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THE APPLICATION OF ENZYME IMMUNOASSAYS TO ASSESS THE EFFICACY OF CHEMOTHERAPY IN GOATS EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA VIVAX*

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SUMMARY

A group of West African Dwarf goats was experimentally challenged with an isolate of *Trypanosoma vivax* 'Y58' stock. The goats were monitored serologically by using antigen-ELISA and Antibody-ELISA before and after Berenil treatment for a period lasting about sixty two days. The mean prepatent period before trypanosomes were detected in their bloodstreams was five days post-infection; circulating antigens and antibodies were detected in the goat sera at an average of four and five days respectively. While parasitaemia fluctuated with time, both antigens and antibodies were consistently detected until after treatment with Berenil. Following treatment, parasites were no longer detected in the bloodstreams. Both the antigens and antibodies dissappeared between 7 and 11 days post-treatment. The study clearly shows that enzyme-immunoassays have values in validating the host immune responses and the efficacy of chemotherapic control methods in animal trypanosomosis.

KEYWORDS: Trypanosomosis, Drugs, Goats, Diagnosis, ELISA

INTRODUCTION

Trypanosoma vivax is one of the three most important pathogenic trypanosomes causing trypanosomosis in livestock in sub-sahara Africa. The other two important species are *T. congolense* and *T. brucei.* A lot of information already exists in the literature concerning the serious impact which trypanosomosis has on livestock production in the region (Swallow, 2000). Inspite of internationally coordinated efforts to control the disease, cases of herds of trypanosusceptible livestock being devastated by the disease are still being reported (Swallow, 2000). Currently, trypanocides remain the principal approach to controlling the disease in animals. The method is not only expensive, but complicated by the fact that the efficacy of these drugs is diminishing fast in some areas due to the development of drug resistance by the parasites.

Central to the need to maximise the efficacy of any international or regional efforts aimed at preventing and controlling trypanosomosis is the availability of sensitive diagnostic techniques to accurately determine the prevalence of the disease in animals. Recently, enzyme immunoassays have been developed which are now the preferred techniques for the diagnosis of animal trypanosomosis in view of the high sensitivities they offer (Masake and Nantulya, 1991). This study was therefore designed to evaluate the usefulness of enzyme immunoassays in monitoring the disease and consequently assessing the efficacy of chemotherapy in goats experimentally challenged with *T. vivax.*

MATERIALS AND METHODS

Experimental animals

Ten West African Dwarf (WAD) female goats which were in-house bred were used for the infection. The goats aged between six and eight months and their weights ranged from 15 to 18kg. Prior to experimental infection, the goats were tested for previous exposure to trypanosome infection by parasitological and serological methods. They were also de-wormed with anthelminthics. The animals were housed in a fly-proof isolation pens and fed *ad. libitium.*

Trypanosome

An isolate of T. vivax stock - ILRAD Y58 (Leaflang *et. al.,* 1976) was used for the experimental infection. The stock was initially maintained by serial passage in mice until required for the goat inoculation.

Experimental design

The experimental goats were randomly divided into two groups of five goats each, housed in separate fly-proof pens. One group was used for the trypanosome inoculation while the other served as control. Each goat in the experimental group was first bled before inoculating with $1x10^6$ trypanosomes (diluted with sterile normal saline) intravenously through the jugular vein. The infected goats were monitored routinely and when its packed cell volume (PCV) values were observed to be very low, they were treated with Berenil (Diminazene aceturate, Hoechst, Germany) at a dose of 7mg/kg body weight. After drug treatment the animals were further monitored until the termination of the experiment.

Monitoring of infections

Following inoculation, all goats were initially bled daily until parasites were detected and then twice weekly for the determinations of PCV (%), parasitaemia, and the levels of antibody and antigen productions. The buffy-coat technique (BCT) as described by Murray *et. al.,* (1977) was used to detect the levels of parasitaemia and PCV.

The serum samples were separated from blood cells and stored until required for serology.

The *T. vivax* antigen used for the antibody-ELISA was prepared as described by Ijagbone *et. al.*, (1989) and the ELISA performed following the technique described by Luckins and Mehlitz (1979). The ELISA kit used for the Antigen ELISA was obtained from the International Atomic Energy Agency (IAEA) laboratory, Vienna, Austria and the test performed according to the method described by Nantulya and Lindqvist (1989). Mean optical density (OD) readings of immunoassays of the serial serum samples were determined and used to determine the pattern of antigen and antibody productions in the infected goats pre- and post-treatment.

RESULTS

Parasiteamia

The average prepatent period was 5 days post-infection. Generally, parasitaemia was low in all the infected goats, producing only few peaks until the animals were treated (Fig. 1). In the routine blood examinations, trypanosomes were not often detected by the BCT and no single animal died as a result of the infection before and after treatment on day 30 post-infection although they appeared weak. Treatment with Berenil was generally effective since all the

Trypanosomosis animals treated were BCT negative during subsequent blood examinations until the termination of the experiment. Anaemia was associated with infection as indicated by the low PCV values recorded among the infected goats (Fig. 2) and which coincided with the onset of parasitaemia in their blood. The lowest average PCV was 14% before treatment and there was a general upward trend after treatment although it never reached the pre-infection values.

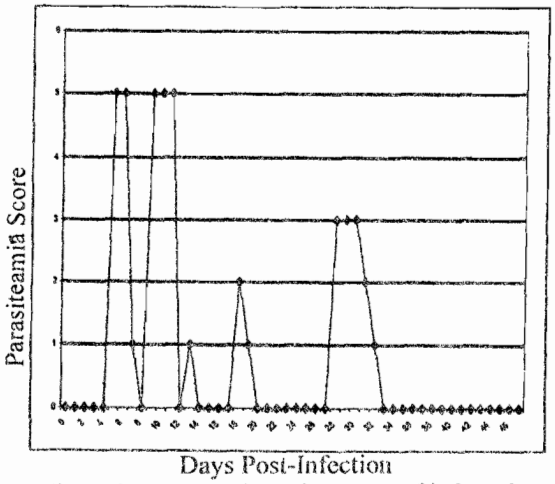

Fig. 1: Mean Parasiteamia scores of infected Goats

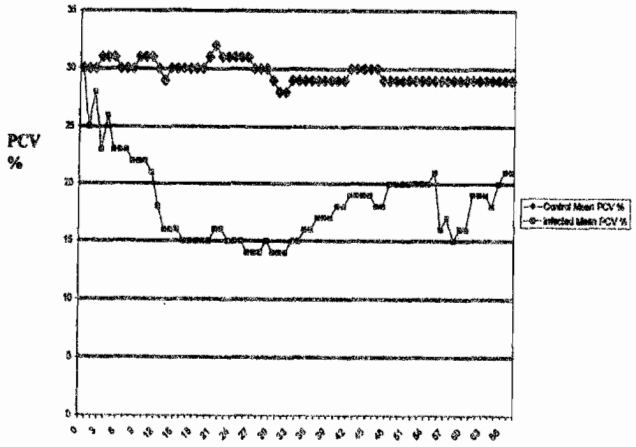

Fig. 2: Mean PCV Values of control and Infected Goats

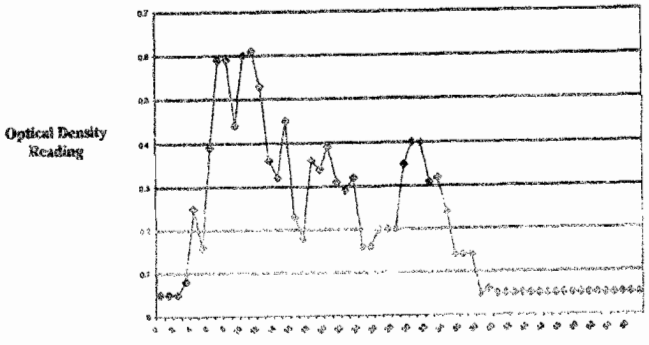

Fig.3: Mean AG-ELISA OD Values of Infected Goats

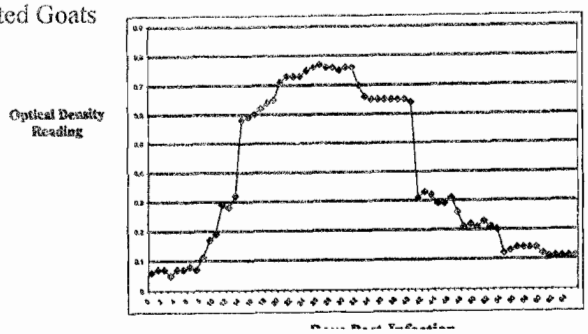

Fig.4: Mean AB-ELISA OD Values of Infected Goats

Detection of antigens and antibodies

Circulating antigens were detected by the Antigen-ELISA 4 days post-infection coinciding with the patency of parasitaemia in most of the infected goats (Fig. 3). Thereafter, the ELISA values showed a progressive increase until day 23 post-infection. The ELISA consistently gave positive readings which fluctuated, even on occasions when parasites were not demonstrable in the peripheral blood. After chemotherapy Ag-ELISA values decreased rapidly and fell to pre-infection values within 7 days post-treatment.

With regards to antibody levels, ELISA values were detected as from day 8 post-infection and increased steadily until day 26 post-infection (Fig. 4). After chemotherapy, Antibody-ELISA values stabilised for a while and declined after 11 days post-treatment. The decline continued until the end of the experiment, although values were still higher than pre-infection levels even when parasites when the trypanosomes were no longer detected in the sera.

DISCUSSION

Seromonitoring of the experimental goats afforded the detection of the circulating antigens and antibodies following infection. The detection of antigens and antibodies in the serum of infected goats even on occasions when trypanosomes were not detected in the peripheral bloodstreams before treatment confirms the sensitivity of immunoassay techniques over parasitological detection methods as earlier reported by other workers (Rae and Luckins, 1984; Masake and Nantulya, 1991). After the period of pre-patency, progressive increases in the levels of antigens and antibodies were recorded, which reached peak levels and thereafter declined even before treatment with Berenil.

This trend was more pronounced in the Ag-ELISA values. The pattern of infection observed appears to be consistent with host-parasite relationship in trypanosomiasis. There was the early systemic proliferative parasite phase, resulting in the parasitaemic peak and corresponding increase in antigenic levels. Expectedly, the antitrypanosomal antibodies produced might have eliminated most of the parasites from the bloodstreams, thus reversing the course of infection. Host antibodies have been known to be trypanocidal in function (Nantulya *et. al.*, 1985).

Another factor that could have been responsible for the low levels of observed parsitaemia might have been as a result of the trypanotolence nature of WAD goats used for the experiment which are capable of resisting and controlling parasitaemia (Murray *et al.*, 1979).

After treatment, ELISA values completely subsided, and no recurrent peaks of antigens and antibodies were subsequently observed. Parasites were also not detected in the bloodstreams. Recurrent phenomenon in trypanosomal infections is regarded as an indication of exhaustion of the antigen reportoire responsible for antibody production. According to Wilson and Gunningham (1970), non-occurrence of antibody peaks is suggestive of self-cure or successful chemotherapeutic treatment as was the case in this study.

The use of enzyme-immunoassays was therefore successful in monitoring the immune response and assessment of the efficacy of drug chemotherapy in the infected goats.

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