Infectivity and Pathogenicity of Sokoto (Northern Nigeria) Isolate of Trypanosoma evansi in West African Dwarf Goats

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Abstract: Based on the previous reports on the existence of variations in the infectivity and pathogenicity of Trypanosoma evansi in the field, sixteen West African Dwarf (WAD) goats were experimentally infected (I.V) with \((2.0\times10^6)\) Sokoto isolate of Trypanosoma evansi. Clinical signs, temperature, hematological, gross and histopathological changes in responses to the experimental infection were determined in the goats. All the infected goats developed parasitaemia 3 days post infection (dpi) with slight increase in temperature in the infected goats, decreased mean PCV % in the infected groups to as low as 18±1.47 from 22.50±0.87. There were leucopenia and lymphocytosis in both infected and uninfected groups. Serous atrophy of fat around the heart and kidneys was observed in goats from two groups (A and B and a goat from group (C) revealed pale liver. Microscopically, there were no pathological lesions in the organs of the sacrificed goats from both infected and uninfected groups. The ability of the parasite to be infective and pathogenic to mice and rat after serial passaging through WAD goats was also tested and proven to be infective and pathogenic. It was discovered that the parasite disappeared from both peripheral circulation and body tissues/organs after 14 days. It was observed that infection of WAD Goats with Trypanosoma evansi (Sokoto isolate) does not produce noticeable clinical signs, gross and histopathological lesions and it is self-limiting.

Key words: Infection, infectivity, pathogenicity, trypanosomosis

INTRODUCTION

Trypanosoma evansi is responsible for a disease known as “Surra”, and the most widespread pathogenic trypanosome globally (Luckins, 1988). The parasite has a wide range of hosts and is pathogenic to many species of domestic and wild animals (Franke et al., 1994; Herrera et al., 2002). It is primarily transmitted mechanically by biting flies like Tabanus, Stomoxys, Haematopota, Lyperosia and Chrysops (Luckins, 1999a; Fraser, 1909). In Africa, camel is the most important host (Dia et al., 1997), but cattle have also been reported as the next most highly susceptible animal (Mahmoud and Gray, 1980). Generally, the disease may take an acute, subacute, or chronic course. The course of an infection and its severity depends on a number of factors including species of the host and the strain of Trypanosoma evansi involved (Losos, 1980).

The pre-patent period of the disease (Surra) varies according to the animal species affected and the immune status of such animals (Ilemobade, 1971). The control of trypanosomosis rely principally on chemotherapy and chemoprophylaxis using salts of three compounds; Diminazene, Homidium and Isometamidium (Leach and Roberts, 1981; ILRAD, 1990). In addition, Quinapyramine, Suramin and recently, Cymelarsan are generally used for therapy and prophylaxis of T. evansi (Zhang et al., 1991; Ndoutamia et al., 1993). Although T. evansi is primarily a parasite of camels, there is likelihood that animals herded together with camels especially small ruminants may become infected with T. evansi (Ngeranwa et al., 1993). Information is lacking on the disease distribution and prevalence of infection of T. evansi in different livestock species in Africa. However, it have been reported from experimental studies that small ruminants are fully susceptible to infection with T. evansi (Anosa, 1974; Whitelaw et al., 1985; Shamak et al., 2006), with the course of infection and severity depending on some factors such as the host species and the strains of the parasite.

Trypanosomosis is the may factor limiting livestock productivity in large areas of humid and sub-humid Africa. Direct losses as a result of trypanosomosis in terms of meat and milk, traction power and programmes to control the disease have been put at US$ 500 million annually while the indirect losses on crop agriculture and human welfare arising from farmers inability to keep livestock in areas with great trypanosomosis risk have been estimated at US$ 5 billion/annum (ILRI, 1997). In sub-Saharan Africa, especially in Nigeria, small

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ruminants form an important part of livestock industry, and because of the search for source of animal proteins for the rapidly growing human population in developing countries, attention has been shifted to goat rearing as an additional source of milk and meat (Yanan et al., 2007).

In Nigeria, extensive work has been done on animal trypanosomosis (Anosa, 1977; Saror, 1975; Sackey, 1998), but little has been done on infection due to *T. evansi* especially in West African Dwarf goats. Since there is variation in the pathogenicity of *T. evansi* strains and corresponding differences in response of animals to infection, it is desirable that a strain (Sokoto isolate) proven to be pathogenic to Savannah goat be evaluated for infectivity and pathogenicity in the trypanotolerant WAD goats predominant in the southern part of Nigeria. The present experimental study was conducted to estimate the infectivity and pathogenicity of Sokoto isolate of *Trypanosoma evansi* in West African Dwarf (WAD) goats in Zero grazing conditions.

**MATERIALS AND METHODS**

This experiment was conducted between March and August, 2008 at Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

**Source of Trypanosoma evansi:** The parasite was isolated from naturally infected camels at slaughter in Sokoto township abattoir. Two rats were used to preserved the parasite and transported by road from Sokoto to Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

**Experimental goats:** Twenty two (22) West African Dwarf (WAD) goats of both sex and aged between 2-4 years were purchased from Kafanchan market in Southern part of Kaduna state and transported by road to Zaria.

On arrival, they were ear-tagged and physically examined for Ectoparasites, screened for haemoparasites using thin blood smear, wet mount and buffy coat methods and helminthic infections using flotation technique. They were all dewormed with albendazole orally at therapeutic dose rate of 7.5 mg/kg body weight and treated against coccidiosis using amprolium at therapeutic dose rate of 7.5 mg/kg body weight in drinking water for 5 days. All the animals were free of Ectoparasites, and those with evidence of *Anaplasma ovis* were treated with long acting oxytetracycline (Tridox L.A, Farvet Bladel Holland) at a dose rate of 20 mg/kg body weight. They were kept in arthropod-proof pen and fed with groundnut hay, grains offal (Dusa) and digiteria hay. Water and salt licks were given *ad libitum*.

The animals were examined for haemoparasites and intestinal helminthes once weekly for the 5-week period of conditioning. They were re-screened for parasitic infections a day prior to experimental infection and were found negative. Base-line parameters of rectal temperature, Packed Cell Volume (PCV), total and differential white blood cell counts were taken during the conditioning period.

After the conditioning period, the sixteen surviving goats were randomly divided into four groups (A-D) of four goats each. The groups were treated as follows; Group A consist of four infected goats out of which two were treated with Diminazene accurate at the dose rate of 3.5 mg/kg body weight on day 21 post-infection (chronic phase, with possibility of the parasites being in tissues/organs). The treatment meant for day 7 (acute phase) was not given because the animals were aparasitaemic. Two of the goats in group B were similarly treated with isometamidium Chloride at the dose rate of 0.5 mg/kg body weight. Group C and D goats served as positive (infected untreated) and negative (uninfected untreated) controls, respectively.

**Experimental Infection:** Eight (8) adult albino rats were used as donors. The rats were sourced from the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. They were screened and certified negative for haemoparasites using the thin blood smears. The rats were then inoculated intraperitoneally with *T. evansi* parasitaemic blood to multiple the parasites. At 4 days post-inoculation, patent parasitaemia was established and became massive by haematocrit centrifuge technique and at more than 20 trypanosomes per microscopic field. They were bled via the ocular vein into a flat-bottom flask containing EDTA as anticoagulant dispensed at 1 mg/kg and was later diluted with phosphate buffered saline glucose.

Each of the goats in groups A-C was infected through the intravenous inoculation of 2 mL of blood containing 2.0×10⁶ *Trypanosoma evansi* as quantified using the improved Naubauer haemocytometer (Petana, 1963) with gram’s of iodine as diluent.

**Post-infection monitoring:** All the experimental goats were monitored daily through the observation of physical appearances, gait, posture, possible discharges from eyes and nose, cutaneous edema, size of superficial lymph nodes and appetite.

The following parameters of all the experimental goats were monitored. Daily rectal temperature was taken with the aid of clinical digital thermometer, parasitaemia was determined every 3 days using wet mount and buffy coat as described by Murray et al. (1983). Packed Cell Volume (PCV), was determined twice weekly. Total and differential white blood cell counts were determined according to Schalm et al. (1995).
Gross and histopathological examination: At day 42 post infection, one of the treated goats in groups A and B, as well as one animal each from groups C and D was picked at random, sacrificed and necropsied. Samples of the spleen, lymph node, brain, bone marrow, liver, heart, kidney, testicle, ovary, uterus, vagina and lungs were taken, fixed in 10% formal saline, embeded in paraffin wax, cut at 5 m thickness and stained with Heamatoxylin and Eosin (H and E) as described by Drury and Wallington (1976). Tissue sections were examined under light microscope (Olympus Optical Co. ltd., Olympus Tokyo, Japan).

Organs impression smears: Lymph nodes, liver, lungs, kidney, brain, spleen, testicles, heart, uterus, ovary, vagina, vulva and bone marrow of necropsied goats were collected and impression smears were prepared from them.

Evaluation of tissue-dwelling ability of the parasite: Two rats and two mice were inoculated with 0.1 mL of parasitaemic blood at a score of +++ on day 3 pi of the goats. Seven (7) dpi when all the infected goats became parasitaemic, two mice were sub-inoculated with the blood of the infected goats and monitored. On day 21 pi of the goats, two rats per infected group were also sub-inoculated with parasitaemic blood and monitored. Six weeks (42 days) pi, homogenates of liver, spleen, lungs, lymph nodes and heart were prepared, diluted with saline and inoculated into mice with two mice being used for each goat. The mice were monitored for parasitaemia and clinical signs of the infection for 6 weeks.

Statistical analysis: The data obtained from the study were summarized as means standard error (Means±SE), while differences between and within the means were analyzed using one way ANOVA using SAS system.

RESULTS

Following the experimental infection of the goats, apart from variations in the rectal temperatures observed, there was no obvious clinical sign. Both the infected and control goats appeared clinically normal. There was a slight rise (39.03±0.23) in the mean rectal temperature between days 3-6 post-infection (pi) in some of the infected goats especially those in group A. Most of the infected goats in other groups maintained rectal temperatures that were within the normal range (38.40±0.15) throughout the period of observations, (Fig. 1).

Parasitaemia: All the infected goats developed parasitaemia 3 days post infection (pi). The parasite disappeared from peripheral circulation of all the infected goats 7 days pi. There was a resurgence on day 11 pi in seven (7) of the infected goats although at low-parasitaemia (+). Four (4) of the infected goats also showed a resurgence of parasitaemia on day 13 pi. It was observed that only one (1) goat remained parasitaemic at 14 dpi and by 16dpi the parasites disappeared from the peripheral blood of all the infected goats and remained so throughout the observation period. All the goats in the uninfected control group remained aparasitaemic throughout the period of the experiment, (Fig. 2).

Hematology: The mean PCV values of all the infected groups showed gradual decrease from the first week of infection to the last week (6th wk) of the observation period. Group A, had its lowest mean PCV value at 5th
week pi from the initial 22.50±0.87 to 18.00±1.47. For group B, the lowest mean value of 20.50±1.44 from the initial value of 25.00±2.71 was recorded at the 4th week pi, while in group C lowest mean value of 18.75±2.06 from the initial value of 29.25±1.49 was observed on the last week (week 6) of the experiment. However, there was a continuous gradual increase in the mean PCV values throughout the period of study from 22.50±2.53 to 23.00±2.68 in the uninfected control group (Fig. 3).

The mean leucocytes counts of all the groups recorded decrease in value throughout the period of the observations. Generally, the mean values ranged between 10.40±1.79 and 6.30±0.32. There was no statistical significant difference (p>0.05) between the values of the infected and uninfected control throughout the period of the experiment (Fig. 4).

There was slight and insignificant (p<0.05) increase in the mean values of the Eosinophils from the 1st week of infection to the 5th week pi in absolute number in most of the infected goats (Fig. 5a, b. Goats in the uninfected control group showed a slight decrease in Eosinophils throughout the period of observations (Fig. 5c). The mean lymphocyte values (65.25±8.23 to 71.50±9.43) increase slightly in all the groups including the uninfected control (Fig. 5d). Monocytes were, however, scanty and were observed between weeks 1 to 5 pi only (Fig. 5e), while basophiles were completely absent throughout the experiment.
**Fig. 5c:** Eosinophils counts of WAD goats pre and post-infection with *T. evansi* (Sokoto isolate)

Eosinophil counts of WAD goats infected with 2.0 x 10^6 *T. evansi* (Sokoto isolate). Two of the infected goats in groups A (●) and B (▲) were treated at day 21; while groups C (x) and D (■) animals served as positive and negative controls, respectively.

**Pathology:** The main post mortem findings in the goats from groups A & B were serous atrophy of fat around the heart and kidneys, while sacrificed goat from group C revealed pale liver. However, the post mortem examination of the animal in the control group did not reveal any significant gross lesions. There were no histopathologic lesions seen in any of the organs studied.

**DISCUSSION**

The findings from these studies revealed that the strain of *Trypanosoma evansi* (Sokoto isolate) used was infective but mildly pathogenic for the WAD goats.

Previous studies (Mohammed, 2000) showed the isolate to be highly pathogenic for the Savannah Brown goats, mice and rats. Studies by other researchers have also shown that horses, sheep, and Kano Brown goats are susceptible to *Trypanosoma evansi* infection (Stephen, 1986; Audu *et al*., 1999; Shehu *et al*., 2006).

The pyrexia recorded at the early phase of the experimental infection (3-6 dpi), which coincided with peak of parasitaemia may probably be as the result of individual variations in susceptibility of the goats to the parasite as earlier reported, that severity and susceptibility depend on the animal species and immune status of the animals infected (Ilemobade, 1971; Herrera *et al*., 2004). It is possible that those that showed high pyrexia were those with weak immunity.

Parasitaemia observed 3 days post-infection in all the infected goats agreed with earlier report on Yankassa sheep by Audu *et al.* (1999). However, the pre-patent period appeared shorter than what was reported by Anosa and Isoun (1976), Sharma *et al.* (2000) and Shehu *et al.* (2006). The disappearance and resurgence of parasitaemia at different intervals of the experimental monitoring may be as a result of dynamic of antigenic variation activity of the parasites as earlier reported (Jones and Mckinell, 1984).

The decrease in the haematocrit values, which coincided with the peak parasitaemia, observed in the infected goats indicates low-grade anemia. Anemia has been reported to be the most important pathogenic features of trypanosomosis, which is apparently caused by the destruction of red blood cells in some organs like the liver, spleen and lymph node (Tizard, 1998; Sharma *et al*., 2000; Cadioli *et al*., 2006). Further more,
the anemia observed in this study was not as low as those reported for trypanosusceptible animals. In a typical trypanotolerance phenomenon, pathogenic *Trypanosoma* species infection does not usually result in anemia (Murray et al., 1982; Murray and Dexter, 1988).

Leucopenia and lymphocytosis, which were associated with the present study have also been reported in trypanosusceptible breeds of cattle and goats (Anosa et al., 1992; Dargantes et al., 2005), showing that there was a level of immune compromise by the goats to the parasites. However, the self cure phenomenon (i.e., animals undergo spontaneous recovery with out treatment) and absent of noticeable gross and histopathologic lesions that were observed from this study had also been reported in different breeds of trypanotolerant cattle by other researchers (Mwangi et al., 1993). The self-cure phenomenon may be due to genetic resistance to the strain of trypanosomus used as been reported to occur in certain breeds of livestock and species of wild life (Murray et al., 1982). It may indicate the ability of the goats to mount effective immunity to limit and eliminate the parasite. This characteristic can be of great benefit to livestock farmers in areas where trypanosomosis is the major factor militating against livestock productivity. In such areas, the use of trypanotolerant livestock can be one of the methods of enhancing livestock productivity (Enwezor and Lawal, 2003). The finding may also serve as a stimulant to researchers in searching for the genes that confer trypanotolerant and enhance productivity in endemic areas.

Conclusion could not be reached on the two drugs used on which is the most effective in treatment of *Trypanosoma evansi* infection because of the self-cure phenomenon observed in all the infected goats before administration of the drugs.

**CONCLUSION**

The following conclusions could be drawn from the study:

- Infection of WAD goats with *Trypanosoma evansi* (Sokoto isolate) produces little or no clinical signs.
- The parasitaemia with *Trypanosoma evansi* in WAD (Sokoto isolate) goats is self-limiting.
- Transient mild anaemia is associated with *Trypanosoma evansi* (Sokoto isolate) infection in WAD goats.
- Infection of WAD goats with *Trypanosoma evansi* (Sokoto isolate) does not produce noticeable gross and histopathologic lesions

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