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DOI: 10.5455/jva.20151019011507

Online version is available on: www.grjournals.com
Parasitaemia in Rabbit Bucks Experimentally Infected with *Trypanosoma Brucei Brucei*

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**Abstract**

This study was designed to determine the levels of parasitaemia on 20 healthy domesticated rabbit bucks infected with *Trypanosoma brucei brucei* over a period of eight weeks. Ten out of the 20 bucks were infected with 1 ml of the parasite containing 1 X 10⁶ trypanosomes and known as infected group A, while the remaining ten bucks were left uninfected as group B. The infected bucks were monitored for six weeks while the others served as control post infection. Parasitaemia was present at day six in the group A animals with mean values of 2.90±0.31, after which there were fluctuations in the levels of parasitaemia with the lowest level of parasitaemia at day 34 having mean values of 0.60±0.16 and peak parasitaemia reached at day 10 post infection having mean value of 3.00±0.33. The overall mean parasitaemia was 1.99 ± 0.25.

**Keywords:** Rabbit bucks, parasite, *Trypanosoma brucei brucei* infection, peak, parasitaemia.

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Received on: 05 Oct 2015
Revised on: 15 Oct 2015
Accepted on: 19 Oct 2015
Online Published on: 30 Oct 2015

Introduction

Animal protein is very vital as it contains all the essential amino acids needed for the growth, development and maintenance of human life and a shortage of this protein is prevalent in most parts of Africa where it is estimated that on the average 10g of animal protein is consumed per day as compared to the recommended daily intake of 35g (FAO, 1986; Ugwu, 2007). Obioha (1992) and Egbunike (1997) observed that the level of consumption of meat and other sources of animal protein in Nigeria is estimated at about 6.0-8.4g per head per day which is about 20g less than the minimum requirement by the National Research Council of the United States of America and is also less than about 13.5g per day prescribed by the WHO.

The need to meet up with the demand for animal protein for the rapidly increasing human population in the developing countries (Allen, 1993; Ahemen and Bitto, 2007) has necessitated the search for alternative sources of this protein. Amongst other sources of animal protein, the rabbit, poultry and pigs are the most common viable options, mainly due to their short generation intervals (Ozor and Maduekwe, 2001; Adebayo and Sorungbe, 2002; Owen et al., 2008).

African animal trypanosomosis (AAT) also known as Nagana is a Zulu word meaning “to be depressed” (WHO, 2001; Courtin et al., 2008). It is a disease complex caused by tsetse-fly transmitted Trypanosoma congolense, T. vivax, T. brucei and T. simiae or mixed infections with one or more of these trypanosomes of the family Trypanosomatidae, genus Trypanosoma (Kamuanga, 2003; Courtin et al., 2008). Animal trypanosomosis in Africa poses one of the most serious veterinary problems in the world causing a major constraint to the agricultural and socio-economic development of tropical Africa (Gasser, 1963; Ilomobade, 1981; Llewellyn et al., 1987; Ikede, 1989). While most other animal diseases have been successfully controlled during this century, trypanosomosis continues to pose a major threat to animal production in sub-Saharan Africa (Pepin and Meda, 2001; WHO, 2001; Kamuanga, 2003; Bawa et al., 2005), despite the age-long attempts to control it (Omotainse et al., 2000).

Trypanosomosis is a disease of great economic importance in livestock in the world because it affects important working animals such as: Buffalo, cattle, camels, horses and other animals, wildlife, can also be infected (OIE, 2008).

In other animals such as rabbits, trypanosomosis also has a chronic course, it can present with anorexia, apathy, pale mucous membranes and edema of eyelids and ears. Infected animals show irregular peaks of parasitaemia for long periods (Da Silva et al., 2009). Thus, these animals are good models for chronic infections due to low peak of parasitaemia and longtime of infection, as occurs in cases of natural infection.

Over the course of infection, an initial prepatency period occurs between the inoculation of parasites in a healthy animal and their detection in the blood or tissue fluids, by direct microscopic observation. Following the prepatency period, the disease progresses in two phases; an acute phase, characterized by high levels of parasitaemia and noticeable clinical symptoms, and a chronic phase, characterized by low parasitaemia which can either lead to emaciation or become clinically unapparent with undetectable changes in variables such as body temperature and haematocrit count (Fernández et al., 2009).

Objective

The objective of this work was to determine the levels of parasitaemia in rabbit bucks experimentally infected with Trypanosoma brucei brucei.

Materials and Methods

Acquisition and Management of Experimental Animals

Twenty (20) domesticated adult rabbit bucks weighing an average of 2.0 ± 0.8 kg were used for the study. The rabbit bucks were acquired from a rabbit try within Zaria metropolis of Kaduna State. The rabbit bucks were allowed to acclimatize for 14 days, were dewormed with Levamisole Hcl (0.1mg/kg) and treated for other diseases such as Eimeria staedia with Embazine ® forte (1g/5kg)
before the commencement of the experiment. The rabbit bucks were randomly assigned into two groups; control and infected groups consisting of ten rabbit bucks respectively.

They were kept in fly proof cages and given access to growers mash and water was provided ad libitum. Stabilates of *T. brucei brucei* were acquired from the Department of Parasitology and Entomology of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, who also sourced it from National Institute for Trypanosomosis Research, Kaduna state. Before infecting the rabbits, the trypanosomes were maintained by serial syringe passages in white rats, and periodically checked for the viability of the parasite. Blood was obtained from the passaged rats by tail bleeding into normal saline and the parasitaemia adjusted to $1 \times 10^6$ trypanosomes per milliliter (ml) by the method of Herbert and Lumsden, 1976. Each rabbit in Group B was inoculated intraperitoneally with 1ml of saline diluted blood containing $1 \times 10^6$ trypanosomes *T. brucei brucei*, while Group A rabbits served as uninfected control.

Blood was collected from all the rabbits by venipuncture of the ear vein. The site for the venipuncture was aseptically prepared and swabbed with methylated spirit and blood was collected into heparinised capillary tubes, about 30% of each capillary tube was left unfilled and the open end of each of the tubes was carefully sealed with flame. The tubes were then loaded into a microhaematocrit centrifuge (Hawksley, England) and centrifuged at 15,000 rpm for 3 minutes.

Parasitaemia was monitored throughout the course of the experiment using Haematocrit Centrifugation Technique and Thin blood smear.

*Thin blood smears.* A drop from the blood was put on a clean glass slide and these were made as in the case of blood smears. The slides were fixed in methanol for 3-5 minutes and stained with Giemsa stains for 25-30 minutes then were read using an oil immersion objective at x100 for identification of trypanosomes (OIE, 1997).

**Concentration methods:** Buffy coat examination (also called the Woo method, OIE, 1997). The microhaematocrit heparinised capillary tubes opened at both ends. Blood was taken up at one end by capillary attraction, until about three-quarters of the length of the tube is filled. The other end of the tube was then sealed over a burner (taking care not to char the blood) or by the use of plasticine. The tubes were then placed in the grooves of the rotor plate, with the sealed end outwards (to prevent the blood from being thrown out during centrifugation); the cover was closed and screwed down, and the timing set and spun at high speed (15,000 rpm) for three to five minutes. After centrifugation, the tubes were removed, care being taken that it remains known to which animal each of the tubes corresponds (OIE, 1997).

The tubes were then examined for the presence of trypanosomes by placing them on a microscope slide in a slot form. Immersion oil was placed on the capillary tube, over the region of the tubes where the parasites, if present, are concentrated and will be visible (buffy coat and buffy coat/plasma junction) (OIE, 1997).

**Statistical Analysis**

Data generated on parasitaemia was expressed as mean ± standard error of the mean (SEM). Unpaired t-test was used to test for differences between groups using Graph pad prism version 5.0. Values of P<0.05 were considered statistically significant.

**Results**

The infected rabbit bucks had an average prepatent period of six days post infection which gradually rose to a peak by the 10th day post infection thereby followed by a fluctuating parasitaemia and anorexia. The mean parasitaemia was $1.99 \pm 0.25$. This is illustrated in figure 1.
Discussion and Conclusion

The *Trypanosoma brucei brucei* used in this study caused clinical trypanosomosis in all the infected rabbits showing a marked pathogenicity in consistence with the findings in *Trypanosoma congolense* infected rabbits (Takeet and Fagbemi, 2009), *Trypanosoma congolense* infection in rats (Egbe-Nwiyi et al., 2005) and *Trypanosoma evansi* infection in rabbits (Ramírez-Iglesias et al., 2012). The infection did not cause any death which is presumed to be due to the strain of the parasite used in this work.

The infected rabbit bucks had an average prepatent period of six days post infection which gradually rose to a peak by the 10th day post infection thereby followed by a fluctuating parasitaemia and anorexia which has also been reported to be characteristic of trypanosomosis (Ramírez-Iglesias et al., 2012). Emaciation in the infected bucks was consistent with the findings of Ogunsanmi et al., 1994, Omotainse et al., 1994 which was reported to be as a result of the trypanolytic crisis which occurs in the peripheral blood of the infected host in the early stage of the disease (Seifert, 1996). Ogwu 1983, Agu and Bajeh, 1986 reported an eventual disappearance of parasites from peripheral circulation which was also observed during the course of this experiment 28 days post infection.

This study has indicated that *T. brucei brucei* is pathogenic to rabbit bucks and could be detrimental to the efforts to increase animal protein, and the socio-economic wellbeing of the tsetse endemic areas in the country where rabbit farming is important.

There should also be improvements in the level of infectious diseases research which should be based on newer basic science techniques including molecular diagnostics and areas of genetic algorithms and ant colony optimization to combinatorial optimization problems (Markand et al., 2013, Markand et al., 2015 and Rajappa et al., 2012).

References


