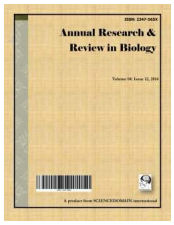

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Effect of *Peste des petits ruminants* Vaccination on Clinico-haematological Parameters of West African Dwarf Sheep Experimentally Infected with *Trypanosoma congolense*

Ijeoma Chekwube Chukwudi[1*], Boniface Maduka Anene[1], Cornelius C. Chukwu[1], Ikenna Onyema Ezeh[2] and Kenneth Ikejiofor Ogbu[3]

[1]Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria.
[2]Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, Nigeria.
[3]Department of Animal Health, Federal College of Animal Health and Production Technology, Vom, Plateau State, Nigeria.

Authors' contributions

This work was carried out collaboratively by the all authors. Authors BMA and CCC designed the experiment. Author ICC drafted the manuscript, performed the statistical analysis. Also, author ICC conducted the research and laboratory experiments with authors IOE and KIO. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The research was conducted to determine the clinico-haematological parameters and impacts of *Peste des petits ruminants* (PPR) vaccination and trypanocidal treatment in *Trypanosoma congolense* infected West African Dwarf (WAD) sheep.
Methodology: Twenty (20) WAD sheep were grouped into five (5) (A-E), each containing four (4) sheep. Group A (Gp A) was the unvaccinated and uninfected control. Groups B, C, D and E were first vaccinated with PPR vaccine, after which D and E were infected with *T. congolense* one (1)

**Corresponding author: E-mail: ijeoma.adieme@unn.edu.ng, tuk2didi@yahoo.com;*

week post-vaccination, and then C and D were treated three (3) weeks post-infection.
Results: A prepatant period of 12-14 days was recorded. The infection was characterized by fluctuating parasitaemia and pyrexia, decreased appetite, slight pale mucous membrane, starry hair coat and enlargement of prescapular and perfermoral lymph nodes. There was no significant change (P>0.05) in the mean body weight and pulse rate of the infected sheep. Decreased packed cell volume (PCV), haemoglobin concentration (Hb conc) and total erythrocyte count (TEC) were observed in the infected sheep. Leucocytosis associated with lymphocytosis which was similar in the infected and uninfected sheep were observed in all the vaccinated sheep starting from two weeks post vaccination. The vaccination had no effect on clinical (temperature, pulse rate, weight gain) and red blood cell (mean PCV, Hb conc and TEC) parameters. Following treatment with diminazene aceturate, the infected and treated sheep became aparasitaemic within 24 hours post treatment and there was no relapse infection. The declines in the clinical and haematological parameters of the infected sheep were reversed by treatment.
Conclusion: Vaccination caused a marked leucocytosis due to lymphocytosis in both infected and uninfected animals and also had no impact on the clinical parameters assessed which is an indication that PPR vaccination had no untoward effect on the animals.

Keywords: Peste des petits ruminants vaccination; diminazene acturate; clinico-haematology; Trypanosoma congolense; sheep.

ABBREVIATIONS

PPR= Peste des petits ruminants; WAD= West African Dwarf; Gp= Group; PCV= Packed cell volume; Hb conc= Haemoglobin concentration; TEC= Total erythrocyte count; TLC= Total leukocyte count; ALC= Absolute leukocyte count, DA= Diminazene aceturate.

1. INTRODUCTION

African animal trypanosomosis is a disease caused by a group of protozoan parasites of the genus *Trypanosoma.* They are known to cause serious disease in man and livestock in Africa and are well known for persistent infection of the blood and induction of profound immunosuppression [1]. Trypanosomosis has been described as the commonest and most threatening disease of ruminant livestock in regions where tsetse fly (the vector organism) is prevalent [2]. Control in Africa and particularly Nigeria, relies principally on chemotherapy and chemoprophylaxis using mainly diminazene, homidium and isometamidium [3,4]. Despite this, treatment of trypanosomosis is faced with challenges of drug resistance due to wrong use of drugs, and the presence of few trypanocides [5,6]. Thus trypanosomosis continues to be a great challenge to the livestock industry due to failures at various control strategies [7,8].

Various reports [9-11] have documented clinical and haematological findings of *Trypanosoma* infected livestock species in Nigeria and also there are reports on the effect of trypanosomosis on certain vaccines in livestock [12-14].

Vaccination of small ruminants with the homologous *Peste des petits ruminants* (PPR) vaccine is the most effective way to control PPR disease [15,16] which is a highly contagious viral disease of small ruminants such as sheep and goats [17,18] with high morbidity and mortality rates of 100% and 90%, respectively [17]. It is endemic in Nigeria, particularly in the South Eastern region [19,20] and dwarf breeds of sheep and goats are particularly susceptible to PPR [21,22].

It is therefore conceivable that vaccination of sheep with PPR vaccine may influence the clinico-haematological outcome and profile of same with possible natural trypanosome infection, hence the study.

This study thus has its objective to document the effects of PPR vaccination and trypanocidal treatment on the clinical and haematological parameters of *Trypanosoma*-infected West African Dwarf (WAD) sheep.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Twenty (20) adult male West African Dwarf (WAD) sheep weighing between 9-13 kg were used in this experiment. They were acclimatized for 2 (two) weeks during which routine treatment developed at NAPRI [23] and modified by Aye

[24] were applied. The sheep were kept in the fly-proof well ventilated space. They were fed on cut and carry (dry) grasses consisting of guinea grass (*Panicum maximum),* elephant grass (*Pennisetum purpureum*) as is the usual practice in this eco-zone. Water was available *ad libitum*. Each sheep was identified using neck tag. The experimental sheep were handled in compliance with the guidelines for the humane treatments of animals during experimentation in the University of Nigeria.

2.2 Trypanosomes

CT70 strain of *Trypanosoma congolense* was obtained from the Nigeria Institute for Trypanosomosis Research (NITR) Vom, Plateau State, Nigeria. The parasites were first isolated from a cow in Zaria and were maintained in rats. They were passaged in donor rats from where the experimental sheep were infected.

2.3 Vaccine/Antigen

Peste des petits ruminants homologous vaccine (PPRV 75/1) was obtained from the Nigeria Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. A 50-dose vial of the vaccine was reconstituted with 50 ml of distilled water and each animal received 1 ml subcutaneously in accordance to the manufactures recommendation.

2.4 Drug Treatment

Diminazene aceturate (Trypanzen®, Veterinary Pharmaceutical, Pantex Holland) was reconstituted according to the manufacturer recommendation by dissolving a sachet containing 2.36 g of diminazene aceturate in 12.5 ml of distilled water. The volume administered was calculated from their body weight at the dose of 7 mg/kg via the intramuscular route.

2.5 Infection of Experimental Sheep

Infected blood from the donor rats were obtained from the retrobulbar plexus via the median canthus of the eyes into a sample bottle containing ethylene diamine tetra acetate (EDTA). Infected blood was then diluted in phosphate buffer saline (PBS). Estimated 1.0×10^6 *T. congolense* suspended in 0.5 ml of PBS was used to infect each sheep via intravenous route. The quantity of parasite inoculated was estimated using the rapid matching method of Herbert and Lumsden [25].

2.6 Experimental Design

The twenty (20) WAD sheep were assigned to five groups (A - E) of four sheep each. Group A sheep were not vaccinated nor infected while all sheep in groups B - E were vaccinated against PPR. Sheep in groups D and E were inoculated with *T. congolense* one week post vaccination. Sheep in groups C and D were treated with 7 mg/kg diminazene aceturate intramuscularly 3 weeks post infection and repeated at 2 weeks later.

2.7 Blood Sample Collection

Blood sample (3 ml) was collected via jugular venipuncture into vacutainer tubes using EDTA as an anticoagulant from all experimental sheep prior to the commencement of the study and thereafter weekly for haematology.

2.8 Parameters

2.8.1 Parasitaemia

The parasites were detected by wet blood film [26] and buffy coat dark phase contrast microscopy method [27], while counts were estimated using the rapid matching technique of Herbert and Lumsden [25].

2.8.2 Clinical parameters

Rectal temperature, pulse rate and body weight were determined [28].

2.8.3 Clinical signs

The sheep were observed twice daily throughout the experimental period for clinical signs of disease (e.g. palpable lymph node, body condition/weight changes, appetite, colour of mucous membrane, capillary refill time, ocular discharges, behaviour e. t. c.) as well as mortality.

2.8.4 Haematology

Packed cell volume, haemoglobin concentration, total erythrocyte counts, total leukocyte counts and differential leukocyte counts [29,28] were determined.

2.9 Statistical Analysis

Data obtained were subjected to One-way analysis of variance (AVOVA). Probability of less than 0.05 ($p \leq 0.05$) were considered significant and variant means were separated using Duncan multiple range test [30].

3. RESULTS AND DISCUSSION

3.1 Parasitaemia

All the infected sheep became parasitaemic by day 14 post infection (PI) and the parasitaemia was sustained until treatment on day 21 PI (Table 1). The parasites cleared in the infected and treated group (D) within 24 hours of treatment and remained aparasitaemic throughout the experiment. The infected untreated sheep (group E) remained parasitaemic till the end of the experiment with fluctuating parasitaemia.

3.2 Rectal Temperature

There was no significant (P>0.05) alteration in the mean rectal temperature of the vaccinated uninfected sheep (Groups B and C|) compared with the unvaccinated uninfected sheep (Group A) throughout the experiment (Fig. 1). The mean rectal temperature of the infected sheep (Group D) were significantly (P<0.05) higher compared with the uninfected groups (A, B and C) starting from week 2 up to week 4 of the experiment. From week 5 (i.e. 1 week post re-treatment), no significant (P>0.05) difference in the mean rectal temperature occurred between group D and groups A, B and C unlike group E (infected and untreated).

3.3 Pulse Rate

There was no significant (p>0.05) difference in the mean pulse rate of the sheep all through the experiment (Fig. 2).

Table 1. Parasitaemia of WAD sheep immunized against PPR and infected with *Trypanosoma congolense* and treated with diminazene aceturate

Day	Group A	Group B	Group C	Group D	Group E
*0	0/4	0/4	0/4	0/4	0/4
**7	0/4	0/4	0/4	0/4	0/4
19	0/4	0/4	0/4	3/4	2/4
20	0/4	0/4	0/4	4/4	4/4
21	0/4	0/4	0/4	4/4	4/4
***28	0/4	0/4	0/4	4/4	4/4
35	0/4	0/4	0/4	0/4	3/4
****42	0/4	0/4	0/4	0/4	4/4
49	0/4	0/4	0/4	0/4	2/4
56	0/4	0/4	0/4	0/4	3/4
63	0/4	0/4	0/4	0/4	2/4
70	0/4	0/4	0/4	0/4	4/4

*Nos of parasitaemic animal in the group/ Total Nos of animal in the group, *****Immunization day, ****** Infection day; ******* 1st treatment day; ********2nd treatment day, Group A: Unvaccinated and uninfected; Group B: Vaccinated and uninfected; Group C: Vaccinated, uninfected and treated with 7mg/kg DA; Group D: Vaccinated, infected and treated with 7mg/kg DA; Group E: Vaccinated, infected and untreated*

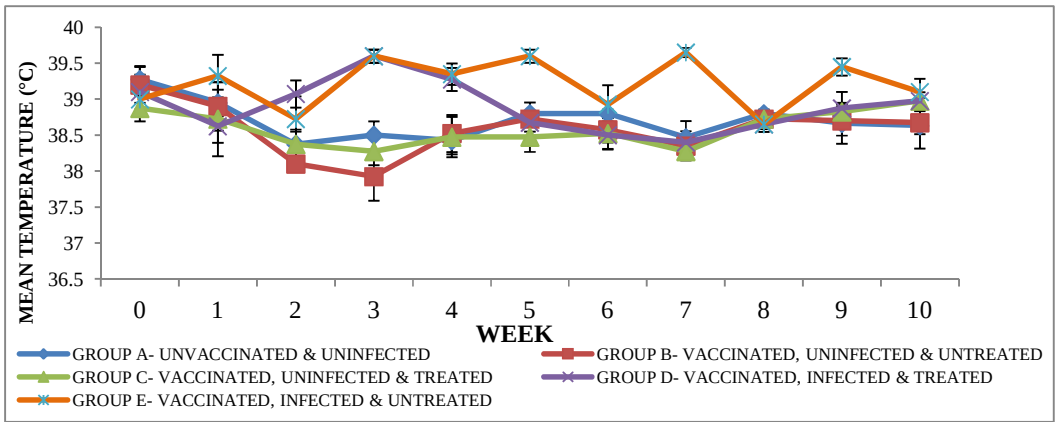

Fig. 1. Mean Temperature (℃) of WAD sheep immunized against PPR and infected with *Trypanosoma congolense* and treated with diminazene aceturate

3.4 Weight Gain

There was no significant (p>0.05) difference in the weight of the sheep (Fig. 3).

3.5 Clinical Signs

The clinical signs initially observed in the infected groups were pyrexia, decreased appetite and starry hair coat. Following treatment, these signs gradually disappeared in the treated group (D) while it continued in the untreated group (E) with additional clinical signs of pale mucous membrane, fluctuating pyrexia and enlargement of the prescapular and prefemoral lymph node.

3.6 Red Blood Cell Parameters

3.6.1 Packed cell volume (PCV)

There was no significant (P>0.05) difference in the mean PCV of the sheep across the groups at week 1, 2 and 3 (Fig. 4). By week 4, there was a significant (P<0.05) decrease in the mean PCV of sheep in groups D and E when compared with groups A, B and C. The PCV was also significantly (P<0.05) decreased in group C when compared with group A but was comparable to group B. From week 5 (i.e. 1 week post re-treatment) PCV in the infected groups (D and E) continued to be significantly (P<0.05) lower than

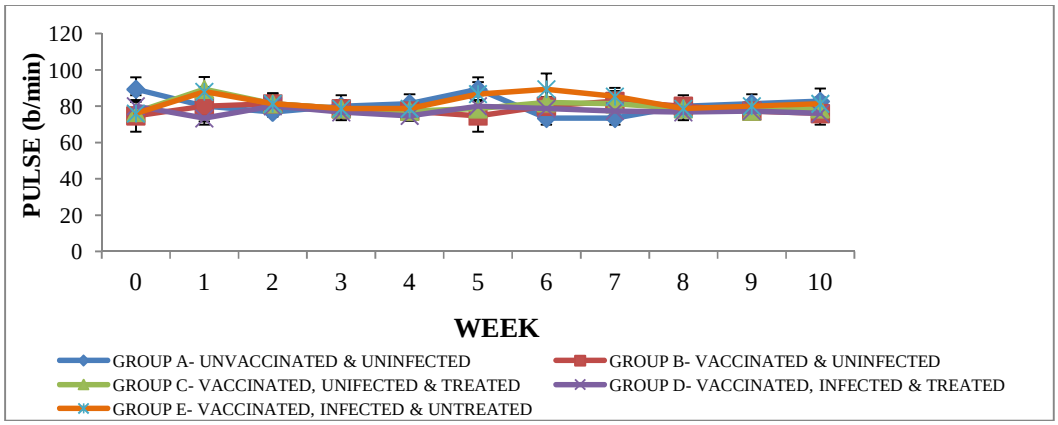

Fig. 2. Pulse Rate (beats/min) of WAD sheep immunized against PPR and infected with *Trypanosoma congolense* and treated with diminazene aceturate

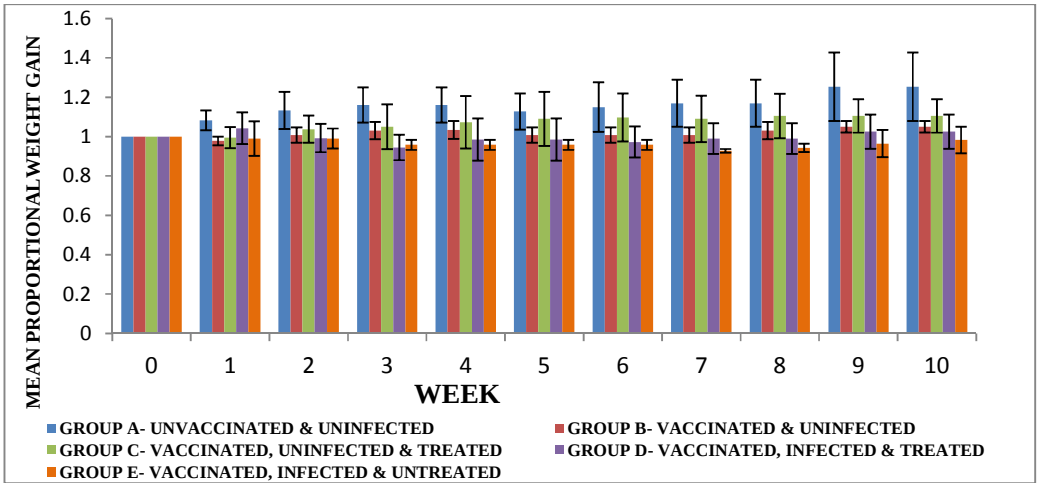

Fig. 3. Mean Proportional Weight Gain of WAD sheep immunized against PPR and infected with *Trypanosoma congolense* and treated with diminazene aceturate

in the uninfected (A, B and C), but it was significantly (P<0.05) higher in group D than E. By week 7 there was a significant (P<0.05) decrease in the mean PCV of vaccinated, uninfected and treated sheep (group C) when compared with vaccinated, uninfected and untreated sheep (group B), but was similar with group D (vaccinated, infected and treated). From week 8 to the end of the study, mean PCV in group D unlike group E did not differ significantly (P>0.05) from the uninfected groups (A, B and C).

3.6.2 Haemoglobin Concentration (Hb Conc)

There was decrease in the mean Hb concentration in groups D and E which was significant (P<0.05) in group E by week 2 (Fig. 5). By week 3, there was a significant (P<0.05) increase in the mean Hb concentration in group D compared with the uninfected groups (A, B and C). By week 4, there was a significant (P<0.05) decrease in the mean Hb concentration in the infected groups (D and E) when compared with the uninfected groups (A, B and C), and in group C when compared with group A. By week 5, Hb concentration of the groups (D and E) were comparable but group E was significantly (P<0.05) lower than groups A, B and C whereas group D was comparable to group B and C but differed significantly (P<0.05) from group A. From week 6 to the end of the experiment, there were significant (P<0.05) decreases in the mean Hb concentration in Group E when compared to group D and also with the control groups A, B and C. Group D did not significantly (P>0.05) differ from the uninfected groups (A, B and C), except on week 8 when it was significantly (P<0.05) lower than group A and B.

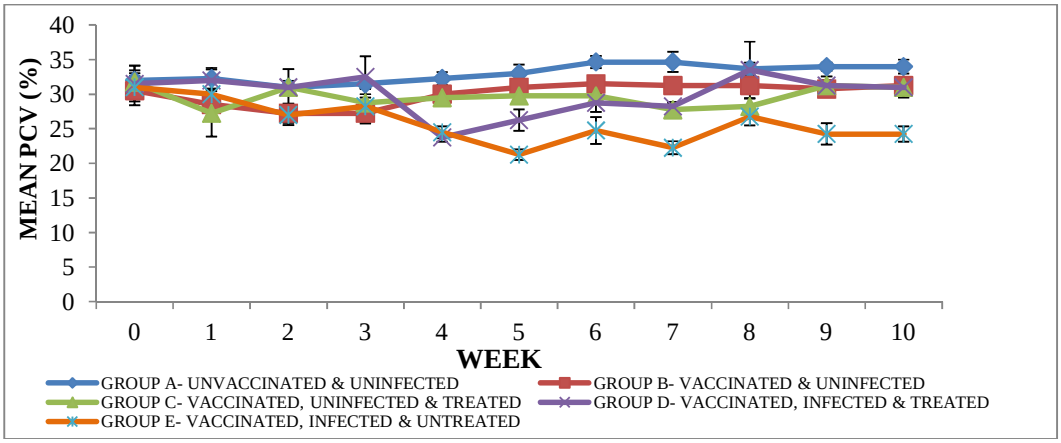

Fig. 4. Mean packed cell volume (%) of WAD sheep immunized against PPR and infected with *Trypanosoma congolense* and treated with diminazene aceturate

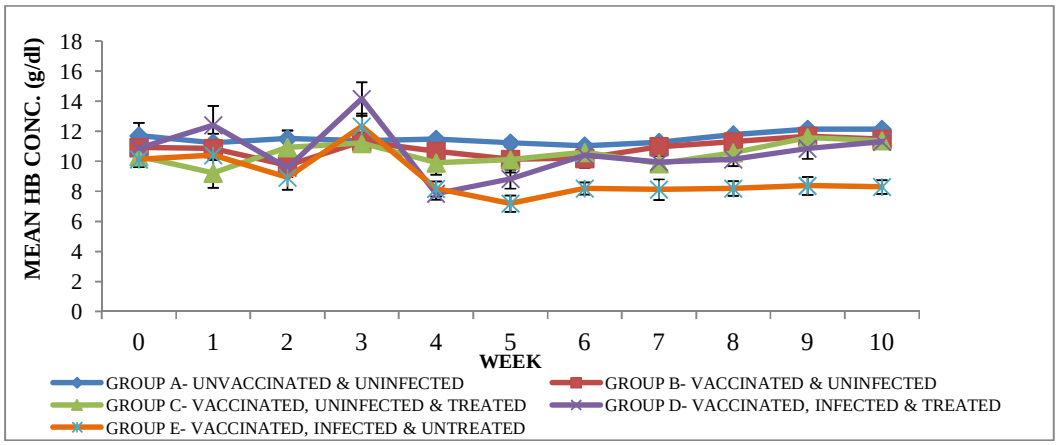

Fig. 5. Mean haemoglobulin concentration (g/dl) of WAD sheep immunized against PPR and infected with *Trypanosoma congolense* and treated with diminazene aceturate

3.6.3 Total Erythrocyte Count (TEC)

There was no significant (P>0.05) difference in the TEC of the sheep across the groups from week 1 to 3 (Fig. 6). From week 4 to 6, there was significant (P<0.05) decrease in the TEC of the infected groups (D and E) when compared with the uninfected groups (A, B and C). From week 6 to the end of the experiment, there was a significant (P<0.05) decrease in the mean TEC of sheep in Group E when compared to group D and also with the control groups A, B and C. On week 7 and 8, TEC in group D was comparable with the vaccinated uninfected groups (B and C), but significantly (P<0.05) lower than group A. From week 9 to the end of the experiment, group D did not significantly (P>0.05) differ from the uninfected groups (A, B and C).

3.7 White Blood Cell Parameters

3.7.1 Total Leukocyte Count (TLC)

There was significant (P<0.05) increase in the TLC count of the vaccinated groups (B, C, D and E) compared with group A starting from week 2 to week 8 (Fig. 7). There was no significant (P>0.05) difference between the TLC in the vaccinated and infected groups (D and E) and vaccinated uninfected groups (B and C). Also, there was no significant (P>0.05) difference between the infected treated (D) and the infected untreated (E) as well as in the uninfected treated group (C) and the uninfected untreated group (B) From week 9 to the end of the experiment, there was no significant (P>0.05) difference in the mean TLC count of sheep across the groups.

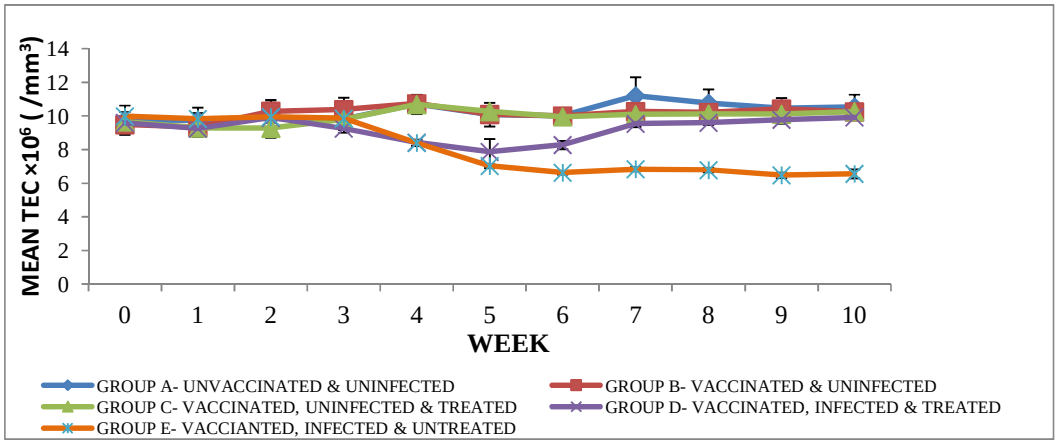

Fig. 6. Mean total erythrocyte count ×10^6 (/mm^3) of WAD sheep immunized against PPR and infected with *Trypanosoma congolense* and treated with diminazene aceturate

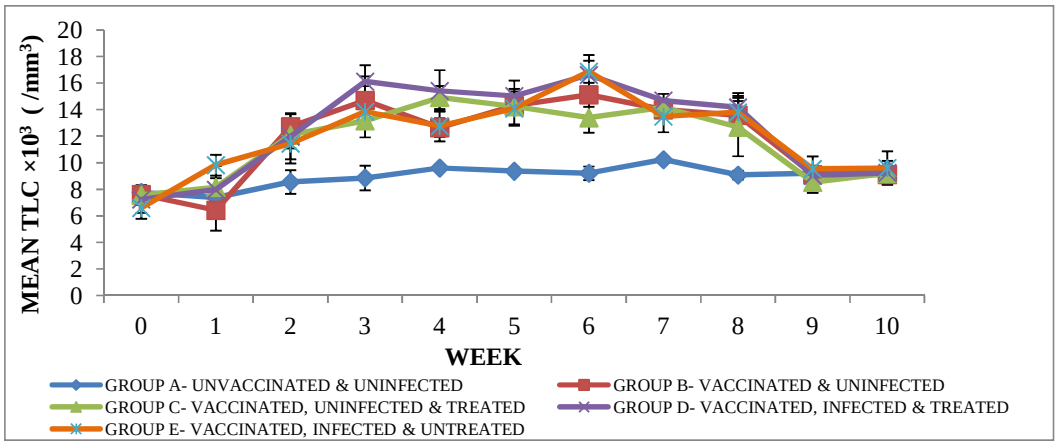

Fig. 7. Mean total leukocyte count ×10^3 (/mm^3) of WAD sheep immunized against PPR and infected with *Trypanosoma congolense* and treated with diminazene aceturate

3.7.2 Absolute Lymphocyte Count (ALC)

There was a significant (P<0.05) increase in the mean absolute lymphocyte count (ALC) in the vaccinated groups (B, C, D and E) compared with the unvaccinated group (A) beginning from week 1 to week 8 of the experiment (Fig. 8). There was no significant (P>0.05) difference between the ALC in the vaccinated and infected groups (D and E) and vaccinated uninfected groups (B and C). Also, there was no significant (P>0.05) difference between the infected treated (D) and the infected untreated (E) as well as in the uninfected treated group (C) and the uninfected untreated group (B). From week 9 to the end of the experiment, there was no significant (P>0.05) difference in the mean ALC across the groups.

4. DISCUSSION

Experimental infection of WAD sheep with *Trypanosoma congolense* was successful with a prepatent period of 12-14 days. This is in agreement with Hill et al. [31] and Kilekoung et al. [32] who reported a prepatent period (PP) of 12 and 14 days in bovine species, respectively. It contrasts with the findings of Katunguka-Rwakishaya et al. [33] who reported a prepatent period of 7-9 days in sheep infected with *Trypanosoma congolense*. *Trypanosoma congolense* is generally reported to have a longer PP than other African animal trypanosome species. A PP of 3-6 days was reported in sheep [34] and 3 days in WAD goat infected with *T. evansi* [35]. Adeiza et al. [36] reported 4.6 days in *T. brucei* infected goats and 5.3 days in *T. vivax*-infected goats. Besides the trypanosome species, the PP and parasitaemia have been reported to vary according to host's immune status [37,32], diagnostic technique used in detecting the parasite [38,32], strain, virulence and infective dose of the parasite [11] and also the species and breed of host infected [37]. It was found that the number of parasites inoculated influenced not only the PP but also the height and duration of parasitaemia [38]. Also serum xanthine oxidase, serum catalase and trypanosome specific immune responses have been reported to play roles of regulation of the level of parasitaemia in the Cape buffalo [39].

The fluctuations of parasitaemia observed at different intervals in the untreated sheep may be attributed to the host's ability to produce trypanolytic substances which may destroy some of the parasites [40]. Furthermore, the parasite's ability to switch its surface coats leading to the formation of new antigenic types that may be unaffected by the prevailing host antibodies may have contributed to the disappearance and resurgence of parasitaemia [40,35]. It must also be recognised that parasitaemia can vary by orders of magnitude during the course of 24 hours, while the source of blood can also affect parasite detection, e.g. *T. congolense* is more readily detected in peripheral blood than in jugular blood [41].

The infected treated sheep became aparasitaemic within 24 hours post treatment and there was no relapse infection throughout the experiment. The absence of relapse in this study may be attributed to the high dose of diminazene aceturate (7.0 mg/kg body weight) and repeat treatment two weeks later. The recommended

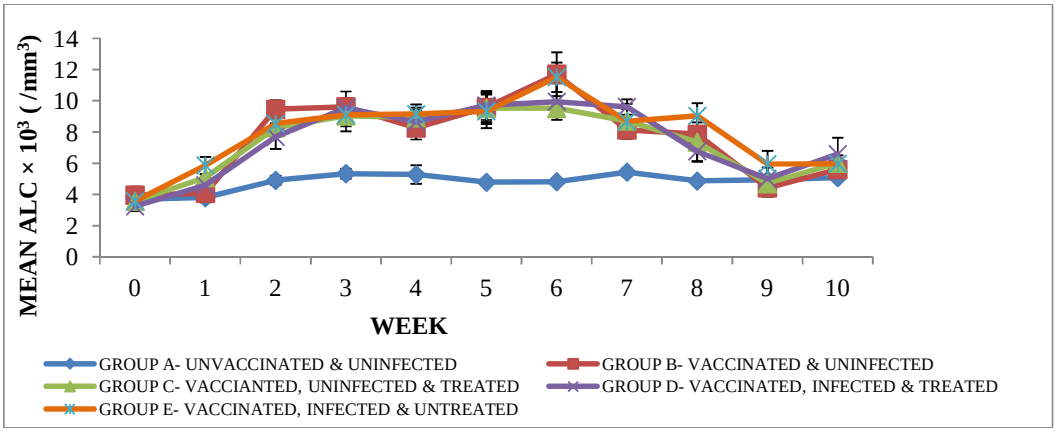

Fig. 8. Mean absolute lymphocyte count ×10³ (/mm³) of WAD sheep immunized against PPR and infected with *Trypanosoma congolense* and treated with diminazene aceturate

dose for the treatment of *T. congolense* infection is 3.5 mg/kg body weight once. The decision to use a high and repeated dose of diminazene aceturate was informed by the growing records of diminished sensitivity of parasites to trypanocides [42,43,4].

Clinical signs of pyrexia, anorexia, slightly pale mucous membrane, starry hair coat, were observed following manifestation of trypanosomes in the blood of the infected sheep. These were similar to those reported in goats, sheep, cattle, Indonesian buffalo and Brazelian Pantanal [34,44]. However, these clinical sign gradually disappeared following treatment, showing that the drug used was able to clear the parasites from the blood and also avert the signs. But these clinical signs persisted in the untreated sheep coupled with fluctuating pyrexia, enlargement of the prescapular and prefemoral lymph nodes. This agrees with the findings of Omotainse and Anosa [45]. Death was not recorded. It has been reported that the severity of the clinical manifestations is dependent on the species and strain of the infecting trypanosome, breed of the animal involved [46]. Stress, such as poor nutrition or concurrent disease, plays a prominent role in the disease process, and under experimental conditions, where these factors may be markedly reduced, it is difficult to elicit severe clinical disease [47].

Pyrexia, observed from week 2 of the experiment, is a recognized clinical symptom of trypanosomosis in animal [48,49,34,44]. It has been reported that the severity of pyrexia depends on the animal species and immune status of the animal infected [37]. Fever in trypanosomosis has been reported to be due to the metabolism of tryptophan to tryptophol by trypanosome parasites and that the accumulation of tryptophol in pharmacology doses in animals is responsible for rectal temperature changes or feverish conditions in host response to heterologous antigens [50]. Also it has been reported to be mediated by the release of pyrogenic cytokines such as tumour necrosis factor (TNF), interleukin (IL-1, IL-6) and interferons (IFNs) into the blood stream in response to exogenous pyrogens (trypanosomes parasites) [51]. The trypanocide administered normalized the temperature as observed from week 5 in this experiment.

The absence of body weight loss in the infected sheep agrees with the findings of Mwangi et al. [52], Bisalla et al. [53] and Ogbaje et al. [35] who reported that trypanosome infection in WAD goat did not produce noticeable clinical sign and gross lesion. This contrasts with other reports that trypanosomosis causes loss of weight [54,48]. It has been found that weight changes in trypanosomosis are markedly influenced by the levels of protein intake, and high intake allows infected animals to grow at same rate as uninfected controls provided energy intake is adequate whilst low energy levels can exacerbate the adverse effects of trypanosomosis on body weight [55]. The observed non significant changes in weight between the infected and the control sheep used in this experiment may be due to the fact that the animals were on a good plain of nutrition with high quality protein in the diet which help in overcoming the weight decreasing effect of trypanosomosis.

The observed non significant change in the pulse rate of infected sheep in the face of anaemia and fever could be attributed to the trypanotolerance trait of the WAD sheep, a trait which may have enabled the animals to contain the cardiovascular effects of trypanosome parasites.

The decrease in the red cell parameters (Hb conc, PCV and TEC) observed in this study signifies anaemia which is a cardinal symptom of trypanosomosis [34,11,56].

The immunization of WAD sheep with PPR homologous vaccine (Nigeria strain 75/1) also had no effect on the red blood cell and clinical parameters showing that the vaccination is safe for use. This is in agreement with the reports of Aikhuomobhogbe and Orheruata [57] and Banik et al. [58].

Immunization resulted in leucocytosis which was evident by week 2 post vaccination (PV) and lasted up to week 8 PV. The leucocytosis observed was associated with lymphocytosis. This is in agreement with the findings of Aikhuomobhogbe and Orheruata [57] in goats vaccinated with PPR vaccine. The lymphocytosis observed in the vaccinated sheep implies that there was a cell mediated immune response. This is in agreement with Aikhuomobhogbe and Orheruata [57]; Das et al. [59]; Banik et al. [58] and Rojas et al. [60] and it shows that the PPR vaccine has actively stimulated the lymphocytes and engendered humoural immune response [59,58].

Leucocytosis observed in the vaccinated infected was comparable to that of vaccinated uninfected sheep. It would thus appear that defective function rather than absolute numbers of cell is crucial to the immunosuppression associated with trypanosomosis. This view is supported by the report of Murray et al. [61] that the immunosuppression caused by trypanosomosis was closely associated with the presence of the living trypanosomes possibly mediated through a B-lymphocyte defect.

5. CONCLUSION

Vaccination of WAD sheep against PPR using the homologous PPR vaccine (Nigerian 75/1 strain) caused a marked leucocytosis due to lymphocytosis in both infected and uninfected animals. Vaccination had no impact on the clinical parameters assessed which is an indication that vaccination had no untoward effect on the animals. Clinical signs of the *T. congolense* infection were not severe and the pulse rate and body weight were not altered by the infection. Diminazene aceturate effectively cleared the parasites from the blood of the infected sheep.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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