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COMPARATIVE APPLICATION OF ANTIGEN DETECTION ENZYME-LINKED IMMUNOSORBENT ASSAY AND BUFFY COAT PARASITOLOGICAL TECHNIQUE FOR DIAGNOSIS OF BOVINE TRYPANOSOMOSIS IN NIGERIA.

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SUMMARY

Antigen-detection enzyme-linked immunosorbent assay (Ag-ELISA) and buffy coat parasitological technique (BCT) were employed for the diagnosis of bovine trypanosomiosis in trade cattle slaughtered at the Bodija Municipal abattoir in Ibadan, Oyo State, between March and November, 2002 and in some sedentary herds located in Oyo, Ondo and Kwara States of Nigeria between September and November, 2002. The results obtained by the BCT showed that the prevalence rates of the disease were: 8.5%; in trade cattle and 9.2%; 14.6%; 16.8% in herds in Oyo, Ondo, and in Kwara State respectively. Comparatively, the use of Ag-ELISA gave higher prevalence rates as follows: trade cattle 16%; Oyo State 16.4%, Ondo 25.0% and Kwara 25%. At species level *Trypanosoma congolense* was the most frequently detected parasite by both the BCT and Ag. ELISA, revealing a shift in the occurrence of the species in cattle against the background that *T. vivax* is the most commonly found trypanosome species in Nigeria. With these results, Ag. ELISA has an obvious role in the epizootiological studies of bovine trypanosomiosis in Nigeria.

KEYWORDS: Tryposomosomosis, Bovine, ELISA, Buffy-coat, Diagnosis

INTRODUCTION

Bovine trypanosomiasis is still a major threat to livestock production in many parts of sub-Saharan Africa. However, in Nigeria records show that for the past three decades, the disease has been diminishing in cattle (Ikede 1986; Anosa 1991). This decline has been attributed to a number of factors which include vehicular transportation of trade cattle across the country thus reducing contact with the vector (tsetse fly), ecological changes brought about by large scale farming and irrigation which distort the natural habitats of the fly and organized disease and vector control programmes of the Government.

Hitherto, the diagnostic tools employed in the surveys of the disease in Nigeria were mostly based on the Standard Trypanosome Detection Methods (STDM) which comprise wet mount, thin and thick stained blood films and animal inoculation. The well known limiting factor associated with this conventional parasitological detection approach is that the methods sometimes yield false results particularly in chronic infections because of the ability of the trypanosomes to evade the blood stream of the hosts from where they are usually detected (Nantulya, 1990).

With the availability of improved diagnostic tools such as enzyme immunossay techniques, it is therefore necessary to re-appraise the status of the disease in cattle in Nigeria. In line with this objective, this study was undertaken to

utilise the antigen-detection enzyme linked immunoassay (Ag- ELISA) in comparison with the conventional buffy coat technique (BCT) for field diagnosis of bovine trypanosomosis in three states of Nigeria and in trade cattle at the Ibadan municipal abattoir, Oyo State, Nigeria.

MATERIALS AND METHODS

Blood sampling

Trade Cattle

Bodija municipal abattoir, belonging to the Ibadan North Local Government, Oyo State, served as the source of trade cattle from which blood and serum samples were obtained. The breeds of cattle normally slaughtered at the abattoir are mostly the White Fulani Zebu which originates from the far Northern States and neighbouring countries. Samples were collected in the months of March through November 2002 (nine months). Blood was collected from the severed jugular vein of slaughtered cattle for serum into dry and sterile Universal and Bijoux bottles (containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Thereafter, smears were made from the EDTA blood, fixed with methanol and Giemsa-stained.

Packed Cell Volume (PCV) values were determined for each blood sample to determine the degree of anaemia, and the buffy coat was examined for trypanosomes. Serum samples were separated and kept at -20^0C until required for the enzyme immunoassay.

Sedentary herds

The cattle were traditionally managed herds located in Oyo and Ondo States in South-Western Zone and Kwara State in the North Central Zone of Nigeria where trypanosomosis is known to be endemic. Majority of the herds were the White Fulani Zebu breed and while a few were Muturu and Keteku. These States fall in the derived Savanna vegetation zone which is usually infected by *Glossina tachinoides and G. palpalis* (Davies 1977).

Cattle at each zone were sampled during a single visit from September to November 2001. Blood samples for serological and parasitological examinations were collected from amongst the cattle and on the spot, direct blood smears were made, while suspected cases were examined by met-mount microscopical examination for trypanosomes directly. Samples were then transported to the laboratory in iced-packs. In the laboratory, the sera were separated and stored at -20^0C in deep freezer until analysed, while parasitological examinations were carried out immediately.

Laboratory examination of samples

Parasitology

Unclotted blood samples collected from abattoir and sedentary herds were first examined for the presence of trypanosomes by the buffy coat technique (BCT) as described by Murray *et. al.* (1977). Briefly, capillary tubes were filled with the test blood samples with both ends of the tubes sealed with plasticine. The tubes were placed in the haematocrit centrifuge and centrifuged for ten minutes after which the packed cell volumes of the samples were read and recorded. Thereafter the tubes were cut into two pieces with a diamond marker at the junction of the buffy coat layer between the packed red blood cells and the plasma fluid. The layer was dropped on a glass slide for wet-mount examination for trypanosomes and smearing. The smears were subsequently stained with Giemsa and examined for trypanosomes and other blood parasites.

Serology

Screening of sera for the presence of trypanosome antigens was carried out with the FAO/IAEA/ILRAD Ag-ELISA kits, obtained from the International Atomic Energy Agency (IAEA) Vienna, Austria. The test was carried out following the procedure as described by Nantulya and Lindqvist (1989). A sample was considered positive for trypanosome antigens if the optical density (OD) value measured at 414 nm was 0.050 (Absorbance) compared with reference values obtained for the standard serum sample supplied with the text kit.

RESULTS

Parasitological findings

Abattoir samples

Out of the total number of 2,257 blood samples collected from trade cattle at the abattoir for the period of nine months, 196 animals (8.7%) had diagnosable parasitaemia as shown in Table1. *Trypanosoma congolense* was the most common species found in 108 among the infected (55%), followed by *T. vivax* found in 70 animals (34%) and *T. brucei* was found in only 10 animals (5%). Eight cattle (4%) had mixed infections of *T. congolense* and *T. vivax*. *Trypanosoma* were detected in animals throughout the months covered during the investigation. Other blood parasites defected were eight cases of *T. theileri* and 12 of *microfilaria* in the samples.

Sedentary herds

Sampling

A total of 911 blood samples were obtained from the different herds in the three states visited. Table II shows the results of the laboratory analyses carried out on the samples. Out of the total 305 cattle sampled in 11 herds in Oyo State, 28 (9.2%) were infected as detected by BCT with *T. congolense* accounting for 68% while *T. vivax* represented only 32% of the infections. *T. brucei* was not detected in Oyo State. In Ondo State, 43 of the 296 cattle bled in eight different herds were infected with trypanosomes giving an infection rate of 14.6%. *T. congolense* was the predominant species detected (62.8%) followed by *T. vivax* (32.6%) and *T. brucei* (4.6%) Among the samples obtained from ten herds in Kwara State, 52 of the 310 cattle (16.8%) were positive. *T. vivax* was the most frequently detected species (51.9%) followed by *T. congolense* (36.5%), and *T. brucei* (7.7%). Two cases of mixed infections of *T. vivax* and *T. congolense were* recorded.

TABLE 1: Frequency of trypanosome infections in Trade Cattle slaughtered between March and November 2002 at the Bodija abattoir detected by Buffy coat method

Month	Sample size	Tc	Tv	Tb	MixedTc/Tv	Tt	Mf	Total	(Rate)
March	308	16	7	1	0	0	0	24	(7.8%)
April	261	9	4	0	2	1	1	15	(5.7%)
May	212	10	3	1	0	3	3	14	(6.7%)
June	137	7	0	2	0	1	1	9	(6.5%)
July	102	7	2	0	0	1	0	9	(8.8%)
August	319	19	11	0	2	0	2	32	(10.0%)
Sept.	342	13	16	1	3	0	3	33	(9.6%)
Oct.	313	11	17	1	0	2	0	29	(9.2%)
Nov.	263	16	10	4	1	0	2	31	(11%)
Total	2257	108	70	10	8	8	12	196	(8.7%)

Tc = *T. congolense;* Tv = *T. vivax;* Tb = *T. brucei;* Tt = *T. theileri;* Mf = *microfilaria*

TABLE II: Prevalence of bovine trypanosomosis in some sedentary herds located in three States of Nigeria from September - November 2002

State	No. of herds	Sample size	Tests	No. positive (%)	Tc(%)	Tryps. Spp. Detected		
						Tv (%)	*Tb(%)*	*Tc/Tv(%)*
Oyo	11	305	BCT	28(9.2)	19 (68)	9 (32)	0	0
			Ag. ELISA	50 (16.4)	21 (42)	14 (28)	1(0.2)	14 (28)
Ondo	8	296	BCT	43 (14.59)	27 (63)	14(33)	2(0.5)	0
			Ag. ELISA	74 (25.0)	32(43)	22(30)	5(0.7)	15(20)
Kwara	10	310	BCT	52 (16.8)	19(37)	27(52)	4(8)	2(4)
			Ag. ELISA	78 (25.0)	31(40)	27(35)	(6-8	14(18)
Total		911						

BCT = Buffy coat technique
Ag. ELISA= Antigen-detection enzyme-linked immunosorbent assay

The overall infection rate, as detected by buffy coat and stained smear examinations across the three states was 12.5% with *T. congolense* (52.8%) , followed by *T. vivax* (40.6%) and *T. brucei* (4.9%). Comparatively Kwara State had the highest infection rate of 16.8%, followed by Ondo State (14.6%) and Oyo State (9.2%).

Antigenaemia (Antigen-detection)

Abattoir samples

Circulating antigens of trypanosomes were detected in a total number of 362 serum samples representing 16% of the total trade cattle sampled. An analysis of the results shows that 164 (out of 196) animals with patent parasitaemia had circulating antigens in their sera, indicating an apparent agreement of 83%. One hundred and sixty-six out of the parasitologically negative samples had circulating antigens of trypanosomes in their sera. *Trypanosoma congolense* was again the predominant trypanosome species detected in the Ag-ELISA accounting for 45% of the infections (163/362), followed by *T. vivax* in 33% (111/362) and *T brucei* 9% (32/362). Mixed infections involving *T. congolense* and *T. brucei* were detected in 30 sera (8%), while *T. congolense* and *T. vivax* were together detected in 19 cases of the infections (5.2%).

In comparison with buffy coat technique (BCT), antigen-detection ELISA yielded higher trypanosome positive results, indicating that the test picked more reactors which were previously undetected by the parasitological method. In both the tests performed, *T. congolense* consistently remained the most frequently detected trypanosomes, followed by *T. vivax* and *T. brucei* among cattle slaughtered at Bodija, Ibadan. The overall prevalence of trypanosomosis at the abattoir was found to be 12%.

Sedentary herds

A breakdown of the pattern of infection as determined by Ag-ELISA showed that 78 (25.0%) of 310 sera tested positive for circulating trypanosomal antigens (Table II) in Kwara State; 50 (16.4%) of 305 sera were positive in Oyo State; while 74 (25.0%) of 296 sera tested positive in Ondo State.

Overall, there was 88% agreement between parasitaemia and antigenaemia, whilst 20% of the negative parasitaemic samples had circulating antigens. *T. congolense* had the highest circulating antigens (42%), followed by *T. vivax* (31%), and then *T. brucei.* But mixed infections of *T. congolense* and *T. vivax* represented 20%.

DISCUSSION

The results of the parasitological investigations on trade cattle arriving for slaughter at the Bodija abattoir indicated a prevalence of 8.7% which agrees with previous findings at the same abattoir. Reynolds and Opasina (1987) examined 3,727 blood samples during a-one-year survey period and found 313 (8.4%) infected with trypanosomes. A year later, Opasina and Ekwuruke (1988) reported 8.5% infection rate in a similar survey at the same abattoir. Based on parasitological results obtained in this study, it would appear that no reduction in the prevalence of trypanosomosis among trade cattle has occured during the past decade. Concerning sedentary herds, parasitological results indicating between 10 and 17% infection rates across the surveyed compared well with previous studies carried out in the same areas (Ikede *et. al.*, 1987).

The inadequacies of parasitological methods as means of diagnosis of trypanosomosis have long been recognised (Gray, 1965; Molyneux, 1975; Luckins and Mehlitz, 1979; Nantulya, 1990; Luckins, 1993). In view of this, results based on the parasitological investigations may not reflect the true infection rates of the disease. Applying Ag-ELISA, higher infection rates were recorded both in trade cattle (11.4 - 18%) and sedentary herds (20 - 23%). Ag-ELISA has been shown to be a more valuable tool of diagnosis due to its high sensitivity (Luckins, 1993; Nantulya, 1990). The results of the present study support these findings.

The explanation for the vast differences between the sensitivity of parasitological and serological techniques is that they measure different elements. While the parasitological techniques detect only trypanosomes present in the peripheral blood circulation, the Ag-ELISA on the other hand detects soluble antigens released by trypanosomes into the surrounding fluids (Luckins, 1993). The number of trypanosomes in peripheral circulation, however, is often too small in chronic infections for detection by parasitological techniques, despite the fact that there may be many trypanosones in other body organs such as spleen, liver, and lymphnodes (Nantulya, 1990). The sensitivity of these tests does not therefore depend upon parasite numbers in peripheral circulation, hence, the high sensitivity even when there may be no detectable parasitaemia.

The results of the ELISA applied in this study re-emphasize the importance of trypanosomosis as a major constraint on livestock production in Nigeria. The recorded high prevalence of the disease removes the erroneous belief that bovine trypanosomosis is becoming insignificant in Nigeria (Ikede, 1986). Therefore, the testing of sera for the presence of trypanosome antigens in the body fluids of the cattle could provide additional although indirect information on bovine trypanosomosis in the country. Furthermore, since the assays detect specific antigens of the trypanosomes, the causative species can then be determined. Relatively, it was revealed in this study that *T. congolense* was the most prevalent trypanosome in both the sedentary herds and trade cattle. However, more surveys need to be carried out in other parts of the country to verify this trend.

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