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Comparative Analysis of the Dark Ground Buffy Coat Technique (DG) and Enzyme-Linked Immunosorbent Assay (ELISA) in the Diagnosis of Trypanosomosis in Cattle

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Target Audience:

Abstract

The prevalence of typanosome infection in 65 cattle reared under expensive system of management was determined using the dark ground buffy coat (DG) technique and the enzyme-linked immunosorbent assay (ELISA). The DG technique showed that there were 18 positive cases (27.69%) of total number of animals, made up of 6 of Trypanosoma congolense, 7 of T. Vivax and 3 of T. Brucei. Other cases were those of mixed infections mainly one case of which all of 3 Trypansomes species were present, and another in which T. vivax, 12 of T. brucei and 2 cases of T. brucei were 30, (46.15% positive cases made up of 6 of T. congolense, 7 of T. vivax, 12 of T. brucei and 2 cases of T. brucie mixed with T. Conglolese. The ELISA technique did not detect any incidence of mixed infections in which the 3 species of trypanosomes were present.

Keywords: *ELISA, Buffy coat, Trypanosomosis, cattle*

Description of Problem

Trypanosomosis is one of the most important constraints to livestock production in the humid and sub-humid tropics of Africa(1). It has therefore becomes very pertinent that various measures have to be taken for early detection, treatment and control of this disease in livestock. This assertion has led to emergence of various control measures such as eradication of tsetse fly, chemoprophylaxis and achemotherapy (2,3). The control of this deadly disease also depends on the quality of diagnostic techniques such as ELISA (45,6,) in use.

The superior ability of infected trypanotolerant animals like the N'dama to control parasitaemia, more often results in low parasitaemia during infection in this group of animals (7). This type of infection may therefore be undetectable by even the best parasitoltogical test. Serology has been reported to offer a diagnostic alternative in such cases (8). However, the disadvantage of most serological technique is that they tend to have low specificity and unable to differentiate between species and between current and previous infection(9). The development of the highly sensitive ELISA offers an opportunity in which these disadvantages can be significantly reduced. In this study, attempt is made to compare the dark ground buffy coat and ELISA techniques so as to establish the desirability of using both techniques in arriving at a definitive diagnosis.

Materials and Methods

The blood of sixty-five white Fulani breed of Cattle in Orieran and Iresa-Adu in Surulere Local Government Area of Oyo State were screened in this investigation. These animals were reared under extensive system of management such that they were constantly exposed to tsetse bite for a blood meal both during the day and at night.

Blood samples were collected by jugular puncture into haperinised capillary tubes. The samples kept on ice were examined for trypanosome parasites some few hours after collection by the DG technique as described by Murray et al (10) with slight modification. Positive samples were stained with Giemsa. Trypanosome species identification was based on speed, types of movement elicited as well as morphology of the stained organism. Antigen detection in sera samples was done using antigen-ELISA technique (6,15). Antigen-ELISA technique is the qualitative detection of the presence or absence of species specific trypanosomal antigen in the sera of the animals.

Results and Discussion

The prevalence of trypanosome infection among the 65 white Fulani cattle as determined by the DG buffy coat method and ELISA technique is presented in table 1. Six, 3 and 7 animals were infected exclusively by T. congolense, T. brucei and T. vivax, while another animal had a mixed infection of T. vivax and T. congolense. Thus, the overall prevalence of trypanosomosis in the folck studies using the DG technique was 27.6%. When the same samples were examined by ELISA technique 7, 6 and 12.

Table 1: Prevalence of Trypanosome among 65-white fulani breed of cattle as detected by DG and ELISA techniques.

Species of Trypanosome DG			ELISA	
	Number Positive	%Positive	Number Positive	%Positive
T. congelense	6	9.23	6	9.23
T. vivax	7	10.77	7	10.77
T.brucie	3	4.62	12	18.46
T.congolense+T.brucei	0	0	3	4.62
T.congelense +T.vivax	0	0	0	0
T.brucei+T.vivax	1	1.59	2	3.08
T.congolense+T.brucei+T.vivax	1	1.59	0	0
Negative.	0	0	0	0
Total	18	27.69	30	46.15

samples showed specific antibodies to T.vivax, T. congolense and T. brucei respectively (Table 1). The distribution of "mixed" infections as detected by ELISA test showed that 2 animals had T. brucei and T. vivax while 3 other animals had T. brucei andT. Congolense. Animals infected with T. congolense had the highest antibody titres as reflected by the higher optical density (O.D) readings.

The results of the present investigation indicate that some of the white Fulani cattle reared under the extensive system of management at Surulere Local Government Area of Oyo State were infected with trypanosomes. The ELISA technique showed that a larger proportion of the herd was infected with trypanosomes (46.2%) whereas the DG buffy coat only detected a lower number of 27.7% in the stock. It is clear that flock experience with the infection was higher than the parasitological results would lead one to believe. Combining the 2 techniques mean that better result can be obtained as against using one of the techniques.

Bovine trypanosomosis in these herds was mostly caused by T. vivax and T. brucei, and closely follwed by T. congolense. Nine cases were diagnosed by DG for T. vivax. For T. brucei 5 cases were diagnosed using DG. For T. congolense 7 cases were diagnosed by DG technique.

The result of this investigation confirmed the findings that T. vivax is predominant during dry season. This survey was carried out during the dry season in the derived Savannah zone of Nigeria to which the Surulere local government Area falls into (9). It is also reported that there may be preponderance of T. vivax all the year round (11). The relatively lower preponderance of T. congolense in this result also confirmed other findings (9,12) that T. congolense was found to be preponderant during the wet season. High level of T. brucei was detected in this study. ELISA studies showed that 12 of the 17 cases were detected by this technique. This is so because T. brucei is tissue - invasive, hance cannot be easily detected by DG technique (2).

The implication of this is that while ELISA was able to detect the trypanosome in some samples, it was not so with the other technique and vice-versa. The lower rate of infection observed in several reports (13, 14) would have been due to the use of routine parasitological techniques which would have missed out some infection when compared to ELISA (15). However, the inability of serological test to differentiate between active and non-active trypanosomosis in natural infections still remains a major limiting factor against their routine use (5).

From the above, it thus appears practically impossible to accurately detect all infected animals in a field situation where dynamic cycle of infection, reinfection, super-infection and remission occurs. Therefore, the use of 2 or more diagnostic techniques that are sensitive and have the potentials of field application to a large number of sample cannot be overemphasized in diagnosis of animal trypanosomosis. The antigen-ELISA so far appears to be a promising field diagnostic tool in conjunction with other standard parasitological methods (15, 16).

Conclusion and Applications

This study has shown that the use of more than one diagnostic tool in arriving at definitive diagnosis of trypanosomosis is desirable. It is therefore suggested that this should be given adequate attention if desired result of effective control of this all-important disease is to be ralized.

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