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RESEARCH

The clinical effect of experimental infection with *Trypanosoma congolense* on Dutch belted rabbits

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Abstract

The clinical effect of *Trypanosoma congolense* infection on Dutch belted (does) rabbits was investigated. Sixteen Dutch belted rabbits weighing between 1.6 and 1.8 kg were grouped into two groups of eight each. Animals were accessed for packed cell volume (PCV), total leucocyte count (TLC), rectal temperature (RT), heart rate (HR), and body weight (BW) before infection as well as 18, 25, and 58 days post inoculation (PI). The level of parasitaemia was estimated on a weekly basis and was graded by number of parasites/field. There was a significant difference ($P < 0.05$) in the mean PCV between treatment and control groups of the rabbits on all days PI. The other parameters were not significantly different between uninfected controls and treatment group although the rectal temperature fluctuated. The mean PCV of infected rabbits was $36.0 \pm 0.53\%$, $35.3 \pm 0.19\%$, and $28.0 \pm 0.89\%$ at days 18, 25, and 58 PI, while for uninfected, the mean PCV was $40.8 \pm 0.11\%$, $41.8 \pm 0.19\%$, and $41.3 \pm 0.08\%$ across the same time periods. Parasitaemia was detected at 6th day PI and remained high to the end of the study. The study suggests that the use of haematinics and anti-pyrexia treatments as part of disease management for rabbits would be useful.

Keywords *Trypanosoma congolense* · Packed cell volume · Dutch belted · Rabbits · Africa

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Introduction

African trypanosomes are protozoan parasites transmitted by various species of flies of the genus *Glossina* (cyclically), and *Stomoxys* or *Tabunus* (mechanically). They cause devastating medical, veterinary, and socioeconomic disorders that have a negative impact on Sub-Saharan Africa's economic development (Aksoy et al. 2017). Each parasite species is adapted to certain climatic and ecological parameters that correspond to the habitat of the tsetse fly vector (*Glossina* spp.), often known as the "belt" of tsetse flies (Shaw et al. 2014). In humans, the disease is known as "sleeping sickness" or human African trypanosomiasis (HAT) and "nagana" or animal African trypanosomiasis (AAT) in domestic animals (Courtin et al. 2008). HAT is caused by two distinct *Trypanosoma brucei* subspecies: *Trypanosoma brucei gambiense* (chronic form) found in western and central Africa, and *Trypanosoma brucei rhodesiense* (acute form) prevalent in eastern and southern Africa (Ruiz et al. 2015). *T. congolense*, *T. brucei*, and *T. vivax* are the most common trypanosomes that cause

disease in domestic animals (Morrison et al. 2016). The bovidae and suidae families of game animals are major reservoirs of infections with minor clinical indications, but the disease in domestic animals is severe often with high fatality (Yaro et al. 2016). The severity of infection with various species of trypanosomes is dependent on the parasite's highly sophisticated immune escape mechanisms, which allow them to manipulate the entire host immune response to evade elimination via antigenic variation of the variant surface glycoproteins (VSG) (Stijlemans et al. 2016). The most common pathogen in livestock is *Trypanosoma congolense* (Henry et al. 2004). Studies have demonstrated phagocytosis of *T. brucei* or *T. congolense* by macrophages in the presence of immunological sera in vitro (Henry et al. 2004). Although several studies have used *T. vivax* or *T. brucei* and have been conducted in rats, cattle, sheep, and goats, there is very little research on rabbits. In Nigeria, rabbits are non-traditional meat source in many households and their husbandry is becoming increasingly important for a variety of reasons, including low initial investment and high fecundity. Trypanotolerant rabbits would result in increased rabbit production and more protein available to the population. Therefore, this study aimed to investigate clinical effect of experimental infection with *T. congolense* in Dutch belted rabbits.

Materials and methods

Trypanosomes, animals, and experimental infection

The *T. congolense* Kaura strain used for the study was isolated from cattle in Kaduna State and was passaged in mice. Sixteen Dutch belted rabbits (does) between 7 and 12 months of age were used for the experiment. The base line parameters for mean TLC (10^9/L), RT (^{0}C), HR (/min), and BW (kg) were recorded. The rabbits were placed in two groups (infected = T) and (control = C) of eight rabbits each. The infected group was inoculated 1 ml T. *congolense* suspension equivalent to 4×10^9 trypanosomes in diluted mouse blood used as a donor via intra-peritoneal injection (Eisler et al. 2001). Parasitaemia was confirmed by wet mount microscopy of blood collected from the rabbits' ear veins on alternate days. On days 18, 25, and 58 PI, blood was taken from the rabbits' ear veins into ethylene diamine tetra acetic acid (EDTA) tubes to determine PCV and TLC. A suspended scale and sac were used to weigh all of the rabbits on days 18, 25, and 58 PI. Rectal temperature and heart rate were measured using a digital thermometer and a stethoscope respectively, every other day including days 18, 25, and 58 PI. Level of parasitaemia was estimated on a weekly basis and was graded by number of parasites/field.

Statistical analysis

Data collected was analyzed using the *T*-test (independent samples test) in SPSS version 23 and the value was regarded significant at $P < 0.05$. Data was presented as mean ± standard error of mean (SE).

Results and discussion

The mean PCV, TLC, RT, HR, and BW of the rabbits before the experiment and the mean values of infected and uninfected animals at days 18, 25, and 58 PI, as well as level of parasitaemia in the infected group are shown in Table 1. The values before inoculation showed that they were within the normal range, an indication that the rabbits used in the study were in good health. The values in the treated group showed some variations from the control group; however, no significant differences ($P < 0.05$) were recorded between them. The values for mean PCV decreased significantly ($P < 0.05$) in the treated group compared to the untreated control group on day 18, 25, and 58 PI until the end of the trial. Parasitaemia was observed on the 6th day PI and the level was represented based on the number of trypanosomes found in the microscopic field (Table 1).

In this study, anemia was observed in infected rabbits, as revealed by decreased PCV PI. This finding agrees with Toma et al. (2008) that anemia is a common pathological feature associated with trypanosomiasis. Egbe – Nwiyi et al. (2005) recorded significantly lower PCV in infected rats ($P < 0.05$) compared to uninfected rats. Similarly, Toma et al. (2008) reported anemia in rabbits infected with *T. congolense*. Studies have shown that RBCs from infected mice exhibited increased osmotic fragility and a different fatty acid membrane composition than RBCs from uninfected mice (Cnops et al. 2015). The change in RBC fragility was not due to interferon (IFN), but rather by host-derived factors such as tumor necrotic factors (TNF) (Balogun et al. 2014; Szempruch et al. 2016). In addition, parasite-derived substances such as sialidases in *T. congolense* infections and extracellular vehicles (EVs) in *T. brucei* infections may contribute to RBC alteration (Szempruch et al. 2016). The mechanisms by which the parasite causes anemia are complex and poorly understood, but most likely include differences in erythropoietic potential and hemodilution factors involved in erythrolysis and eryhtrophagocytosis (Habila et al. 2012). The regulation of erythropoietin homeostasis, the host's potential to raise neutralizing antibodies against secreted trypanosome virulent factors, and the general mechanism involved in inflammation control such as the

Table 1 Mean (±SEM) of PCV, TLC, HR, RT, and BW of rabbits before and after inoculation with *T. congolense* in treated and untreated control groups and level of parasites per field in the treated group

Mean values	Before inoculation	Days/post inoculation		
Parameters	BI	18 PI	25 PI	58 PI
PCV (%)	41.3 ± 0.03[a]	36.0 ± 0.53[a]	35.3 ± 0.19[a]	28.0 ± 0.89[a]
TLCx10^9/L	5.2 ± 0.05	5.5 ± 0.08	3.9 ± 0.08	3.4 ± 0.09
RT (^{0}C)	38.4 ± 0.06	38.3 ± 0.07	39.2 ± 0.09	39 ± 0.10
HR (bpm)	143.0 ± 0.71	141 ± 0.96	143 ± 1.22	144 ± 1.13
BW (kg)	1.7 ± 0.04	1.7 ± 0.07	1.5 ± 0.07	1.6 ± 0.06
Parasites/field	-	++	+++	++++
Control group				
PCV (%)	42.3 ± 0.04[a]	40.8 ± 0.11[b]	41.8 ± 0.19[b]	41.3 ± 0.08[b]
TLCx10^9/L	5.3 ± 0.03	5.9 ± 0.05	4.2 ± 0.08	3.5 ± 0.09
RT (^{0}C)	38.6 ± 0.06	38.0 ± 0.12	38.2 ± 0.11	38.8 ± 0.08
HR (bpm)	145.0 ± 56	149 ± 0.59	144 ± 0.94	149 ± 1.27
BW (kg)	1.7 ± 03	1.6 ± 0.07	1.6 ± 0.07	1.80 ± 0.07

There was a significant difference ($P < 0.05$) in the mean PCV between treated and untreated control groups of the rabbits on all days PI and other parameters were not significant

Key: B1, before inoculation; P1, post inoculation

[*]Values with different superscripts are statistically significant

+, 1–10/field; ++, 11–50/field; +++, 51–100/field; ++++, > 100/field

regulation of the interferon-gamma and interleukin (IFNγ/IL-10) balance, as well as other cytokines are also thought to play a role (Stijlemans et al. 2018). The hallmarks of anemia are arguably the most well-known and well-described features of AT (African trypanosomiasis) to date. The rectal temperature of infected rabbits in the present study showed fluctuating pyrexia, as trypanosomiasis has been linked to variable pyrexia in animals (Ogwu and Njoku 1986). Low body weight, anemia, and stress associated with immune complex reactions could cause anoestrus in females resulting to poor reproductive performance. The presence of trypanosomes in the blood samples of infected animals was first observed six days post inoculation, and the report was consistent with previous reports that *T. congolense* prefers the capillary bed (Losos and Ikede 1972). Reports showed that phagocytosis of trypanosomes by kupfer cells, mediated by actively produced antibodies, was first noticed on day 5 post infection in mice infected with *T. congolense*, and greatly increased on day 6 post infection (Henry et al. 2004). Given the 6 days incubation period observed in the present study and that of Toma et al. (2008), who reported an 8 days incubation period in rabbits, a 7-day incubation period for *T. congolense* may be considered the average incubation period. However, the length of the incubation time may not be determined by the animal species involved but the species of the trypanosomes. As the parasites multiplied, the PCV level decreased and remained low, resulting in pale mucous membranes and chronic anemia. The present study reaffirmed that *T. congolense* induced severe anemia in infected rabbits and suggests that in addition to trypanocides and antipyretics, haematinic therapy be given priority in the treatment of rabbits infected with *T. congolense*.

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Data availability The data that were generated or analyzed in the course of this study were included in the article.

Declarations

Ethics approval Approval was obtained from the University of Abuja Ethical Committee on Animal Use (UAECAU) (UAECAU/2022/008). The procedures used in this study adhered to the tenets of the Declaration of UAECAU.

Consent to participate Not applicable.

Consent for publication All authors have agreed to this final version of the manuscript and have given their consent for its publication in the *Parasitology Research* journal.

Conflict of interest The authors declare no competing interests.

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