Comparative study on the pathogenic effects of Diminazine aceturate sensitive and resistant isolates of Trypanosoma congolense in goats

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Abstract

The study was conducted in goats to compare the pathogenic effects of Trypanosome congolense known to be resistant and sensitive to Diminazine aceturate at 7 mg/kg, respectively. Fifteen male goats, 6 to 10 months of age were randomly divided into three groups. The first group (Group A) was artificially infected via intravenous route with the Diminazine aceturate resistant T. congolense isolate while the second group (Group B) received Diminazine aceturate sensitive T. congolense isolate and the third group was left non-infected control (Group C). The goats were clinically monitored for 11 weeks and blood samples were collected three times a week for parasitological and hematological examinations. The mean body temperature was found to fluctuate between (37.2ºC±0.24 ºC and 39.0 ºC ±0.30 ºC 37.5ºC±0.10 ºC and 40.0ºC±0.48 ºC in groups A and B, respectively with statistically significant (p<0.05) differences. A mean parasitaemia score of 2.5±0.48 and 4.0±0.0.50 was recorded in groups A and B, respectively with significant variation (p<0.05). The mean packed cell volume was also varied between 27.0%±0.48% to 18.5%±0.36% and 26.0%±0.25% to 17.4%±0.0.47% in groups A and B, respectively with statistically significant (p<0.05) difference. A marked decline in mean packed cell volume and raise in parasitaemia was noticeable in group B as compared to group A. The difference in packed cell volume among the groups was statistically significant (p<0.05). In the control group, the mean body temperature and packed cell volume recorded was in the physiological range throughout the trial period. The present study disclosed that in spite of differences in the level of parasitaemia, anemia was found to be the prominent clinico-pathological finding in both Diminazine aceturate resistant and sensitive isolates of T. congolense infected goats.


http://dx.doi.org/10.4314/evj.v16i1.5
Introduction

Trypanosomiasis is a protozoan disease caused by various species of Trypanosomes belonging to the genus Trypanosoma which are found in the blood and tissue of their host and generally transmitted by haematophagus arthropod vector (Jordan, 1986). It is one of the most researched diseases in Africa and is known to be a major constraint to livestock production and their products on approximately 10 million km² of land, covering 37 countries (FAO, 2000).

The most important species of Trypanosome affecting sheep and goat are Trypanosoma congolense, Trypanosoma vivax and Trypanosoma brucei. Moreover, species such as Trypanosome simae can also affect sheep and goats (Putt et al., 1980, Radostitis et al., 2006). Trypanosomes show different degree of pathogenicity among the domestic animals which they parasitize. There are a number of trypanocidal drugs available for treating and preventing the disease in endemic areas. These drugs have being used for over many years. Furthermore, as drugs are not always available, are expensive, problem of under dosing and use of some drugs for both prophylaxis and chemotherapy could lead to the occurrence of drug resistance (Sewell and Brocklesby, 1990).

There are observable differences in the pathogenicity between trypanocidal drug sensitive and resistant stocks of trypanosome as reported by different workers. More specifically, there was an observation that Trypanosoma congolense strains which had broken through prophylactic drugs appear in the blood in a very low number at an irregular interval, and were of mild pathogenicity (Hassen, 1996). However, these are limited information on the pathogenic effects of Trypanosoma congolense in small ruminants. Therefore, the major objective of the present study was to compare the pathogenic effects of Diminazine aceturate sensitive and resistant Trypanosoma congolense field isolates in goats under laboratory condition.
Materials and methods

Study description and animals

This study was conducted at the Faculty of Veterinary Medicine, Debre Zeit on a total of fifteen male indigenous goat breed, 6-10 months of age, purchased from an area free of tsetse infestation. During the adaptation period of one month, hematological examinations were done to screen the goats for trypanosomosis and other haemoparasites. Besides, the packed cell volume was monitored during this period. The goats were all treated with Fenbendazole (7.5mg/kg) against helminthosis, and sprayed with acaricide (CBM 8). The goats were ear tagged. They all were kept in one barn during the study period. During the day time, they were let to graze on the nearby field. Water supply was ad libitum.

Study isolates

The study was done with two Trypanosoma congolense isolate, which are identified to be sensitive and resistant to the therapeutic dose of Diminazine aceturate at 7 mg/kg body (Hassen, 1996), respectively.

Experimental design and study protocol

The goats were randomly divided into three groups (Table 1). The first two groups were artificially inoculated intravenously with Trypanosoma congolense isolates and the third group was left as control. Passages were done from donor mice previously infected intraperitonially with the trypanosome isolates. The mice were anaesthetized with chloroform and blood collected from the heart into heparinized syringe. The goats were inoculated with the mice blood with high parasitaemia \(10^4-10^5\) trypanosomes/0.5 ml through the jugular vein. The goats were clinically monitored for twelve weeks. Accordingly, the mucous membrane was examined, the rectal temperature of the goats was recorded and blood samples were taken every morning three times a week for laboratory examination.
Table 1. Experimental design and study protocol.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No of goats</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>5</td>
<td>Resistant to the therapeutic dose of Diminazine aceturate at 7 mg/kg body</td>
</tr>
<tr>
<td>Group B</td>
<td>5</td>
<td>Sensitive to the therapeutic dose of Diminazine aceturate at 7 mg/kg body</td>
</tr>
<tr>
<td>Group C</td>
<td>5</td>
<td>Control</td>
</tr>
</tbody>
</table>

**Laboratory examination**

**Measurement of packed cell volume**

About 5ml blood was collected from the jugular vein directly into a heparinized tube and mixed gently. Following the packed cell volume measurement was done according to (Woo, 1970) and the value was expressed as a percentage.

**Estimation of parasitaemia**

Dark ground buffy coat examination were performed according to the technique indicated by (Murray et al., 1977) for the estimation of parasitaemia. Accordingly, blood was taken in a capillary tube and centrifuged at 1200 rpm for 5 minutes. The tube was cut 1mm below the buffy coat layer and 1cm above the plasma layer, the content was expressed on the slide and covered with slip. The smear was examined under X40 objectives. 20 microscopic fields were examined for a smear and the level of parasitaemia was estimated according to Paris et al (1982).

**Data analysis**

Data such as temperature, packed cell volume and parasitaemia were stored in Microsoft Excel spreadsheet for each group. Data were analyzed using repeated measure analysis of variance in SPSS version 15.0 (2000). The p value for the significant level was considered to be 0.05.

**Results**

**Clinical examination**

Three course of the disease was observed during the study in both infected groups; acute, sub acute and chronic were observed in both infected groups. In group A, the goats showed acute and chronic courses of illness. The acute form was characterized by early onset of illness including lethargy, depression, fever,
anemia within 2 weeks post infection. The chronically sick goats remained parasitaemic up to 11 week post infection.

In group B, a sub acute and chronic course of the disease was developed. The sub acute form of the infection lasted 4 to 8 weeks post infection. During this period there were signs of anemia, weakness and lethargy. In the chronic form, the goats survived with different levels of parasitaemia up to 11 weeks post infection.

**Temperature**

The mean rectal temperature has been fluctuating between 37.2°C±0.24 ºC and 39.0 ºC ±0.30 ºC in group A while it has been fluctuating between 37.5°C±0.10 ºC and 40.0°C±0.48 ºC in group B. The difference in mean rectal temperature between the groups was statistically significantly (p<0.05). In the control group, the mean rectal temperature was maintained within the range 37.0ºC±0.24 ºC and 37.5 ºC ±0.10 ºC throughout the study period (Figure 1).

![Figure 1. Mean weekly temperature of infected and control groups.](image)

**Parasitaemia**

Initial parasitaemia was developed 15 days post infection and the first peak mean parasitaemia of 2.5±0.48 occurred four weeks after infection in group A. The subsequent parasitaemic waves were lower than that initial peak parasitaemia score and the parasite populations were scanty, the average parasitaemia score was 1.28+ during the periods following the initial peak parasitaemia. In group B, however, the parasitaemia developed within 6-9 days post infection. The first peak parasitaemia ±0.50 in DG score was discovered in the third week post infection. Following the first peak of parasitaemia, there
were series of parasitaemia waves with lower scores, the average being 2.6 + (Figure 2). This peak parasitaemia in group B was significantly higher (p<0.05) than that of group A.

![Figure 2. Mean weekly parasitaemia (DG score) of infected groups.](image)

**Packed cell volume (pcv)**

In group A, a decrease in mean packed cell volume from the mean initial packed cell volume of 27%±0.48% to 24.9%±0.41% was registered at the third week post infection. There was a significant decline (p<0.05) in the packed cell volume to 18.5%±0.36% at the fifth week post infection. In group B, there has been decline in mean packed cell volume to 22.2% ± from the initial mean packed cell volume of 26%±0.25% after two week post infection. Further decline up to 16.2%±0.10% was recorded at week four (Figure 3). The decline in the packed cell volume was found to be significant (p<0.05).

![Figure 3. Mean weekly packed cell volume of infected and control groups.](image)
Figure 4. Mean weekly packed cell volume versus parasitaemia for groups A.

Figure 5. Mean weekly packed cell volume versus parasitaemia for groups B.

**Discussion**

Depending on such factors like the concentration of trypanosomes in an inocculum, the strain of the trypanosome and the nature of the host species, trypanosomiasis could have an acute episode lasting for few days and a chronic stages running for longer period of time which may be interrupted by periodic incidents of severe illness or could undergo a spontaneous recovery. In this study three types of clinical pictures were observed during the entire study period. These are acute form, sub acute form and chronic form. Accordingly, the clinical observation in this study showed that in group A, the infected sick goats could not resist the initial peak parasitaemia which was followed by a
rapid decline in the mean haematocrit value at week five post infection. In the sub acute form, the goat survived the first peak parasitaemia but the infection persisted with occurrence of subsequent waves of parasitaemia. These waves of parasitaemia have led to the progressive decline in packed cell volume. As a result, the goats become weak, depressed, and severely anaemic as seen by the decline of the mean haematocrit of 16.2% at week 5 post infections, especially in group B.

As it is the case in most African trypanosomical infections, the initial systemic proliferation of the two isolates reach a peak and then declined. Remissions were short lived and the goats developed a fluctuating chronic parasitaemia up to the end of the study period. The survivors of the infection in both groups have eliminated most of the parasites in the initial parasitaemia, but some of the parasites manage to survive up to the end of the study period. Thus a chronic fluctuating parasitaemia developed in both group A and group B. Similar observations in small East African breed of goat known as the Imbo breed (Mutayoba et al., 1989).

The significantly elevated mean body temperature (p<0.05) that was observed in group B could be attributed to the peak parasitaemia scores, although the extent of the rise in body temperature may or may not reflect the degree of parasitaemia. A similar significant elevation of body temperature at peak parasitaemia observed in group could be attributed to the level of parasitaemia. In all the infected goats, the body temperature fluctuated between 37.2°C±0.24 ºC -40.0°C±0.48 ºC. However, higher temperature peak were occasionally encountered in chronic cases which were not associated with rising parasitaemia. The higher mean body temperature in group B could be attributed to the higher mean parasitaemia levels in the group. The mean body temperature of the control goats in the entire study weeks were in the range of 37.0°C±0.24 ºC and 37.5 ºC ±0.10 ºC.

In group B, parasitaemia has developed in 6-9 days post infection. This finding is not in agreement with the findings of Burundi et al (1994) in which they found an average prepatent period of 3 days post infection for Diminazine aceturate sensitive stock of Trypanosoma congolense. The difference could be attributed to difference in the dose of the innoculum, the no of passages of the Trypanosome stocks, and the age of the goats. Although it is difficult to explain the exact cause and the mechanism underlying the higher parasitaemia scores registered in in group B during the subsequent weeks as compared to group A, it could be due to the difference in the multiplication
rate of the isolates. The individual difference among the goats in response to the parasites multiplication could also be account for the difference in the level of parasitaemia. Similar observations were reported by Mamman et al (1995) who observed that Diminazine-resistant *Trypanosoma congolense* occur at low levels in trypanosome populations.

In group A, the goats became parasitemic within 11-13 days post infection. The finding is almost similar to that of Burundi *et al.* 1994, who reported a prepatent period of 14 days in Diminazinace aceturate resistant *Trypanosoma congolense* stock in mice. The decline in the level of parasitaemia in both isolates especially from week 5 onwards can be attributed to the ability of the host to eliminate most of the population of the parasite following the initial peak parasitaemia.

Anemia is recognized as the most important clinical manifestation of naturally occurring and experimentally induced animal trypanosomiasis (*Murry and Dexter, 1988*). In this study, the artificially induced caprine trypanosomiasis resulted in the marked decline of the hematocrit value as compared to the control goats indicating that anemia is the major pathology as in large animals. There is lack of information regarding the events occurring between the time of inoculation and challenge and the appearance of the parasite in the circulation. Similar observation was reported by Stephen (1986).

The mean packed cell volume of group B was lower than of group B. This could be attributed to the series of highest parasitemic waves occurred during these weeks in the group. In both infected groups the mean packed cell volume has shown a slight improvement from week 5 onwards. This can be attributed to the tendency of the host to reduce the parasite load in the latter weeks of infection. Naturally, it is said that young animals are tolerant to trypanosome infection. Besides, the environmental stress was minimum which may contribute to the improvement in the mean packed cell volume. The control group has a mean packed cell volume ranging 27.0%±0. 20% to 28.2%±0.17% through out the study period.

There was an assertion that drug resistant isolates cause mild anemia (Stephen, 1986). The result of the present study affirmed that there was a difference in the level of anemia between the two groups as evaluated by mean packed cell volume. Diminazinaceturate sensitive *Trypanosoma congolense* isolates have showed higher parasitaemia scores as compared to Diminazine aceturate resistant *Trypanosoma congolense* isolates. In conclusion, anemia as indicated
by decline in packed cell volume was found to be the main pathology in both infected groups. All the animals had developed the disease and there was notable distinction in the clinical pictures of the disease between the groups. The present study has certain limitations which such as small sample size, it was a controlled experiment and the isolates were highly passaged in mice which might have reduced the pathogenecity of the parasites which could in turn affect their pathogenic effects. These points inquire further study with low passed isolates in larger sample animals, preferably under field condition.

References


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