

Abortion and its probable cause in gilts experimentally infected with *Trypanosoma brucei*

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ABSTRACT

This paper intends to report abortion in gilts and its probable cause in an experimental infection with *T. brucei*. Twelve domestic crossbred female piglets aged eight weeks and two boars aged eight months were purchased from piggeries in Samaru Zaria, Nigeria and housed in clean fly proof pens. When the piglets attained puberty, they were divided into two groups, six experimental and six uninfected control. During their third estrus period, all the gilts were bred randomly by the two boars whose fertility had been previously determined. When the gilts were between 35 and 39 days pregnant, the ones in the experimental group were inoculated each with two milliliters of the infected blood containing 1.8×10^6 parasites via the anterior vena cava. The gilts in the control group were not inoculated. All the inoculated pigs developed clinical trypanosomosis characterized by fever, pale mucous membranes, anorexia, dullness, reduction in feed intake, reduced weight gain, weight loss, emaciation, short and moist cough, moist rales, mucopurulent ocular discharges, hyperemia of the skin, lethargy, un-coordinated movements, posterior paresis, recumbency and death of two infected gilts. The gilts in the control group carried their pregnancies to term and farrowed normally but those in the experimental group had their pregnancies terminated between 40 and 58 days post breeding. The probable cause of loss of pregnancy of these gilts was pyrexia.

Keywords: *Trypanosoma brucei*; gilts; infection; abortion

INTRODUCTION

Pigs are susceptible to *Trypanosoma simiae*, *T. brucei*, *T. congolense*, and *T. suis* (Losos, 1986; Sekoni, 1994; Seifert, 1996). However such infections are usually considered to be of little pathogenic and economic significance except infection by *T. simiae* (Onah, 1991).

Trypanosoma brucei causes clinical disease and sub clinical infections which impair reproduction in both the male and female pigs in single and mixed infections. The clinical signs exhibited by infected pigs are intermittent fever, high and eventually fluctuating parasitemia, increasing degrees of anemia, progressive loss of weight and condition, anorexia, nervous signs, paresis and wobbling of the hind legs, dehydration, hyperemia, petechial hemorrhages, ascites and death (Omeke and Ugwu, 1991; Onah and Uzoukwu, 1991; Otesile *et al.*, 1992; Allam *et al.*, 2012). The reproductive abnormalities attributed to these infections in pigs include lowered reproductive capacity of the infected boars (Omeke, 1991; Omeke, and Onuora, 1992),

anestrus in sows (Onah, 1991), delayed puberty, depressed estrus and impaired fertility in gilts (Omeke and Rawlings, 1998), posterior paresis in gilts (Allam *et al.*, 2006; Allam *et al.*, 2012).

The studies carried out to determine the impact of trypanosomosis on pigs is very scanty. Also, very little attention is being paid on the significance of the disease among this animal *species* (Waiswa, 2005).

MATERIALS AND METHODS

Experimental animals

Twelve domestic crossbred female piglets aged eight weeks were purchased from two piggeries in Zaria and housed in clean fly proof pens in the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. The piglets were ear notched for identification and screened for endo- and ecto-parasitic infections. Baseline hematological data were obtained and the piglets were treated against nematodes and ecto-parasites such as mites with Ivermectin (Ivomec^{Rx}) at the dose rate of 200 µg/kg body weight subcutaneously. The piglets were fed on compounded diet of 18% crude protein comprising of maize, 36.8%; soya bean, 5%; ground nut cake, 23.5%; rice bran, 30%; beniseed cake, 2%; bone meal, 2%; premix, 0.2%; table salt, 0.5%, with water provided *ad-libitum* throughout the period of the study.

The trypanosome used

The trypanosome used in this study was obtained from the Nigerian Institute of Trypanosomiasis and Onchoserchiasis Research Vom, Nigeria. It was originally isolated from a pure natural infection in cattle in Federe, Kaduna state Nigeria. The parasite was inoculated into mice and transported to the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria.

Experimental design

When the gilts were 7 months old they were randomly divided into two groups of 6 experimental and 6 controls.

Evaluation of fertility of the boars

The semen of the two boars was collected and analysed to establish their fertility.

Mating

During the third estrus period, all the gilts were bred randomly by the fertile boars.

Determination of pregnancy

From day 21 after mating, all the gilts were observed closely for signs of pregnancy. Ultrasonography and progesterone assay was done to confirm the pregnancies of the gilts.

Inoculation of animals

Prior to inoculation of the gilts, the infected blood of the mice was inoculated into eight rats. The parasitemia in the rats was monitored and classified according to the method described by Woo (1969). When peak parasitemia was attained, the rats were anesthetized in a jar of chloroform and the bled via the heart. All the blood collected was pooled with ethylene diamine tetra acetic acid (EDTA) as anticoagulant

in a beaker. About 2 milliliters of the infected rat blood containing approximately 1.8×10^6 *T. brucei* organisms were inoculated into each animal in the experimental group via the anterior vena cava while the ones in the control group were left intact.

Clinical examination and blood sampling for parasitemia and packed cell volume (PCV)

Following inoculation, all the gilts were clinically examined, weighed their rectal temperatures taken and their blood samples collected daily. The blood samples were put into tubes that contain EDTA as anticoagulant and were examined for levels of parasitemia and PCV. The values obtained were then recorded. This continued until all the infected animals were positive for trypanosomes. Subsequently, all the gilts were clinically examined twice weekly, their rectal temperatures taken, weighed and their blood in anticoagulant was used to determine PCV levels and parasitemia.

Blood sampling for progesterone assay

The blood samples intended for progesterone assay were collected from the gilts via the anterior vena cava on the days that they were bred (day 0) and subsequently at three days interval till the termination of the experiment. The blood was put into test tubes which do not contain anticoagulant. The serum samples obtained were stored at -20°C and were later analyzed for progesterone concentration using progesterone specific Radio Immuno Assay (RIA) kits prepared by the Animal Production Unit FAO/IAEA Agriculture Laboratory Agency's Laboratories Seibersdorf Austria.

RESULTS

All the infected gilts developed parasitemia 2 to 3 days post infection (p.i.). Peak parasitemia was attained in 4 of the gilts on day 7 p.i. While in the remaining two pigs, peak parasitemia was attained on days 35 and 42, respectively. Thereafter, there were fluctuations in the levels of parasitemia which continued till the end of the study. Two of the gilts died on day's 42 and 92 p.i., respectively, during which time their levels of parasitemia were high (Fig. 1).

The rectal temperatures of the infected gilts started rising from the 3rd day p.i. This was observed in one of the gilts which had a temperature of 39.8°C . The rise in temperatures of the infected animals was followed by fluctuations. The highest temperature noticed during the study was 40.3°C on day 21 p.i. The temperatures of the infected animals were significantly higher than those of the control ($P < 0.05$), (Fig. 2). There was a gradual decrease in the PCV values of the infected pigs. This started from day 21 p.i. in one of the pigs. The lowest PCV value obtained during the study was 17%. This occurred on day 70 p.i. The mean PCV values of the infected animals were significantly lower than those of the control ($P < 0.05$), (Fig. 3). The infected animals from day 28 p.i were observed to be gaining less weight than the ones in the control group. The infected animals were gaining an average of 0.71 kg every two weeks whereas the controls were gaining an average of 1.5 kg during the same period. By day 42 p.i the infected animals started losing weight while the control gained weight steadily (Fig. 4). The difference in the weights between the infected and the control was found to be statistically significant ($P < 0.05$).

Other clinical signs observed in the infected gilts were dullness, reduction in feed intake, reduced weight gain, weight loss, emaciation, short and moist cough, moist rales, mucopurulent ocular discharges, hyperemia of the skin, lethargy, un-coordinated movements, posterior paresis, recumbency and death of two

infected gilts. The boars that were used to breed the gilts were found to be fertile. Their semen was creamy in color and viscous. About 95% of the sperm cells were alive, normal and highly motile.

All the gilts in this study became pregnant. This was evident by non return to estrus during the next expected estrus post breeding. The visual and behavioral signs of estrus were absent in all of them. Ultrasonography of the gilts between 35-39 days pregnant revealed the presence of embryonic vesicles. Also the levels of progesterone increased steadily in the serum of all the gilts after they were bred. The levels of progesterone of the control animals remained high until at parturition. However the levels of progesterone of the infected gilts dropped suddenly during the gestation period. The sharp drop in the progesterone levels signified the end of pregnancy in the infected gilts. This occurred between days 6 and 18 p.i. During this time their fetuses were between 43 and 55 days old. The levels of progesterone of the gilts are summarized in (Fig. 5). The gilts in the control group carried their pregnancy to term and they all farrowed normally.

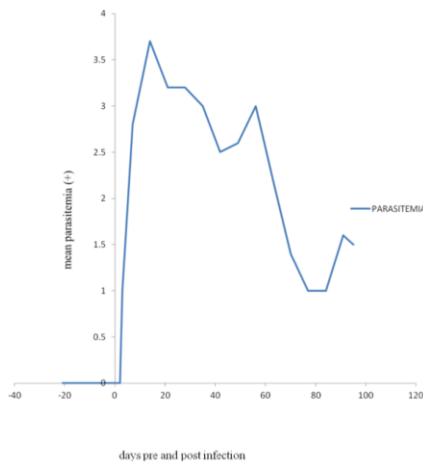


Fig. 1. Mean levels of parasitemia of *T. brucei* infected gilts.

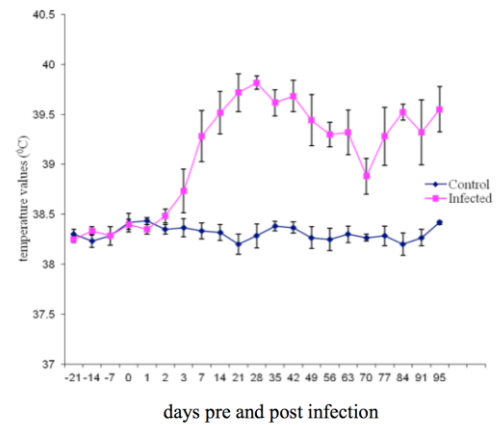


Fig. 2. Mean temperature changes of *T. brucei* infected and control gilts.

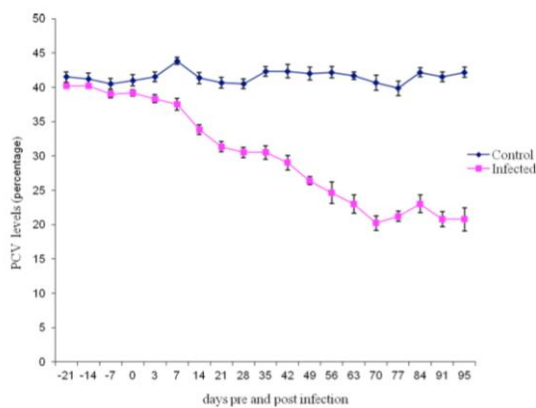


Fig. 3. Mean packed cell volume of *T. brucei* infected and control gilts.

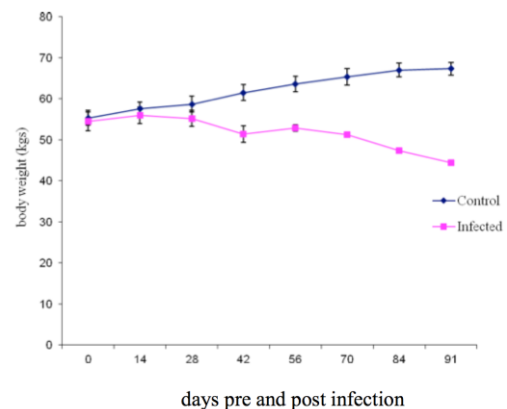


Fig. 4. Mean bodyweight changes of *T. brucei* infected and control gilts.

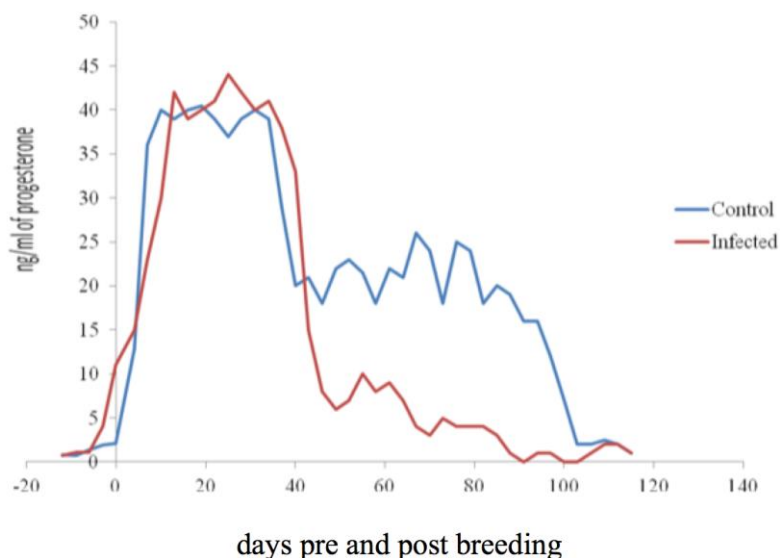


Fig. 5. Mean progesterone levels of control and *T. brucei* infected gilts.

DISCUSSION

The *T. brucei* used in this experiment caused clinical trypanosomosis in all the infected gilts. Two of the infected pigs died as a result of the infection. This could be because the animals used for this experiment were inoculated only once with the organism and they were fed with high quality diet and they had no detectable concurrent infection. Usually under field conditions in Nigeria, animals are under continuous trypanosome challenge in endemic areas, their nutrition is usually poor; they sometimes have endo- and ecto-parasites and other concurrent infections. These factors make animals highly susceptible to trypanosomes (Ogwu, 1983). However the factors were almost completely eliminated in this experiment which could have accounted for the low fatality noticed.

The rectal temperatures and parasitemia which were persistently high and were later followed by fluctuations are common findings in animals with trypanosomosis. Anemia which is indicated by the drop in PCV values was also observed during the course of this infection. It is the most consistent feature of trypanosomosis caused by *T. vivax*, *T. congolense* and *T. brucei* (Anosa, 1983a; Anosa, 1983b; Anosa, 1988).

The pregnancies of the infected gilts were terminated in the second trimester due to the infection with *T. brucei*. This is similar to reports obtained by previous workers in other livestock infected with trypanosomes (Ogwu, *et al.*, 1986; Bawa *et al.*, 2000).

The factors responsible for abortion in animals infected with trypanosomosis include: stress on the fetus and the dam, high and fluctuating fever, fetal hypoxia due to anemic condition of the dam, transplacental fetal infection and reduced levels of plasma progesterone (Ogwu, 1983). In this study, the gilts lost their pregnancies, very early into the infection. During which time the extent of invasion of the ovaries, fallopian tubes, and the uterus by the parasite or the damage of these organs could not have caused

termination of pregnancy in these gilts. Also, the levels of PCV indicated that the animals were not anemic. The pyrexia experienced by the gilts during the infection could have caused the abortion in the infected gilts.

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