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Clinical Observation on Three Nigerian Breeds of Sheep Experimentally Infected with *Trypanosoma Vivax*

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ABSTRACT

Livestock production has been continually challenged with trypanosomes of which *Trypanosoma vivax* is the most pathogenic cause and the least studied in sub-Saharan Africa. Five sheep of each of the three Nigerian breeds, viz: West African Dwarf (WAD), Yankassa and Ouda were intravenously infected with 2.5×10^6 *Trypanosoma vivax* per milliliter and monitored until the Packed Cell Volume (PCV) declined to 15% when they were treated with diminazene aceturate. Three animals of each breed served as an uninfected control. The parameters monitored were parasitemia, clinical signs, bodyweight, and PCV. Levels of parasitemia were not significantly different across the breeds. Thirteen out of fifteen infected sheep had fever three days *pi*, followed by signs of pale mucous membranes, enlarged lymph nodes, serous nasal discharge, loss of weight, and dullness at various days *pi* in all the infected sheep. There were no significant ($p>0.05$) differences between the weight of all infected animals in the three breeds. The mean PCV of infected sheep was significantly ($P<0.05$) lower than that of non-infected sheep as from 14 days *pi* to 21 days *pi* in all the breeds of sheep. The least decline in PCV occurred in WAD sheep (20.92%) compared to Yankassa (31.65%) and Ouda (30.36%). The field strain of mouse-infective *T. vivax* used had 99% homology with the diagnostic antigen gene of *T. vivax* (L25129 T and U43183) from GenBank. All the three Nigerian breeds of sheep are susceptible to *T. vivax* infection and WAD sheep are more tolerant (resilience) to *T. vivax* infection than Yankassa and Ouda sheep.

1. INTRODUCTION

In sub-Saharan Africa, sheep is increasingly becoming a major source of animal protein contributing about 30% to total meat consumption (Ahmed and Egwu, 2014). Sheep contribute considerably to the rural economies, not only as a source of protein (meat and milk), hides for the leather industry and manure for crop production; but also provide readily disposable income for small holder farmers (Ng'ayo *et al*., 2005; Okaiyeto *et al*., 2008). Estimates from the National Bureau of Statistics showed that there are 36.4 million sheep in Nigeria (NBS, 2016). As of 2006, 1229 breeds of sheep (which include 995 local breeds, 134 regional transboundary breeds and 100 international transboundary breeds) have been identified (FAO, 2007), out of which four breeds were credited to Nigeria. These include West African Dwarf (WAD), Yankassa, Uda and Balami. Diseases such as animal trypanosomosis, however, pose a great risk to their production.

Trypanosomosis is a major threat and constraint to livestock development in sub Saharan Africa. It is a globally occurring haemoparasitic disease that cuts

across many species of mammals and its severity depends on infected animal *species*, age, and *species* of trypanosomes (Van den Bossche and Delespaux, 2011; Spickler, 2018). The three most important tsetse transmitted trypanosomes are: *Trypanosoma congolense, T. vivax*, and *T. brucei*. Trypanosomes are transmitted cyclically by Tsetse flies **(***Glossina*) and *T. vivax,* which is one of the most pathogenic trypanosomes, can as well be transmitted mechanically by flies such as *Tabanids and Stomoxys* (Duffy *et al*., 2009). The clinical manifestation of trypanosomosis include pyrexia, anaemia, weight loss, enlarged lymph nodes, reduced milk production, high mortality, and abortion (Desquesnes, 2004; OIE, 2018; Silva *et al*., 2013). The control of trypanosomosis has been based on tsetse fly control, chemoprophylaxis, chemotherapy, and the use of trypanotolerant livestock (Holmes, 2013). In times past, most extensive studies evaluating the biological and economic impacts of trypanosomosis in livestock, were carried out in cattle but recent studies have shown that sheep and goats raised or reared in high tsetse fly challenged areas succumb to trypanosomosis and caused great economic loss (Irungy *et al*., 2002; Masiga *et al*., 2002; Dhonde *et al*., 2008; Batista *et al*., 2009). There is need to increase food animal production to meet up the protein requirements of the growing population and sheep that have faster growth rates and shorter reproductive cycles; will greatly contribute to the realization of this goal. However, sheep production and productivity in the tropics has been continually challenged with trypanosomes; of which *Trypanosoma vivax* is one of the most pathogenic strains and the most difficult to cultivate in the laboratory and consequently limited biological studies/investigations into this parasite. This paper presents the clinical observations on three Nigerian breeds of sheep experimentally infected with *T. vivax* that grew successfully in mice.

2. MATERIALS AND METHODS

2.1. Location of study and Experimental Animals: The study was carried out in the Experimental Animal House of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria. Twenty-four rams of three Nigerian breeds of sheep viz: West African Dwarf (WAD) (8), Yankassa (8) and Ouda (8), aged between 12-24 months and weighing between 14.0 and 29.0kg were used in this study. The animals were obtained locally in Nigeria from Abeokuta and Ibadan. On arrival, the animals were tagged and screened for blood, faecal, and skin parasites, and appropriate treatments were administered based on the laboratory results. The rams were also vaccinated against Peste des Petits Ruminant (PPR) using the PPR vaccine (National Veterinary Research Institute, NVRI, Vom, Nigeria). Also, they were acclimatized for four weeks before they were challenged with *T. vivax.* From each breed, five animals were infected and three were not infected (negative control). Each breed was housed separately in a fly- proof pen partitioned into six units (control inclusive) with a fenced holding yard. A tsetse trap was positioned in the holding yard throughout the experiment. They were fed basal diets consisting of forages in the morning and concentrates (16%) in the afternoon. Salt lick and water were provided *ad libitum*. The animals were protected from ticks and other biting insects during the study using cypermethrin pour-on every two weeks. An anthelmintic was used during the study to prevent interference of the infection with helminthosis.

2.2. Trypanosome Stock:

Field isolate of *T. vivax* from cattle slaughtered in Abeokuta abattoir was used. Each trypanosome positive blood sample was inoculated into two mice intra-peritoneally. Six mice were inoculated altogether. Parasitemia was checked daily using Haematocrit Centrifugation Technique (HCT). The first parasitemia was seen on the 5th day of inoculation and it became high on the 8th day. Blood sample was collected through the tail vein into EDTA tube. DNA was extracted from the aliquoted part of the blood using Quick-gDNA™ Mini-Prep (Zymo Research Corporation, Irvine, CA, USA and molecularly typed for trypanosomes using ITS primer (Njiru *et al*., 2005). The result was positive for *T. vivax.* This was further confirmed using specie specific (ILO 1264 and ILO 1265) primer set for *T. vivax.* The *T. vivax* positive mouse was bled through the retro-orbital plexus and the blood (0.5ml) was passage into 2 mice. To expand the isolate, the mice were bled intra-orbitally, and a Red Sokoto goat weighing 13kg with PCV of 27% was infected with 0.2 ml of mice blood through the jugular vein. The goat became parasitemic on the 8th day post-infection and on day 11 when the parasitemia was high, the rams were infected.

2.3. Trypanosome detection by PCR

PCR amplification was performed in 20 µl final reaction volume containing the equivalent of 20 ng of genomic DNA 10mM Tris-HCl, pH 8.3, 1.5mM $MgCl_2$, 50 µM KCl, 200 µM each of dNTPs, 40ng of each of the primers and 1 unit of Taq DNA polymerase

(Bioneer, Inc. Alamada CA USA). The reactions were placed in a MJ MINI^{TM} Personal Thermocycler model PTC-1148 (Biorad, Hercules, CA, USA). The reaction conditions were as follows: For its: initial denaturation of the DNA at 95^{o}C for 5 minutes, followed by 35 cycles of 95^{o}C for 1 minute, 58^{o}C for 1 min and 72^{o}C for 1 min with final extension at 72^{o}C for 10 min.

For ***T. vivax***; initial denaturation at 94 ^{o}C for 4 minutes followed by 35 cycles of 94^{o}C for 4 minutes followed by 35 cycles of 94 ^{o}C for 30s, 60^{o}C for 45s, and 72^{o}C for 30s followed by final extension at 72^{o}C for 5 minutes.

Gel electrophoresis procedure: Ten microliters of the PCR products were electrophoresed through 1% agarose gel in 1x TBE (89 mM Tris, 89mM boric acid 1 mM EDTA) at 90V for 80minutes, along with 10 µl of GENEMate Quanti-Marker 100bp DNA ladder (BioExpress, Kaysville, UT, USA). Gels were stained with GelRed® Nucleic Acid stain (Phenix Research Products, Candler, NC, USA) at 5 µl/100 ml of the agarose gel suspension. After electrophoresis, PCR products were visualized on a UV transilluminator and were photographed using an Alphalmager HP System (Protein Simple, Santa, Clara, CA, USA). A positive control (with reference DNA) and a negative control (without DNA) were included with each set of reactions. PCR primers and their source references are given below: The generic primers set that target the internal transcribes spacer 1 (ITS 1) was (CF: 5'-CCGGAAGTTCACCGATATTG-3' and BR: 5'-TGCTGCGTTCTTCAACGAA-3' (Njiru *et al*., 2005). While the primer set for *T. vivax* was ILO 1264 and ILO 1265 and its primer sequence 5'-3" was CAGCTCGCCGAAGGCCACTTGGCTGGG and TCGCTACCACAGTCGCAATCGTCGTCTCAAGG (Masake *et al*. (1997).

The PCR products obtained were sent for partial sequencing in the core laboratory of Cornell University using forward primer. The obtained sequences were BLAST for homology search.

2.4. Experimental Design and Sample Collection: Five rams from each breed were infected with 0.5ml of goat blood containing 2.5 x 10^6 *T. vivax* per milliliter through the jugular vein. While three animals of each of the three breeds served as the uninfected control. The Protocols used were approved by the Ethical Committee of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria. Just before infecting the animals with *T. vivax*, 4ml of blood was collected from the jugular vein of each of the animals to determine the baseline haematological values and were screened negative for trypanosomes. All animals were sampled twice-weekly post-infection.

2.5. Clinical signs: The temperature of infected animals were taking daily and were also physically checked for any observable signs of infection and such were documented.

2.6. Determination of Live Weight: The live weight of all the animals were measured twice weekly with a hanging scale.

2.7. Determination of Packed Cell Volume: The PCV of each of the blood samples was determined in the laboratory as described by Kelly (1984).

2.8. Detection of trypanosomes and Estimation of Parasitemia: Haematocrit centrifugation technique (HCT) and wet mount were used to detect trypanosomes and the parasitemia was estimated using the buffy coat parasitemia scoring system as described by Paris *et al* (1982).

Statistical analysis

The data is presented as mean ± SD, the difference in means was compared using one-way ANOVA using GraphPad Prism 7.

3. RESULTS

3.1. Identification of Trypanosome: The trypanosomes obtained from mice (these mice were earlier inoculated with trypanosome-positive blood from cattle) exhibited rapid movement across the field in both wet mount and buffy coat under the microscope. PCR test and sequencing revealed that the Trypanosomes had 99% homology with those *T. vivax* sequences (Accession numbers: U43183 and L25129) in GenBank.

3.2. Parasitaemia: Parasitaemia was seen three days *pi* in all sheep except in one WAD and one Ouda which eventually became parasitaemic four days *pi*. The mean parasitaemia of *T. vivax* infected sheep are presented Figure 1. The parasitaemia was not significantly different (P>0.05) in all breeds and it ranges between 2.5×10^5 and 2.5×10^6 organisms per ml of blood.

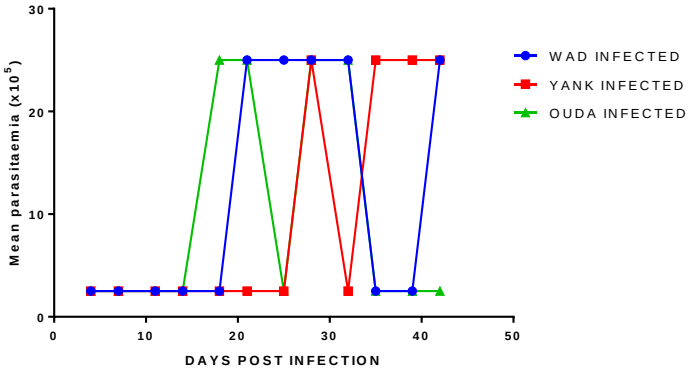

Figure 1: Mean parasitaemia (×10^5) of sheep experimentally infected with *T. vivax*

3.3. Clinical signs: The first observable clinical sign of trypanosomosis was fever in 10 of the 15 infected animals three days *pi*. Other signs became apparent 7 to 10 days *pi*. The signs include pale mucous membranes, enlarged lymph nodes, serous nasal discharges, loss of weight and dullness in a few of the animals. At 14 days *pi*, the mucous membrane of one of the infected Yankassa was very pale (PCV was 14%) and it was treated with diminazene aceturate (7mg/kg) to forestall unnecessary suffering. One Ouda ram became recumbent 34 days *pi* and was immediately treated with diminazene aceturate but died the following day. At 42 days *pi*, the infection phase of the experiment was terminated. But as at that time, two WAD, two Yankassa, and one Ouda sheep had not been treated with diminazene aceturate because their PCV values were above 15%. Other infected sheep were treated at different days *pi* using 15% PCV as the inclusive criterion. It was also observed that seven (7) rams that had been treated earlier had a relapse of infection and had to be treated again with 14mg/kg b.w of diminazene aceturate. The mean (range) days to relapse were 21.75 ±7.09 (17-32), 26.0±7.07 (21-31) and 21±00 (21) for Ouda, WAD, and Yankasa respectively.

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3.4. Live Weight: The mean liveweight of *T. vivax* infected sheep are presented Figure 2. There were no significant ($p>0.05$) differences observed among the three breeds in the loss of body weight. There were also no significant ($p>0.05$) differences between the weight gains of all non-infected animals in the three breeds.

3.5. Packed Cell Volume: The mean PCV of non-infected and *T. vivax* infected sheep are presented Figure 3. The mean PCV of infected sheep was significantly ($P<0.05$) lower than that of non-infected sheep as from 14 days *pi* to 21 days *pi* in all the breeds of sheep. The most significant decrease was noticed on day 21 post-infection. By 28 days *pi*, the PCV of infected sheep declined by 20.92%, 30.36% and 31.65% for WAD, Ouda, and Yankassa respectively from pre-infection PCV value. The least decline occurred in WAD sheep

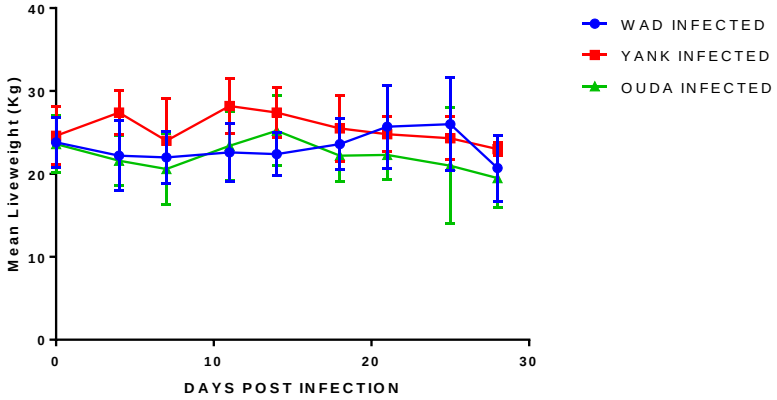

Figure 2: Mean Live weight values (Kg) of sheep experimentally infected with *T. vivax*

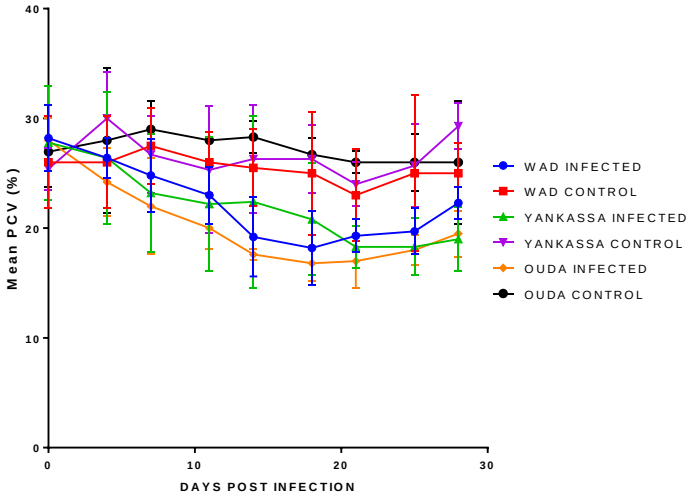

Fig. 3: Mean PCV values (%) of sheep experimentally infected with *T. vivax*

4. DISCUSSION

The paper presents the clinical observations on three Nigerian breeds of sheep experimentally infected with *T. vivax* that was successfully raised in mice. The development of parasitemia was detected three days *pi* in all the infected sheep except one Ouda and one WAD. The parasitemia started undulating from day 18 *pi* until when infection was terminated when the PCV of any of the infected animal reached 15% and was treated or till when the experiment was finally terminated on day 42 *pi*. This is similar to the report of Nereida and Armando (2019) in which sheep experimentally infected with *T. vivax* had undulating parasitemia during the first 28 days after infection. The clinical manifestations observed in infected sheep such as fever, anaemia, pale mucous membranes, enlarged lymph nodes and loss of weight agree with observations of Desquesnes, (2004); Silva *et al*. (2013). The live weight values of the infected and non-infected sheep were not significantly ($p< 0.05$) affected across breeds. This agrees with the report of Bengaly *et al*. (1993) that observed no significant difference in the decrease body weight between Djallonke and Sahelian Fulani sheep and goats experimentally infected with trypanosomes. A similar report was also reported in Albino rats experimentally infected with *T. brucei* that showed no significant changes in body weight (Ezeh *et al*., 2016).

There was a steady decline in the PCV of all the infected sheep and it reached a significant level from day 14 to day 28 post-infection. This agrees with the findings of Anosa *et al*. (1983), Taylor and Authie, (2004) and Marcothy *et al*. (2008) that anaemia is a cardinal sign of trypanosomosis. Also, Nereida and Armando (2019) reported a progressive reduction in the haematocrit values of sheep experimentally infected with *T. vivax*. Reduction in PCV values in small ruminants naturally infected with trypanosomes was also reported by Samdi *et al*. (2008); Kebede *et al*. (2009); Kebede *et al*. (2016). The WAD sheep had the lowest percentage decrease from pre-infection value PCV (20.92%) and this may be an indication that WAD sheep are more tolerant to trypanosomes than Yankassa and Ouda sheep. This agrees with the results of experimental infections of small ruminants with *T. congolense* that showed that the PCV values in Djallonke sheep and WAD goats were significantly less affected than those in non-trypanotolerant animals (Adah *et al*., 1993; Bengaly *et al*, 1993; Geerts *et al*., 2009). The *T. vivax* employed in this study grew successfully in mice contrary to the general belief that it is difficult to cultivate *T. vivax* in laboratory animals. This belief has limited experimental investigations into this parasite. The in-vivo mouse-infective *T. vivax* ever reported was Y486 and its derivatives (Gibson, 2012). The obtained sequence of the mouse-infective *T. vivax* used in this study, had 99% homology with L25129 *T. vivax* and U43183 *T. vivax* isolates that were present in the GenBank.

5. CONCLUSION

In conclusion, all three Nigerian breeds of sheep are susceptible to *T. vivax* infection. Among the breeds

studied, WAD sheep were able to maintain the least reduction in PCV from pre-infection values. The *T. vivax* isolate used in this study successfully grew in mice.

Small ruminants should be included in the trypanosomosis control programmes organized by the government because they could serve as reservoirs of trypanosomes to other animals especially cattle.

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