MONITORING OF A TSETSE AND TRYPANOSOMOSIS CONTROL PROGRAMME IN PLATEAU AND BAUCHI STATE, NIGERIA, USING ANTIGEN ELISA AND PARASITOLOGICAL TECHNIQUES

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Abstract

MONITORING OF A TSETSE AND TRYPANOSOMOSIS CONTROL PROGRAMME IN PLATEAU AND BAUCHI STATE, NIGERIA, USING ANTIGEN ELISA AND PARASITOLOGICAL TECHNIQUES.

Between July 1994 and January 1995 a total of 1153 samples were collected from cattle in Plateau and Bauchi State, Nigeria, and analysed for the presence of trypanosome infections using parasitological (Buffy Coat Technique [BCT] and blood film smears) and serological techniques (Ag-ELISA). A simple random sampling technique was employed. Tsetse flies and other insects were trapped during the same period using NITSE and biconical traps. Twenty two tsetse flies (6 *Glossina p. palpalis*, 3 *G. longipalpis* and 13 *G. tachinoides*) were caught, identified and dissected to check for trypanosomal infections. The results obtained using parasitological techniques showed an average prevalence rate in the two states surveyed of 3.4%. The antigen-capture ELISA technique (Ag-ELISA) was used to analyse 280 serum samples which were negative for trypanosomes when checked by BCT. Of these samples none were positive for *T. congolense* and 4 (1.4%) were detected positive for *T. brucei*. A subset of 120 samples was analysed for the presence of *T.vivax* and 3 (2.5%) were found to be positive. The relative specificity of the Ag-ELISA for *T. brucei*, *T.congolense* and *T. vivax* was 98.5%, 100% and 97.5%, respectively.

1. INTRODUCTION

African trypanosomosis has been recognized as a major constraint to livestock production in Nigeria, and concerted efforts have been made to control it. The main vector of the disease is the tsetse fly (*Glossina* spp.). There are four species of importance in the transmission of trypanosomosis in Nigeria, *G. palpalis*, *G. longipalpis*, *G. morsitans morsitans* and *G. tachinoides*. The disease in cattle is caused in Nigeria by *Trypanosoma vivax*, *T. brucei* and *T. congolense*. Tsetse and trypanosomosis were recently detected on the Jos Plateau, previously thought to be too high in altitude for the survival of tsetse flies [1, 2]. Furthermore, an outbreak of *T. vivax* infection in cattle was reported from an area near lake Chad, a supposedly tsetse free zone [3].

As far back as 1930 control measures against tsetse were initiated, including bush clearing, spraying with insecticides, the use of traps and more recently biological control methods e.g. the release of sterilize d insects. Each technique has had various levels of success in tsetse eradication in the country. Parasitological techniques used for diagnosis in Nigeria included the examination of stained blood smears and wet blood films. However, these techniques have a limited sensitivity, especially for detecting chronic trypanosome infections. The recent introduction of the Buffy Coat Technique (BCT) has improved diagnostic capabilities [4]. Recently, another approach to diagnosis was reported based on the detection of trypanosomal antigens circulating in the blood of infected cattle [5]. Improvement in the diagnosis of bovine trypanosomosis using more sensitive immunoassay techniques could make a significant contribution to the monitoring of disease control in Nigeria. The objective of the present study was to use the antigen capture Enzyme Linked Immunosorbent Assay (Ag-ELISA) in the monitoring of a tsetse eradication campaign in Plateau and Bauchi State, Nigeria.

2. MATERIALS AND METHODS

2.1. Parasitological survey

A survey for the prevalence of trypanosome infections in 50 herds of cattle was conducted during the wet and dry season between July 1994 and January 1995. Fourteen local government areas

(LGAs) in Plateau and Bauchi State in three different vegetational zones (montane, northern guinea savannah and southern guinea savannah) were surveyed. Fourty herds located in 13 LGAs in Plateau State and 10 herds in one LGA in Bauchi State were visited (Table I). A total of 1175 blood samples were collected for parasitological and serological analysis. The parasitological techniques used were the BCT and thin blood smears. Mice and rats were subinoculated for species confirmation. The packed red cell volume (PCV) was determined. Various breeds of cattle were sampled including White Fulani, Sokoto Gudali, Wadara, Friesians and crossbreds. A simple random sampling strategy was used to collect blood samples by jugular venepuncture into vacutainer tubes (heparinized and non-heparinized). Samples were transported to the laboratory in coolboxes. Blood smears were prepared immediately following bleeding and fixed with methanol. Serum samples were separated from clotted blood and stored at -20°C awaiting analysis.

TABLE I. PREVALENCE OF BOVINE TRYPANOSOMOSIS IN PLATEAU AND BAUCHI STATE, NIGERIA, AS DIAGNOSED BY PARASITOLOGICAL TECHNIQUES

State	LGA	Veg. zone	No. of animals sampled	No. pos.	% pos.	*T.v.*	*T.c.*	*T.b.*	N.I.
Plateau	Jos north*	n.guinea	61	10	16.3	3	7	-	-
	Jos south	montane	547	8	1.5	1	7	-	-
	Bassa		37	0	0	-	-	-	-
	Barkinladi		20	0	0	-	-	-	-
	Mangu		31	3	9.7	-	3	-	-
	Pankshin		31	0	0	-	-	-	-
	Wase	n.guinea	67	3	4.9	1	-	-	2
	Shendam	s.guinea	49	5	10.2	-	1	-	4
	Lafia	s.guinea	49	4	8.2	1	3	-	-
	Keffi	s.guinea	30	0	0	-	-	-	-
	Akwanga	s.guinea	49	2	4.1	1	1	-	-
	Kanam	s.guinea	23	0	0	-	-	-	-
	Nassarawa	s.guinea	43	0	0	-	-	-	-
Bauchi	Tafawa Balewa	n.guinea	116	7	6.0	7	-	-	-
Total			1153	42	3.6	14	22	0	6

LGA = local government area.
Veg. zone = vegetational zone.
No. = number; No. pos. = number of animals detected positive.
T.v. = *Trypanosoma vivax*; *T.c.* = *T. congolense*; *T.b.* = *T. brucei.*
N.I. = trypanosome species not identified.
* sampling site at the boundary between Plateau and Bauchi State.
n. guinea = northern guinea savannah; s. guinea = southern guinea savannah.

2.2. Serological survey

Serum samples were screened for the presence of trypanosomal antigens using the FAO/IAEA antigen capture Enzyme Linked Immunosorbent Assay (Ag-ELISA) and the FAO/IAEA manual, a modification of the protocol described previously [5]. A threshold value of 10 percent positivity (PP value) was established. The cut-off point of 10% was obtained by measuring the average optical density reading of serum samples from animals which had PCV values ≥28 and which were parasitologically negative.

2.3. Entomological survey

Two types of traps, the NITSE [6] and biconical trap [7] were used in three vegetational zones and in 14 LGAs of Plateau and Bauchi State between July 1994 and January 1995. Traps were placed around the herd and near the watering point closest to the herd. The traps were checked at regular intervals during a period of 24 hours. The tsetse caught in the traps were transferred to special fly cages and transported to the nearest veterinary laboratory for identification and dissection.

3. RESULTS

3.1. Parasitological results

Using standard parasitological techniques 42 (3.4%) of the 1175 samples were found to be positive for trypanosomes (Table I). More samples were detected to be positive by BCT than by thin blood smear technique. *Trypanosoma vivax* and *T. congolense* were detected in both Plateau and Bauchi state. *T. brucei* was not detected using the thin blood smear or the BCT. Of the 42 samples, 14 (33.33%) were positive for *T. vivax*, while 22 (53.33%) were positive for *T. congolense*. Parasites in six out of the 42 samples could not be identified due to poor quality of the blood smears.

3.2. Serological results

A total of 280 samples were tested by Ag-ELISA for *T. brucei* and *T. congolense* and a subset of 120 samples were screened for the presence of *T. vivax*. Initial results indicate that four animals (1,4%) were detected positive for *T. brucei* (Table II), none for *T. congolense* (Table III) and 3 (2.5%) were positive for *T. vivax* (Table IV).

TABLE II. BUFFY COAT TECHNIQUE AND ANTIGEN ELISA RESULTS FOR *TRYPANOSOMA BRUCEI*

			BCT results		Total
			Pos	Neg	
ELISA	results	Pos	0	4	4
		Neg	0	271	271
		Total	0	275	275

Relative specificity = 271 / 275 x 100 = 98.5 %.
pos. = positive; neg. = negative.

3.3. Entomological results

As a result of the entomological survey 22 tsetse flies were caught using either NITSE or biconical traps. The species were identified as 13 *G. tachinoides* (caught in Jos north), 6 *G. palpalis*

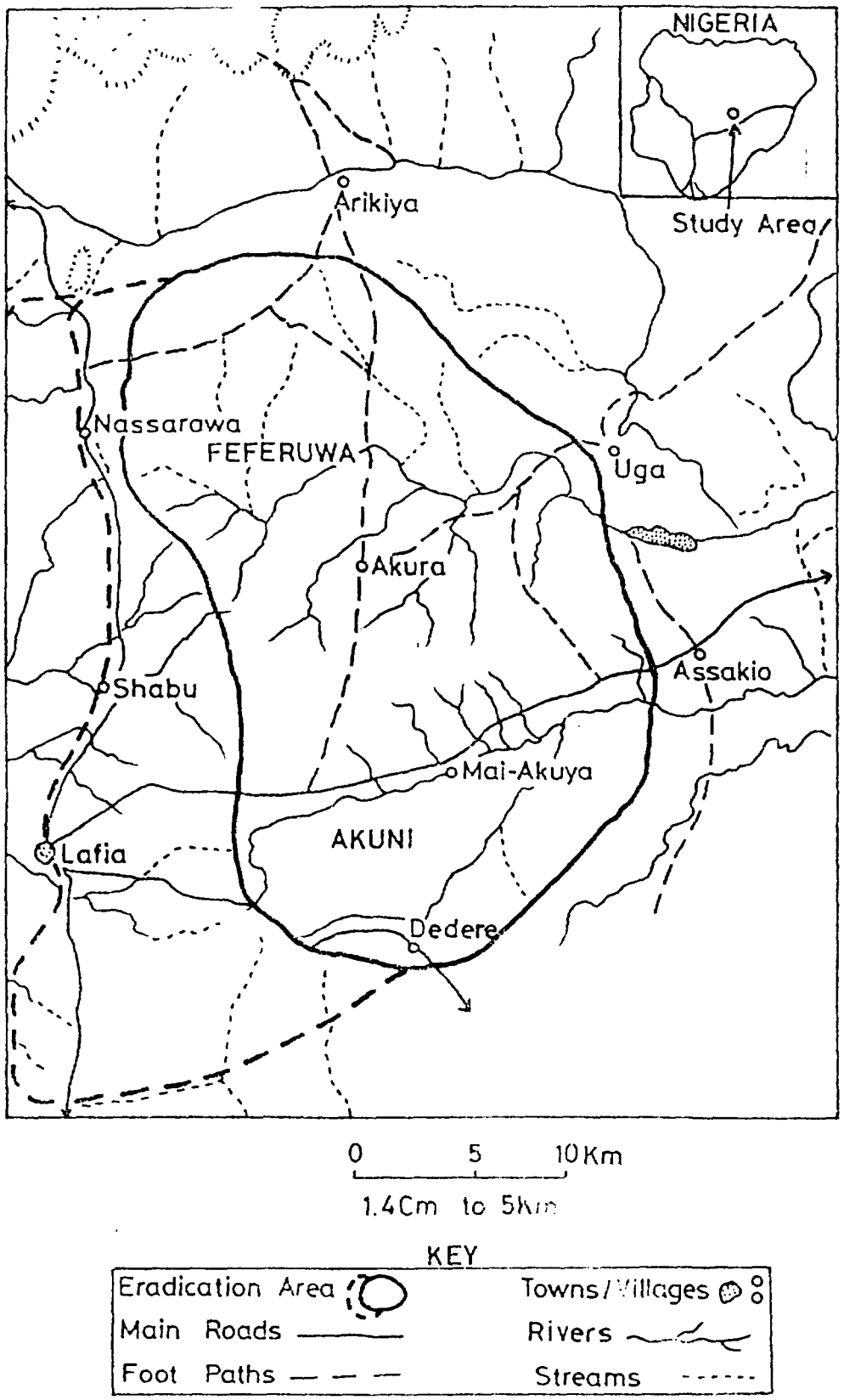

FIG.1. Map showing the area where the sterile insect technique was used from 1980 to 1987 to eradicate Glossina palpalis *in the Lafia Local Government Area, Plateau State, Nigeria.*

palpalis (caught in Lafia and Keffi) and 3 *G. longipalpis* (caught in Lafia; Fig.1). All tsetse flies were dissected, but did not harbour trypanosome infections. Other biting flies caught in the traps included *Tabanus* spp., *Stomoxys* spp., *Haematopotes* spp., *Chrysops* spp. and *Haematobia* spp.

TABLE III. BUFFY COAT TECHNIQUE AND ANTIGEN ELISA RESULTS FOR *TRYPANOSOMA CONGOLENSE*

			BCT results		Total
			Pos	Neg	
ELISA	results	Pos	0	0	0
		Neg	0	280	280
		Total	0	280	280

Relative specificity = 280 / 280 x 100 = 100 %.
pos. = positive; neg. = negative.

TABLE IV. BUFFY COAT TECHNIQUE AND ANTIGEN ELISA RESULTS FOR *T. VIVAX*

			BCT results		Total
			Pos	Neg	
ELISA	results	Pos	0	3	3
		Neg	0	116	116
		Total	0	119	119

Relative specificity = 116 / 119 x 100 = 97.5 %.
pos. = positive; neg. = negative.

4. DISCUSSION

The results obtained in this survey confirm previous reports stating that the Jos Plateau is no longer tsetse and trypanosomosis free [1, 2]. In those areas where tsetse flies were not caught but trypanosomes were detected in cattle, infection could have been due to mechanical transmission by other biting insects (*Stomoxys* spp. and *Tabanus* spp.).

When comparing the parasitological and Ag-ELISA results of our study, the Ag-ELISA detected 2.5% (7 of the 280 samples) positive, while the BCT found 3.4% (42 of the 1175 animals) to be infected. The Ag-ELISA was 6 to 7 times more sensitive than the BCT. This result confirms previous findings, which showed the Ag-ELISA to be 4 times more sensitive than the BCT [8]. Although, the BCT is reported to be one of the best methods for diagnosing trypanosome infections in animals [4], our results show the Ag-ELISA to be a reliable and sensitive technique for disease diagnosis [5]. Since the Ag-ELISA detects trypanosomal antigens, it is a useful tool for monitoring the effect of trypanosomosis and tsetse control programmes in endemic areas such as Nigeria. In addition, the test could be useful in large scale epidemiological surveys in Nigeria.

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