$.

III. RESULTS AND DISCUSSION

A. Clinical Signs

Swelling on the face: This was observed two to three weeks post-infection in group five (diminazene aceturate and vitamin- A three weeks post-infection). Nervous signs also manifested in some infected animals of groups three and five that started receiving treatment two weeks post-infection. The nervous symptoms include circling, muscle spasms, and torticollis after prostration. Lethargy and Anorexia mainly occurred in group five from day 18 post-infection, making them eat very little. This lethargy or somnolence disappeared following treatment with diminazene aceturate. Also observed were fluctuating pyrexia, pale mucous membranes, increased respiratory and pulse rates, enlargement of pre-scapular and pre-femoral lymph nodes, and emaciation.

A mortality rate of 25% was recorded in groups two, three, and five during the second week and 33% in group five during the third week. Death first occurred as early as two weeks post-infection in groups two, three, and five.

B. Vital parameters

➢ *Parasitemia*

Table 1 shows the result of the mean group parasitemia of experimental animals. Parasitemia occurred in all the infected groups from four to six days post-infection (PI). Parasitemia progressed steadily in the infected groups until treatment commenced.

By week 3, the mean parasitemia of group five (diminazene aceturate and vitamin A) three weeks post PI was significantly higher ($P<0.05$) when compared with those of other infected and treated groups (groups two, three, four, and six).

There was zero parasitemia in all the infected and treated groups from week four to the end of the study (week 12).

➢ *Rectal Temperature*

Table 2 presents the result of the experimental animals' mean group rectal temperature. The infection caused a significant rise in the rectal temperature in infected groups two to six compared to group one (uninfected and untreated) from week one to week four. In both weeks two and three, the mean rectal temperatures of group two were significantly ($P<0.05$) higher than those of group 3 (diminazene aceturate and vitamin A and Levamisole two weeks PI). There was no significant difference ($P=0.01$) between the infected and untreated groups from weeks eight to nine.

➢ *Proportional body weight changes*

The result of the mean group, proportional weight changes of experimental animals, is shown in Table 3. The infection did not cause significant differences ($P>0.05$) in the mean proportional body weights across the groups from weeks one to six compared to the uninfected control group. By week six, there was a significant increase in group three's mean proportional body weight (Diminazene Aceturate and Levamisole) compared to group four. But not groups one (uninfected and untreated) but not groups two and three. From week 11 to the end of the study, there was a significant increase ($P<0.05$) in the mean proportional body weight of group five when compared with the other infected and treated groups (two, three, four, and six).

➢ *Packed cell volume*

The result of the mean group packed cell volume (PCV) of experimental animals is shown in Table 4. The mean PCV of all the infected groups (2, 3, 4, 5, and 6) was significantly lower ($P<0.05$) when compared with that of the uninfected control (group 1) from week 1 to the end of the experiment. There was a significant decrease (P) in PCV in groups 4 (Diminazene Aceturate and Vitamin A 2 weeks PI) and 5.

➢ *Hemoglobin Concentration*

The result of the mean group hemoglobin concentration (HbC) of experimental animals is shown in Table 5. By week six, the mean HbC was significantly ($P<0.05$) highest in group four.

By week seven, the mean HbC of groups three, five, and six were not significantly ($P>0.05$) different when compared with group one but were significantly higher ($P<0.05$) than those of groups two and four. The HbC of group 2 was significantly higher than that of group 4. By week 12, the mean HbC of all the infected and treated groups (2, 3, 4, 5, and 6) were significantly lower ($P<0.05$) when compared with that of the uninfected control group (1).

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TABLE 1: Mean Group Parasitemia of WAD Sheep Experimentally Infected with *T. brucei* and Treated with Diminazene Aceturate and Levamisole/Vitamin A/Both and Vaccinated against Brucellosis

Experimental Period (weeks)	GROUP 2 DA 2 weeks PI	GROUP 3 DA & levamisole Two weeks PI	GROUP 4 DA & Vit. A Two weeks PI	GROUP 5 DA & Vit. A Three weeks PI	GROUP 6 DA & Vit.A & levamisole Two weeks PI.
0*	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
1	14.73(3.85)^{a}	81.25(47.51)^{a}	19.00(5.00)^{a}	90.00(54.38)^{a}	30.00(11.86)^{a}
2**#	36.71(8.85)^{ab}	66.00(30.09)^{b}	64.75(22.31)^{b}	86.00(7.00)^{b}	84.25(19.89)^{b}
3***	0.00(0.00)^{a}	0.00(0.00)^{a}	0.00(0.00)^{a}	36.71(16.50)^{b}	0.00(0.00)^{a}
4	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
5	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
6	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
7	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
8	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
9	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
10	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
11	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
12	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)

Different Superscripts (a, b, c) in a row indicate a significant difference between the means of the groups at (P<0.05)
* Experimental Infection ** Early treatment *** Late treatment
# Vaccination with *Brucella abortus* S1

TABLE 2: Mean Group Rectal Temperature of WAD Sheep Experimentally Infected with T. brucei and Treated with Diminazene Aceturate and Levamisole/Vitamin A/Both and Vaccinated against Brucellosis

Experimental Period (weeks)	GROUP 1 Uninfected, Untreated	GROUP 2 DA Two weeks PI	GROUP 3 DA & levamisole Two weeks PI	GROUP 4 DA & vit. A Two weeks PI	GROUP 5 DA & vit. A Three weeks PI	GROUP 6 DA & vitamin A & Levamisole Two weeks PI
0*	38.68(0.50)^{a}	39.05(0.13)^{a}	38.80(0.11)^{a}	39.00(0.18)^{a}	39.05(0.12)^{a}	38.88(0.75)^{a}
1	39.08(0.15)^{a}	39.93(0.37)^{b}	40.80(0.09)^{c}	40.83(0.85)^{c}	40.65(0.65)^{c}	40.34(0.65)^{c}
2**#	39.25(0.12)^{a}	40.63(0.88)^{c}	40.00(0.26)^{b}	39.93(0.48)^{b}	40.93(0.67)^{c}	39.88(0.21)^{b}
3***	38.85(0.65)^{a}	39.97(0.33)^{b}	39.17(0.32)^{a}	39.30(0.20)^{a}	39.85(0.15)^{b}	39.03(0.06)^{a}
4	39.15(0.16)^{a}	39.00(0.06)^{a}	39.07(0.22)^{a}	39.35(0.22)^{ab}	39.85(0.05)^{b}	39.98(0.18)^{a}
5	38.98(0.31)^{a}	38.90(0.06)^{a}	39.30(0.15)^{a}	39.25(0.20)^{a}	39.20(0.20)^{a}	39.08(0.08)^{a}
6	39.03(0.33)^{a}	39.27(0.37)^{ab}	40.00(0.06)^{b}	39.03(0.17)^{a}	38.90(0.00)^{a}	39.28(0.16)^{ab}
7	38.85(0.06) ^{a}	39.67(0.23)^{b}	39.70(0.10)^{b}	38.98(0.03)^{a}	38.95(0.05)^{a}	39.18(0.16)^{a}
8	39.55(0.19)^{a}	39.47(0.29)^{a}	39.83(0.07)^{a}	39.35(0.20)^{a}	39.90(0.10)^{a}	39.43(0.20)^{a}
9	39.45(0.26)^{a}	39.43(0.30)^{a}	39.27(0.22)^{a}	39.13(0.19)^{a}	39.70(0.20)^{a}	39.35(0.18)^{a}
10	39.75(0.25)^{a}	39.30(0.17)^{ab}	38.87(0.32)^{b}	39.40(0.15)^{ab}	39.55(0.05)^{ab}	39.30(0.25)^{ab}
11	39.25(0.24)^{a}	39.27(0.37)^{a}	39.57(0.28)^{a}	39.57(0.17)^{a}	38.85(0.05)^{a}	39.38(0.18)^{a}
12	39.60(0.24)^{a}	39.50(0.20)^{a}	39.67(0.28)^{a}	39.28(0.16)^{a}	39.60(0.10)^{a}	39.05(0.10)^{a}

Different Superscripts (a, b, c) in a row indicate a significant difference between the means of the groups at (P<0.05)
* Experimental Infection; ** Early treatment; *** Late treatment
# Vaccination with *Brucella abortus* S19.

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Table 3 Mean Group Proportional Body Weight Changes of WAD Sheep Experimentally Infected with T. brucei and Treated with Diminazene Aceturate, Levamisole/Vitamin A/Both and Vaccinated against Brucellosis

Experimental Period (weeks)	GROUP 1 Uninfected, Untreated	GROUP 2 DA Two weeks PI	GROUP 3 DA & levamisole Two weeks PI	GROUP 4 DA & vit. A Two weeks PI	GROUP 5 DA & vit. A Three weeks PI	GROUP 6 DA & vit. A levamisole Two weeks PI.
0*	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}
1	1.28 (0.29)^{ab}	1.34 (0.03)^{ab}	1.40 (0.06)^{ab}	1.21 (0.06)^{a}	1.42 (0.08)^{b}	1.35 (0.1)^{ab}
2**#	1.34 (0.05)^{ab}	1.22 (0.08) ^{ab}	1.34 (0.08)^{ab}	1.18 (0.05)^{a}	1.42 (0.06)^{b}	1.30 (0.09)^{ab}
3***	1.31 (0.05)^{a}	1.36 (0.07)^{a}	1.34 (0.09)^{a}	1.12 (0.07)^{a}	1.34 (0.14) ^{a}	1.30 (0.09)^{a}
4	1.34 (0.05)^{a}	1.15 (0.05)^{a}	1.19 (0.06)^{a}	1.13 (0.07)^{a}	1.33 (0.24) ^{a}	1.32 (0.10)^{a}
5	1.33 (0.09)^{a}	1.33 (1.12)^{a}	1.27 (0.09)^{a}	1.12 (0.07)^{a}	1.22 (0.22) ^{a}	1.32 (0.10)^{a}
6	1.33 (0.09)^{ab}	1.31 (0.10)^{ab}	1.45 (0.08)^{b}	1.08 (0.07)^{a}	1.33 (0.18)^{ab}	1.35 (0.10)^{ab}
7	1.34 (0.05)^{a}	1.42 (0.14)^{a}	1.51 (0.05)^{a}	1.00 (0.07)^{b}	1.40 (0.25)^{a}	1.30 (0.09)^{ab}
8	1.38 (0.08)^{a}	1.42 (0.14)^{a}	1.51 (0.05)^{a}	1.00 (0.07)^{b}	1.36 (0.21)^{a}	1.28 (0.11)^{ab}
9	1.37 (0.09)^{ab}	1.44 (0.16)^{ab}	1.53 (0.07)^{b}	1.04 (0.09)^{a}	1.39 (0.25)^{ab}	1.26 (0.12)^{ab}
10	1.34 (0.05)^{ab}	1.42 (0.14)^{bc}	1.51 (0.05)^{bc}	1.04 (0.09)^{a}	1.71 (0.00)^{c}	1.26 (1.24)^{ab}
11	1.38 (0.08)^{a}	1.42 (0.14)^{a}	1.51 (0.07)^{a}	1.20 (0.06)^{a}	1.86 (0.00)^{b}	1.35 (0.10)^{a}
12	1.38 (0.8)^{ab}	1.44 (0.16)^{ab}	1.53 (0.07)^{b}	1.15 (0.11)^{a}	1.86 (0.00)^{c}	1.30(0.09)^{ab}

Different Superscripts (a, b, c) in a row indicate a significant difference between the means of the groups at (P<0.05)

* Experimental Infection ** Early treatment *** Late treatment

\# Vaccination with Brucella abortus S19.

Table 4 Mean Group PCV (% ± Standard Error) of WAD Sheep Experimentally Infected with T. brucei and Treated with Diminazene Aceturate, Levamisole/Vitamin A/Both and Vaccinated against Brucellosis

Experimental period (weeks)	GROUP 1 Uninfected, Untreated	GROUP 2 DA Two weeks PI	GROUP 3 DA & levamisole Two weeks PI	GROUP 4 DA & vit. A Two weeks PI	GROUP 5 DA & vit. A Three weeks PI	GROUP 6 DA & vit. A & levamisole Two weeks PI.
0*	35.50(1.26)^{a}	31.75(3.07)^{a}	33.75(3.33)^{a}	33.25(3.77)^{a}	34.75(3.902)^{a}	31.75(2.95)^{a}
1	37.50(0.96)^{a}	25(4.12)^{b}	22.50(3.80)^{b}	21.50(3.40)^{b}	25.25(1.93)^{b}	21.75(3.9)^{b}
2**#	39.50(0.50)^{a}	19.67(4.18) ^{b}	18.67(0.33)^{b}	21.25(3.20)^{b}	22.67(1.45)^{b}	21.50(3.20)^{b}
3***	39.50(0.29)^{a}	22.33(2.85)^{b}	20.00(2.89)^{b}	18.50(2.53)^{b}	22.50(0.50)^{b}	20.50(2.63)^{b}
4	38.75(0.48)^{a}	22.00(2.52)^{b}	22.67(1.76)^{b}	18.25(2.32)^{b}	20.50(0.5)^{b}	22.75(2.63)^{b}
5	38.00(0.48)^{a}	25.00(1.53)^{b}	27.33(1.45)^{b}	18.00(2.27)^{c}	19.00(0.00)^{c}	23.25(1.60)^{bc}
6	38.50(0.29)^{a}	24.67(0.33)^{cd}	28.00(1.15)^{bc}	22.75(1.15)^{d}	26.00(1.00)^{bcd}	39.00(1.47)^{b}
7	37.50(0.29)^{a}	26.30(0.33)^{d}	30.00(1.15)^{bc}	22.00(1.08)^{e}	32.00(2.00)^{b}	28.25(0.75)^{cd}
8	38.25(0.29)^{a}	28.67(0.33)^{bc}	27.33(1.15)^{c}	23.75(1.08)^{d}	30.50(2.00)^{b}	27.50(0.75)^{c}
9	38.00(0.48)^{a}	26.00(0.58)^{c}	30.00(1.15)^{b}	24.00(0.48)^{c}	29.50(0.50)^{b}	30.00(0.71)^{b}
10	38.00(0.00)^{a}	24.66(0.33)^{d}	32.00(1.15)^{b}	23.75(0.25)^{d}	29.00(0,00)^{c}	29.75(0.85)^{c}
11	36.50(0.65)^{a}	26.67(1.20)^{d}	32.00(0.58)^{b}	25.25(0.25)^{d}	29.50(0.50)^{c}	29.25(0.48)^{c}
12	37.50(0.29)^{a}	26.67(0.88)^{de}	31.33(0.67)^{b}	26.00(0.58)^{e}	28.50(0.50)^{cd}	29.75(0.85)^{bc}

Different Superscripts (a, b, c) in a row indicate a significant difference between the means of the groups at (P<0.05)

* Experimental Infection ** Early treatment *** Late treatment

\# vaccination with Brucella abortus S19.

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Table 5: Mean Group Hemoglobin Concentrations (g/dL± SE) of WAD Sheep Experimentally Infected with T. brucei and Treated with Diminazene Aceturate, Levamisole/Vitamin A/Both and Vaccinated against Brucellosis

Experimental period (weeks)	GROUP 1 Uninfected, Untreated	GROUP 2 DA Two weeks PI	GROUP 3 DA & levamisole Two weeks PI	GROUP 4 DA & vit, A Two weeks PI	GROUP 5 DA & vit. A Three weeks PI	GROUP 6 DA & vit. A & levamisole Two weeks PI.
0*	9.65(0.09)^{a}	9.7(0.14)^{a}	9.75(0.10)^{a}	8.98(0.41)^{a}	9.83(0.66)^{a}	9.63(0.90)^{a}
1	10.10(0.14)^{a}	8.70(0.12)^{b}	8.68(0.12)^{b}	7.0(0.18)^{c}	8.85(0.57)^{b}	8.05(0.61)^{bc}
2**#	10.88(0.43)^{a}	11.17(0.44)^{a}	11.83(0.95)^{a}	8.43(0.19)^{b}	8.83(0.17)^{b}	11.83(0.31^{a}
3***	11.50(0.65)^{a}	11.17(0.44)^{a}	11.17(0.74)^{a}	8.05(0.50)^{b}	7.40(0.40)^{b}	11.98(0.18)^{a}
4	12.75(0.25)^{ab}	10.17(0.44)^{c}	11.83(0.52)^{b}	10.08(0.15)^{c}	10.40(0.50)^{c}	13.13(0.16)^{a}
5	12.88(0.13)^{a}	11.33(0.67)^{c}	13.27(0.39)^{ab}	10.00(0.08)^{d}	14.25(0.25)^{b}	13.93(0.15)^{b}
6	12.53(0.21)^{ab}	10.67(0.33)^{d}	11.73(0.37)^{b}	9.73(0.15)^{e}	14.00(0.20)^{c}	12.90(0.31)^{a}
7	12.70(0.12)^{a}	11.00(0.29)^{b}	12.17(0.49) ^{a}	10.05(0.16)^{c}	12.65(0.15)^{a}	12.80(0.27)^{a}
8	13.30(0.12)^{a}	12.03(0.57)^{b}	12.07(0.54)^{b}	10.70(0.12)^{c}	14.00(0.20) ^{a}	13.40(0.13)^{a}
9	14.00(0.00)^{a}	12.00(0.50)^{b}	12.27(0.64)^{b}	10.70(0.12)^{c}	14.00(0.20)^{a}	13.90(0.12)^{a}
10	14.53(0.21) ^{a}	11.63(0.37)^{c}	13.23(0.43)^{b}	10.78(0.10)^{d}	13.75(0.25)^{b}	13.75(0.13)^{b}
11	14.63(0.13) ^{a}	11.43(0.47)^{c}	12.67(0.23) ^{b}	10.08(0.34)^{d}	13.00(0.00) ^{b}	13.05(0.24)^{b}
12	14.95(0.50)^{a}	11.77(0.43)^{d}	13.23(0.43) ^{b}	12.18(0.17)^{cd}	12.75(0.25)^{bc}	13.10(0.27)^{b}

Different Superscripts (a, b, c) in a row indicate a significant difference between the means of the groups at (P<0.05)
* Experimental Infection ** Early treatment *** Late treatment
# Vaccination with Brucella abortus S19.

➢ *Red Blood Cell Count ($X10^3$/ml ± SE)*

The result of the mean group total red blood cell counts of experimental animals is shown in Table 6. By weeks 5 to 9, there was no difference in the mean TRBC count across the groups. By week 12, the TRBC counts of groups 1, 2, and 4 were significantly lower (P>0.05) when compared with those of groups 5 and 6 but not group 3.

➢ *White Blood Cell Count ($X10^3$/µL ± SE)*

The result of the mean group total white blood cell (WBC) count (x103/ µL ± S.E.) of experimental animals is shown in Table 7. By weeks one and two, all of the infected and treated groups (2, 3, 4, 5, and 6) had WBC counts that were substantially higher (P<0.05) than group one's (uninfected control). When compared to the other experimental groups by week three, the mean WBC count of group three (Diminazene Aceturate and Levamisole, two weeks PI.) was considerably lower (P>0.05). By weeks four and 11, the groups had no discernible change.

➢ *Absolute Neutrophil Count (X103/µL ± SE)*

Table 8 shows the result of the mean absolute neutrophil counts of the various groups. By week one, there was a significant increase (P<0.05) in the mean absolute neutrophil count of groups two and three (Diminazene Aceturate) when compared with that of group one (uninfected control) but not groups four, five, and six. There was no significant change between the experimental groups from week five to the end of the study[10].

➢ *Absolute Lymphocyte Count (x103/µL ± SE)*

Table 9. shows the mean absolute lymphocyte counts of the various experimental groups. By week 10, the mean absolute counts of all the infected and treated groups (2, 3, 4, 5, and 6) were significantly lower than that of the uninfected control group. By week one, there was a significant increase (P<0.05) in the mean absolute lymphocyte count of groups two (Diminazene Aceturate) and four (Diminazene Aceturate, Levamisole, and Vitamin A) when compared with group one (untreated control) but not group 3 (Infected with Trypanosoma brucei and treated with diminazene aceturate and Levamisole).

➢ *Absolute Monocyte Count (x103/µL ± SE)*

Table 10 shows the result of the mean absolute monocyte count of the various groups. From the beginning to the end of the study, there was no significant difference (P>0.05) in the mean absolute counts of the experimental groups except by week 2. Group 4 (Diminazene Aceturate & Vitamin A) had significantly higher monocyte counts than the other experimental groups over two weeks.

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Table 6: Mean Group Total Red Blood Cell Counts (x106/µL ± SE) of WAD Sheep Experimentally Infected with T. brucei and Treated with Diminazene Aceturate, Levamisole/Vitamin A/Both and Vaccinated against Brucellosis

Experimental period (week)	GROUP 1 Uninfected, Untreated	GROUP 2 DA Two weeks PI	GROUP 3 DA & levamisole Two weeks PI	GROUP 4 DA vit. A Two weeks PI	GROUP 5 DA & vit. A Three weeks PI	GROUP 6 DA & vit. A & levamisole Two weeks PI.
0*	12.26 (1.50)^{a}	12.91 (1.14)^{a}	11.79 (1.31)^{a}	11.74 (1.17)^{a}	12.44 (1.07)^{a}	11.32 (1.08)^{a}
1	11.95 (1.26)^{a}	11.06 (0.76)^{ab}	8.72 (0.55)^{c}	9.08 (0.43)^{bc}	10.01 (0.47)^{abc}	8.62 (0.23)^{c}
2**#	13.36(0.95)^{a}	8.44(0.26)^{b}	8.90(0.52)^{b}	9.05(0.13)^{b}	8.71(0.11)^{b}	9.11(0.07)^{b}
3***	12.85 (0.67)^{a}	10.45 (0.73)^{bc}	9.51 (0.30)^{c}	11.37 (0.35)^{ab}	10.00 (0.30)^{bc}	10.52 (0.28)^{bc}
4	13.89 (0.39)^{a}	9.65 (0.41)^{b}	10.69 (1.28)^{b}	11.38 (0.24)^{b}	10.73 (0.15)^{b}	11.14 (0.78)^{b}
5	13.80 (0.36)^{a}	10.70 (0.61)^{b}	11.12 (1.19)^{b}	11.86 (0.10)^{ab}	11.41 (0.36)^{ab}	11.51 (1.09)^{ab}
6	13.30 (0.50)^{ab}	11.27 (0.67)^{b}	11.50 (0.87)^{ab}	13.35 (0.18)^{ab}	13.62 (0.35)^{a}	12.06 (0.78)^{ab}
7	13.33 (0.20)^{ab}	12.70 (0.35)^{ab}	12.61 (0.38)^{a}	13.90 (0.10)^{ab}	14.00 (0.00)^{b}	13.15 (0.65)^{ab}
8	13.37 (0.24)^{a}	11.93 (0.93)^{b}	13.46 (0.32)^{a}	13.17 (0.17)^{ab}	14.25 (0.25)^{a}	13.74 (0.22)^{a}
9	13.94 (0.60)^{ab}	13.21 (0.45)^{a}	13.98 (0.29)^{ab}	13.63 (0.88)^{ab}	14.45 (0.11)^{b}	14.24 (0.28)^{b}
10	13.95 (0.18)^{a}	14.00 (0.00)^{a}	13.96 (0.04)^{a}	13.72 (0.15)^{a}	14.78 (0.22)^{b}	14.73 (0.23)^{b}
11	14.21 (0.26)^{a}	14.10 (0.11)^{a}	14.06 (0.60)^{a}	14.12 (0.19)^{a}	14.83 (0.18)^{b}	14.61 (0.08)^{b}
12	14.23 (0.13)^{a}	14.34 (0.17)^{a}	14.52 (0.06)^{ab}	14.27 (0.30)^{a}	15.00 (0.00)^{b}	14.97 (0.35)^{b}

Different Superscripts (a, b, c) in a row indicate a significant difference between the means of the groups at (P<0.05)

* Experimental Infection ** Early treatment *** Late treatment

\# Vaccination with Brucella abortus

Table 7: Mean Group Total White Blood Cell Counts (x103/µL ± SE) of WAD Sheep Experimentally Infected with T. brucei and Treated with Diminazene Aceturate, Levamisole/Vitamin A/Both and Vaccinated against Brucellosis

Experimental period (week)	GROUP 1 Uninfected, Untreated	GROUP 2 DA Two weeks PI	GROUP 3 DA & levamisole Two weeks PI	GROUP 4 DA & vit. A Two weeks PI	GROUP 5 DA & vit. A Three weeks PI	GROUP 6 DA & vit. A & Levamisole 2 weeks PI.
0*	11.07 (0.50)^{a}	10.81 (0.76)^{a}	10.76 (0.29)^{a}	10.54 (0.53)^{a}	10.37 (0.69)^{a}	9.31 (0.94)^{a}
1	10.31 (0.65)^{a}	15.33 (0.24)^{b}	14.95 (0.66)^{b}	14.89 (0.83)^{b}	14.54 (0.87)^{b}	14.95 (0.92)^{b}
2#**	11.01 (0.19)^{a}	16.96 (0.58)^{bc}	16.42 (1.04)^{b}	16.23 (0.77)^{b}	19.20 (1.2)^{c}	17.36 (0.49)^{bc}
3***	10.55 (0.66)^{a}	22.40 (0.40)^{d}	7.82 (0.42)^{b}	10.93 (0.98)^{a}	12.7 (0.30)^{a}	15.35 (0.48)^{c}
4	11.22 (0.28)^{a}	12.61 (1.70)^{a}	11.13 (0.35)^{a}	11.17 (0.45)^{a}	10.14 (0.94)^{a}	11.38 (0.18)^{a}
5	11 (0.35)^{ab}	13.73 (2.04)^{a}	7.23 (0.15)^{c}	7.90 (0.88)^{bc}	10.3 (1.50)^{bc}	8.42 (0.42)^{bc}
6	11.26 (0.13)^{a}	9.83 (0.80)^{ab}	8.01 (0.58)^{b}	7.90 (0.73)^{b}	9.15 (0.85)^{b}	8.2 (0.38)^{b}
7	9.76 (0.34)^{a}	9.08 (0.82)^{a}	7.88 (0.51)^{a}	7.85 (0.74)^{a}	9.35 (0.95)^{a}	8.53 (0.32)^{a}
8	9.57 (0.25)^{ab}	8.36 (0.89)^{ab}	7.49 (0.33)^{b}	7.53 (0.34)^{b}	10.00 (1.40)^{a}	8.98 (0.69)^{ab}
9	9.25 (0.28)^{ab}	9.80 (1.17)^{a}	6.50 (0.64)^{b}	7.05 (0.38)^{ab}	7.4 (1.40)^{ab}	6.60 (1.23)^{b}
10	10.51 (0.64)^{a}	9.20 (0.83)^{ab}	6.63 (0.45)^{c}	6.93 (0.41)^{bc}	7.50 (1.00)^{bc}	6.28 (1.01)^{c}
11	10.56 (0.68)^{a}	7.59 (0.62)^{b}	6.77 (0.32)^{b}	6.23 (0.48)^{b}	7.30 (0.70)^{b}	5.63 (0.68)^{b}
12	10.01 (0.52)^{a}	7.33 (0.44)^{b}	6.87 (0.32)^{b}	6.18 (0.38)^{b}	7.05 (0.55)^{b}	5.98 (0.37)^{b}

Different Superscripts (a, b, c) in a row indicate a significant difference between the means of the groups at (P<0.05)

* Experimental Infection ** Early treatment *** Late treatment

\# Vaccination with Brucella abortus S19

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Table 8: Mean Group Absolute Neutrophil Count (x103/µL ± Standard Error) of WAD Sheep Experimentally Infected with T. brucei and Treated with Diminazene Aceturate, Levamisole/Vitamin A/Both and Vaccinated against Brucellosis

Experimental period (weeks)	GROUP 1 Uninfected, Untreated	GROUP 2 DA Two weeks PI	GROUP 3 DA & levamisole Two weeks PI	GROUP 4 DA & vit. A Two weeks PI	GROUP 5 DA & vit. A Three weeks PI	GROUP 6 DA & Vit. A & levamisole Two weeks PI.
0*	4.93(0.37)^{a}	4.85(0.42)^{a}	5.03(0.33)^{a}	4.85(0.14)^{a}	4.01(0. .55)^{a}	3.69(0.55)^{a}
1	4.94(0.55)^{a}	7.28(0.14)^{bc}	8.02(1.22)^{c}	5.06(0.19)^{a}	5.77(0.33)^{ab}	4.81(0.07)^{a}
2#**	5.03(0.74)^{a}	5.45(2)^{a}	4.52(1.57)^{a}	4.89(0.60)^{a}	5.02(1.81)^{a}	5.10(0.57)^{a}
3***	4.71(0.41)^{ab}	7.44(2.49)^{a}	2.17(0.75)^{b}	3.31(0.35)^{b}	2.22(1.30)^{b}	5.65(0.21)^{ab}
4	5.47(0.20)^{a}	5.42(1.89)^{a}	4.48(1.50)^{ab}	4.97(0.78)^{ab}	1.62(0.96)^{b}	4.96(0.22)^{ab}
5	5.57(0.63)^{ab}	6.33(2.26)^{b}	2.81(0.94)^{ab}	3.77(0.89)^{ab}	1.72(1.04)^{a}	3.17(0.70)^{ab}
6	5.41(0.64)^{a}	4.25(1.49)^{ab}	3.66(1.23)^{ab}	4.18(0.84)^{ab}	1.66(0.97)^{b}	3.29(0.29)^{ab}
7	4.67(0.49)^{a}	4.20(1.44)^{a}	3.41(1.16)^{a}	4.15(0.83)^{a}	1.87(1.09)^{a}	4.22(0.24)^{a}
8	4.44(0.52)^{a}	3.05(1.23)^{a}	3.01(1.01)^{a}	3.40(0.28)^{a}	2.04(1.19)^{a}	4.44(0.35)^{a}
9	4.40(0.53)^{a}	3.69(1.28)^{a}	2.70(0.93)^{a}	3.97(0.38)^{a}	1.77(1.05)^{a}	3.15(0.63)^{a}
10	4.70(0.44)^{a}	3.55(01.21)^{ab}	2.95(1.00)^{ab}	3.89(0.39)^{ab}	1.80(1.05)^{b}	3.00(0.52)^{ab}
11	4.74(0.49)^{a}	2.43(0.87)^{ab}	2.73(0.93)^{ab}	3.22(0.50)^{ab}	1.53(0.95)^{b}	2.53(0.34)^{ab}
12	4.40(0.49)^{a}	2.30(0.85)^{ab}	3.04(1.02)^{ab}	3.52(0.24)^{ab}	1.57(0.94)^{b}	2.84(0.36)^{ab}

Different Superscripts (a, b, c) in a row indicate a significant difference between the means of the groups at (P<0.05)

* Experimental Infection ** Early treatment *** Late treatment

\# Vaccination with Brucella abortus S19.

Table 9: Mean Group Lymphocyte Count (x103/µL ± Standard Error) of WAD Sheep Experimentally Infected with T. brucei and Treated with Diminazene Aceturate, Levamisole/Vitamin A/Both and Vaccinated against Brucellosis

Experimental period (weeks)	GROUP 1 Uninfected, Untreated	GROUP 2 DA Two weeks PI	GROUP 3 DA & levamisole Two weeks PI	GROUP 4 DA & Vit. A Two weeks PI	GROUP 5 DA & Vit. A Three weeks PI	GROUP 6 DA & Vit. A & levamisole Two weeks PI.
0*	6.14(0.33)^{a}	5.96(0.60)^{a}	6.02(0.38)^{a}	5.99(0.74)^{a}	5.13(0.61)^{a}	5.58(0.56)^{a}
1	5.37(0.65)^{a}	7.98(0.29)^{bc}	6.85(1.27)^{ab}	9.68(0.77)^{c}	8.40(0.74)^{bc}	10.05(0.90)^{c}
2#**	5.92(0.70)^{a}	6.74(2.42)^{ab}	7.56(2.57)^{b}	11.35(0.81)^{c}	9.06(03.41)^{b}	11.84(0.82)^{cd}
3 ***	5.84(.39)^{a}	9.20(3.09)^{a}	3.60(1.21)^{a}	7.52(0.71)^{a}	3.95(2.33)^{a}	7.20(2.48)^{a}
4	5.58(0.25)^{a}	3.97(1.46)^{a}	3.82(1.31)^{a}	4.30(1.49)^{a}	3.24(1.88)^{a}	6.30(.39)^{a}
5	5.40(0.52)^{a}	3.71(1.39)^{a}	2.48(0.82)^{a}	3.78(0.27)^{a}	3.29(1.93)^{a}	4.76(0.65)^{a}
6	5.63(0.55)^{a}	2.89(0.98)^{a}	9.17(6.99)^{a}	3.60(.28)^{a}	2.81(1.63)^{a}	4.79(0.55)^{a}
7	5.09(0.47)^{a}	2.29(0.78)^{a}	2.35(0.88)^{a}	3.59(0.30)^{a}	2.75(1.60)^{a}	4.14(0.23)^{a}
8	5.02(0.48)^{a}	2.86(1.00)^{a}	2.49(0.83)^{a}	3.97(0.18)^{a}	2.88(1.70)^{a}	4.38(0.45)^{a}
9	4.76(0.32)^{a}	3.52(1.28)^{ab}	2.10(0.73)^{b}	2.95(0.22)^{ab}	1.85(1.10)^{b}	3.26(.55)^{ab}
10	5.81(0.30)^{a}	3.21(1.11)^{b}	1.95(0.65)^{b}	2.90(0.23)^{b}	1.88(1.10)^{b}	3.11(.46)^{b}
11	5.72(0.36)^{a}	3.21(1.09)^{b}	2.17(0.81)^{b}	2.95(0.38)^{b}	2.11(1.22)^{b}	3.01(0.35)^{b}
12	5.62(0.14)^{a}	2.98(1.03)^{b}	2.07(0.78)^{b}	2.60(0.36)^{b}	1.88(1.08)^{b}	3.20(0.26)^{b}

Different Superscripts (a, b, c) in a row indicate a significant difference between the means of the groups at (P<0.05)

* Experimental Infection ** Early treatment *** Late treatment

\# Vaccination with Brucella abortus S19.

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Table 10: Mean Group Monocyte Counts (x103/µL ± Standard Error) of WAD Sheep Experimentally Infected with T. brucei and Treated with Diminazene Aceturate, Levamisole/Vitamin A/Both and Vaccinated against Brucellosis

Experimental period (weeks)	GROUP 1 Uninfected, Untreated	GROUP 2 DA Two weeks PI	GROUP 3 DA & levamisole Two weeks PI	GROUP 4 DA & Vit. A Two weeks PI	GROUP 5 DA & Vit. A Three weeks PI	GROUP 6 DA & Vita. A levamisole Two weeks PI.
0*	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00 (0.00)	0.00(0.00)	0.00(0.00)
1	0.00(0.00)[a]	0.00(0.00)[a]	0.00(0.00)[a]	0.00 (0.00)[a]	0.03(0.04)[a]	0.00(0.00)[a]
2#**	0.00(0.00)[a]	0.00(0.00)[a]	0.00(0.00)[a]	0.08 (0.05)[b]	0.00(0.00)[a]	0.00(0.00)[a]
3 ***	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00 (0.00)	0.00(0.00)	0.00(0.00)
4	0.00(0.00)[a]	0.00(0.00)[a]	0.00(0.00)[a]	0.00 (0.00)[a]	0.02(0.02)[a]	.03(0.03)[a]
5	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00 (0.00)	0.00(0.00)	0.00(0.00)
6	0.50(0.50)[a]	0.02(0.02)[a]	0.07(0.04)[a]	0.08 (0.08)[a]	0.02(0.02)[a]	0.04(0.02)[a]
7	0.00(0.00)[a]	0.00(0.00)[a]	0.06(0.04)[a]	0.08 (0.07)[a]	0.00(0.00)[a]	0.04(0.02)[a]
8	0.00(0.00)[a]	0.02(0.10)[a]	0.17(0.17)[a]	0.02 (0.02)[a]	0.02(0.02)[a]	0.05(0.02)[a]
9	0.00(0.00)[a]	0.00(0.00)[a]	0.02(0.02)[a]	0.08 (0.08)[a]	0.00(0.00)[a]	0.07(0.04)[a]
10	0.00(0.00)[a]	0.02(0.02)[a]	0.02(0.02)[a]	0.07 (0.08)[a]	0.00(0.00)[a]	0.07(0.03)[a]
11	0.25(0.25)[a]	0.00(0.00)[a]	0.02(0.02)[a]	0.06 (0.05)[a]	0.00(0.00)[a]	0.02(0.01)[a]
12	0.00(0.00)[a]	0.00(0.00)[a]	0.00(0.00)[a]	0.06 (0.06)[a]	0.00(0.00)[a]	0.03(0.03)[a]

Different Superscripts (a, b, c) in a row indicate a significant difference between the means of the groups at (P<0.05)
* Experimental Infection ** Early treatment *** Late treatment
# Vaccination with Brucella abortus S19.

➢ *Humoral Immune Response To Brucella Abortus Antigen*

Antibodies were detected in the sera of all vaccinated antigen-challenged animals one week post-vaccination. Depression of the titer was observed only in the fifth week of the study in groups 5 (Diminazene Aceturate and Vitamin A 3, weeks PI) and 6 (Diminazene Aceturate, Levamisole, and Vitamin A, two weeks PI). The titers increased by week six and persisted till the end of the studies. All samples agglutinated up to the last tube up to 1:10240.

Table 11 Brucella abortus Antibody Titres (IU/µL) OF WAD Sheep Experimentally Infected with T. brucei and Treated with Diminazene Aceturate, Levamisole/ Vitamin A/Both and Vaccinated against Brucellosis

SAMPLE GROUP NO	*WK. 0	WK 1	**# WK. 2	*** WK 3	WK 4	WK 5	WK 6	WK 7	WK 8	WK 9	WK 10	WK 11	WK 12
1A	0	0	0	80	80	80	80	80	80	80	80	80	10240
1B	0	0	0	80	80	80	80	80	80	80	80	80	10240
1C	0	0	0	80	80	80	80	80	80	80	80	80	10240
1D	0	0	0	80	80	80	80	80	80	80	80	80	10240
2A	0	0	0	80	80	80	80	80	80	80	80	80	10240
2B	0	0	0	80	80	80	80	80	80	80	80	80	10240
2C	0	0	0	80	80	80	80	80	80	80	80	80	10240
2D	0	0	-	-	-	-	-	-	-	-	-	-	-
3A	0	0	0	80	80	80	80	80	80	80	80	80	10240
3B	0	0	0	80	80	80	80	80	80	80	80	80	10240
3C	0	0	-	-	-	-	-	-	-	-	-	-	-
3D	0	0	0	80	80	80	80	80	80	80	80	80	10240
4A	0	0	0	80	80	80	80	80	80	80	80	80	10240
4B	0	0	0	80	80	80	80	80	80	80	80	80	10240
4C	0	0	0	80	80	80	80	80	80	80	80	80	10240
4D	0	0	0	80	80	80	80	80	80	80	80	80	10240
5A	0	0	-	-	-	-	-	-	-	-	-	-	-
5B	0	0	0	-	-	-	-	-	-	-	-	-	-
5C	0	0	0	80	80	40	80	80	80	80	80	80	10240
5D	0	0	0	80	80	20	80	80	80	80	80	80	10240
6A	0	0	0	80	80	20	80	80	80	80	80	80	10240

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6B	0	0	0	80	80	40	80	80	80	80	80	80	10240
6C	0	0	0	80	80	10	80	80	80	80	80	80	10240
6D	0	0	0	80	80	20	80	80	80	80	80	80	10240

Group 1: Uninfected and untreated Group 2: Treated with Diminazene aceturate Group 3: Treated with Diminazene aceturate and Levamisole 2 weeks post-infection. Group 4: Treated with Diminazene aceturate and Vitamin A 2 weeks post-infection. Group 5: Treated with Diminazene aceturate and Vitamin A 3 weeks post-infection. Group 6:Treated with Diminazene aceturate Levamisole and Vitamin A 2 weeks post-infection.

* Experimental Infection ** Early treatment *** Late treatment
# Vaccination with *Brucella abortus* S19 - Absence (death) of animal in the group.

Table:12 Positive Response to Brucella abortus Antigen/Number of Surviving WAD Sheep Experimentally Infected with T. brucei and Treated with Diminazene Aceturate, Levamisole/Vitamin A/Both and Vaccinated against Brucellosis

Experi Mental period (week)	**GROUP 1 Uninfected, Untreated**	**GROUP 2 DA Two weeks PI**	**GROUP 3 DA & levamisole Two weeks PI**	**GROUP 4 DA & Vit. A Two weeks PI**	**GROUP 5 DA & Vit. A 3 weeks PI**	**GROUP 6 DA, Vit A & Levamisole Two weeks PI.**
0*	0/4	0/4	0/4	0/4	0/4	0/4
1	0/4	0/4	0/4	0/4	0/4	0/4
2**#	0/4	0/4	0/4	0/4	0/4	0/4
3***	4/4	3/4	3/4	4/4	2/4	4/4
4	4/4	3/4	3/4	4/4	2/4	4/4
5	4/4	3/4	3/4	4/4	2/4	4/4
6	4/4	3/4	3/4	4/4	2/4	4/4
7	4/4	3/4	3/4	4/4	2/4	4/4
8	4/4	3/4	3/4	4/4	2/4	4/4
9	4/4	3/4	3/4	4/4	2/4	4/4
10	4/4	3/4	3/4	4/4	2/4	4/4
11	4/4	3/4	3/4	4/4	2/4	4/4
12	4/4	3/4	3/4	4/4	2/4	4/4

* Experimental Infection ** Early treatment *** Late treatment
# Vaccination with *Brucella abortus* S19 on day ten post-infection.

C. Discussion

Parasitemia was established on the 4th day and was evident in all the infected animals six days post-infection (PI). The early presence of parasitemia is in line with the findings of Mohammed et al., who recorded a pre-patent period of 3-4 days in T. congolense-infected sheep. Starting treatment three weeks PI with vitamin A combined with diminazene aceturate may have aided in the complete cure observed.

There was no significant change in the group's mean body weight during the experiment, confirming the treatment's efficacy and evidence of recovery. This result agrees with the findings of Mwangi et al.[11], who reported no differences in weight in cattle. High protein intake allows infected animals to grow at the same rate as the uninfected. Treatment with Levamisole has been reported to improve weight gain when used as an immunomodulator and administered at repeated doses of 2.5mg/kg[12].

Significant reductions occurred in the infected sheep's total RBC, PCV, and Hb counts. Declines in erythrocytic indices in this study were, however, reversed by treatment. The severity of anemia has been shown to depend on the level and duration of parasitemia in infected animals [13]

The neutrophil count decreased progressively from one-week post-treatment until the end of the experiment in groups treated with diminazene aceturate and vitamin A three weeks PI.

There were no significant changes in the eosinophil, basophil, and monocyte counts of infected and uninfected animals. However, these findings do not agree with an earlier report of eosinopenia and monocytosis in T. brucei infected deer-mice and T. vivax-infected sheep[14].

A mortality rate of 25% was recorded in groups two, three, and five in the second week of infection. In the third week of the disease, mortality rose to 33% in group five. High mortality has also been reported in dogs and protein-deprived mice in T. evansi and T. Cuzi infection [15]. However, there was no death in the group treated with Levamisole. Zero mortality could have resulted from early treatment before infection reached the late stage, as in the case of groups two, three, four, and six.

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D. Conclusion

Immunomodulators' use in treating West African Dwarf sheep infected with Trypanosoma brucei and vaccinated against brucellosis increased erythrocytic indices and reduced Total White Blood Cell count. High titres of Brucella abortus antibody recorded in all vaccinated animals up to the last week of the study indicate that trypanosomiasis did not suppress antibody production in these animals. Therefore, Ovine trypanosomiasis should be treated with diminazene aceturate and either vitamin A or levamisole or diminazene aceturate, vitamin A and Levamisole.

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