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Original Research

Self-cure of *Trypanosoma brucei brucei* infection in West African Dwarf sheep

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ABSTRACT

This study investigated the natural course of parasitaemia in West African Dwarf (WAD) sheep experimentally infected with *Trypanosoma brucei brucei* parasites and the potential for spontaneous regression without any therapeutic intervention. Ten (10) adult West African Dwarf sheep of both sexes were assigned to two groups of five sheep each. One of the groups was inoculated with approximately 1×10^6 trypanosome parasites per animal, intravenously, while the second group served as uninfected control. Blood samples were collected daily until parasitaemia was established and for 7 days post establishment of parasitaemia and thereafter weekly till the end of the experiment in order to monitor the course of parasitaemia. The clinical signs, packed cell volume (PCV) and rectal body temperature were monitored. A pre-patent period of 28 days was observed with peak mean parasitaemia of 81.60 ± 27.71 million trypanosome parasites, on day 43 post inoculation. The parasitaemia declined in 60 % of the sheep from day 141 post-infection (PI) and reduced to 0.34±0.02. Clinical signs observed include weakness, mild anorexia and pale mucous membranes. The 40 % of animals that manifested clinical signs eventually died and their postmortem findings were indicative of severe anaemia and dehydration. Monitoring the sheep which recovered (for parasitaemia and clinical signs) up to 141 days PI showed there was no relapse. The *Trypanosoma b. brucei* infected WAD sheep used in the study were able to contain both parasitaemia and fever and recovered from the infection.

Keywords: Self-cure, *Trypanosoma brucei brucei*, West African dwarf sheep

INTRODUCTION

Trypanosomosis, caused by the protozoan parasites of the genus '*Trypanosoma*' is a major health concern for livestock in sub-Saharan Africa, causing substantial economic losses, and a threat to food security (Barret *et al*., 2003; Rashid *et al*., 2008; Muhanguzi *et al*., 2015). The disease is found mainly in sub-Saharan Africa, between latitude 14^0N and 29^0S (Samdi *et al*., 2010). Human infections with trypanosomes are called Human African Trypanosomosis (HAT) while animal infections are known as African Animal Trypanosomosis (AAT). These infections are more prevalent in rural areas (Hoet *et al*., 2007). Several trypanosome species cause diseases in animals. *Trypanosoma brucei brucei*, *Trypanosoma congolense* and *Trypanosoma vivax* cause disease in livestock called by the Zulu name, *Nagana, derived from N'gana'* (Maser *et al*., 2003). Domestic livestock including bovines, ovines, caprines, equids, camelids and suids are susceptible to infection with one or more of these *Trypanosoma* species and can lead to acute and/or chronic forms of the wasting disease, resulting in high morbidity and mortality (IICAB, 2009).

Trypanosome parasites infect the bloodstream, lymphatic system, and various organs, leading to systemic damage; death occurs due to severe pancytopaenia and other pathologies (Dunn *et al*., 2022; Maxfield and Bermudez, 2022). A wide range of symptoms is usually observed including weight loss, anaemia, fever, lethargy, infertility and ultimately death if left untreated (Giordani *et al*., 2016). Other clinical findings in trypanosomosis include pyrexia, lymph node and spleen enlargement, ataxia, lethargy, weight loss, oedema, immunosuppression, abortion and decrease in milk production. Haematological changes including anaemia, leukopaenia and increased immunoglobin levels have been reported in trypanosomoses (Sulaiman and Adeyemi, 2010; Sivajothi *et al*., 2015).

Anaemia is a prominent pathological feature of trypanosomoses (Taylor and Authie, 2004) and, in conjunction with other systemic lesions, can contribute to death through eventual congestive heart failure. Fever is a

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cardinal clinical sign of trypanosomosis (Bezie *et al*., 2014; Giordani *et al*., 2016). Undulating pyrexia often exists in trypanosomoses and has direct relationship with fluctuating parasitaemia (Mbaya *et al*., 2009; Desquesnes *et al*., 2013; Desquesnes *et al*., 2022). Fever is less pronounced in the chronic stage of trypanosomosis as well as in carrier animals in which parasitaemia also becomes very low or even undetectable (Spickler, 2018).

Trypanosomes are transmitted cyclically by inoculation of infective metacyclic stages of the parasite into blood vessels of susceptible hosts during blood meals by Glossina spp (Desquesnes *et al*., 2013). Mechanical transmission of trypanosomes is possible when a tsetse begins a blood meal on an infected host and ends it on another host; provided the time between the two meals is short enough to ensure the survival of parasites in the insect mouthparts (Moloo *et al*. 2000). Mechanical transmission through biting flies, such as Tabanids (horse flies) and Stomoxys (stable flies) and by vampire bats is also possible for Trypanosoma vivax and is recognized in parts of Africa, including some regions of Ethiopia, Chad, and Sudan (Ahmed *et al*., 2016). Mechanical transmission of *T. congolense*, which causes the most severe disease in ruminants, has been shown under experimental conditions (Desquesnes and Dia, 2003) and can therefore not be excluded from contributing to its spread in Africa (Desquesnes *et al*. 2009).

Successive waves of parasitaemia are known features of trypanosomoses, commonly caused by antigenic variation (Mbaya *et al*., 2007; Stijlemans *et al*., 2018). Parasitaemia in susceptible animals may be influenced by the number of parasites inoculated, stressors such as nutrition/starvation, inter-current/concurrent infections, host immune competence and the pathogenicity of the strain of trypanosome species (Taylor and Authie, 2004; Elshafie *et al*., 2018).

Persistence of parasitaemia occurs due to trypanosome parasites modulating or evading the host's immune responses, possibly due to antigenic variation of their variant surface glycoproteins (VSG) (Croos, 2003) as well as refuge points with subsequent lapses in infections such as cerebrospinal fluid and aqueous humor (Frevert *et al*., 2012). These variant surface glycoproteins are very immunogenic and trypanosome parasites take advantage of the VSG abundance and immunogenicity to evade or hide from recognition by the hosts' immune system (Horn, 2014; Mugnier *et al*., 2016). This variation is responsible for the recurrence of parasitaemia.

Low, infrequent, or absence of parasitaemia is associated with the late phase (recovery) of anaemia during which erythrocyte values begin to return towards pre-infection values and other pathological changes undergo resolution (Mbaya, *et* al., 2012; Stijlemans *et al*., 2018) leading to self-recovery as commonly encountered in trypanotolerant animals (Mbaya *et al*. 2009).

Self-cure, in the context of trypanosome infection, refers to sheep's innate ability to recover from trypanosome infection without external intervention, ultimately clearing the parasite from the bloodstream. This phenomenon challenges conventional wisdom, which often necessitates costly, toxic, and sometimes, labour-intensive treatments, to combat trypanosomosis in livestock (Wurochekke *et al*., 2004; Deterding *et al*., 2005). Most of the available trypanocides, also, have the tendency to elicit drug resistance (Legros *et al*., 2002).

Unraveling the underlying mechanism behind the self-cure process could revolutionize the current approach to managing trypanosomosis, offering a ray of hope for livestock farmers. Therefore, the present study aimed to explore the innate resilience of the WAD breed of sheep to *T. b. brucei* infection. This research holds promise for the agricultural sector and highlights the importance of preserving indigenous livestock breeds' genetic diversity and adaptive traits in the face of infectious diseases.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Ten (10) adult West African Dwarf (WAD) sheep of both sexes weighing between 10kg to 25 kg were used for this study. They were purchased from Ariam market in Ikwuano LGA of Abia State, Orba market in Nsukka, Enugu State and Udua Ekponwa market in Akwa Ibom State. On arrival, they were kept in the animal house belonging to Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The sheep were acclimatized for two months before the commencement of the study. During this period, they underwent prophylactically treatment for endoparasites and ectoparasites with Albendazole (Tuyil Pharm Ind. Ltd., Nig), Ivermectin, Tick and Flea Powder (Propets Product & Serv., Nig) and long-acting Oxytetracycline (TETROXY LA®, Bimeda, Holland). Blood samples were collected from the animals to screen for the presence of trypanosomes. The sheep were fed with guinea grass (*Panicum maximum*) and elephant grass *(Pennisetum purpureum*), kitchen wastes including plantain peel, banana peels, yam peels, and dried cassava (*Manihot esculentum*). In addition, commercial dry preparations containing a mixture of cereal bran, husks of legumes and flour powder were also fed to the sheep. Feed and water were provided *ad libitum*. Ethical Approval for conduct of the work was obtained from the College of Veterinary Medicine Research Ethics Committee of Michael Okpara University of Agriculture, Umudike (MOUAU/CVM/REC/202109).

TRYPANOSOME PARASITES INOCULATION

Trypanosoma brucei brucei used in this study was local isolate obtained from a clinically infected dog presented to the Veterinary Teaching Hospital, University of Nigeria, Nsukka. The isolate was identified in the Department of Veterinary Parasitology and entomology, University of Nigeria, Nsukka. The parasites were maintained in a donor albino mouse before infection of the experimental animals. Each infected sheep was inoculated intravenously through the jugular vein with 2ml of saline-diluted blood containing 1×10^6 of the trypanosome parasites. The number of infective trypanosomes was determined using the rapid matching method of Herbert and Lumsden (1976). Parasitaemia was monitored in each of the infected sheep daily post-infection (PI) till patency and weekly thereafter till the end of the experiment using the wet mount and buffy coat microscopy as described by Woo (1970) and Murray *et al*. (1977).

EXPERIMENTAL PROCEDURE

The sheep were assigned into two groups of five (5) sheep per group. Group I was uninfected untreated control and group II was infected untreated.

MONITORING OF PARASITAEMIA

Blood samples obtained from sheep infected with trypanosomes were examined daily until parasitaemia was established in all infected groups and thereafter weekly till the end of the experiment. The wet blood film method (Woo, 1970) was used for initial detection of parasitaemia. Micro-haematocrit buffy-coat method was used to confirm infection/parasitaemia (Murray *et al,* 1977) when parasites were absent in wet blood film. The degree of parasitaemia was estimated by the rapid matching method (Herbert& Lumsden, 1976).

STATISTICAL ANALYSIS

Data obtained were computed into means and the Student's t-test was used to compare means of parasitaemia. The software SPSS version 22.0 was used to perform the statistical test. The level of precision was held at 95 % and $P \leq 0.05$ was set for significance.

RESULTS

Parasitaemia was observed in infected animals 28 days post-infection and progressed rather slowly before declining set in. The mean parasitaemia of the infected group, at onset (day 28 PI), was 7.32±1.08. The mean parasitaemia of the infected group increased to 35.72±2.36 on day 36 PI but rose to a peak of 81.60±27.71 on day 43 PI. From days 50 to 57 PI the mean parasitaemia declined progressively from 2.25 ± 0.150 to 0.50±0.00 in the infected group. The mean parasitaemia kept fluctuating between 0.43±0.44 and 0.34±0.02 from day 64 PI till the end of the study on day141 PI. (Table I)

There was also no significant (P>0.05) difference in the mean rectal temperature of trypanosome-infected sheep when compared with the uninfected control group throughout the period of study (Table II).

The mean packed cell volume of the infected group was significantly (P≤0.05) lower than the uninfected group from day 28 PI to day 43 PI, afterwards, there was no significant (P>0.05) variation in PCV of the two groups till the end of the experiment (Table III). Furthermore, 40 % mortality was recorded in this study, two sheep from the infected group died on day 35 PI.

Table I: Parasitaemia ± SEM ($\times 10^6$/ml) in West African Dwarf Sheep infected with Trypanosoma brucei brucei parasite.

Trypanosome brucei - infected WAD Sheep

Day post infection	Infected Untreated	Uninfected untreated
28	7.32±1.08	0.00 ± 0.00^b
30	18.38±2.71	0.00 ± 0.00^b
32	21.51±2.25	0.00 ± 0.00^b
34	25.45±2.39	0.00 ± 0.00^b
36	35.72 ± 2.36^a	0.00 ± 0.00^b
43	81.60 ± 27.71^a	0.00 ± 0.00^b
50	2.25 ± 0.15^a	0.00 ± 0.00^b
57	0.50 ± 0.00^a	0.00 ± 0.00^b
64	0.43 ± 0.03^a	0.00 ± 0.00^b
71	0.37 ± 0.05^a	0.00 ± 0.00^b
78	0.43 ± 0.03^a	0.00 ± 0.00^b
85	0.37 ± 0.05^a	0.00 ± 0.00^b
92	0.43 ± 0.03^a	0.00 ± 0.00^b
99	0.43 ± 0.03^a	0.00 ± 0.00^b
106	0.34 ± 0.02^a	0.00 ± 0.00^b
113	0.43 ± 0.03^a	0.00 ± 0.00^b
120	0.43 ± 0.03^a	0.00 ± 0.00^b
127	0.43 ± 0.03^a	0.00 ± 0.00^b
134	0.34 ± 0.02^a	0.00 ± 0.00^b
141	0.34 ± 0.02^a	0.00 ± 0.00^b

Data presented as means ± SEM. Different Superscripts $^{(a, b)}$ in a row indicate significant difference between the means of the groups at (P<0.05).

Table II: Rectal temperature (^{0}C) of West African Dwarf Sheep infected with Trypanosoma brucei brucei parasite.

Days post inoculation	Infected untreated	Uninfected Untreated
28	39.53±0.27^a	39.25±0.31^a
30	39.40±0.49^a	39.00±0.11^a
32	39.68±0.21^a	39.47±0.32^a
34	39.30±0.45^a	39.35±0.39^a
36	38.85±0.69^a	38.50±0.26^a
43	38.08±0.37^a	37.78±0.13^a
50	37.70±0.60^a	37.55±0.17^a
57	37.75±0.38^a	37.70±0.29^a
64	39.01±0.41^a	39.18±0.06^a
71	38.63±0.40^a	39.18±0.06^a
78	38.83±0.28^a	39.50±0.32^a
85	39.10±0.43^a	39.00±0.41^a
92	38.53±0.51^a	39.45±0.31^a
99	38.25±0.23^a	39.08±0.41^a
106	38.18±0.27^a	38.45±0.39^a
113	38.98±0.46^a	38.43±0.26^a
120	39.25±0.27^a	39.43±0.26^a
127	38.80±0.65^a	38.88±0.45^a
134	38.78±0.39^a	39.40±0.23^a
141	38.70±0.55^a	39.00±0.45^a

Data presented as means ± SEM. Different Superscripts (a, b) in a row indicate significant difference between the means of the groups at ($P \leq 0.05$)

DISCUSSION

Parasitaemia in sheep used in this study was established on day twenty-eight (28) post-inoculation and was evident in all infected sheep on day 30 PI. The average pre-patent period of 29 days observed in sheep in this study is in contrast with 5 days earlier observed in *T. brucei* infected WAD sheep (Akpan *et al*., 2017), 3.5 days in *T. congolense* infected sheep (Mohammed *et al*., 2009), 7 days in *T. brucei* infected Yankasa rams (Wada *et al.,* 2020) and 6 days in *T. vivax* infected Zebu cattle (Dagnachew *et al*., 2015. This result is similar to 20 days pre patent period observed in *T. evansi* infected Yankasa ram (Wada *et al*., 2020).

The long prepatent period observed in infected sheep in this study could be due to low virulence and pathogenicity of the trypanosome isolates used (Wada *et al.,* 2020). It is possible that the locally sourced sheep used resisted the parasites. This ability to eliminate trypanosome infections or parasites, observed among experimentally challenged ruminants in this study may also explain the zero trypanosome prevalence recorded earlier in a survey conducted in the study area (Akpan *et al*., 2021).

The decrease in parasitaemia observed in the infected group of sheep could be attributed to recession after peak of parasitaemia and natural resistance to trypanosomes by trypanotolerant breeds (Naessens *et al*., 2002; Maganga *et al*., 2018). Trypanotolerant breeds of sheep and goats include the Djallonke, West African Dwarf, Guinea and Casamance breeds (Missohou *et al*., 2006). This decline in parasitaemia continued after peak parasitaemia until it became barely detectable, which is an indication of self-cure. This result corroborates the works of Malatji (2022). Some trypanotolerant animals may exhibit self-cure and eliminate the trypanosome organisms while others may remain persistently infected but maintain productivity and show few or no signs of illness (Spickler, 2018). This trypanotolerance could be due to innate resistance and natural genetic manipulation overtime, to adapt to tsetse bites.

The absence of significant difference in the mean rectal temperature of sheep between infected and uninfected groups throughout the period of study is in contrast with most reported observations in trypanosomosis of sheep (Mohammed *et al*., 2009; Akpan *et al*., 2017). However, fluctuation of pyrexia in trypanosome-infected animals has been reported by many researchers (Obidike *et al*., 2005) in dogs, (Eze *et al*., 2006) in pigs, (Akpa *et al*., 2008) in dogs, (Mohammed *et al*., 2009) in sheep, (Sivajothi *et al*., 2014) in cattle and (Akpan *et al*., 2017) in WAD sheep. Since pyrexia has direct relationship with parasitaemia (Mbaya *et al*., 2009), it could be that the sheep used are of the

Table III: Packed Cell Volume (%) of West African Dwarf Sheep infected with *Trypanosoma brucei brucei* parasite.

Days Post Infection	Infected Untreated	Uninfected Untreated
28	14.00±0.41^b	25.75±0.48^a
36	17.00±1.08^b	26.25±0.75^a
43	23.00±1.73^{ab}	27.25±1.11^a
50	20.00±0.58^b	27.00±0.71^a

Data presented as means ± SEM. Different Superscripts $^{(a, b)}$ in a row indicate significant difference between the means of the groups at ($P \leq 0.05$).

trypanotolerant breeds, capable of containing both parasitaemia and fever. This showed in their parasitaemia, with the infection level reducing among the infected animals even without any treatment.

The significant decline in PCV observed in the infected sheep is indicative of anaemia and is in agreement with earlier reports that trypanosomosis causes anaemia (Bengaly *et al*., 2002, Abenga *et al*., 2005; Nweze *et al*., 2011; Laohasinnarong *et al*., 2015).

Anaemia in trypanosomosis has been reported to be haemolytic in nature (Mbaya *et al*., 2012). The expanded and active mononuclear phagocytic system (MPS) plays a major role in haemolytic anaemia of trypanosomosis as a result of erythrophagocytosis which develops soon after infection and continues through the various stages of the disease (Mbaya *et al*., 2012; Osuagwuh, 2014). The severity of anaemia depends on the level and duration of parasitaemia in trypanosome-infected animals. The anaemia observed post-infection might also have been associated with activation of the mononuclear phagocytic system due to increased demand on the system to remove dead RBCs, tissue cells, trypanosomes, antigen-antibody complexes and to participate in immune responses (Stijlemans *et al*., 2018; Onyilagha & Uzonna, 2019).

CONCLUSION

The gradual decrease in level of parasitaemia until barely detectable in the infected group of sheep as recorded in this study suggests self-cure, and is as a result trypano-tolerant nature of the WAD breed of sheep used. The high survivability of the infected sheep supports the report that WAD sheep are able to contain both fever and parasitaemia.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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