IAEA-TECDOC-925

# Application of an immunoassay method to improve the diagnosis and control of African trypanosomosis

*Proceedings of the Workshop on Epidemiological Tools for Monitoring Trypanosomosis and Tsetse Control Programmes organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and the IAEA Department of Technical Co-operation held in Addis Ababa, Ethiopia, 17–28 April 1995*

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The originating Section of this publication in the IAEA was:

Animal Production and Health Section
International Atomic Energy Agency
Wagramerstrasse 5
P.O. Box 100
A-1400 Vienna, Austria

APPLICATION OF AN IMMUNOASSAY METHOD TO IMPROVE
THE DIAGNOSIS AND CONTROL OF AFRICAN TRYPANOSOMOSIS

IAEA, VIENNA, 1997
IAEA-TECDOC-925
ISSN 1011–4289



Printed by the IAEA in Austria
February 1997

## FOREWORD

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture supports the introduction and use of nuclear and related techniques in developing countries through co-ordinated research programmes. A co-ordinated research programme (CRP) entitled "Improving the diagnosis and control of trypanosomosis and other vector-borne diseases of African livestock using immunoassay methods" was executed from 1987 to 1993 with funding from the Directorate General of Development Co-operation (DGIS) of the Netherlands. A total of 12 reports by the various Research Contract holders were compiled and published as a Technical Document (IAEA-TECDOC-707) together with conclusions, recommendations and reports by experts in the field.

Assistance to the Research Contract holders of the above mentioned CRP was continued by providing expert services, equipment and fellowship training through a Regional Technical Co-operation Project (IAEA TCP RAF/5/028) for an additional two years from mid-1993 to mid-1995. Financial assistance for this TCP was obtained from a voluntary contribution by the Overseas Development Agency of the United Kingdom and from funds allocated by the IAEA Department of Technical Co-operation for this purpose. The overall aim remained the same as in the previous CRP, i.e. to support national animal disease research institutes belonging to the national agricultural research systems (NARS) in their efforts to improve their capability to diagnose animal trypanosomosis. The more specific aim of the TCP was not only to strengthen disease diagnostic capabilities in the various laboratories in Africa, but also to promote the installation of serum banks, to assist in disease data analysis and to introduce simple epidemiological techniques.

This publication contains the results presented by the participants at the FAO/IAEA/ILRI Epidemiology Workshop on the Monitoring of Trypanosomosis and Tsetse Control Programmes held at the International Livestock Research Institute (ILRI) in Addis Ababa, Ethiopia, from 17 to 28 April 1995.

FAO and IAEA wish to express their sincere appreciation to the participants of the workshop for the keen interest and the diligent work during the project period resulting in the scientific reports as presented in this publication. The two organizations also wish to acknowledge the support and expertise given by various experts, G. Duvallet, M. Eisler, I. Frame, P. Lessard, A.G. Luckins and C. Ooijen during the project period. The officer responsible for this publication is R.H. Dwinger of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

## *EDITORIAL NOTE*

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# THE USE OF AN IMMUNOASSAY METHOD TO IMPROVE THE DIAGNOSIS OF AFRICAN TRYPANOSOMOSIS AND ITS APPLICATION TO MONITOR DISEASE CONTROL PROGRAMMES: A SUMMARY


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## 1. TRYPANOSOMOSIS

### 1.1. Importance of the disease

Cattle, goats, sheep and camels provide smallholder farm families in Africa with important sources of nutritious food - milk and meat - as well as with cash income through the sale of animal products. In addition, livestock provide tractive power for transport of goods or for the cultivation of land. Furthermore, the animals produce manure, which can be used as fuel or fertilizer. Efforts to increase livestock production in sub-Saharan Africa have been hampered by seasonal shortages of fodder, a multitude of livestock diseases as well as the lack of a land-tenure system. One of the most intractable diseases affecting all species of livestock in Africa is trypanosomosis. Infection with the protozoan parasite results in high mortality in acute cases and in a severe loss of production in chronic cases, thus, effectively retarding agricultural development in large areas of the continent. Three species of parasites are of importance in ruminants, i.e. *Trypanosoma brucei*, *T. congolense* and *T. vivax*. The parasites are transmitted by an insect vector, the tsetse fly (genus *Glossina*), which occurs over an area of 11 million km$^2$ of Africa, about 37% of the continent, affecting both animals and humans in 40 different countries [1]. There are 23 extant species of *Glossina* in Africa, each one with its particular habitat requirements and behavioural patterns [2]. Because of the complexity of this disease-vector inter-relationship it is not surprising that little progress has been made in controlling the disease since the discoveries by Bruce late in the nineteenth century of the links between tsetse flies, trypanosomes and disease [3].

### 1.2. Diagnosis of the disease

The traditional diagnostic techniques for detecting trypanosomes in animals have been the microscopic examination of wet blood films or of thin and thick bloodsmears following fixation and Giemsa staining. The development of concentration methods such as the microhaematocrit centrifuge technique (MHCT) or the buffy coat technique (BCT) using a phase contrast/darkground microscope has improved diagnosis considerably [4, 5]. All these parasitological techniques are very specific (a positive result indicates the true presence of a trypanosome and few false positives are encountered), but generally lack sensitivity (failure to detect the parasite and, thus, the occurrence of false negatives). However, in chronically infected animals low parasitaemias often occur, which could not be detected by the most sensitive technique, the BCT method (Table I).

In 1971 the enzyme-linked immunosorbent assay (ELISA) was developed [6] and various applications of the technique soon proved to be highly sensitive and specific as well as reliable and flexible methods for screening large numbers of samples. The technique was modified for the detection of trypanosomal antigens and applied for the diagnosis of *T. vivax*, *T. congolense* and *T. brucei* infections in cattle [7]. In experimental infections the test proved to be a sensitive technique for the diagnosis of animal trypanosomosis [8, 9]. Moreover, indications were that the test was well suited for application in developing countries [10]. Consequently, the ELISA was transferred to the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture for distribution to various African research institutes for extensive validation under tropical conditions.

## 1.3. Control of the disease

There are currently three methods available to control trypanosomosis: the administration of trypanocidal drugs, the exploitation of trypanotolerant animals and various techniques to control the insect vector.

Chemotherapy of infected animals or chemoprophylaxis of animals at risk is a successful method and widely used. However, it needs close veterinary supervision and depletes African governments of much needed foreign exhange. Moreover, resistant organisms have been reported on numerous occasions and relapses are not uncommon due to organisms hiding in the central nervous system and other privileged sites where the parasites are inaccessible to the veterinary drugs.

TABLE I. LOWEST SENSITIVITY LEVELS OF DIFFERENT PARASITOLOGICAL TECHNIQUES IN DETECTING *T. CONGOLENSE*, *T.VIVAX* AND *T. BRUCEI*

| Trypanosomes per ml | BCT | | | MHCT | | | Wet film | | | Thick film | | | Thin film | | | Mouse |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tc | Tv | Tb | Tc | Tv | Tb | Tc | Tv | Tb | Tc | Tv | Tb | Tc | Tv | Tb | Tb |
| $5.0 \times 10^4$ | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | ND |
| $2.5 \times 10^4$ | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | ND |
| $5.0 \times 10^3$ | + | + | + | - | + | + | - | - | - | - | - | + | - | - | - | + |
| $2.5 \times 10^3$ | + | + | - | - | + | + | - | - | - | - | - | - | - | - | - | + |
| $5.0 \times 10^2$ | + | + | - | - | - | + | - | - | - | - | - | - | - | - | - | + |
| $2.5 \times 10^2$ | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |

Table has been modified from [11]; BCT = buffy coat technique using a darkground/phase contrast microscope [5]; MHCT = microhaematocrit centrifuge technique [4]; Mouse = mouse sub-inoculation; Tc = *Trypanosoma congolense*; Tv = *T. vivax*; Tb = *T. brucei*; ND = not done.

Trypanotolerant animals are able to survive and produce meat and milk for the rural population in the tsetse infested areas of Africa, while the trypanosensitive breeds either succumb to the disease or have to be maintained through costly chemotherapeutic measures. Regular monthly collection of health and production parameters of trypanotolerant N'Dama cattle under traditional management conditions and high tsetse challenge showed that the productivity compared favourably with that of breeds elsewhere in Africa [12].

The third method is aimed at controling or eradicating the vector of the disease, the tsetse fly. In the beginning of the century bush clearing and elimination of wildlife were used to destroy the habitat and the nutritional source of the insects. However, these methods proved to be only temporary solutions and are nowadays not accepted. Since the 1950s insecticides have been used on a large scale, either by ground or aerial spraying. However, some compounds have been reported to have detrimental effects on the environment [13] and only a limited number of insecticides and a few specialized methods of applying them can achieve the objective of effective control at an acceptable cost [14]. Recent improvements to trap design and the simultaneous use of odour attractants, have led to the employment of insecticide impregnated traps and electric screens for tsetse control operations [15]. A biological and environmentally safe method of vector control is the sterile male release technique [16] in which artificially sterilized males compete with wild tsetse for mating opportunities with females, since only one mating per lifetime is usual in female tsetse. This technique is effective when insect population densities are low and aims at total eradication of the vector. However, it is essential that a successful eradication be sustained by the effective prevention of tsetse reinvasion from adjacent non-cleared areas. The most recent and successful approach to tsetse control is integrated pest management (IPM) using a variety of techniques such as stationary targets and insecticide treated livestock to reduce the population density, the sterile insect technique to eradicate tsetse or the use of trypanotolerant livestock if eradication is not feasible.

## 2. TECHNICAL ASSISTANCE

### 2.1. The Regional Technical Co-operation Project (RAF/5/028)

With assistance from the Government of the Netherlands an FAO/IAEA Co-ordinated Research Programme (CRP) was initiated in 1987 to transfer ELISA technology to a number of African research institutes and to improve the diagnosis of trypanosomosis in particular. The biological reagents were assembled in a ready-to-use kit form, consumables and training were provided and small research projects for field validation of the ELISA were initiated. This resulted in a Technical Document (TECDOC), in which the results of the CRP were presented [17]. Following the successful completion of the CRP, the IAEA Department of Technical Co-operation and the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture provided support for a Regional TCP (RAF/5/028) to strenghten disease diagnosis in nine National Agricultural Research Systems (NARS) and to introduce improved diagnostic techniques in five additional institutes. In addition, the TCP provided equipment to install serum banks, fellowships for additional training in data analysis and expert missions to resolve specific problems. The scientific results obtained by the various researchers are presented in this TECDOC.

### 2.2. Participating institutes

The majority of the institutes that participated in the original FAO/IAEA CRP entitled "Improving the Diagnosis and Control of Trypanosomosis and other vector-borne Diseases of African Livestock using Immunoassay Methods" were assisted for an additional two years by the IAEA Department of Technical Co-operation through the TCP RAF/5/028. At the initiation of the TCP five institutes in Burkina Faso, Cameroon, Côte d'Ivoire, Ethiopia and Nigeria were added, while two institutes in Morocco and the Gambia, were removed from the list being supported during the biennium (Table II).

TABLE II. AFRICAN INSTITUTES ASSISTED BY THE IAEA DEPARTMENT OF TECHNICAL CO-OPERATION THROUGH TCP RAF/5/028

| Country | Research institute | Principal investigator |
|---|---|---|
| Burkina Faso | CIRDES | Z. Bengaly |
| Cameroon | LANAVET | C. Ndamkou |
| Côte d'Ivoire | LANADA | A. N'Depo |
| Ethiopia | NTTICC | N. Tewelde |
| Ghana | Central Veterinary Lab. | C. Doku |
| Kenya | KETRI | W. Olaho-Mukani |
| Mali | Lab. Central Veterinaire | O. Diall |
| Nigeria | NVRI | S. Ajayi |
| Senegal | ISRA/LNERV | M. Seye |
| Sudan | University of Khartoum | E. Elamin |
| Uganda | LIRI | N. Okuna |
| United Republic of Tanzania | ADRI | H. Mbwambo |
| Zambia | CVRL | L. Sinyangwe |
| Zimbabwe | CVL | R. Ries |

## 2.3. Workshop

A workshop was organized in order to enable the principal investigators to present the research results of the previous year and discuss the workplans as proposed for the coming year. In addition, training in the epidemiological analysis of parasitological, serological and haematological data sets was provided to the participants by using a computer software package (Epi Info version 6). The workshop was held at the ILRI facilities in Addis Ababa, Ethiopia, from 17-28 April, 1995 and was attended by 18 researchers from Africa (see list of participants). Five scientists were invited to lecture on subjects such as basic statistics, epidemiology, diagnosis and pathogenesis of trypanosomosis, rapid rural appraisal procedures, geographical information systems, guidelines for handling and storing serum samples. Moreover, time was devoted to obtaining "hands-on" experience in the use of a computer and in particular to using the software package for the epidemiological analysis of each participant's own data set.

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# IMPROVEMENTS ON AN ELISA TO DETECT TRYPANOSOMAL ANTIGENS AND ITS USE AS A MONITORING TOOL IN TSETSE AND TRYPANOSOMOSIS CONTROL PROGRAMMES


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**Abstract**

IMPROVEMENTS ON AN ELISA TO DETECT TRYPANOSOMAL ANTIGENS AND ITS USE AS A MONITORING TOOL IN TSETSE AND TRYPANOSOMOSIS CONTROL PROGRAMMES.

Monoclonal antibodies directed at epitopes of *Trypanosoma brucei*, *T. congolense* and *T. vivax* have been used to capture and detect trypanosomal antigens in bovine blood samples using an enzyme-linked immunosorbent assay (ELISA) developed elsewhere. The test has been transformed in a ready-to-use kit format for distribution among a network of 15 African research institutes. The specificity of the test was assessed under experimental and field conditions and found to be 96% (± 2%) for *T. brucei*, 99.5% (± 1%) for *T. congolense* and 99% (± 1%) for *T. vivax*. Following a validation period under field conditions, adjustments were made to the protocol to increase the sensitivity of the ELISA and to improve the suitability of the test for laboratory use under African conditions.

Presently the ag-ELISA is being applied in conjunction with conventional parasitological techniques such as the buffy coat technique (BCT) to monitor progress in various tsetse and trypanosomosis control programmes and in a tsetse eradication effort in the United Republic of Tanzania, on the island of Zanzibar. The two tests complement each other, since infections not detected by one test may be detected by the other. In general, the serological test tends to produce more false negatives during subacute infections, while the parasitological techniques tend to produce more false negatives during chronic infections. Since the sensitivity of the ELISA is not optimal, research efforts at the FAO/IAEA Agriculture and Biotechnology Laboratory will be focused on improving this aspect. However, these efforts are severely hampered by the lack of a diagnostic test that can be used as a "gold standard". The use of the polymerase chain reaction for verifying doubtful test results and as a possible candidate for a "gold standard" to diagnose trypanosomosis are discussed. Finally, future plans are outlined to initiate the use of geographical information systems to assess the impact of tsetse control and eradication programmes on land use and disease distribution.

## 1. INTRODUCTION

Traditionally trypanosomosis in animals has been diagnosed by laborious microscopic examination of individual blood samples, initially thin and thick Giemsa stained smears, later wet films. Concentration methods were developed in the seventies using a haematocrit centrifuge. As a result the diagnosis of the disease was improved and more animals were detected to be infected with trypanosomes. These techniques, the Woo method [1] and the buffy coat technique (BCT) [2], had as an additional advantage that the anaemia of the animal could be assessed simultaneously by measuring the packed red cell volume percentage. However, although the specificity of the techniques was good (very few false positives were encountered), the sensitivity was insufficient. The lower detection limit of the most sensitive technique (the BCT) was reported to be between 100 and 1000 trypanosomes/ml blood [3]. This proved to be insufficient, since trypanosomosis in cattle is often encountered under field conditions as a chronic disease with low levels of circulating parasites in the blood [4].

The discovery of monoclonal antibodies [5] and the use of ELISA technology provided an additional diagnostic tool for testing large numbers of samples with a reasonable accuracy of detecting infected animals.

## 2. MATERIALS AND METHODS

Monoclonal antibodies directed at epitopes of *Trypanosoma brucei*, *T. congolense* and *T. vivax* have been used to capture and detect trypanosomal antigens in bovine blood samples using an enzyme-linked immunosorbent assay (ELISA) developed elsewhere [6]. The test has been transformed in a ready-to-use kit form for distribution among 15 African research institutes.

## 3. RESULTS

The specificity of the test was assessed under experimental and field conditions and found to be 96% (± 2%) for *T. brucei*, 99.5% (± 1%) for *T. congolense* and 99% (±1%) for *T. vivax*. Following a validation period under field conditions [7], minor adjustments were incorporated in the protocol to increase the sensitivity of the ELISA and improve the robustness of the test for use under laboratory conditions in Africa. The following assay adjustments have been made:

(1) the use of high binding plates
(2) constant shaking for 15 minutes at 37°C
(3) dilution of control and test serum samples
(4) addition to the serum diluent buffer of 0.5% normal mouse serum as a liquid phase blocking agent
(5) addition to the conjugate diluent buffer of 1% bovine serum albumin as a liquid phase blocking agent
(6) use of tetramethylbenzidine chromogen and hydroxide peroxide substrate
(7) use of non corrosive 1M phosphoric acid solution used as a stopping solution
(8) incorporation of standardized internal quality control samples such as a strong positive, moderate and negative antigen control as well as a conjugate control
(9) expression of results as percent positivity relative to the strong positive antigen control
(10) use of a specialized computer software program developed for the ELISA kit.

Presently the ag-ELISA is being applied in conjunction with conventional parasitological techniques such as the buffy coat technique (BCT) to monitor progress in various tsetse and trypanosomosis control programmes in Africa and in a tsetse eradication effort in the United Republic of Tanzania, on the island of Zanzibar.

## 4. DISCUSSION

The sensitivity of the ELISA was not satisfactory since it failed several times to detect animals with subacute infections. This lack of sensitivity might be inherent to the characteristics of the test as it is presently used. Of importance are the size and the nature of both antibody and antigen. For example, the monoclonal antibodies used for detecting *T. brucei* and *T. congolense* are of the IgM isotype, which are on the one hand more sensitive (due to a higher avidity because of the pentameric structure), but on the other hand more fragile than the IgG isotype. The latter characteristic has implications for the repeatability of the test results. Another feature of the test is that the same antibody is being used to capture as well as to detect the antigen, which might lead to a competition between the capture and detecting antibodies for a binding spot on the antigenic site (the epitope). Furthermore, the trypanosomal antigen to be detected by the antibodies is an internal unsecreted molecule released only following destruction of the parasite by the host immune system. Consequently, the amount of circulating antigens during the initial (subacute) phase of infection is low and might be below the detection threshold of the test. Conversely, during later stages of the disease the formation of immune complexes might mask the antigenic determinants recognized by the monoclonal antibodies used in the test. Furthermore, the size and the nature of the antigen are of importance for the performance of the test. Studies have shown the *T. vivax* antigen to be an 8-kDa peptide [8] and the *T. congolense* antigen to be a protease, while the *T. brucei* antigen has not yet been characterized.

It is intended to focus research efforts at the FAO/IAEA Agriculture and Biotechnology Laboratory at improving the sensitivity of the test. However, these efforts are severely hampered by the lack of a diagnostic test that can be used as a "gold standard".

The polymerase chain reaction (PCR) is known to be a very sensitive test, but will detect false positives if insufficient controls are being used during the sampling and testing procedures. For trypanosomosis in particular this test would be ideally suited as the "gold standard". It would have to verify doubtful samples, which have been detected positive by ELISA, but have not been found positive parasitologically in order to distinguish the true from the false positives. At the same time it would be useful if the PCR technique could be employed to detect infected animals that have tested negative in the ELISA and BCT due to insufficient sensitivity of these latter two tests (to detect the

false negatives). The practical significance of the PCR would be that in disease eradication programmes it is of great importance to detect remaining foci of infection (to detect the false negatives). It would be equally important to unmask the false positives, which would assist in giving an indication when to stop eradication efforts. In collaboration with the University of Glasgow, United Kingdom, and the International Livestock Research Institute (ILRI), Kenya, investigations will be initiated to incorporate the PCR in the diagnostic methods for monitoring disease control.

As an additional tool for assessing the effect of tsetse control programmes and for analysing the geographical and environmental implications of these interventions, we intend to use computerized systems such as geographical information systems (GIS). GIS will allow the input, storage, manipulation, analysis and display of geographically referenced data. Data sets of importance for assessing tsetse and trypanosomosis control programmes should contain information on climate, vegetation, geographical features (such as altitude, soils), human, animal and vector populations and socio-economic influences. Thus, one can assess the effects of disease interventions on land use, animal distribution or the environment. Moreover, GIS can be used to map disease distribution when geographically referenced disease prevalence data are available. Similarly, the influence and dynamics of changes in disease incidence can be predicted and disease risk maps can be produced for various geographical sites.

## ACKNOWLEDGEMENTS

We wish to acknowledge the intellectual assistance of Dr. J.D. Dargie and Dr. R. Geiger. The research and extension work was supported by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and the FAO/IAEA Agriculture and Biotechnology Laboratory at Seibersdorf with additional support from the Ministry for Overseas Development Collaboration (DGIS) of the Government of The Netherlands and the Overseas Development Agency of the United Kingdom.

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# PREVALENCE OF TRYPANOSOMOSIS IN CATTLE IN BURKINA FASO

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**Abstract**

PREVALENCE OF TRYPANOSOMOSIS IN CATTLE IN BURKINA FASO.

The specificity of the Antigen ELISA was determined by examining 320 bovine serum samples from a tsetse free area and found to be high for the three trypanosome species pathogenic to cattle. By using parasitological techniques in conjunction with the Ag-ELISA it was possible to greatly increase the number of animals found positive for trypanosomes in a tsetse endemic area. For example *T. brucei* was detected in a region where it had not been encountered previously. The Ag-ELISA will be used to monitor the efficacy of past and on-going tsetse control programs.

## I. INTRODUCTION

Burkina Faso is a landlocked country located in West Africa with a surface area of 375 000 km$^2$. It has a human population of 9 million and a livestock population of 4 000 000 cattle, 4 500 000 sheep, 5 000 000 goats, 400 000 donkeys and horses, 1 000 000 pigs and 25 000 camels [1]. In the subhumid zone of Burkina Faso trypanosomosis in cattle is a disease of great importance, but information on prevalence based on large scale field sampling is not available. Consequently, the need arose to introduce a reliable diagnostic system for epidemiological surveys and for the assessment of the impact of tsetse control programs on trypanosomosis transmission in cattle. The objectives of the present study were threefold. Firstly to generate information on the prevalence of trypanosomosis in cattle through extensive surveys of cattle populations in various ecological environments. Secondly, to monitor the effectiveness of tsetse control programs, which have been carried out by CIRDES since 1990. Finally, to train scientists from countries near Burkina Faso in the use of Ag-ELISA technology.

## 2. MATERIALS AND METHODS

In the south-west of Burkina Faso five provinces were considered of importance for the assessment of the present situation of animal trypanosomosis in the country [2]. Four areas were randomly selected for sampling in Kénédougou Province and three in Comoé Province each [3]. Initial surveys were conducted to collect general information, to estimate the prevalence of trypanosomosis and to calculate the sample sizes. A three stage sampling method was designed: simple random sampling at the village level, simple random sampling at the herd level and systematic sampling at the animal level. Sample sizes were 521 samples in Kénédougou Province and 200 in Comoé Province (Table I). Sampling will continue in the remaining three Provinces of Houet, Bougouriba and Mouhoun.

Blood was collected for the investigation of parasites by buffy coat technique (BCT) and Giemsa stained thin smears. Serum samples were collected to detect circulating trypanosomal antigens using the Ag-ELISA.

With regard to the impact assessment of tsetse control programs two sites were selected for sampling: a pastoral zone at Samorogouan and one at Yallé. In Samorogouan a tsetse control program has been carried out from 1990 to 1992 using insecticide pour-on and traps. A study to monitor the effectiveness of the tsetse control program was carried out by bleeding at the beginning of the study and at two-month intervals. In Yallé the same protocol was followed as in Samorogouan, except that the cattle were bled at four-month intervals, animals were treated with Berenil® at the beginning of the study and insecticide application was at two-month intervals with Deltametrhin® 1%. All samples were examined by BCT and Ag-ELISA.

*FIG.1. Map of Burkina Faso indicating the study area.*

TABLE I. CATTLE POPULATIONS IN THE SELECTED STUDY SITES

| Location | Houet | Poni | Kénédougou | Samorogouan Ranch 1 | Samorogouan Ranch 2 | Yallé |
|---|---|---|---|---|---|---|
| Cattle population | 174 000 | 145 000 | 52 000 | 3 000 | 1 500 | 1 500 |
| Sampling size | ND | 200 | 521 | 91 | 82 | 300 |
| Total no. of sera | ND | 200 | 521 | 728 | 656 | 1300 |

ND = not done.

## 3. RESULTS

To determine the specificity of the Ag-ELISA test, 320 cattle were sampled from a tsetse free area. The specificity was calculated to be 100% for *T. brucei*, 100% for *T. congolense* and 99.7% for *T. vivax* (Table II).

TABLE II. COMPARISON BETWEEN THREE TECHNIQUES TO DETECT TRYPANOSOMES IN 320 SAMPLES FROM A TSETSE FREE AREA (DORI REGION)

| Test | *T. brucei* | *T. congolense* | *T. vivax* |
|---|---|---|---|
| BCT | 0 | 0 | 0 |
| Thin smear | 0 | 0 | 1 |
| Ag-ELISA | 0 | 0 | 0 |

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

From a trypanosomosis endemic area (Kénédougou Province), 521 cattle were examined for the presence of parasites or trypanosomal antigens (Table III). A total of 55 animals were found to contain trypanosomes using either one of two parasitological techniques (BCT or thin smears), while 58 animals were detected with the Ag-ELISA to have trypanosomal antigens.

TABLE III. COMPARISON BETWEEN THREE TECHNIQUES TO DETECT TRYPANOSOMES IN 521 SAMPLES FROM AN ENDEMIC AREA (KÉNÉDOUGOU)

| Test | No. of samples positive | T.b. | T.c. | T.v. | T.b./T.c. | T.b./T.v. | T.c./T.v. | T.b./T.c. T.v. |
|---|---|---|---|---|---|---|---|---|
| BCT | 38 | 0 | 23 | 12 | 0 | 0 | 3 | 0 |
| Thin smear | 42 | 0 | 20 | 18 | 0 | 0 | 4 | 0 |
| Ag-ELISA | 58 | 42 | 3 | 10 | 2 | 1 | 0 | 0 |

BCT = Buffy coat technique; Ag-ELISA = Antigen-detection enzyme-linked immunosorbent assay;
T.b. = *Trypanosoma brucei*; T.c. = *Trypanosoma congolense;* T.v. = *Trypanosoma vivax.*

The percent of animals positive for trypanosomes per breed, sex or age category and per geographical zone using either a parasitological or serological technique was examined. It appeared that crossbred animals (Zebu x Baoulé) were more often infected than purebred animals (Table IV).

To monitor the effectiveness of past and on-going tsetse control programs 300 cattle were sampled at an initial bleeding in the pastoral zone of Samorogouan and a similar number in the pastoral zone of Yallé (Sissili), respectively. During subsequent bleedings a total of about 1500 sera were collected in Samorogouan and a total of 1000 sera in Yallé. All samples will be analysed by Ag-ELISA.

TABLE IV. PERCENT OF ANIMALS POSITIVE FOR TRYPANOSOMES USING EITHER A PARASITOLOGICAL OR A SEROLOGICAL TECHNIQUE PER BREED, SEX, AGE OR GEOGRAPHICAL ZONE

| Category | | % positive using BCT or thin smear | % positive using Ag-ELISA | No. |
|---|---|---|---|---|
| Breed | Zebu | 5.6 | 8.9 | 323 |
| | Zebu/Baoule | 16.3 | 15.8 | 171 |
| | Baoule | 6.9 | 6.9 | 29 |
| Sex | female | 7.5 | 10.1 | 348 |
| | male | 14.1 | 14.1 | 149 |
| Age | < 1 year | 10.2 | 12.2 | 49 |
| | 1-2 years | 5.7 | 10.8 | 158 |
| | > 2 years | 11.9 | 10.4 | 269 |
| Geographical | N'Dorola | 6.0 | 6.0 | 149 |
| zone | Orodara | 13.8 | 14.9 | 181 |
| | Samorogouan | 0 | 4.5 | 110 |
| | Koloko | 19.3 | 20.5 | 83 |

BCT = buffy coat technique; Ag-ELISA = antigen-detection enzyme-linked immunosorbent assay;
* No. = number of animals sampled.

## 4. DISCUSSION

Results from the Ag-ELISA indicated *T. brucei* to be present in the region of Kénédougou. Following the use of various stress factors (transport, nutritional) it was possible to produce a patent *T. brucei* infection, which proved to be of a low pathogenicity.

Although results indicated that crossbred Zebu x Baoulé animals were more often infected than purebred cattle, it should be noted that on the one hand Zebu are immediately treated with trypanocidal drugs once found infected and that on the other hand Baoulé are present in very small numbers in the region.

## ACKNOWLEDGEMENTS

We are grateful to the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and CIRAD-EMVT for the financial support and technical advice provided during the study.

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# BOVINE TRYPANOSOMOSIS IN NORTH PROVINCE OF CAMEROON

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**Abstract**

BOVINE TRYPANOSOMOSIS IN NORTH PROVINCE OF CAMEROON.

The results of the examination of 2959 bovine blood samples collected from four divisions of North Province of Cameroon showed a prevalence of 1.72 for *T. brucei*, 0.98 for *T. congolense* and 4.03 for *T. vivax* using parasitological techniques, such as the buffy coat technique (BCT) and the microhaematocrit centrifugation technique (MHCT). Prevalence rates in tsetse infested areas were higher than in tsetse free areas for *T. brucei* and *T. congolense*, but not for *T. vivax*. The Antigen ELISA was used to detect trypanosomal antigens in serum samples of a subset of the same animals. By using the Ag-ELISA many more animals were detected positive for *T. brucei* and *T. vivax*, but not for *T. congolense*, than when just the two parasitological techniques were used. As a matter of fact 90% of the *T. brucei* infections were detected by the Ag-ELISA and 10% by using either the BCT or the MHCT.

## 1. INTRODUCTION

Tsetse eradication campaigns have been carried out in the three northern provinces of Cameroon since 1967. At present the region contains two entomological situations: tsetse infested areas and tsete free areas. The present study had two objectives. The first objective was to draw up a map of bovine trypanosomosis in Northern Cameroon. The second objective was to monitor the tsetse eradication campaign by comparing the various prevalence rates of trypanosomosis in cattle from tsetse free areas with those from tsetse infested areas. The present study reports results obtained in the North Province.

## 2. MATERIALS AND METHODS

The North Province includes 14 subdivisions organized in 4 divisions (Figs 1 and 2). Cattle were examined for the presence of trypanosomes in blood and for trypanosomal antigens in serum samples [1, 2]. The number of cattle bled was stratified by administrative region, with the provincial subdivision being the stratum for the study. A random-cluster sampling scheme was used to select the herds in each subdivision.

As parasitological techniques the microhaematocrit centrifugation technique (MHCT), the buffy coat technique (BCT) and Giemsa-stained smears were used [3, 4]. The Antigen ELISA provided by the Joint FAO/IAEA Division was used as a serological technique. All serum samples collected in the field were labelled with a two-line code indicating in line one the division (two characters) and the subdivision (two characters) and in the second line the herd (a two-figure number) and the animal (a three-figure number) as an indication of the order they were bled (Table I). Thus, the study is creating a large serum bank, which will be available for investigations of other diseases. Serum bank sample numbers were computerized in a dBase III Plus database file including additional information on geographical details,

TABLE I. EXAMPLE OF THE CODING SYSTEM USED FOR IDENTIFYING SAMPLES IN THE SERUM BANK

| Code | Significance |
|---|---|
| BE-GA | From the Garoua Subdivision in the Benue Division |
| 02-045 | Serum sample collected from 45th animal bled in the 2nd herd visited |

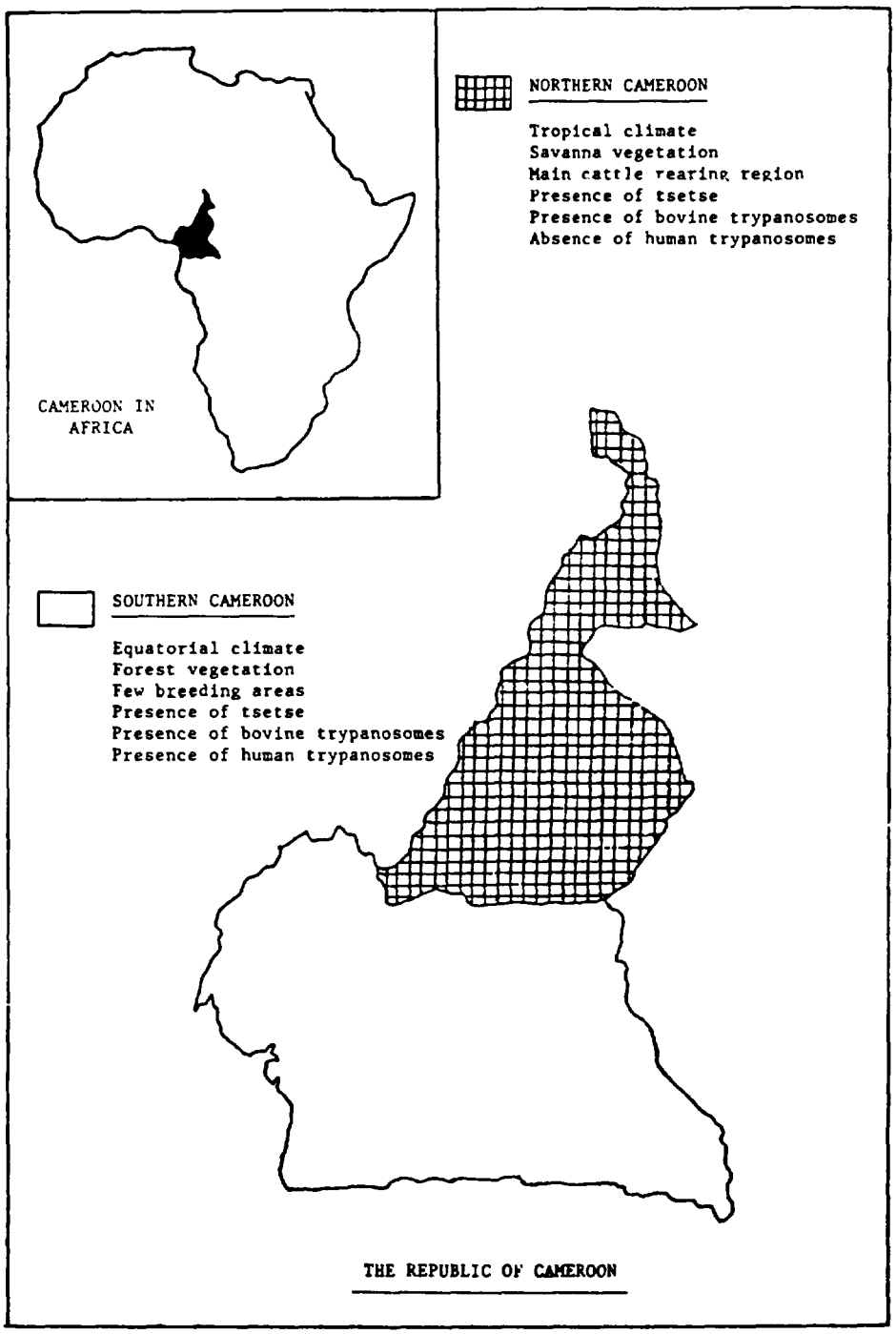


*FIG. 1a. Map of Cameroon showing some differences between the southern and northern parts of the country.*

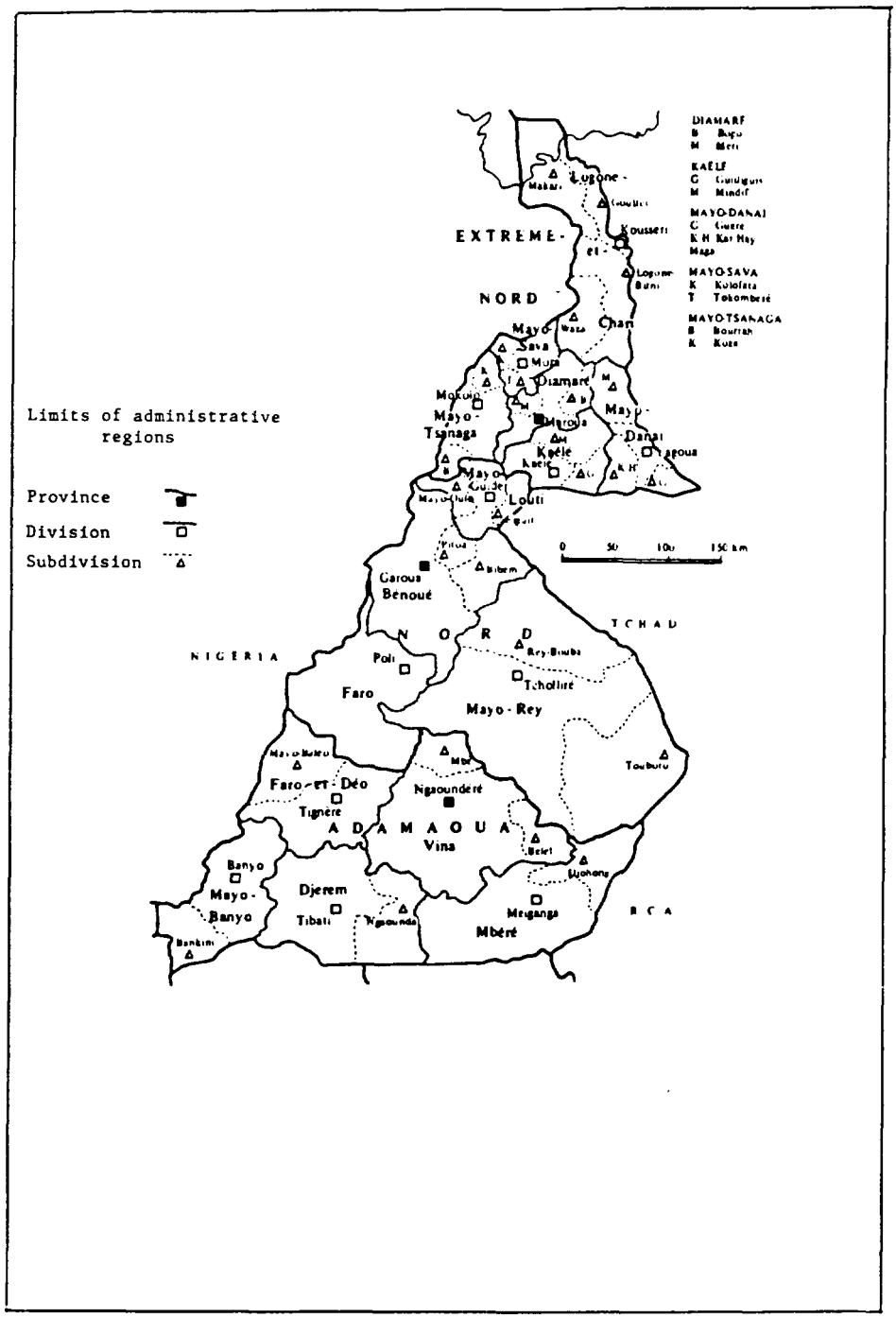


*FIG. 1b. Administrative map of Northern Cameroon.*

animal characteristics (such as sex, age, trypanocidal drug details [5], behaviour of the animal), entomological (presence/ absence of tsetse), parasitological (packed red cell volume [PCV], MHCT, BCT and stained smear values) and serological results (percent positive [PP] values).

## 3. RESULTS

### 3.1. Parasitological results

A total number of 2 959 cattle were examined from November 1994 to January 1995. Using the BCT and MHCT techniques overall prevalence rates were detected for *T. brucei*, *T. congolense* and *T. vivax* of 1.72 %, 0.98 % and 4.03 %, respectively. When considering only the tsetse free areas prevalence rates of less than 1% were detected for *T. brucei* and *T. congolense*, while for *T. vivax* the prevalence rate was 3.87% (Table II).

TABLE II. TRYPANOSOME PREVALENCE RATES IN TSETSE FREE AREAS

| | MHCT & BCT | | | | | |
|---|---|---|---|---|---|---|
| | Positive | | Negative | | Total | |
| Trypanosome species | No. | % | No. | % | No. | % |
| *T. brucei* | 11 | 0.69 | 1590 | 99.31 | 1601 | 100 |
| *T. congolense* | 2 | 0.12 | 1599 | 99.88 | 1601 | 100 |
| *T. vivax* | 62 | 3.87 | 1539 | 96.13 | 1601 | 100 |

BCT = buffy coat technique; No. = number of animals
MHCT = microhaematocrit centrifugation technique

When the prevalence rates were considered for the tsetse infested areas, they were relatively low for the three trypanosome species involved, being 3 % for *T. brucei*, 2 % for *T. congolense* and 4 % for *T. vivax* (Table III).

TABLE III. TRYPANOSOME PREVALENCE RATES IN TSETSE INFESTED AREAS

| | MHCT & BCT | | | | | |
|---|---|---|---|---|---|---|
| | Positive | | Negative | | Total | |
| Trypanosome species | No. | % | No. | % | No. | % |
| *T. brucei* | 40 | 2.95 | 1315 | 97.05 | 1355 | 100 |
| *T. congolense* | 27 | 1.99 | 1328 | 98.01 | 1355 | 100 |
| *T. vivax* | 57 | 4.21 | 1298 | 95.79 | 1355 | 100 |

BCT = buffy coat technique; No. = number of animals.
MHCT = microhaematocrit centrifugation technique.

### 3.2. Antigen ELISA results

Antigen ELISA results can be presented from two of the four divisions that have been sampled in North Province (Table IV). One of the divisions is considered to be tsetse free (Mayo-Louti), while the other one (Mayo-Rey) is infested with tsetse flies. An animal was considered positive if either the MHCT,

the BCT or the Ag-ELISA gave a positive result. Comparisons between the three techniques were made. When comparing the Antigen ELISA technique with the two parasitological techniques (BCT and MHCT), it appeared that the use of the Ag-ELISA increased the sensitivity of the diagnosis of *T. brucei* and *T. vivax* infections in cattle. On the other hand, the sensitivity of the diagnosis of *T. congolense* infections was not increased by using the Ag-ELISA (Table IV).

TABLE IV. COMPARISON OF THE SENSITIVITY (%) OF TWO PARASITOLOGICAL TECHNIQUES (BCT AND MHCT) WITH THE ANTIGEN ELISA

| | Mayo-Louti (tsetse free area) n = 627 | | Mayo-Rey (tsetse infested area) n = 616 | |
|---|---|---|---|---|
| Trypanosome species | Positive by BCT MHCT | Positive by BCT MHCT ELISA | Positive by BCT MHCT | Positive by BCT MHCT ELISA |
| *T. brucei* | 0.64 (4*) | 5.58 (35) | 0.65 (4) | 6.01 (37) |
| *T. congolense* | 0.16 (1) | 0.16 (1) | 1.95 (12) | 1.95 (12) |
| *T. vivax* | 5.26 (33) | 8.93 (56) | 1.79 (11) | 5.03 (31) |

n = number of animals examined.
* between brackets: the number of animals detected positive.
BCT = buffy coat technique.
MHCT = microhaematocrit centrifugation technique

When comparing the two parasitological techniques, the BCT appeared to be more sensitive than the MHCT. Nevetheless, the MHCT was a useful diagnostic method as it allowed to detect some buffy coat-negative animals, in particular cattle infected with *T. brucei* (17.65%). More than half of the animals infected with *T. brucei* or *T. congolense* were detected using both the MHCT and the BCT. On the contrary, only 38% of the cattle infected with *T. vivax* could be detected using parasitological techniques (Table V).

TABLE V. COMPARISON OF THE NUMBER OF SAMPLES DETECTED POSITIVE USING TWO PARASITOLOGICAL TECHNIQUES (MCHT and BCT)*

| Trypanosome species | MHCT | BCT | MHCT & BCT | Total |
|---|---|---|---|---|
| *T. brucei* | 9 (17.65%)† | 13 (25.49%) | 29 (56.86%) | 51 (100%) |
| *T. congolense* | 2 (6.90%) | 11 (37.93%) | 16 (55.17%) | 29 (100%) |
| *T. vivax* | 3 (2.50%) | 70 (58.33%) | 46 (38.33%) | 119 (100%) |

* Total number of samples examined = 2959.
† = between brackets: the percentage of samples detected positive with the technique as compared to the total number of samples found infected with the particular trypanosome species.
BCT = buffy coat technique.
MHCT = microhaematocrit centrifugation technique.

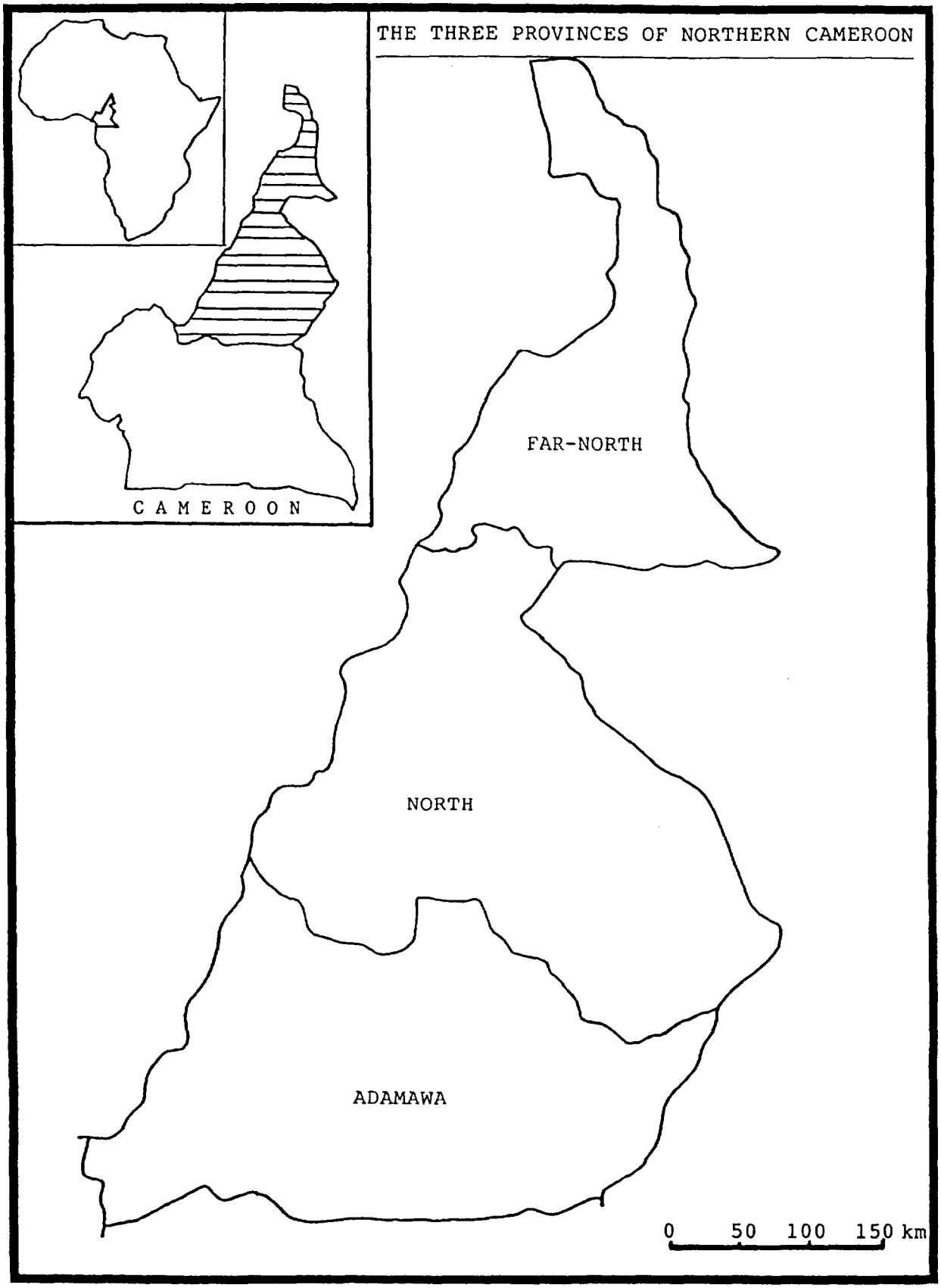


*FIG. 2. Map showing the three provinces of Northern Cameroon*

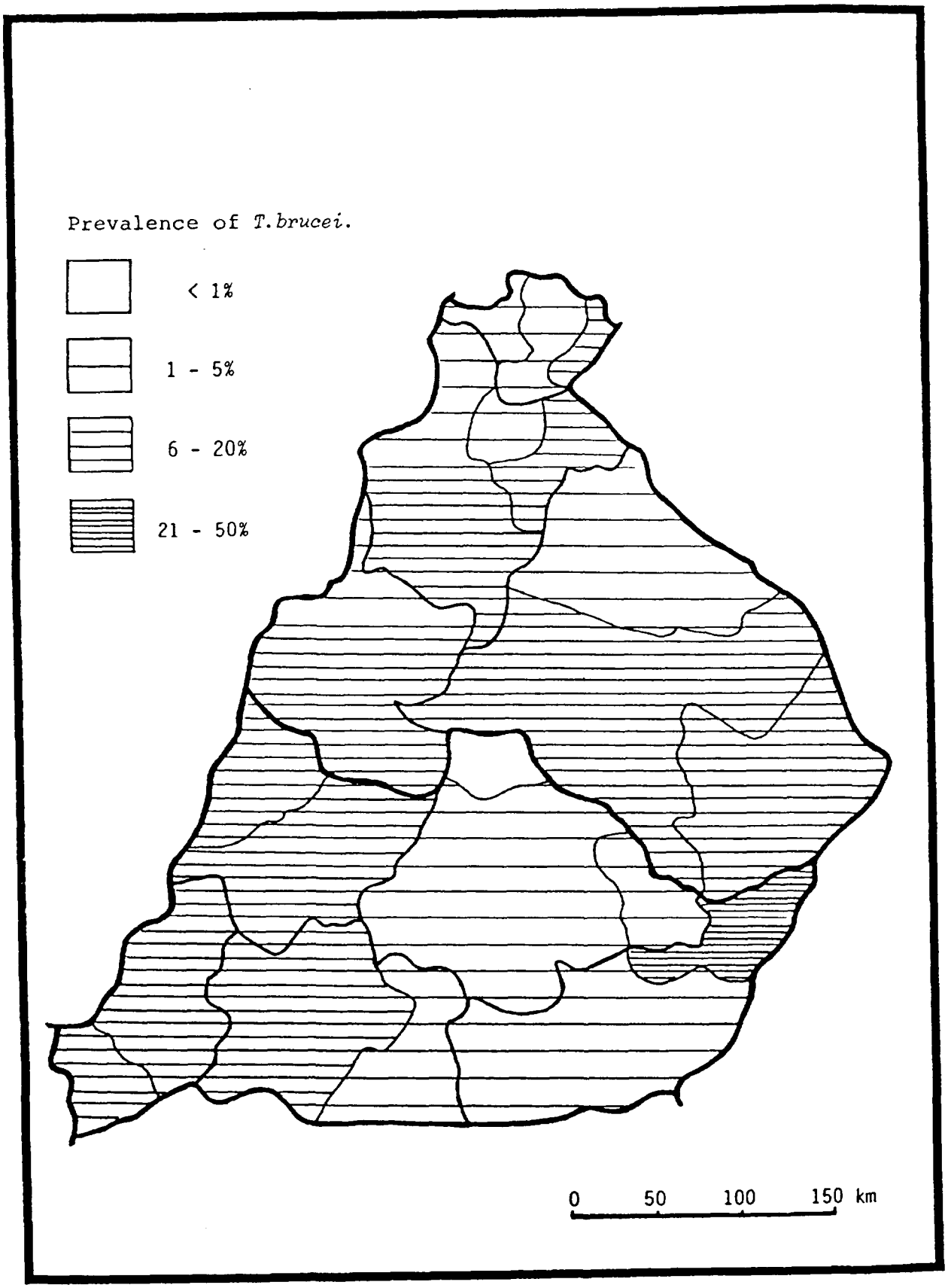


*FIG. 3. Map showing the prevalence of Trypanosoma brucei in Adamawa and North Province in 1995.*

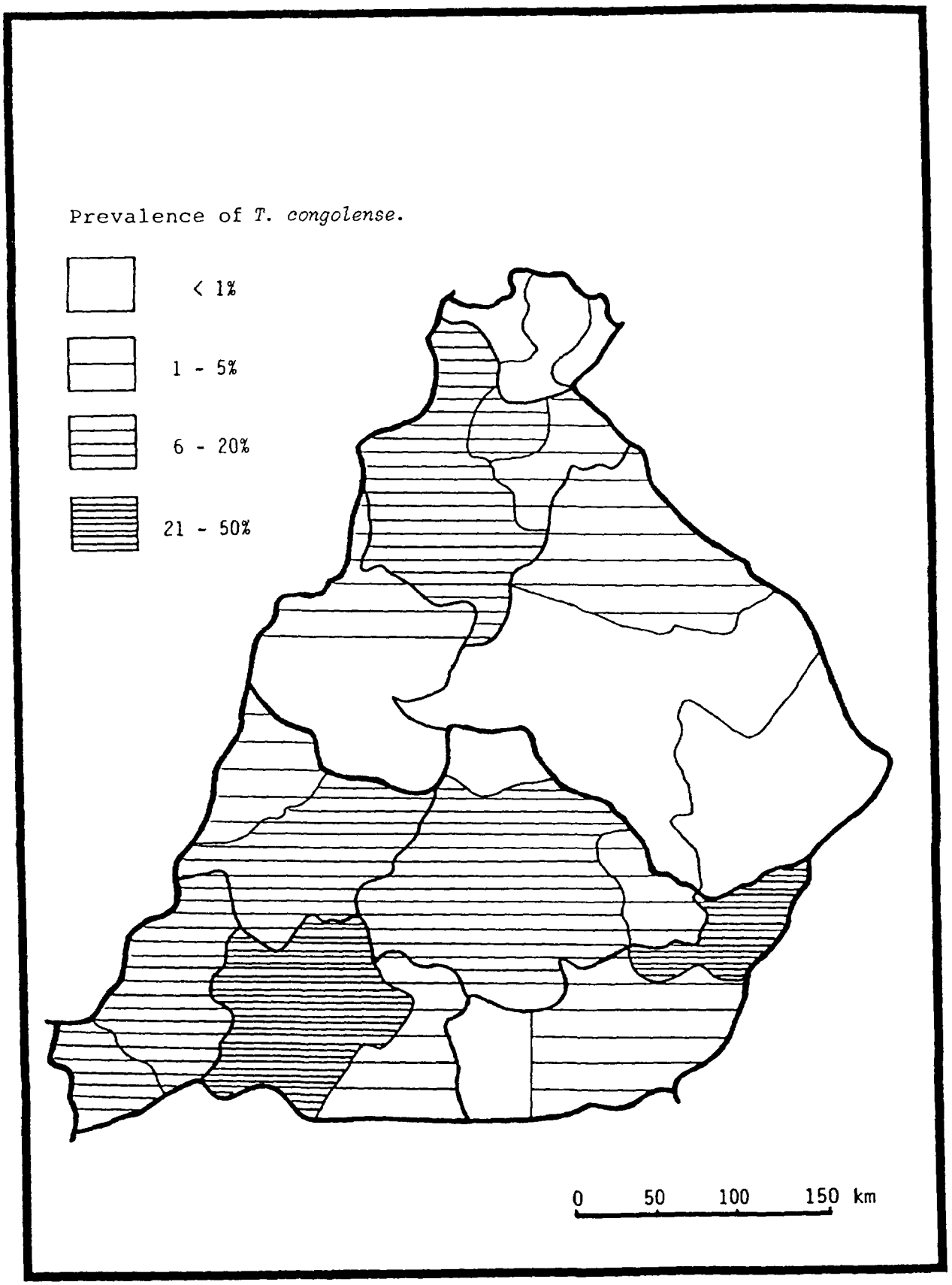


*FIG. 4. Map showing the prevalence of Trypanosoma congolense in Adamawa and North Province in 1995.*

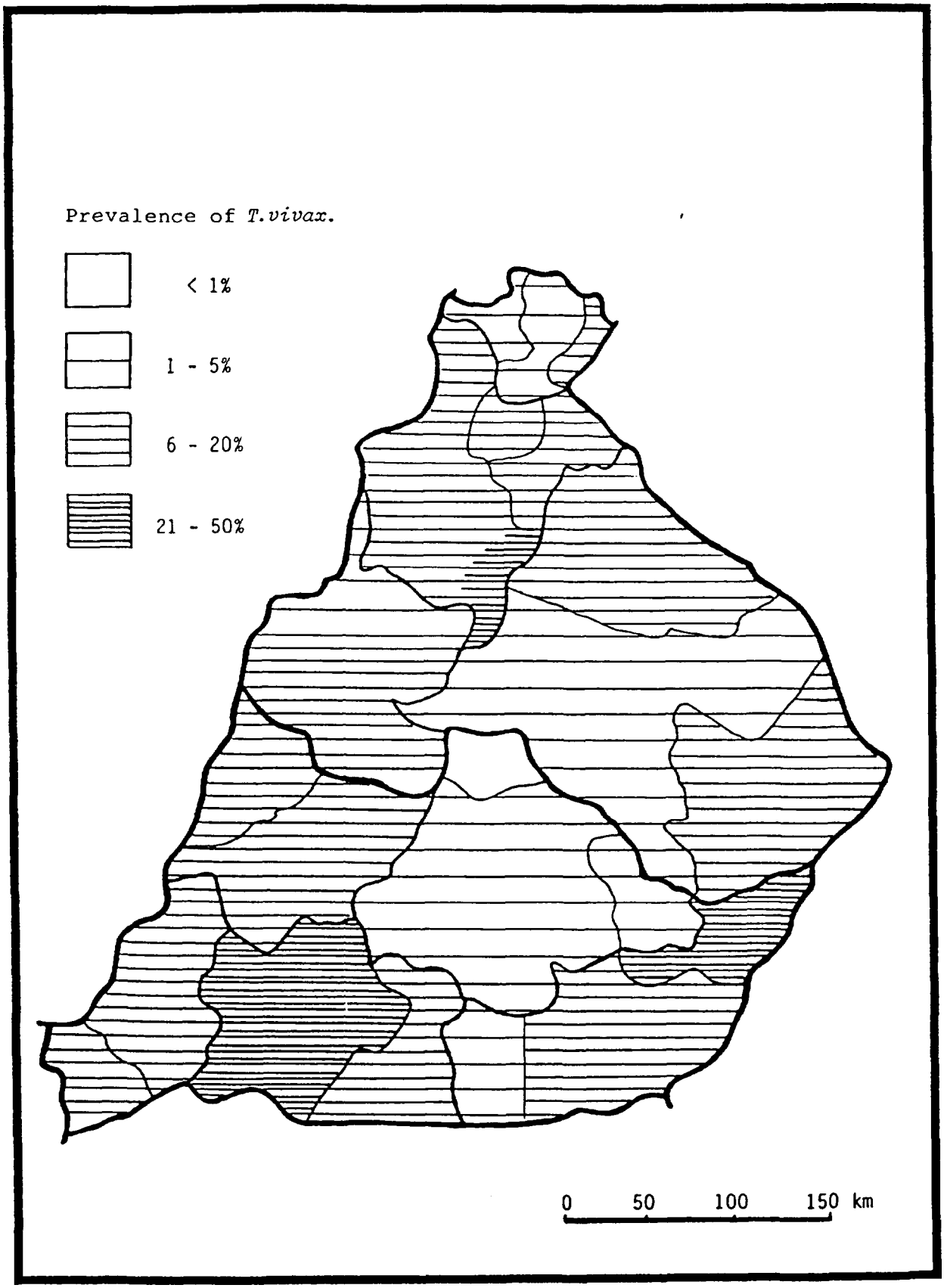


*FIG. 5. Map showing the prevalence of Trypanosoma vivax in Adamawa and North Province in 1995.*

When comparing the parasitological techniques with the Antigen ELISA few samples detected positive with the former technique could be confirmed with the latter test. For example, of the 14 animals detected positive for *T. congolense* using either the BCT or the MHCT, none could be confirmed with the Ag-ELISA. Of the 92 animals found to harbour *T. vivax*, 41 were detected using parasitological techniques, 48 by using the Ag-ELISA and only three were positive in both techniques. On the other hand, the majority of the *T. brucei* infections were diagnosed using the Ag-ELISA (Table VI).

TABLE VI. COMPARISON OF THE NUMBER OF SAMPLES DETECTED POSITIVE USING PARASITOLOGICAL TECHNIQUES AND ANTIGEN ELISA*

| Trypanosome species | Parasiological techniques | Ag-ELISA | Parasit. tech. & Ag-ELISA | Total |
|---|---|---|---|---|
| *T. brucei* | 7 (9.46%)† | 66 (89.19%) | 1 (1.35%) | 74 (100%) |
| *T. congolense* | 14 (100%) | 0 (0%) | 0 (0%) | 14 (100%) |
| *T. vivax* | 41 (44.56%) | 48 (52.17%) | 3 (3.26%) | 92 (100%) |

* Total number of samples examined = 1318.

† = between brackets: the percentage of samples detected positive with the technique as compared to the total number of samples found infected with the particular trypanosome species.

## 4. DISCUSSION

The three trypanosome species pathogenic to cattle in subSaharan Africa were detected in both tsetse infested and "tsetse free" areas of the North Province of Cameroon. The prevalence of *T. congolense* was low in either one of the areas, but significantly higher in the tsetse infested area (1.95%) than in the tsetse free area (0.16%). The prevalence of *T. brucei* was similar in both areas (6%). On the other hand, the prevalence of *T. vivax* was higher in the tsetse free area (8.9%) than in the tsetse infested area (5%). The relatively low trypanosome prevalence rates found in the tsetse infested areas could be explained by the fact that every animal suspected of trypanosomosis is commonly treated with trypanocidal drugs. Nevertheless, the results show clearly that by using the Ag-ELISA in conjunction with parasitological techniques such as the BCT and the MHCT, it was possible to detect many more animals infected with trypanosomes and, thus, to increase the sensitivity of the diagnostic methods (Figs 3, 4 and 5). Furthermore, the results of the survey indicate that the southern part of the North Province of Cameroon, which was declared tsetse free in 1991, seems to be reinfested today [6]. Reinfestation likely occurred due to the presence of wildlife reserves which had not been subjected to tsetse contol methods. The finding of a prevalence rate of 5.58% for *T. brucei* detected in a "tsetse free" area should be corroborated by entomological investigations.

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# THE USE OF ANTIGEN-DETECTION ELISA FOR THE DIAGNOSIS OF BOVINE TRYPANOSOMOSIS IN CÔTE D'IVOIRE

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**Abstract**

THE USE OF ANTIGEN-DETECTION ELISA FOR THE DIAGNOSIS OF BOVINE TRYPANOSOMOSIS IN CÔTE D'IVOIRE.

An Antigen ELISA (Ag-ELISA) detecting circulating antigens of trypanosomes was evaluated in the central region of Côte d'Ivoire for the serodiagnosis of cattle trypanosomosis. Of 1423 sera examined, only 43 were positive in the MHCT/BCT, 105 (7%) were detected using stained blood smears, and 74 (5%) were found positive using the Ag-ELISA. The predominant trypanosome species was *T. brucei*, being present in 84% of the positive samples as detected by the BCT, in 96% using stained blooodsmears, and in 72% by Ag-ELISA. *T.vivax* was detected less frequently.

The serological (ELISA) test did not detect all positive animals as found by the haematological techniques. However, the two techniques should be used in a complementary way to improve the diagnosis of the disease. The results confirm that the prevalence of trypanosomes in cattle is low in the study area. The low prevalence can be due to prophylaxis and therapy of livestock in combination with successsful tsetse trapping.

## 1. INTRODUCTION

Tsetse transmitted trypanosomosis forms a big contraint to livestock production in Côte d'Ivoire since the entire country is infested with tsetse flies. For some years the use of trypanotolerant breeds, in combination with chemotherapy and prophylaxis, has allowed a reduction of economic losses due to the disease. However, diagnosis has been difficult in the past due to the lack of a simple, rapid and sensitive test.

An Ag-ELISA developed at ILRAD [1] and later distributed by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture provides the opportunity to compare the diagnostic capabilities of parasitological with serological tests and to evaluate the effectiveness of tsetse control programmes in the central region of Côte d'Ivoire.

## 2. MATERIALS AND METHODS

### 2.1. Survey area

The survey area is located in the centre of Côte d'Ivoire, near the city of Bouaké between 4° and 6° latitude north and 6.5° and 8° longitude east (Fig. 1). The area comprises 50.000 km$^2$ and is formed by a plain and a plateau of 200-300 m.a.s.l. The climate is dominated by two rainy seasons with an annual rainfall varying between 1100-1600 mm. The temperature varies between 19°C and 34°C. Rainfall is drained by two major rivers, the Bandama and the Comoe. In the study area are approximately 51.000 cattle present.

The vegetation is a sudan savannah type interspersed with gallery forests. The principal vectors of trypanosomosis present in the study area are *Glossina longipalpis, G. palpalis gambiensis, G. morsitans submorsitans, G. medicorum.*

### 2.2. Animals

Initially ten cattle herds were selected and in each herd fifty animals between one and 10 years of age were sampled during the first visit. The cattle were identified with numbered eartags. Seven of the herds were visited on a regular basis, i.e. 4 times with an interval of 3 months. The animals were N'Dama, Zebu or crossbreds (N'Dama x Zebu) and were raised for both milk and meat production.

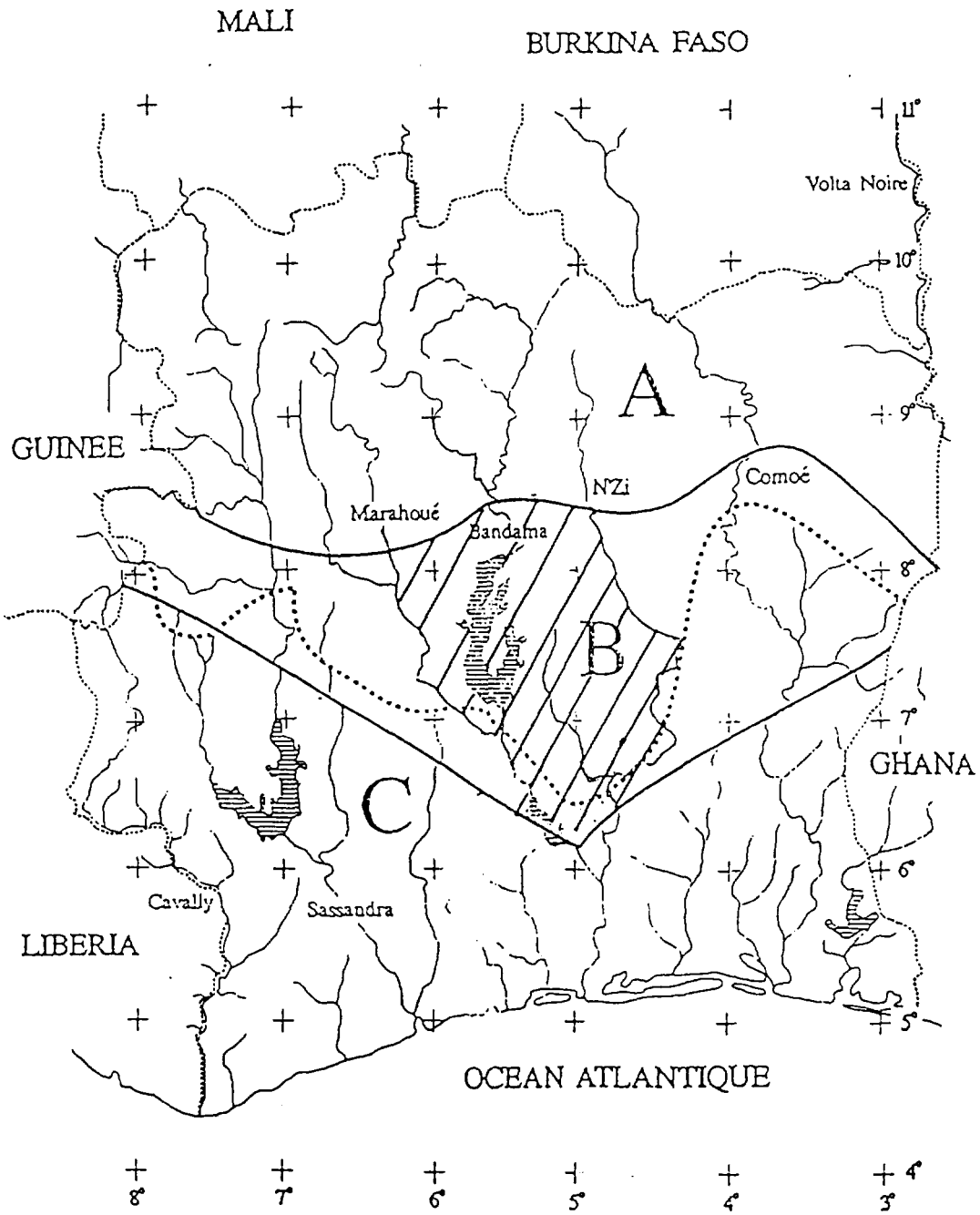


*FIG. 1. Map of Cote d'Ivoire showing the study area (hatched area) where cattle herds were sampled. A = Sudan Savanna type vegetation, B = Transition area, C = Forest zone; Dotted line indicates the southern boundary of Baoulé type cattle, a local trypanotolerant breed.*

### 2.3. Sampling

Peripheral blood was collected from the ear vein (two capillary tubes per animal), centrifuged and examined in the field for packed red cell volume (PCV) levels. The presence of trypanosomes was determined in the field using the microhaematocrit centrifuge technique (MHCT) during the first visit and the Buffy Coat Technique (BCT) during subsequent visits [2]. In addition, thin and thick blood smears were prepared and stained with Giemsa.

Furthermore, serum samples were collected from the jugular vein. Following centrifugation, sera were stored in 1 ml aliquots at -20°C until analysis by Ag-ELISA using the kit supplied by the Joint FAO/IAEA Division.

Rectal faecal samples were collected during the first and second visit from 50 animals, while 10 cattle were sampled during the two following visits. The samples were processed using the Mac Master technique.

Tsetse flies were monitored using Vavoua traps (modified with a short screen and a long cone) and examined by the National Tsetse Control Service at Bouake. The number of traps used depended on the area and vegetation density and ranged from 20 to 50 traps. The traps were located in the pastures, gallery forests and at the border of the forest.

## 3. RESULTS

### 3.1. Parasitological investigation

Microscopic examination of samples from the first visit using MHCT [2] showed a low number of positives (1.8%). During subsequent visits the BCT was used, which resulted in a total of 43 (3%) of the 1423 samples being positive, while 105 (7%) were positive using thin/thick blood smears (Table I).

TABLE I. COMPARISON BETWEEN THREE TECHNIQUES TO DETECT TRYPANOSOMES IN 1423 SAMPLES FROM A CENTRAL STUDY AREA IN CÔTE D' IVOIRE

| Test | No. of samples positive | T.b. | T.c. | T.v. | T.b./ T.c. | T.b./ T.v. | T.c./ T.v. | T.b./T.c./ T.v. |
|---|---|---|---|---|---|---|---|---|
| BCT/ MHCT | 43 | 36 | 1 | 2 | 0 | 4 | 0 | 0 |
| Thin/ thick smear | 105 | 101 | 0 | 1 | 1 | 2 | 0 | 0 |
| Ag-ELISA | 74 | 53 | 2 | 13 | 1 | 4 | 0 | 1 |

BCT = buffy coat technique; MHCT = haematocrit centrifuge technique.
Ag-ELISA = antigen-detection enzyme-linked immunosorbent assay.
T.b. = *Trypanosoma brucei*; T.c. = *Trypanosoma congolense*; T.v. = *Trypanosoma vivax*.

The predominant trypanosome species detected in all three tests was *T. brucei*, followed by mixed infections with *T.brucei / T.vivax* (Tables I, II, III). In two animals *T. theileri* was detected. When monitoring stained blood smears *Babesia bovis* and microfilaria were detected in 43 (3%) and 102 (7%) of the samples, respectively.

### 3.2. Serological investigation

Ag-ELISA detected circulating trypanosomal antigens in 74 (5%) of 1423 sera tested (Table I). As detected by Ag-ELISA 68 (92%) were single infections and 6 (8%) were mixed infections

(Tables I and III). In animals infected with a single species, *T. brucei* antigens were detected most frequently (72%), followed by *T. vivax* antigens (18%). With regard to mixed infections, the combination *T. brucei/T. vivax* was detected in 4 (5%) samples (Tables I and III).

The combined use of BCT/MHCT and Ag-ELISA resulted in 113 (8%) positive animals of the 1423 sera tested (Table I). When the results of stained blood smears were included, the total number of animals detected infected in the study area increased to 196 (14%).

TABLE II. PARASITOLOGICAL RESULTS OF A LONGITUDINAL INVESTIGATION OF CATTLE FROM A STUDY AREA IN CENTRAL CÔTE D'IVOIRE

| | | | Diagnostic test | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Buffy coat technique | | | | Thick/thin smear | | | |
| Date of visit | No. samp. | No. pos. | T.b. | T.c. | T.v. | T.b./ T.v. | T.b. | T.v. | T.b./ T.v. | T.b./ T.c. |
| June 1994 | 500 | 69 | 8 | 1 | 0 | 0 | 60 | 0 | 0 | 0 |
| Sept. 1994 | 316 | 38 | 8 | 0 | 1 | 3 | 22 | 1 | 2 | 1 |
| Jan. 1995 | 308 | 31 | 19 | 0 | 1 | 1 | 10 | 0 | 0 | 0 |
| March 1995 | 299 | 10 | 1 | 0 | 0 | 0 | 9 | 0 | 0 | 0 |

T.b. = *Trypanosoma brucei*; T.c. = *Trypanosoma congolense*; T.v. = *Trypanosoma vivax*.
No. samp. = number of animals sampled.
No. pos. = number of animals tested positive.
Jan. = January; Sept. = September.

TABLE III. SEROLOGICAL RESULTS OF OF A LONGITUDINAL INVESTIGATION OF CATTLE FROM A STUDY AREA IN CENTRAL CÔTE D'IVOIRE

| Date of visit | No. samp. | No. pos. | T.b. | T.c. | T.v. | T.b./ T.c. | T.b./ T.v. | T.c./ T.v. | T.b./ T.c./ T.v. |
|---|---|---|---|---|---|---|---|---|---|
| June 1994 | 500 | 23 | 14 | 0 | 6 | 1 | 0 | 2 | 0 |
| Sept. 1994 | 316 | 20 | 16 | 1 | 2 | 0 | 0 | 1 | 0 |
| Jan. 1995 | 308 | 19 | 14 | 1 | 2 | 0 | 0 | 1 | 1 |
| March 1995 | 299 | 12 | 9 | 0 | 3 | 0 | 0 | 0 | 0 |
| Total | 1423 | 74 | 53 | 2 | 13 | 1 | 0 | 4 | 1 |
| % | | 100 | 72 | 3 | 18 | 1 | 0 | 5 | 1 |

T.b. = *Trypanosoma brucei*; T.c. = *Trypanosoma congolense*; T.v. = *Trypanosoma vivax*
No. samp. = number of animals sampled
No. pos. = number of animals tested positive
Sept. = September; Jan. = January

### 3.3. Coprological investigation

Of the 786 faecal samples investigated, the predominant helminth species detected were strongyles (54%) followed by *Paramphistomum* (12.6%) and coccidia (9%) [Table IV].

TABLE IV. NUMBER OF CATTLE FOUND INFESTED WITH VARIOUS INTESTINAL PARASITES IN CENTRAL CÔTE D'IVOIRE AS DETECTED BY FAECAL WORM EGG COUNTS

| Helminth species | No. of cattle positive | % | Range of egg counts (e.p.g.) |
|---|---|---|---|
| Strongyles | 428 | 54.4 | 50-6500 |
| Cestodes | 23 | 2.9 | |
| Coccidia | 74 | 9.4 | 50-600 |
| Strongyloides | 3 | 0.4 | 50-400 |
| Paramphistomum | 99 | 12.6 | |
| Fasciolides | 5 | 0.6 | |
| Ascarides | 3 | 0.4 | |

e.p.g. = eggs per gram of faeces.

### 3.4. Relationship between PCV values and antigen ELISA

During the third visit the relationship between PCV levels and Ag-ELISA results was investigated. Ag-ELISA negative sera had average PCV values between 31 and 35%, while animals which were found positive in the Ag-ELISA had PCV values ranging between 26 and 30%. Some negative animals had PCV values above 50%, while all positive sera were below 40% (Figs 2a and 2b).

### 3.5. Tsetse fly density

Between 0.2 and 9.4 tsetse/trap/day were counted.

## 4. DISCUSSION

The results obtained from the central part of Côte d'Ivoire show a trypanosome prevalence rate in cattle of 14%. However, during the initial visit diagnostic capabilities were hampered considerably due to the lack of a phase contrast microscope thus underestimating prevalence rate.

Using standard parasitological techniques (BCT/MHCT) 3% of the samples was detected positive. However, the examination of stained blood smears detected 7% of the animals infected, and the Ag-ELISA found 5% of the cattle infected. The results obtained are relatively low, possibly due to low parasitaemia levels. On the other hand, the tsetse fly density in the field was similarly low (0.2 - 9.4 tsetse/trap/day). Moreover, during the dry season, people tend to burn the bush, which has a profound effect on the ecosystem resulting in a strong reduction of the tsetse population.

Parasitological examination detected 63% of the 196 animals infected with trypanosomes. Serological examination was able to detect 61 (31%) of the positive cases. The predominant trypanosome species detected were *T. brucei* and *T. vivax*.

It has been suggested that acute parasitaemias may not be associated with the presence of circulating antigens, since trypanosomes have to be destroyed first by the immune system before release of internal antigens, which are the determinants detected by the monoclonal antibodies used

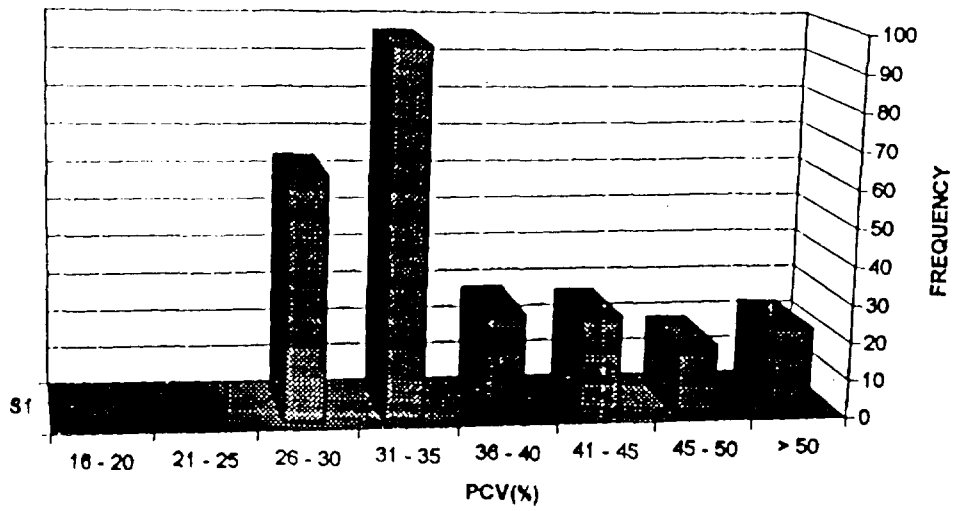


*FIG. 2a. Frequency distribution of packed red cell volume (PCV) values of Antigen ELISA negative cattle at the third herd visit in Côte d'Ivoire.*

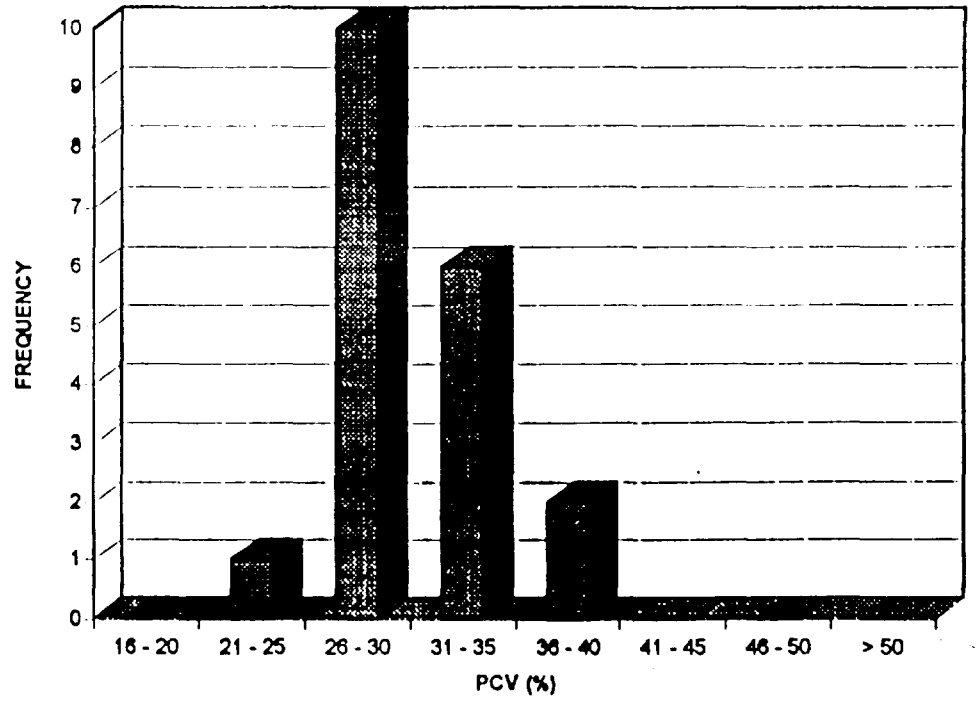


*FIG. 2b. Frequency distribution of packed red cell volume (PCV) values of Antigen ELISA positive cattle at the third herd visit in Côte d'Ivoire.*

in the ELISA [3, 4]. Consequently, the Ag-ELISA is a sensitive test for detecting chronic infections, but less suitable to detect acute infections.

*Babesia bovis* (3%) and *microfilaria* (7%) were detected by stained blood smears, but are unlikely to give cross reactions with the Ag-ELISA [1].

The difference between PCV values of the animals testing negative and positive in the Ag-ELISA was not great, but awaits testing for statistical significance. Moreover, it should be taken into account that the presence of *Babesia* and strongyles can equally exert a depressive effect on PCV values.

In conclusion, the combination of two techniques, one parasitological (BCT) and the other serological (Ag-ELISA), will increase the number of positive cases detected and thus enhance the diagnosis of bovine trypanosomosis [3].

## ACKNOWLEDGEMENTS

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture kindly contributed substantial funding and provided the equipment and reagents used for this research. We would like to thank Dr. Jacob Zinsstag of the C.S.R.S and all the technicians in the laboratories of Bouaké and Bingerville.

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# THE USE OF ANTIGEN ELISA TO MONITOR THE EFFECTIVENESS OF A TSETSE CONTROL CAMPAIGN IN THE UPPER DIDESSA VALLEY, WESTERN ETHIOPIA

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**Abstract**

THE USE OF ANTIGEN ELISA TO MONITOR THE EFFECTIVENESS OF A TSETSE CONTROL CAMPAIGN IN THE UPPER DIDESSA VALLEY, WESTERN ETHIOPIA.

Blood and serum samples were collected from a tsetse free zone in the central highlands of Ethiopia. The samples were collected to determine the specificity and establish percentage positivity cut-off points of the antigen ELISA. Blood samples collected from these areas were negative for trypanosomosis using Standard Trypanosome Detection Methods (STDM). Ag-ELISA, in contrast, detected circulating trypanosomal antigens in 7.6% of the serum samples collected. Similarly, samples were collected from a tsetse infested zone in the upper Didessa valley, western Ethiopia, to assess the sensitivity of the Ag-ELISA. STDM detected trypanosomal infections in the range of 15.8 and 16.7% of blood samples from this zone. On the other hand, Ag-ELISA, indicated the presence of circulating trypanosomal antigens in 38.6% of serum samples tested.

Moreover, Ag-ELISA was used to monitor the effectiveness of a tsetse control campaign in the upper Didessa valley. There were great differences in the prevalence rates of trypanosomosis, as revealed by the STDM and Ag-ELISA, between the tsetse controlled and tsetse infested zones of the upper Didessa valley. Generally, the Ag-ELISA revealed the presence of circulating trypanosomal antigens in only 43.7% of patent infections. Nevertheless, the test detected 318 more cases which were not diagnosed by any one of the STDM used. More interestingly, Ag-ELISA indicated the widespread presence of *T. brucei* in the cattle sampled in all zones.

## 1. INTRODUCTION

In Ethiopia, trypanosomosis is routinely diagnosed in animals using clinical and laboratory findings. However, the clinical signs of trypanosomosis are not pathognomonic for the disease. Consequently, diagnosis often relies on other means such as detecting the parasites. But as trypanosomosis is mostly a chronic disease, the parasitological techniques currently in use are not sensitive enough to detect the majority of infections. Serological assays which detect antibodies directed against trypanosomes are very sensitive but often show too many false positive results due to the persistence of anti-trypanosomal antibodies in the circulation for a prolonged time [1]. In recent years, a diagnostic technique based on monoclonal antibodies and using an enzyme linked immunosorbent assay (Ag-ELISA) was developed at ILRAD to detect circulating trypanosomal antigens [2]. The test is used for the diagnosis of *Trypanosoma vivax*, *T. congolense* and *T. brucei* infections in cattle as well as *T. evansi* infections in camels [3].

The objective of the present study was to establish Ag-ELISA in the NTTICC laboratory in Bedelle and to use the test in combination with standard trypanosome detection methods (STDM) for monitoring the effectiveness of the tsetse control campaign in the upper Didessa valley, Western Ethiopia. Moreover, the test will be used to map the distribution and prevalence rate of trypanosomosis in the country using sera collected as part of the Pan African Rinderpest Campaign (PARC).

## 2. MATERIALS AND METHODS

Random (cluster) sampling was employed to study the prevalence rate of trypanosomosis in cattle herds. Blood and serum samples were collected from three different areas. The areas were categorized as either tsetse free, tsetse infested or tsetse controlled.

The tsetse free zone was located in the central highlands of Ethiopia. Samples were collected from this zone to determine the specificity of the test and to establish the cut-off point for the Ag-ELISA based on local negative bovine sera.

The tsetse infested and tsetse controlled areas were in the upper Didessa valley, western Ethiopia. Blood and serum samples were collected from the two zones once every three months. The

samples from the tsetse infested zone were used to estimate the sensitivity of the Ag-ELISA. Finally, Ag-ELISA was used to monitor the effectiveness of the tsetse control campaign in the upper Didessa valley.

### 2.1. Standard trypanosome detection methods

In the present study, Ag-ELISA was used together with three parasitological techniques referred to as STDM [4]. The STDM employed were thick and thin blood smears and the buffy coat technique (BCT). Thick blood smears were prepared and stained as described by Murray *et al.* [5]. The entire thick blood smear was examined using an oil immersion objective (Leitz NPL 100/1.30). Approximately 200 fields per slide were examined. Thin blood smears were prepared on the same slide next to the thick blood film and used to identify the trypanosome species. The BCT was used as explained by Murray *et al.* [5]. Two capillary tubes were prepared from each sample and were centrifuged on the spot. After measuring the Packed red Cell Volume (PCV) percentage, the contents of the buffy coat were expressed on a microscope slide. The drop of fluid was covered with a cover slip (22 x 22 mm) and examined using a microscope (Leitz NPL 25/0.05).

### 2.2. Antigen ELISA

Serum samples for Ag-ELISA were collected concurrently with the blood samples. Blood was collected from the jugular vein into 10 ml vacutainer tubes without additive. Serum was harvested following centrifugation of the blood samples. Samples were stored at -20$^0$C until used for analysis. The protocol and reagents distributed by the Joint FAO/IAEA Division were used to detect circulating antigens of *T. brucei, T. congolense* and *T. vivax* using Ag-ELISA.

## 3. RESULTS

### 3.1. Tsetse free study area

Blood and serum samples collected from tsetse free areas in the central highlands of Ethiopia were checked for the presence of trypanosomes using STDM and Ag-ELISA (Table I). Using STDM it was not possible to detect trypanosomes in the blood samples collected from the tsetse free areas. On the other hand, using Ag-ELISA 7.6% of the serum samples were found to contain circulating trypanosomal antigens. *T. brucei* accounted for the highest proportion of positive samples, followed by *T. vivax* and mixed trypanosome infections. Single infections due to a *T. congolense* antigenaemia were not detected.

The stained blood smears were negative for other haemoparasites.

TABLE I. DIAGNOSTIC RESULTS OF BLOOD AND SERUM SAMPLES COLLECTED IN A TSETSE FREE AREA IN THE CENTRAL HIGHLANDS OF ETHIOPIA

| | | | Trypanosome species | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Test | No. of samples | No. of pos. | T.b. | T.c. | T.v. | T.b./ T.c. | T.b./ T.v. | T.c./ T.v. | T.b./T.c. /T.v. |
| Stained blood smears | 300 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| BCT | 300 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ag-ELISA | 236 | 18 | 10 | 0 | 4 | 1 | 3 | 0 | 0 |

No.= number; pos.= positives; T.b.= *Trypanosoma brucei*; T.c.= *T. congolense*; T.v.= *T. vivax*
BCT= buffy coat technique; Ag-ELISA= antigen detection enzyme-linked immunosorbent assay

### 3.2. Tsetse infested study area

Samples were collected from a tsetse infested area in the upper Didessa valley where no previous history of tsetse control exists. The area has a high density of tsetse flies (*Glossina morsitans submorsitans* and *G. tachinoides*). The samples were analysed for the presence of trypanosome infections using STDM and Ag-ELISA (Table II). With regard to the STDM, trypanosomes were detected in 15.8% and 16.7% of the samples using stained blood smears and BCT, respectively. In both cases, *T. congolense* was the most frequently detected species followed by *T. vivax*, *T. brucei* and mixed infections. Ag-ELISA, in contrast, detected trypanosomal antigens in 38.6% of the serum samples, with *T. brucei* accounting for the highest proportion of positives followed by mixed infections, *T. congolense* and *T. vivax*.

TABLE II. DIAGNOSTIC RESULTS OF BLOOD AND SERUM SAMPLES COLLECTED IN A TSETSE INFESTED AREA IN THE UPPER DIDESSA VALLEY, WESTERN ETHIOPIA

| | | | Trypanosome species | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Test | No. of samples | No. of pos. | T.b. | T.c. | T.v. | T.b./ T.c. | T.b./ T.v. | T.c./ T.v. | T.b./T.c. /T.v. |
| Stained blood smears | 850 | 134 | 19 | 70 | 43 | 0 | 0 | 2 | 0 |
| BCT | 844 | 141 | 0 | 102 | 38 | 0 | 0 | 1 | 0 |
| Ag-ELISA | 841 | 325 | 176 | 34 | 25 | 58 | 17 | 1 | 14 |

No.= number; pos.= positives; T.b.= *Trypanosoma brucei*; T.c.= *T. congolense*; T.v.= *T. vivax*
BCT= buffy coat technique; Ag-ELISA= antigen detection enzyme-linked immunosorbent assay

### 3.3. Tsetse control study area

Similarly, samples were collected from a tsetse control project area in the upper Didessa valley in Western Ethiopia. The samples were analysed using STDM and Ag-ELISA (Table III). Using stained blood smears and BCT, trypanosomes were detected in 5.7% and 5.6% of the samples, respectively. In both techniques, *T. vivax* proved to be the predominant parasite species detected, followed by *T. congolense*. A single case of *T. brucei* was detected using the BCT, which was confirmed by Ag-ELISA. On the other hand, Ag-ELISA, detected trypanosomal antigens in 12% of the serum samples with *T. brucei* antigens being most frequently detected, followed by *T. congolense*, *T. vivax* and mixed infections.

TABLE III. DIAGNOSTIC RESULTS OF BLOOD AND SERUM SAMPLES COLLECTED IN A TSETSE CONTROLLED AREA IN THE UPPER DIDESSA VALLEY, WESTERN ETHIOPIA

| | | | Trypanosome species | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Test | No. of samples | No. of pos. | T.b. | T.c. | T.v. | T.b./ T.c. | T.b./ T.v. | T.c./ T.v. | T.b./T.c. /T.v. |
| Stained blood smears | 632 | 36 | 0 | 7 | 28 | 0 | 0 | 1 | 0 |
| BCT | 607 | 34 | 1 | 13 | 19 | 0 | 0 | 1 | 0 |
| Ag-ELISA | 632 | 76 | 35 | 24 | 12 | 3 | 2 | 0 | 0 |

No.= number; pos.= positives; T.b.= *Trypanosoma brucei*; T.c.= *T. congolense*; T.v.= *T. vivax*
BCT= buffy coat technique; Ag-ELISA= antigen detection enzyme-linked immunosorbent assay

### 3.4. Comparison between various diagnostic techniques

Of the 174 patent trypanosome infections diagnosed by BCT, 76 cases had circulating trypanosomal antigens as detected by Ag-ELISA (Table IV). On the other hand, Ag-ELISA diagnosed 318 more cases which were not discovered by any of the STDM utilised. Furthermore, the STDM and Ag-ELISA in combination detected a total of 416 trypanosomosis cases.

TABLE IV. COMPARISON BETWEEN THE BUFFY COAT TECHNIQUE (BCT) AND ANTIGEN ELISA WITH RESPECT TO DETECTING TRYPANOSOME INFECTIONS IN CATTLE

| | | BCT results | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| Antigen ELISA results | Positive | 76 | 318 | 394 |
| | Negative | 98 | 950 | 1048 |
| | Total | 174 | 1268 | 1442 |

## 4. DISCUSSION

Antigen detection ELISA appears to indicate the occurrence of trypanosome cases in areas with no previous record of tsetse flies. In Ethiopia, it is not uncommon to find cases of tsetse transmitted animal trypanosomosis at 2000 masl using STDM alone [6]. Previous records of tsetse fly catches indicated the presence of *G. m. submorsitans* at an altitude of 2000 masl near the Finchaa valley, Western Ethiopia, which is at an altitude lower than that of the present study areas [7]. Nevertheless, no additional information is available with regard to animal movements in the region, which might explain the presence or absence of infected animals in the area.

In the present study it was difficult to assess the true specificity of the Ag-ELISA, because it was not certain whether all animals in the tsetse free area were true or false negatives.

Before the start of the tsetse control programme in the upper Didessa valley, the prevalence rate of trypanosomosis using BCT was as high as 60% [8]. The area had been recently invaded by tsetse flies and many susceptible animals were exposed to the disease. Following the implementation of tsetse control operations, trypanosomosis cases were reduced considerably [8]. However, the reclaimed area is small compared to the adjacent tsetse infested area and extension of the control operation has not been possible due to lack of financial resources. Consequently, frequent break downs occurred along the barrier zones. Nevertheless, our results using STDM and Ag-ELISA are in agreement with previous findings, except that more *T. brucei* infections were detected by Ag-ELISA than in other studies. A great difference in trypanosome prevalence rates was detected between the tsetse controlled and tsetse infested areas of the upper Didessa valley using STDM and Ag-ELISA.

Considerable discrepancies were found between the two techniques. Ag-ELISA was not sensitive enough to detect patent infections as diagnosed by the STDM. Although it is not clear why Ag-ELISA was negative in the majority of patent infections, previous reports [9] have shown that the test gave false negative results at the early stage of infection. On the other hand, STDM gave relatively good results at the acute stage but were rather insensitive during the chronic phase of trypanosomosis and, in particular, misdiagnosed infections caused by *T. brucei*. STDM detected trypanosomes present in the peripheral circulation, while Ag-ELISA detected soluble antigens released when trypanosomes disintegrated [9]. Consequently, the sensitivity of the Ag-ELISA did not rely on the level of parasitaemias in peripheral blood and could be high when parasites were not detectable. For this reason, STDM and Ag-ELISA should be used in combination to enhance the diagnostic value of each technique.

Ag-ELISA seemed to detect the presence of *T. brucei* in cattle in the upper Didessa valley. Similar results were obtained examining bovine sera from the Central Region of Ghana from where no trypanosomosis cases had been recorded previously [10]. On the other hand, STDM detected *T. vivax* and *T. congolense* as the most predominant trypanosome species present in cattle in,

respectively, the tsetse controlled and tsetse infested areas of the upper Didessa valley. Although it is difficult to give a scientific explanation for this discrepancy, it should be considered that in Ethiopia there is widespread application as well as serious misuse of trypanocidal drugs. Moreover, the drugs are administered primarily to treat animals infected with *T. vivax* and *T. congolense*, which are considered the most important trypanosome species. This is not only because *T. brucei* causes mild infections in cattle, but also because this trypanosome species is not easily detectable using STDM. Consequently, it can not be discounted that *T. brucei* could be the most widespread chronic infection present among cattle herds in Ethiopia.

More interestingly, Ag-ELISA detected mixed trypanosome infections in many animals which had not been diagnosed as such previously by the STDM. Hornby [11] indicated that mixed trypanosome infections are easily overlooked since the presence of one species can inhibit multiplication of the second species.

## ACKNOWLEDGEMENTS

I am grateful to the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture for financial assistance and technical support by providing equipment, reagents and standardised protocols. I am also indebted to the International Laboratory for Research on Animal Diseases (ILRAD) for training one of the NTTICC laboratory staff members. Finally, I would like to express my sincere gratitude to the Head and all technical staff of the NTTICC for assistance in conducting the research.

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# SEROLOGICAL SURVEY OF AFRICAN ANIMAL TRYPANOSOMOSIS IN GHANA: THE ROLE OF THE ANTIGEN ELISA AS A DIAGNOSTIC TOOL

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**Abstract**

SEROLOGICAL SURVEY OF AFRICAN ANIMAL TRYPANOSOMOSIS IN GHANA: THE ROLE OF THE ANTIGEN ELISA AS A DIAGNOSTIC TOOL.

Preliminary results are presented of the analysis of 3000 serum samples collected from Zebu cattle in the Nabogu valley. Of the 3000 serum samples collected so far, 182 have been tested using the buffy coat technique (BCT) and the antigen ELISA test. A total of 56 samples have been found positive by both techniques. Using the parasitological technique (=BCT) 44 samples were diagnosed to be positive, while the Ag-ELISA detected 19 positive samples. The majority (75%) of cases detected positive by either technique was due to a single infection by *Trypanosoma brucei*.

## 1. INTRODUCTION

African animal trypanosomosis and human sleeping sickness impose a severe constraint on the socio-economic development of the areas infested by tsetse flies. Experience in Ghana showed that the disease in animals often assumed the chronic form hence production losses occurred over long periods of time without farmers being aware. Moreover, trypanosomosis is the most important infectious disease hampering the development of the livestock industry since it holds captive vast areas of productive farm- and grazing land. Over 80% of the cattle population in Ghana is trypanotolerant [1], but it is well known that under high tsetse challenge these breeds do succumb to the disease just like trypanosensitive breeds. The control of the tsetse fly through the use of simple cost effective and environmentally safe techniques and devices offers a long term solution to trypanosomosis control.

The Epidemiolgy Unit of the Veterinary Services Department has started compiling information on the impact of various diseases, including trypanosomosis, on the productivity of livestock in Ghana [2]. At the same time the unit is carrying out an extensive vector survey to update the tsetse distribution map.

The objective of the present study was to collect baseline data on the species, density and distribution of the vector on a nationwide scale. Furthermore, it was intended to determine the prevalence of the disease and its impact on livestock productivity. Moreover, the study was meant to determine low cost but effective vector disease control devices and strategies and to assess the efficacy of the antigen Elisa as a diagnostic tool.

## 2. MATERIALS AND METHODS

Four priority areas were selected for the study (Fig. 1):

(1) Black Volta basin (northwestern Ghana)
(2) Nabogu valley (Pong-Tamale)
(3) Brong Ahafo region
(4) Accra Plains (southern Savannah zone)

The Tsetse and Trypanosomosis Control Unit has since the inception of the National Livestock Services Project collected nearly 3000 serum samples from both the Southern Savannah Zone and the Nabogu Valley. Additional serum samples will be collected from the other two priority areas and analysed together with 10 000 serum samples collected as part of the Pan African Rinderpest Campaign (PARC) already present in the serum bank. The results will be used to determine the prevalence of trypanosomosis in the country.

For the vector surveys biconical and monoconical traps were deployed in the riverine vegetation along the river courses and placed from 200 to 300 meters apart depending on the thickness of vegetation cover. The traps were inspected twice daily for one week in each particular location. Tsetse

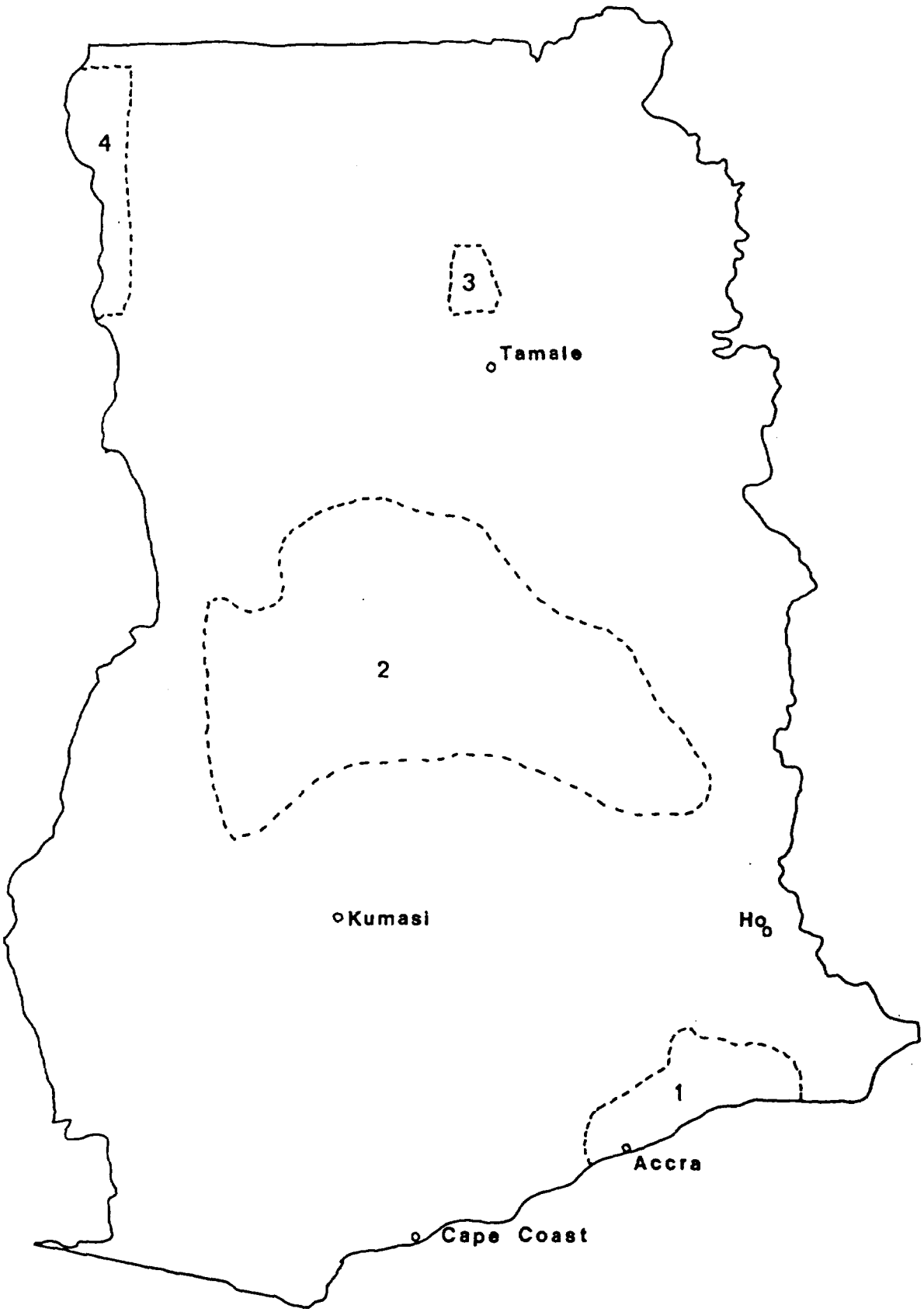


*FIG.1. Map of Ghana showing the National Livestock Services Project's priority areas for tsetse control (indicated by a dashed line): 1= Winneba/Kasoa in the Accra Plains (southern Savannah zone); 2= Brong Ahafo region; 3= Pong-Tamale in the Nabogo valley; 4= Wa in the Black Volta basin (northwestern Ghana).*

fly catches, species and densities (number of tsetse per trap per day) were recorded. Fresh tsetse flies were dissected to determine the age and infection status.

Samples were collected with microhaematocrit tubes from the ear vein, centrifuged and examined for the presence of trypanosomes using the buffy coat technique (BCT). Whenever samples were found to be positive, thin smears were made from the red blood cell/plasma interphase, the number of trypanosomes were scored and the trypanosome species was identified. Blood samples were taken from the jugular vein using vacutainer tubes and kept on the bench for one to two hours. Subsequently, the serum was separated and stored at -20°C for testing using the antigen ELISA test. Samples that were blood tinged were first centrifuged to obtain clear sera.

## 3. RESULTS

The entomological study is still in progress and results will be reported once cattle in all four priority areas have been sampled.

Since January, 1995 nearly 3000 serum samples have been collected, of which 182 samples have been tested. A total of 56 samples have been found positive both by the BCT and the Ag-ELISA test (Table I).

TABLE I. RESULTS OF SAMPLES COLLECTED FROM CATTLE IN NABOGU VALLEY

| Test | No. of samples | No. of positive samples | % positive |
|---|---|---|---|
| BCT | 182 | 44 | 24.4 |
| Ag-ELISA | 182 | 19 | 10.4 |
| Combined BCT / Ag-ELISA | 182 | 56 | 30.8 |

No. = number.
BCT = buffy coat technique.
Ag-ELISA = antigen-detection enzyme-linked immunosorbent assay.

Using the parasitological technique (=BCT) 44 samples were diagnosed to be positive, while the Ag-ELISA detected 19 positive samples. The majority (75%) of the cases detected by either technique was due to a single infection. The predominant trypanosome species detected was *Trypanosoma brucei* (Table II).

TABLE II. DISTRIBUTION OF TRYPANOSOME SPECIES DETECTED IN 182 CATTLE FROM NABOGU VALLEY

| Test | No. of samples positive | T.b. | T.c. | T.v. | T.b./T.c. | T.b./T.v. | T.c./T.v. | T.b./T.c./T.v. |
|---|---|---|---|---|---|---|---|---|
| BCT | 44 | 13 | 5 | 14 | 3 | 6 | 3 | 0 |
| Ag-ELISA | 19 | 11 | 0 | 5 | 1 | 2 | 0 | 0 |

No. = number.
BCT = buffy coat technique.
Ag-ELISA = antigen-detection enzyme-linked immunosorbent assay.
T.b. = *Trypanosoma brucei*; T.c. = *Trypanosoma congolense*; T.v. = *Trypanosoma vivax*.

The prevalence of *T. brucei*, *T. congolense* and *T. vivax* detected during the trials (BCT and Ag-ELISA combined) was 17.6%, 6.0% and 14.8%, respectively (Table III).

TABLE III. PREVALENCE OF TRYPANOSOME SPECIES AS DETECTED BY THE TWO DIAGNOSTIC TECHNIQUES USED

| Trypanosome species | BCT | Ag-ELISA | BCT and Ag-ELISA combined |
|---|---|---|---|
| *Trypanosoma brucei* | 22 | 14 | 32 |
| *Trypanosoma congolense* | 11 | 1 | 11 |
| *Trypanosoma vivax* | 23 | 7 | 27 |

BCT = buffy coat technique.
Ag-ELISA = antigen-detection enzyme-linked immunosorbent assay.

## 4. DISCUSSION

The results obtained so far indicate that, contrary to previous findings, the predominant trypanosome species in the Nabogu valley was *T. brucei* [3].

Moreover, our results indicate that the BCT appears to be more sensitive than the Ag-ELISA. This finding may be due to the fact that the samples were taken from Zebu cattle, a trypanosensitive breed. Consequently, most of the animals were in the acute phase of the disease when parasitaemia is very high but antigenaemia is low. A similar situation occurs in high challenge areas. Another possible explanation for our results is that the quality of the reagents was affected by poor handling while in transit from Vienna to Ghana [4]. Therefore, it is advisable to retest the serum samples using fresh ELISA reagents and compare the results.

## ACKNOWLEDGEMENTS

We wish to acknowledge the assistance of Dr. C.I. Mahaina, Deputy Head of the Tsetse Control Unit, Mr. George Adjetey, Assistant Chief Technical Officer in charge of the serology unit of the Central Veterinary Laboratory and Mr. Peter K. Busie, our typist. We are very grateful to the entire staff of the Tsetse Control Unit for their tireless work performed often under extremely difficult conditions. We are thankful to the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture for financial and technical support.

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# USE OF A TRYPANOSOMAL ANTIGEN ELISA TO MONITOR TSETSE AND TRYPANOSOMOSIS CONTROL PROGRAMMES IN KENYA

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**Abstract**

USE OF A TRYPANOSOMAL ANTIGEN ELISA TO MONITOR TSETSE AND TRYPANOSOMOSIS CONTROL PROGRAMMES IN KENYA.

The capture Antigen-ELISA was used to monitor serum samples originating from three study areas in Kenya. At the Galana ranch the test was used to assess re-invasion of an area previously cleared of *Glossina pallidipes*. In Busia district the Ag-ELISA is being used to monitor the progress of a tsetse and trypanosomosis control programme. At Taita and Tara ranches the capture Antigen ELISA and the buffy coat technique (BCT) were used to evaluate the efficacy of a commercial Cypermethrin® dip for the control of *Glossina pallidipes*.

## 1. INTRODUCTION

Tsetse-transmitted trypanosomosis is endemic in 60% of Kenya's rangelands, which constitute 25% of the country [1]. Though large-scale eradication of tsetse and trypanosomosis remains an elusive goal, the availability of low-cost technologies and the prospects of community participation, give optimism for the smaller, and more managable tsetse and trypanosomosis programmes [2]. This study reports results derived from some of the smaller tsetse and trypanosomosis programmes in Kenya. These programmes included a Deltamethrin® trial in Galana Ranch, a tsetse and trypanosomosis control project on the Kenya-Uganda border in Busia District and a Cypermethrin® trial in Taita/Taru.

The capture Antigen ELISA was used to monitor serum samples originating from Galana ranch. The assay was used to detect trypanosome infections in cattle, which might indicate a possible re-invasion of an area previously cleared of the predominant tsetse species in the area, *Glossina pallidipes*.

In Busia district located at the border with Uganda, the Antigen ELISA was used to evaluate the success of a tsetse and trypanosomosis control programme. A project supported by the Overseas Development Agency of the United Kingdom was initiated in collaboration with the governments of Kenya and Uganda to control tsetse and trypanosomosis along the common border. During the years 1992 and 1993 impregnated targets and traps as well as Pour-on® and Spot-on® were the tsetse control methods used. All animals detected positive for trypanosomes were treated with the trypanocidal drug diminazene aceturate (Berenil®).

In the third study area, at the Taita and Taru ranch, the capture Antigen ELISA and the buffy coat technique (BCT) were used to evaluate the efficacy of a commercial Cypermethrin® dip for the control of *Glossina pallidipes*. The two ranches are adjacent to one another, Taita ranch being located in Taita district, Taru ranch in Kwale district. The ranches are situated in the coastal hinterland of Kenya and are infested with a single species of tsetse fly, *Glossina pallidipes*. Rainfall is low to medium (Fig. 1) and the vegetation is mainly thorn-scrub, a suitable tsetse habitat.

## 2. MATERIALS AND METHODS

### (1) Galana ranch

The tank "E" area was selected for sample collection since it had been cleared of *Glossina longipennis* using Deltamethrin®-impregnated traps. In order to measure the rate of tsetse re-invasion of this area, a clean herd of 100 steers was introduced and monitored weekly by BCT to assess the degree of trypanosome challenge. Concurrently, tsetse trapping was carried out using bi-conical traps on a weekly basis. In addition, serum samples were collected from all animals for the first three months on a monthly basis, and thereafter, once every three months. Serum samples were stored at -20°C for future analysis by capture Antigen ELISA. A herd of 100 steers kept in an area of medium

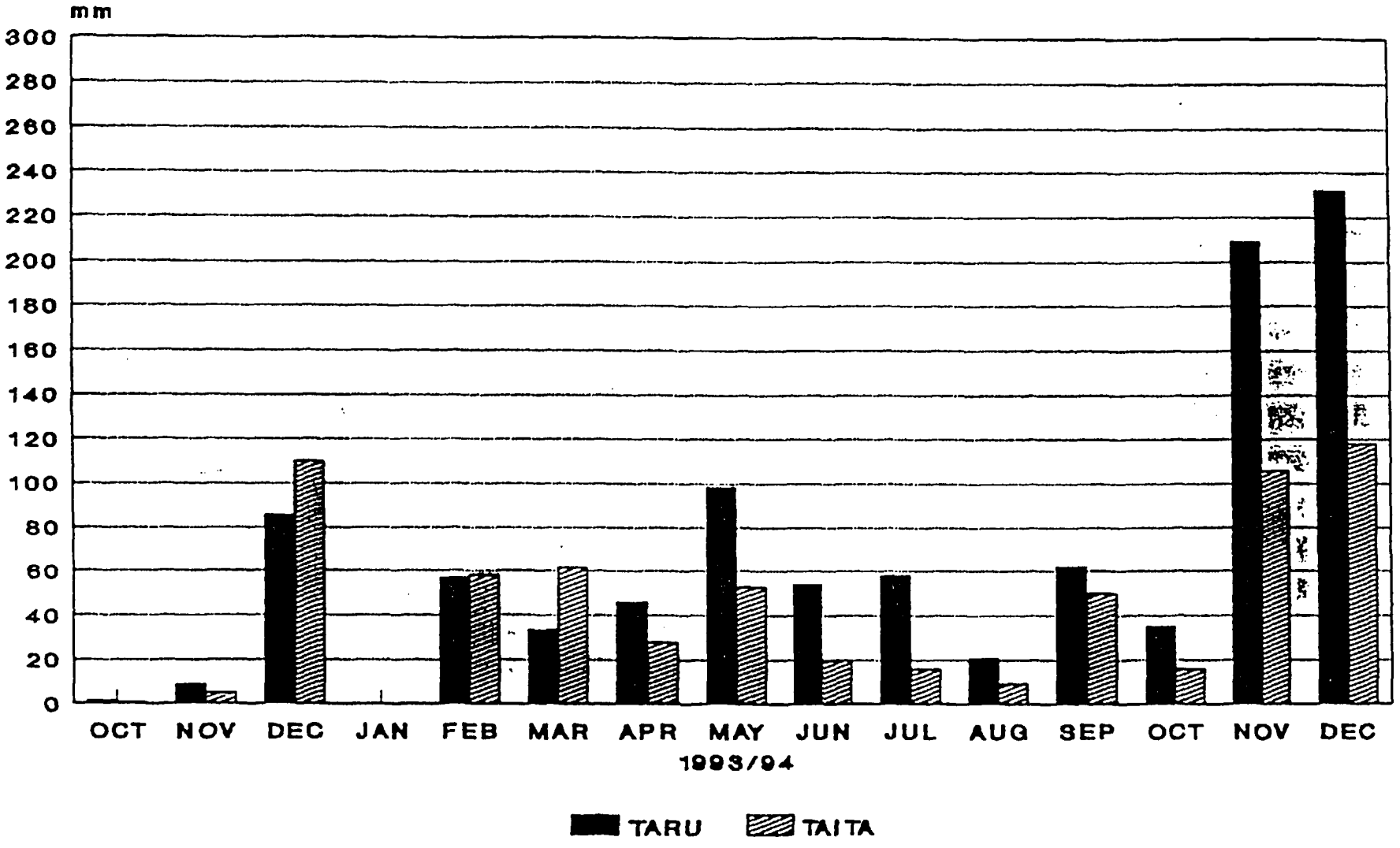


*FIG. 1. Monthly rainfall (mm) on Taita and Taru ranches in 1994.*

tsetse challenge at a distance of about 30 km from the tank "E" area was used for comparative reasons.

(2) Busia district

Three areas, namely Rukada, Apatit and Katelenyan were selected as sites for the assessment of the control programme. As part of the assessment the tsetse population was monitored and cattle were sampled to detect trypanosome infections using parasitological (BCT) and serological (Ag-ELISA) techniques.

(3) Taita and Taru ranch

A herd of 1000 cattle was maintained on Taita ranch and treated weekly with Cypermethrin® using a diptank. Among this herd a sentinel group of 100 steers was monitored weekly by BCT and monthly by Ag-ELISA. On the adjacent Taru ranch at a distance of 10 km, a second sentinel herd of 100 steers was monitored the same way and sprayed with Steladone® weekly. Half of the animals in each sentinel group (50 steers) were treated with diminazene aceturate (Berenil®) on an individual basis when found infected and the other half (50 steers) were treated with homidium chloride (Novidium®) in a block treatment whenever $> 10\%$ of the herd was found to be infected within any 4-week period.

## 3. RESULTS

(1) Galana ranch

A total of 900 serum samples were collected and are awaiting Antigen ELISA analysis. The study forms part of the ODA supported research programme on the epidemiology of animal trypanosomosis. Consequently, the serological results will be combined with data on tsetse challenge as well as BCT scores and haematocrit values for epidemiological analysis.

(2) Busia district

A total of 2400 sera were collected and stored at -20°C for future analysis by Antigen-ELISA. Presently 780 serum samples have been analysed. Complete data analysis is anticipated following the collection of all Ag-ELISA, tsetse, parasitological, and haematological data.

(3) Taita and Taru ranch

To date only 392 of the 4290 serum samples have been analysed by Ag-ELISA. Using parasitological techniques (BCT) 3 samples were detected positive for *T. congolense*, 60 for *T. vivax* and none for *T. brucei*. Using the Ag-ELISA, 7 samples were found positive for *T. congolense*, 26 for *T. vivax* and 73 for *T. brucei* (Table I). A cut-off value of 10 percent positivity (PP) was used for each one of the three trypanosome species. The cut-off value was determined using the ELISA results of a negative cattle population (Table II). BCT results showed a significant difference between the steers at Taita and Taru (Figs 2a and 2b). However, the two sites seem to show a similar trend declining over time in the number of tsetse caught per trap per day (Fig. 3), suggesting a slight positive effect of dipping cattle with Cypermethrin®.

TABLE I. PARASITOLOGICAL AND SEROLOGICAL INVESTIGATIONS OF CATTLE UNDER TSETSE CHALLENGE AT THE TAITA AND TARU RANCHES, KENYA

| Diagnostic test | | | | | |
|---|---|---|---|---|---|
| Buffy coat technique | | | Antigen ELISA | | |
| T.b. | T.c. | T.v. | T.b. | T.c. | T.v. |
| 0 | 3 (0) | 60 (5) | 73 | 7 | 26 |

T.b. = *Trypanosoma brucei*; T.c. = *Trypanosoma congolense*; T.v. = *Trypanosoma vivax*.
( ) = number of samples detected positive using the Antigen ELISA.

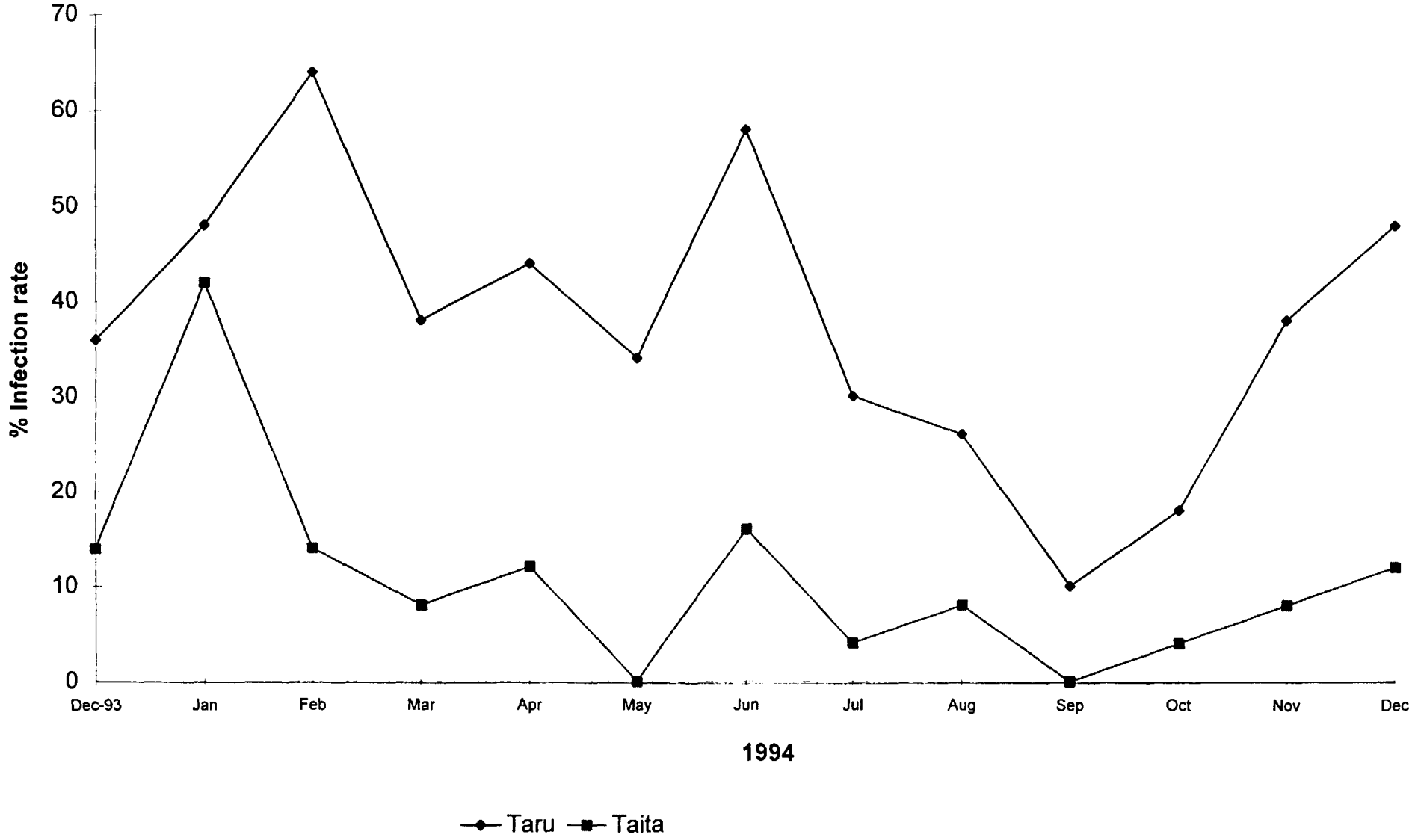


*FIG. 2a. Monthly prevalence of trypanosomosis as detemined by BCT in cattle on Taita and Taru ranches (Berenil group).*

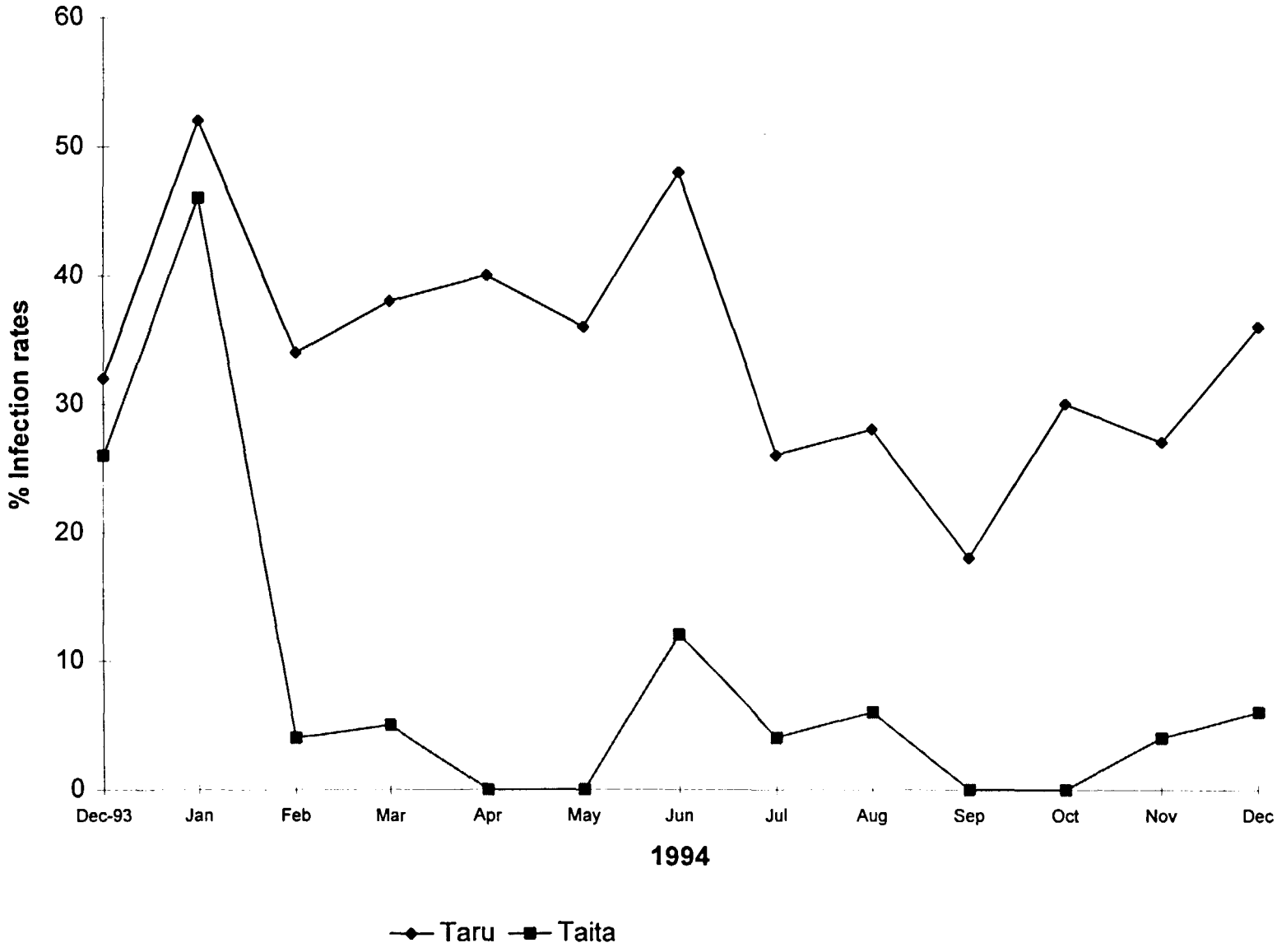


*FIG. 2b. Monthly prevalence of trypanosomosis as detemined by BCT in cattle on Taita and Taru ranches (homidium chloride group).*

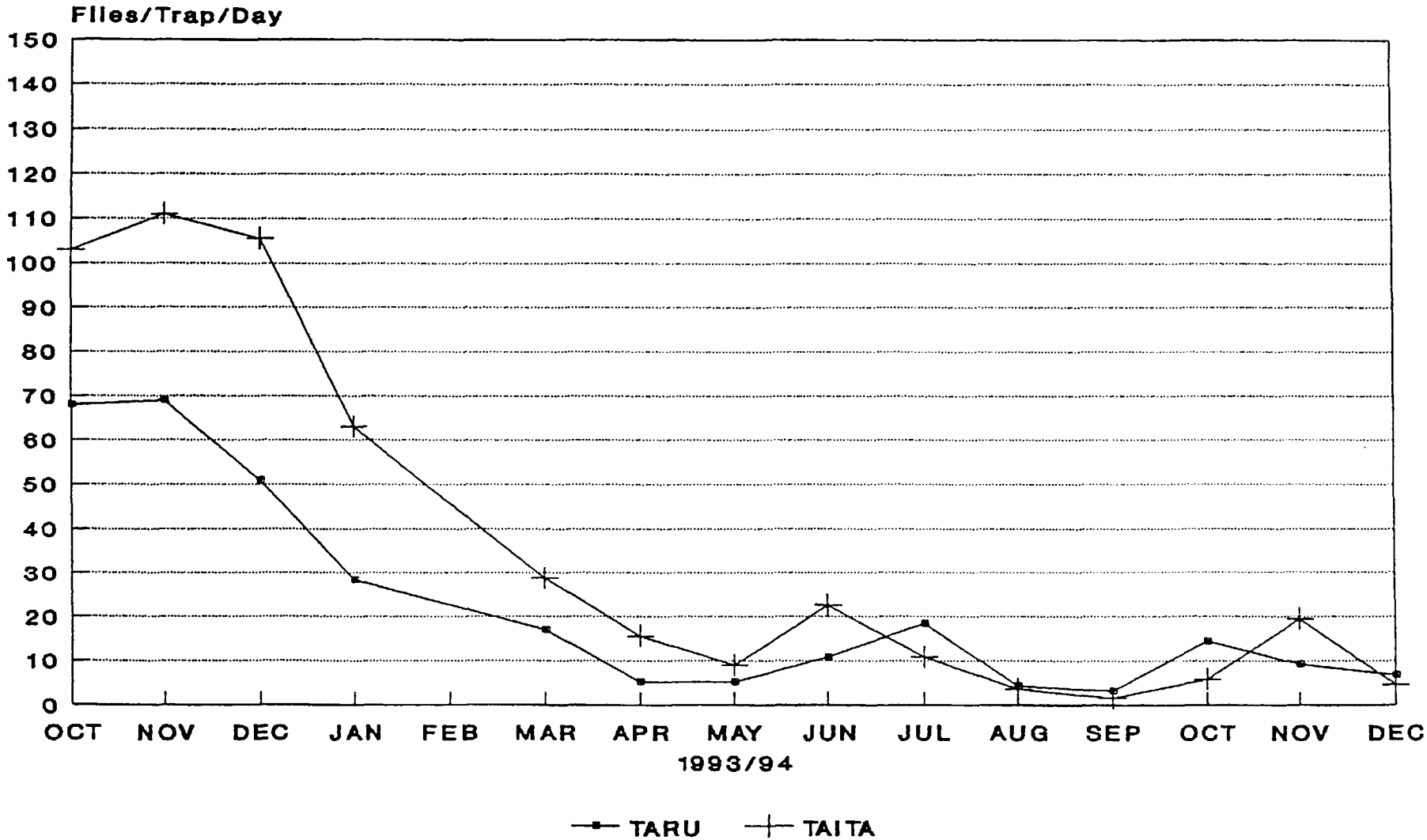


*FIG. 3. Mean tsetse fly catches on Taitu and Taru ranches in 1994.*

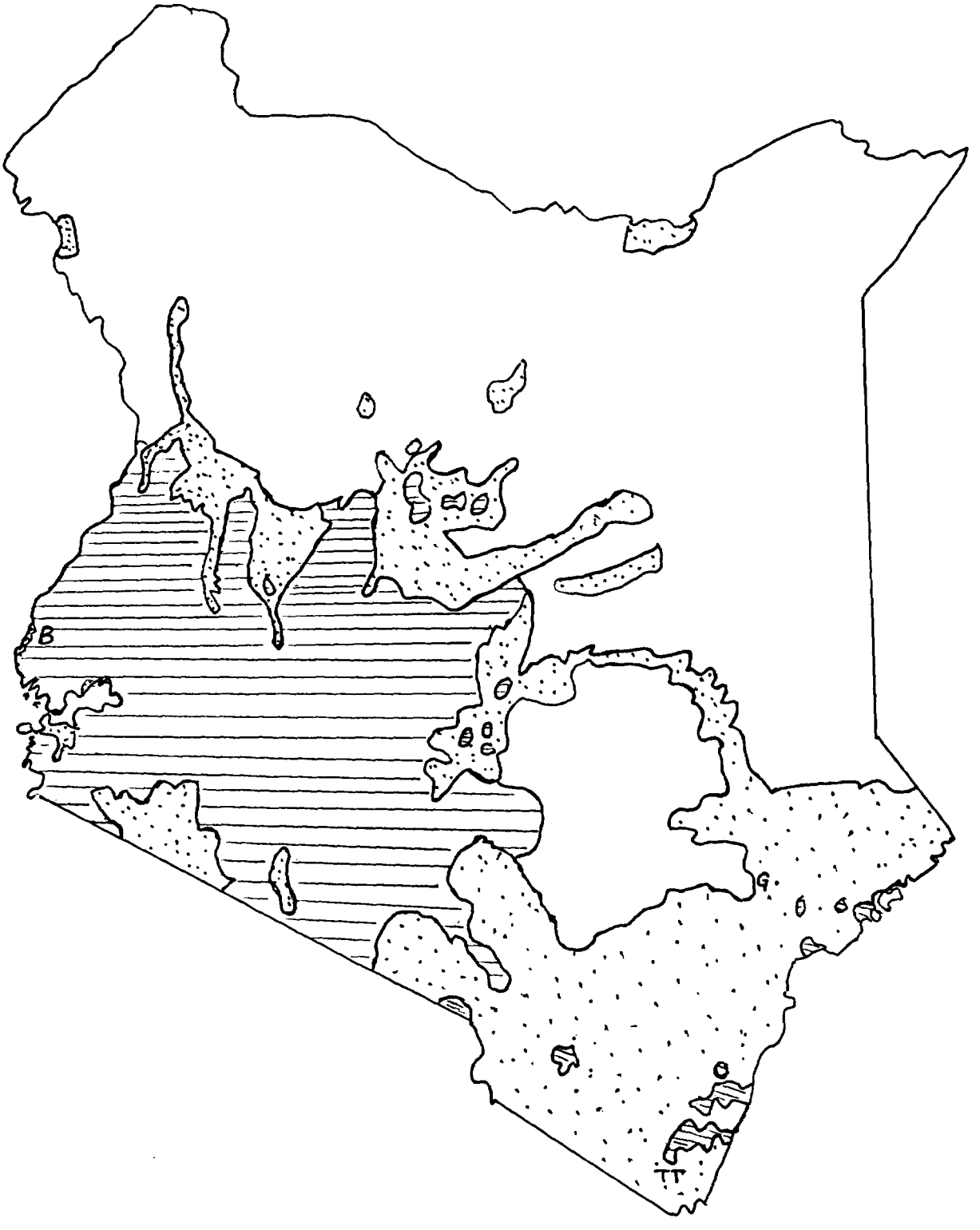


*FIG. 4. Tsetse distribution map of Kenya (1967) showing the study sites: B= Busia; G= Galana ranch; TT= Taita/Taru ranches; hatched area = tsetse free; dotted area = tsetse infested; blank area = unsurveyed.*

TABLE II. PARASITOLOGICAL AND SEROLOGICAL INVESTIGATIONS OF A NEGATIVE CATTLE POPULATION FROM THE KAPITI AND KETRI FARMS

| | | Diagnostic test | | | | | |
|---|---|---|---|---|---|---|---|
| | | Buffy coat technique | | | Antigen ELISA | | |
| Farm | No. | T.b. | T.c. | T.v. | T.b. | T.c. | T.v. |
| Kapiti (ILRI) | 200 | 0 | 0 | 0 | 4 | 0 | 2 |
| KETRI (Muguga) | 60 | 0 | 0 | 0 | 0 | 0 | 0 |

T.b. = *Trypanosoma brucei*; T.c. = *Trypanosoma congolense*; T.v. = *Trypanosoma vivax*.
No. = number of animals sampled.

## 4. DISCUSSION

Since only a small part of the serum samples (1172 of the 7590 samples) have been analysed, the results should be discussed following the analysis of the complete data set. However, preliminary results of the Ag-ELISA test indicate a high specificity (99.2%) and a low sensitivity (8.3%). The sensitivity may require further readjustment, especially with regard to the cut-off values of percent positivity for *T. congolense* and *T. vivax*. As shown in Figures 2a and 2b, parasitological results show evidence of a significant decline in infection rates on Taita Ranch, where Cypermethrin® dip was being used. However, there was no significant difference in tsetse fly catches in the two places. Similar observations were made in an earlier study on Galana Ranch where Deltamethrin® was being used. It was suggested that the lack of difference in fly numbers could be due to re-invasion (Fig. 4) and that the high degree of protection of cattle against trypanosomosis was due to the possible repellant effect of the pyrethroid [3].


## ACKNOWLEDGEMENTS

This work was supported by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, to whom we are very greatful. We specifically thank the KETRI field staff at Galana, Taita/Taru and Busia for technical assistance. We would also like to thank Dr. J.B. Githiori for the graphics. This paper is published with the permission of the Director, KETRI.


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# EVALUATION OF THE ANTIGEN ELISA AS A TOOL FOR ASSESSING THE IMPACT OF TSETSE CONTROL PROGRAMMES ON THE INCIDENCE OF TRYPANOSOME INFECTIONS IN LIVESTOCK

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**Abstract**

EVALUATION OF THE ANTIGEN ELISA AS A TOOL FOR ASSESSING THE IMPACT OF TSETSE CONTROL PROGRAMMES ON THE INCIDENCE OF TRYPANOSOME INFECTIONS IN LIVESTOCK.

The objective of the present study was to determine the suitability of the antigen ELISA as a method for assessing the efficacy of a tsetse control programme, based on the use of traps and screens impregnated with Deltamethrine®. The efficacy of a tsetse control programme can be measured by monitoring the tsetse density on the one hand and by monitoring the infection rate and packed red cell volume (PCV) level of cattle on the other hand. We have used both monitoring methods as reference points to evaluate the Ag-ELISA, by determining how results of the Ag-ELISA correlated with those of the two reference methods. The monitoring was carried out in 8 localities: 4 situated in the tsetse control area and 4 located in a region without tsetse control (untreated area).

The results were collected over a 12-month-study period. In the Deltamethrine® treated area the tsetse density was reduced by nearly 95% after one month of control and virtually no tsetse fly could be caught after 12 months of tsetse control. A similar tendency was observed for the trypanosome infection rate in cattle, which decreased from 6% to 2% in the treated area, but increased from 3% to 10% in the untreated area.

At the begining of the experiment, the average PCV values (29.4%) were identical for animals in the treated and the untreated areas. Following 12 months of tsetse control, the average PCV value of animals in the treated area was 4.7 percentage units higher than of animals in the untreated area. The study of antigenaemia covered the first three months of the experiment. The rate of anigen positivity did not seem to correlate with trypanosome infection rates in cattle, nor with tsetse fly densities. This may be attributed to the low sensitivity of the test, while the specificity as studied on sera from tsetse free areas was high (98%). The monitoring period using the Ag-ELISA should be extended to cover the entire 12-month-study period to verify the preliminary conclusion. In addition, studies on the sensitivity of the test should be carried out.

## 1. INTRODUCTION

Tsetse and trypanosomosis control using insecticide impregnated traps and screens is increasingly being used in different localities in Mali. However, there is a need to monitor the efficacy of the control operations using improved diagnostic techniques, as the currently available tests are known to be of limited sensitivity. The antigen ELISA originally developed at ILRAD in 1989 [1, 2] has been proposed as a suitable test for monitoring tsetse control programmes. The test is based on monoclonal antibodies specific for the three trypanosome species pathogenic in cattle: *T. vivax*, *T. congolense* and *T. brucei* [3]. Subsequently, the test has been validated in 10 different research laboratories in Africa [4]. The results of the validation study called for some refinement of the assay which was done at the FAO/IAEA Agriculture and Biotechnology Laboratory at Seibersdorf. The improved version of the test has been used for validation under various ecological situations. In our case we have been using the test to monitor a tsetse/trypanosomosis control operation in the region of Niena in Mali.

## 2. MATERIALS AND METHODS

### 2.1. Study sites

The sector of Niena has been chosen for a pilot tsetse control programme. It covers 500 km$^2$ and contains approximately 56 000 cattle. Two species of tsetse flies are prevalent: *Glossina palpalis gambiensis* and *G. tachinoides* alongside the river Bagoe and its affluents (Fig. 1). The annual rainfall is 800-1000 mm (Fig. 2).

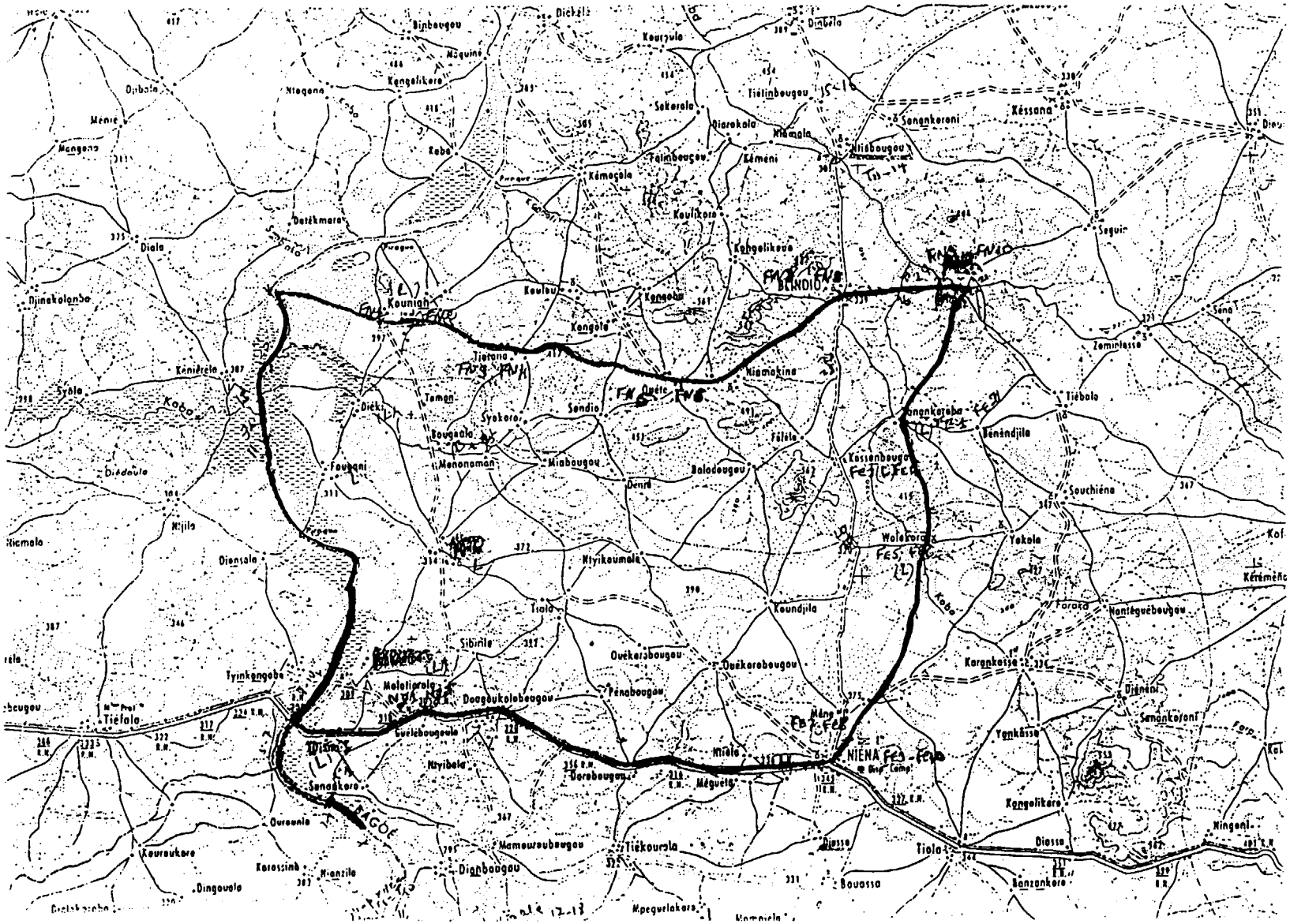


*FIG. 1. Map of the study area for implementing a pilot tsetse control programme near Niena in Mali.*

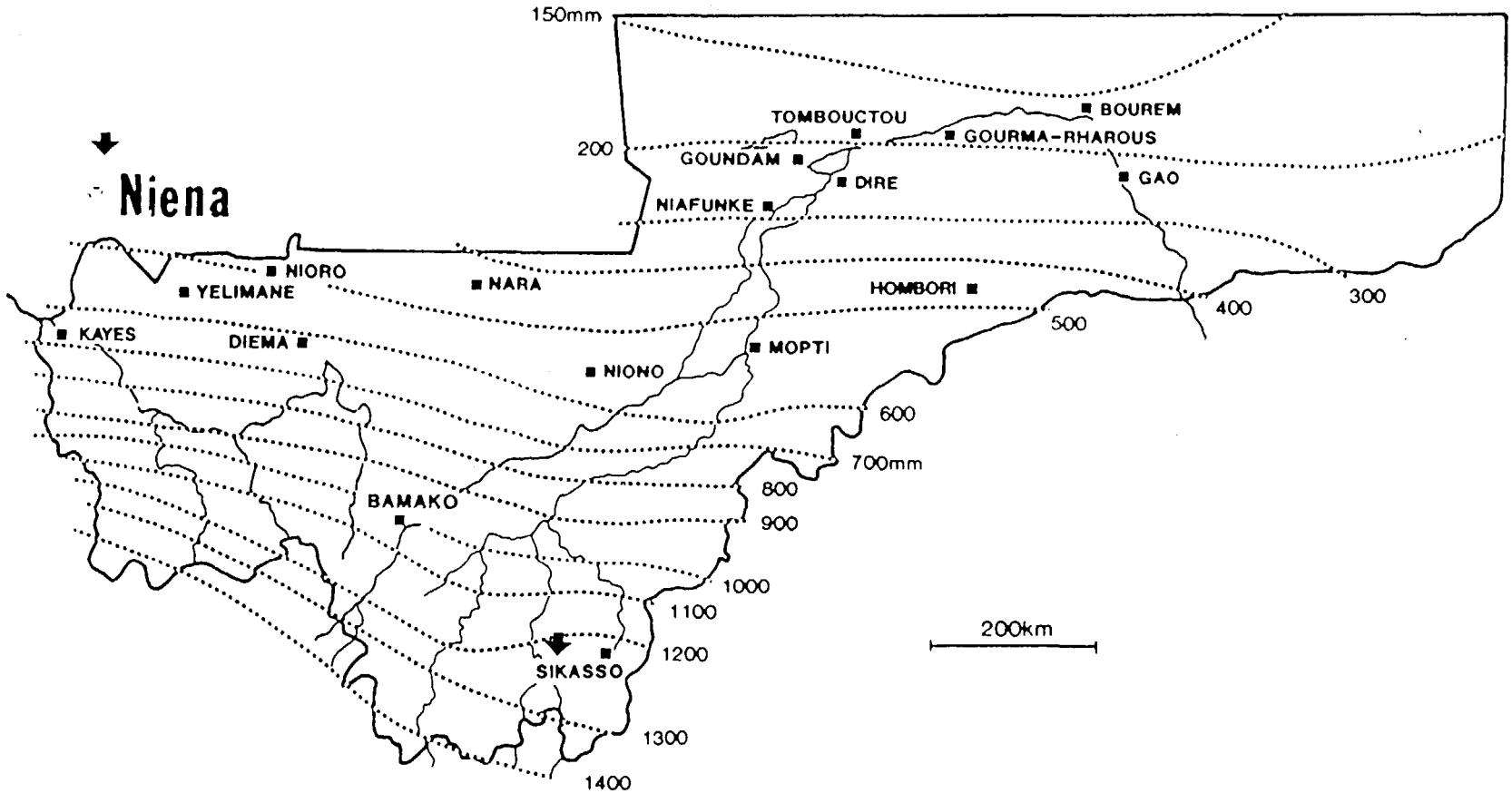


*FIG. 2. Map of Mali indicating the study area (arrow) and the isohyets of the annual rainfall averaged over the period between the years 1922 and 1980.*

### 2.2. Monitoring of tsetse flies

Within the limits of the tsetse control area impregnated traps (biconical and vavoua) were deployed alongside the Bagoe river and its tributaries. Every 1-2 months entomological surveys were carried out using biconical traps to monitor the apparent density of tsetse flies (number of tsetse/trap/day) at selected points on the river banks. For comparitive purposes the same studies were conducted at selected sites in the neighbouring untreated area.

### 2.3. Monitoring of cattle infections

Fifty animals were selected in each of eight villages. Four of the villages were located in the test area, while the four remaining villages were located in the untreated area. The animals were ear-tagged and blood samples were collected every two months for monitoring of parasites and antigens. Parasite detection was done using the buffy coat technique (BCT). Thin blood smears were prepared for the identification of the trypanosome species and for monitoring other blood parasites (*Anaplasma*, *Babesia*, *Theileria*, etc.). Faecal samples were collected at irregular intervals and analysed. Serum samples were collected from the 8 herds for antigen detection. The sera were identified with the animal number, the village name, the date of sampling and a project reference number and stored at -20°C until use. The monitoring of antigens was conducted according to the revised protocol prepared by the IAEA using the biological reagents as developed by ILRAD.

### 2.4. Data analysis

To determine the effect of the tsetse control measures on the prevalence of trypanosomosis, the results obtained from the animals in the treated area at various sampling times were assessed and compared with the results obtained from animals in the untreated area. In addition, a comparison was made between tsetse fly density and trypanosomosis prevalence in both treated and untreated areas on the one hand and antigen positivity on the other hand.

## 3. RESULTS

### 3.1. Monitoring of tsetse flies

Before tsetse control measures were initiated, the apparent tsetse density was relatively high in the treated as well as in the untreated area for both tsetse species (Table I). One month after the beginning of the control programme, tsetse density was reduced by 93% for *G. palpalis gambiensis* and by 98% for *G. tachinoides*. One year after deployment of the targets, in December 1994, no tsetse flies could be captured in the treated area (Table I).

TABLE I. VARIATIONS IN APPARENT DENSITIES OF TSETSE FLIES ( TSETSE/TRAP/DAY) IN BOTH UNTREATED AND TREATED AREAS DURING A TSETSE CONTROL PROGRAMME IN MALI

| Area | Tsetse species | Months before or after start* of control programme | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | -1 | +1 | +3 | +6 | +8 | +11 | +12 |
| Untreated | *G. palpalis*† | 11.55 | 6.15 | - | 2.35 | 1.75 | 9.80 | 3.25 |
| | *G. tachinoides* | 2.40 | 1.30 | - | 0.15 | 0 | 0.30 | 1.90 |
| Treated | *G. palpalis*† | 16.66 | 0.56 | 0.10 | 0.36 | 0.36 | 1.70 | 0 |
| | *G. tachinoides* | 3.70 | 0.03 | 0.05 | 0.16 | 0.16 | 0.06 | 0 |

* The tsetse control programme started in April 1993 in an area of 500 km$^2$ near Niena, Mali.
†*G. palpalis* = *Glossina palpalis gambiensis*.

### 3.2. Monitoring of cattle infections

At the begining of the project the trypanosome prevalence rate in the selected herds was 6.2% in the treated area and 3.4% in the untreated area (Table II). But it should be noted that animals before entering the study had been regularly treated by the owners using either Berenil® or Trypamidium®. This accounts for the low trypanosome infection rate in the cattle at the begining of the experiment. However, once the study was started animals were not treated by the owners unless parasites were detected. During the study a fluctuation of the infection rate in both areas was observed. One year after the beginning of the study, the average prevalence rate was relatively low in the treated area (2%) and the animals had a higher average PCV value (31.3%) than in the untreated area, where the prevalence rate 9% and the average PCV value was 26.6% (Table II).

TABLE II. PARASITOLOGICAL AND HAEMATOLOGICAL RESULTS OF BOVINE SAMPLES FROM 8 VILLAGES SITUATED EITHER IN A TREATED* OR UNTREATED AREA IN MALI

| V. | Months before or after the start* of the control programme | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | -1 | | +1 | | +3 | | +8 | | +11 | | +12 | |
| | P | PCV | P | PCV | P | PCV | P | PCV | P | PCV | P | PCV |
| 1 | 3.6 | 31.7 | 0 | 29.5 | 0 | 45.7 | 0 | 45.7 | 6.8 | 35.7 | 0 | 32.4 |
| 2 | 8.0 | 29.8 | 14.0 | 28.8 | 2.0 | 32.0 | 13.0 | 32.1 | 6.7 | 31.0 | 4.8 | 28.4 |
| 3 | 6.0 | 28.6 | 10.0 | 31.5 | 4.0 | 34.0 | 2.1 | 34.0 | 2.4 | 33.8 | 0 | 33.2 |
| 4 | 7.9 | 27.2 | 2.0 | 29.7 | 0 | 30.7 | 0 | 35.4 | 0 | 31.9 | 3.0 | 31.8 |
| A | 6.2 | 29.4 | 6.5 | 29.9 | 1.5 | 33.1 | 3.7 | 36.8 | 4.2 | 33.2 | 2.0 | 31.3 |
| 5 | 0.9 | 30.6 | 16.0 | 29.8 | 0 | 31.9 | 26.3 | 30.6 | 21.6 | 30.1 | 14.3 | 27.9 |
| 6 | 2.0 | 26.9 | 4.0 | 32.1 | 0 | 31.9 | 0 | 33.0 | 2.8 | 32.3 | 6.2 | 25.7 |
| 7 | 4.0 | 32.0 | 14.0 | 31.2 | 12.5 | 32.4 | 4.3 | 33.5 | 0 | 33.8 | 2.8 | 26.7 |
| 8 | 5.9 | 27.9 | 18.0 | 24.9 | 13.5 | 29.7 | 16.7 | 29.2 | 2.6 | 28.9 | 13.1 | 26.2 |
| A | 3.5 | 29.4 | 13.0 | 29.5 | 6.2 | 31.6 | 10.8 | 31.6 | 6.7 | 31.3 | 9.0 | 26.6 |

* The tsetse control programme started in April 1993 in an area of 500 km$^2$ near Niena, Mali.
1, 2, 3 and 4 are villages in the treated area; 5, 6, 7 and 8 are villages in the untreated area.
V. = Village (numbers correspond with the ones in Table III).
P = prevalence rate of trypanosomes in bovine blood samples.
PCV = packed red cell volume (%).
A = average.

### 3.3. Comparison between entomological, parasitological and haematological data

In the treated area, the suppression of the tsetse population resulted in a decline of trypanosome prevalence rate (Fig. 3). In the untreated area the tsetse density as well as the trypanosome prevalence rate remained elevated (Fig. 4). Initially the average PCV values of animals in treated and untreated areas were similar. However, after the eleventh month of intervention PCV values were lower in the untreated area than in the treated one.

Due to the analysis of an insufficient number of serum samples, the curves of antigen levels were incomplete.

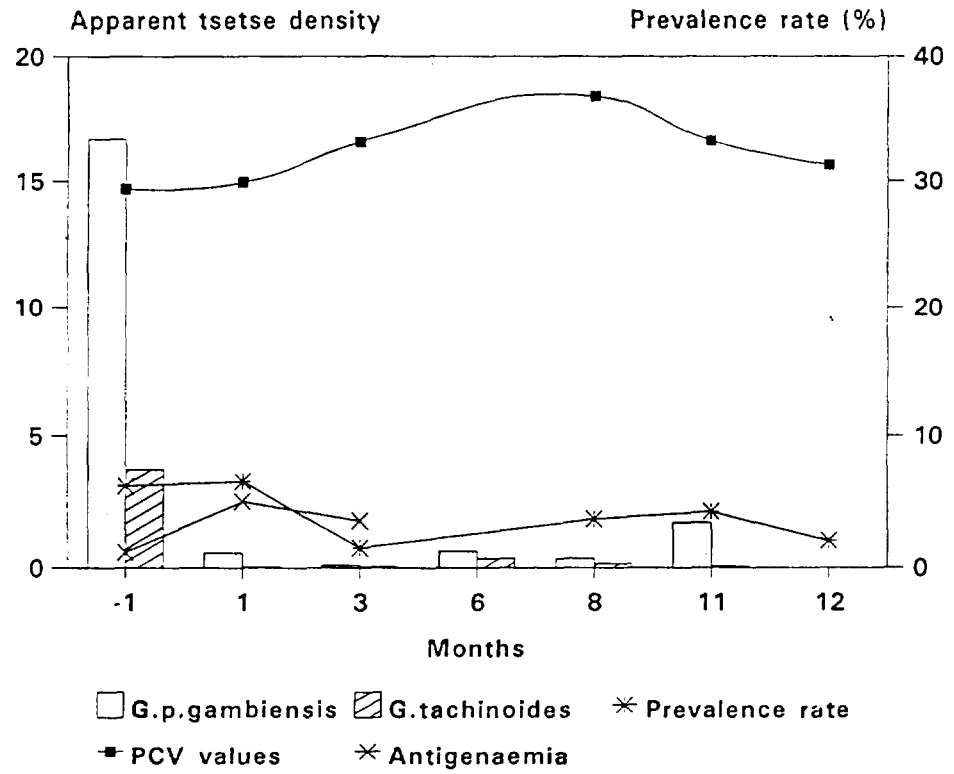


*FIG. 3. Variations in apparent tsetse density and parasite, antigen and PCV values in cattle during a 12-month-observation period following a tsetse control programme in Mali.*

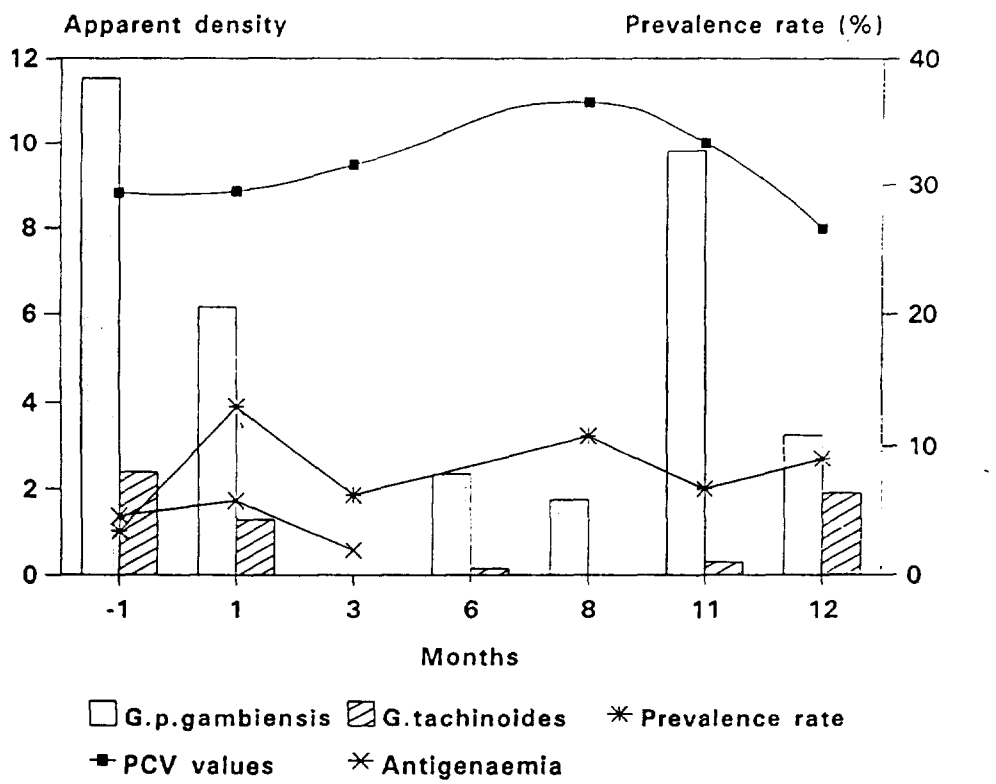


*FIG. 4. Variations in apparent tsetse density and parasite, antigen and PCV values in cattle during a 12-month-observation period in an untreated area in Mali.*

### 3.4. Serological data

A total of 1,021 serum samples were collected during three sampling visits. Following the analysis of the samples the antigen ELISA appeared to be less sensitive than the standard parasitological techniques (Tables II and III). However, the results are incomplete and more samples need to be analysed before a final conclusion with respect to sensitivity can be made.

The specificity of the test has been assessed using sera collected in a tsetse free area in order to determine the positive/negative cut-off point. The specificity was 98% and the cut-off point used was 10 percent positivity (PP).

TABLE III. SEROLOGICAL RESULTS OF BOVINE SAMPLES FROM 8 VILLAGES SITUATED EITHER IN A TREATED OR UNTREATED AREA IN MALI USING THE ANTIGEN ELISA

| Area | | | Months before or after the start* of the control programme | | |
|---|---|---|---|---|---|
| | Village number | Village name | -1 | +1 | +3 |
| Treated | 1 | Titiana | 2† | 19.86 | 9.93 |
| | 2 | Kona | 13.11 | 10.92 | 10.92 |
| | 3 | Pankourou | 3.87 | 1.92 | 3.87 |
| | 4 | N'Tjila | 0.00 | 27.27 | 18.18 |
| | | Average | 4.74 | 14.97 | 10.71 |
| Untreated | 5 | Diassa | 8.43 | 10.56 | 9.93 |
| | 6 | Diomana | 4.68 | 14.04 | 4.68 |
| | 7 | Tonokala | 0.00 | 14.58 | 6.24 |
| | 8 | N'Tiobougou | 42.42 | 30.3 | 0.00 |
| | | Average | 13.86 | 17.37 | 5.88 |

* The tsetse control programme started in April 1993 in an area of 500 km$^2$ near Niena, Mali.
† The results are expressed in percent positivity.

## 4. DISCUSSION

The Niena tsetse control programme has been a success from both an entomological and parasitological point of view. In the treated area a remarkable reduction in the tsetse fly density was found and the trypanosomosis prevalence rate decreased to nearly zero in the monitored herds. In terms of the economic impact of the control programme, the reduction in the utilization of trypanocidal drugs in the area need to be assessed. Similarly, animal production parameters such as weight changes and reproductive performance should be monitored in the animals in the two areas to assess economic impact. The tsetse control programme will continue an additional year after which an evaluation of its impact will be carried out. The use of the antigen ELISA in combination with parasitological techniques will provide a better assessment of the differences in bovine infection rates between the treated and untreated areas.

## ACKNOWLEDGEMENTS

We would like to thank the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture for the financial support and technical advice provided during the study.

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# 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*

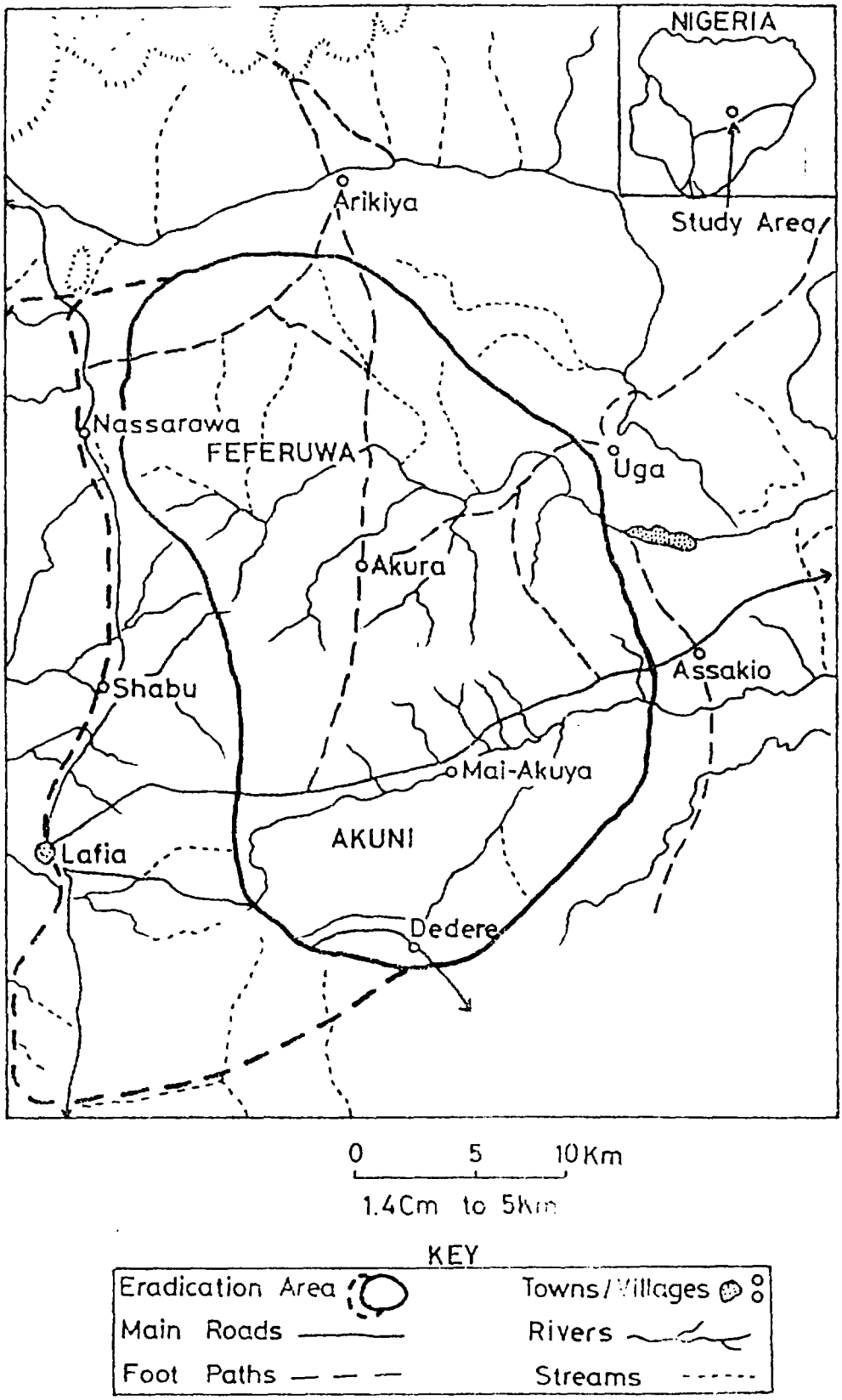


*FIG.1. Map showing the area where the sterile insect technique was used from 1980 to 1987 to eradicate* Glossina palpalis *in the Lafia Local Government Area, Plateau State, Nigeria.*

*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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# DETECTION AU BUFFY COAT TECHNIQUE ET EN ELISA DE SOUCHES DE TRYPANOSOMES SUPPOSEES CHIMIORESISTANTES AU SENEGAL ET CARACTERISATION THERAPEUTIQUE



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**Abstract-Resumé**

DETECTION AND CHARACTERISATION OF TRYPANOSOME STRAINS SUPPOSEDLY RESISTANT TO TRYPANOCIDAL DRUGS IN SENEGAL.

In the region of Sokone cattle are constantly exposed to infections with trypanosomes transmitted by *Glossina morsitans submorsitans* and *G. palpalis gambiensis*. Trypanocidal drugs are widely used by the farmers on the 50.000 cattle present in the region. Consequently, drug resistance has become a major problem. During the present study goats were inoculated with trypanosome strains isolated from infected cattle. Following the appearance of parasitaemia, the animals were treated with either Bérénil®, Samorin® or Ethidium®. The results indicated the parasites were susceptible to Samorin, but one of the *Trypanosoma vivax* strains showed resistance to Bérénil® and Ethidium®.

In addition, the performance of the antigen detection ELISA was compared with that of the Buffy Coat Technique using more than 1000 serum samples from the Sokone region and 100 samples from Northern Senegal infested with tsetse flies. The results showed a very high specificity of 98%. However, additional tests will be necessary to assess the sensitivity properly.

DETECTION AU BUFFY COAT TECHNIQUE ET EN ELISA DE SOUCHES DE TRYPANOSOMES SUPPOSEES CHIMIORESISTANTES AU SENEGAL ET CARACTERISATION THERAPEUTIQUE.

La région de Sokone, située dans le centre-Sud du Sénégal, héberge un cheptel bovin estimé à 50 000 têtes. Du fait de la présence de *Glossina morsitans submorsitans* et *G. palpalis gambiensis*, ces bovins sont soumis à un risque permanent de trypanosomose. Des traitements trypanocides sont souvent pratiqués et ont pu conduire à l'apparition de souches de trypanosomes chimiorésistantes.

La présente étude, menée dans cette région entre avril 1994 et mars 1995, a permis d'inoculer des chèvres avec des souches de trypanosomes prélevées sur des bovins parasités. Un criblage thérapeutique de ces souches a été ensuite effectué avec le Bérénil®, le Samorin® et l'Ethidium®. Les résultats indiquent une sensibilité normale des souches pour le Samorin®, mais l'une de ces souches (*Trypanosoma vivax*), s'est montrée résistante pour le Bérénil® et l'Ethidium®.

Par ailleurs, les performances de la technique ELISA de détection des antigènes sériques trypanosomiens comparée à la technique B.C.T. ont été évaluées sur plus de 1000 sérums bovins de la région de Sokone et sur près de 200 autres sérums de la région Nord du Sénégal indemne de glossines. Les résultats indiquent une bonne spécificité de la technique (supérieure à 98%). Pour ce qui est de la sensibilité, des investigations complémentaires semblent nécessaires.

## 1. INTRODUCTION

La région de Sokone (centre-sud du Sénégal) est quasi-totalement infestée de *Glossina morsitans submorsitans* et *G. palpalis gambiensis*, principales vectrices locales des trypanosomoses. Le cheptel bovin y est estimé à environ 50 000 têtes, généralement des *Diakore* (croisement Zébu-Ndama). Les éleveurs font souvent appel aux agents vétérinaires locaux pour des traitements trypanocides de leurs animaux. L'utilisation intensive et durable du Bérénil® et du Samorin® a fait craindre l'apparition de souches de trypanosomes résistant à ces médicaments. Des investigations menées dans la région en 1991-92 avaient abouti à une forte suspicion de l'existence de telles souches. La présente étude est destinée à se prononcer avec précision. Il s'agit d'effectuer un criblage parasitologique et sérologique sur un grand nombre de bovins de la zone, d'isoler des souches de trypanosomes à partir des bovins trouvés positifs pour les inoculer à des chèvres. Ces chèvres sont

ensuite traitées avec chacun des trois principaux trypanocides vétérinaires (Bérénil®, Samorin® ou Ethidium®). La persistance post-thérapeutique de l'infestation signifie une chimiorésistance à la dose employée pour le traitement. En cas de sensibilité normale, le trypanocide concerné est utilisé sur le terrain pour confirmation des résultats de laboratoire, en attendant la définition d'un programme de lutte approprié.

## 2. MATÉRIEL ET MÉTHODES

Quatre visites ont été faites sur le terrain, à intervalles d' un mois, au cours desquelles les bovins retenus ont été régulièrement examinés pour le diagnostic des trypanosomoses. Ensuite des essais thérapeutiques sur des chèvres inoculées avec les souches isolées de ces bovins ont été effectués au laboratoire pendant 3 mois. Au bout de ce temps, une cinquième puis une sixième visites espacées de 1 mois ont été faites pour confirmer sur les mêmes bovins les résultats des essais thérapeutiques.

### 2.1. Investigation entomologique

Déploiement de pièges de capture "Challier-Laveissière" pendant 24 à 72 heures dans les forêts classées limitrophes des villages où vivent les bovins cibles. Les glossines capturées dans chaque forêt prospectée ont été triées en fonction de l'espèce et du sexe puis comptées.

### 2.2. Investigation parasitologique

Dépistage parasitologique des trypanosomes portant au départ sur 532 bovins numérotés à l'oreille. La Buffy Coat Technique (B.C.T.) [1] a été utilisée pour le diagnostic des trypanosomoses sur le terrain, avec lecture de l'hématocrite suivie de l'examen de l'interphase entre lame et lamelle. La confection des frottis de sang a été irrégulière, en raison du retard de la réception du matériel.

Les actions suivantes ont été réalisées en visites 2 et 3:

- traitement au Samorin®, 0,5 mg/kg, I.M., (solution à 1%) des bovins B.C.T. positif à la visite qui suit celle du diagnostic. De plus, en raison du retard de livraison des réactifs de sérologie, les bovins à faible hématocrite ont subi le même traitement.
- subinoculation de chèvres: prélèvement à la seringue de 5 ml de sang de bovin parasité (B.C.T. positif), injection immédiate, en I.V., à une chèvre.

De retour au Laboratoire, inoculation de 3 autres chèvres avec chaque souche de terrain puis suivi de la parasitémie jusqu'à ++ ou +++. Traitement à ce moment au Bérénil®: solution à 7%, 7,0 mg/kg, I.M., au Samorin®: solution à 1%, 0,5 mg/kg, I.M., ou à l'Ethidium®: solution à 1%, 1,0 mg/kg, I.M. La chèvre inoculée sur le terrain sert en même temps de témoin non traité. Les examens parasitologiques se sont ensuite poursuivis régulièrement.

Les visites 3 et 4 n' ont intéressé que les bovins positifs et ceux à faible hématocrite (211 bovins à la visite 3 et 204 à la visite 4).

Au cours de la visite 5, les bovins numérotés présents dans chaque troupeau ont été répartis en deux lots: un lot traité et un autre servant de témoin non traité.

La visite 6 a permis de contrôler l'effet du traitement en comparant ces deux lots.

### 2.3. Investigation sérologique

Prélèvement I.V. de 5 à 10 ml de sang jugulaire par animal sur vacutainer sec; après 2 heures à la température ambiante et 24 heures à +4°C, centrifugation à 1000 tours/mn pendant 15 minutes, récolte du sérum, adjonction de 0,01% de sodium benzoate dans un volume de 10 $\mu$l; conservation à -20°C jusqu'à l'emploi.

Les subinoculations à partir des bovins séropositifs n'ont pas pu se faire, les réactifs de sérologie nous étant parvenus après la dernière visite de terrain.

## 3. RÉSULTATS ET DISCUSSION

### 3.1. Résultats entomologiques

Il y avait d'importantes différences dans la densité des tsétsé selon les localités et selon l'espèce de glossine. Niokholokho: très faible densité de *G. palpalis* et absence de *G. morsitans;* Karang: densité moyenne pour *G. morsitans,* nulle pour *G. palpalis;* Toubakouta: absence de *G.*

*palpalis* et densité moyenne de *G. morsitans;* enfin Keur Andalla: présence des deux espèces, avec de fortes densités (Tableau I). Ces différences sont essentiellement dues au fait que les forêts concernées sont assez éloignées les unes des autres et offrent des biotopes différents pour les glossines. Nous avons également noté une baisse sensible de la densité des tsétsé à Karang et à Toubakouta par rapport aux prospections de 1991. Cela est sans doute lié aux fréquentes coupes de bois pour l'usage domestique, au défrichement pour l'agriculture, et aux feux de brousse. Enfin, les glossines femelles ont été capturées en plus grand nombre que les mâles, sauf dans la forêt de Toubakouta.

TABLEAU I. RÉSULTATS DES CAPTURES DE GLOSSINES

| Forêt | Nombre de captures | | | | |
|---|---|---|---|---|---|
| | *G. palpalis* | | *G. morsitans* | | Total glossines |
| | Mâles | Femelles | Mâles | Femelles | |
| Keur Andalla | 35 | 54 | 44 | 105 | 238 |
| Toubakouta | 0 | 0 | 39 | 21 | 60 |
| Karang | 0 | 0 | 9 | 12 | 21 |
| Niokholokho | 0 | 2 | 0 | 0 | 2 |
| Total | 35 | 56 | 92 | 138 | 321 |

### 3.2. Résultats parasitologiques

Les résultats parasitologiques indiquent une remarquable constance de la prévalence des trypanosomoses chez les bovins de cette zone lorsqu'on utilise la technique de diagnostic B.C.T (Tableau II). En effet, le taux de 5% obtenu en visites 1-2 (dépistage effectué sur 532 bovins avant les traitements initiaux) puis en visite 5 (3 à 4 mois après ces traitements) avait été trouvé au dépistage initial effectué lors des études de 1991-92.

TABLEAU II. RESULTATS DU DIAGNOSTIC PARASITOLOGIQUE DES TRYPANOSOMOSES BOVINES

| Visite | Nombre de bovins | Espèces de trypanosomes | | |
|---|---|---|---|---|
| | | *T.c.* | *T.v.* | Total |
| 1-2 | 532 | 15 | 12 | 27 (5.07 %) |
| 3 | 211 | 2 | 3 | 5 (2.36 %) |
| 4 | 204 | 0 | 2 | 2 (0.98 %) |
| 5 | 147 | 3 | 5 | 8 (5.44 %) |
| 6 | 126 | 2 | 1 | 3 (2.38 %) |
| Total | 1220 (100 %) | 22 (1.80 %) | 23 (1.88 %) | 45 (3.68 %) |

*T. c.* = *T. congolense.*
*T. v.* = *T. vivax.*

Avec les épreuves sérologiques par ELISA-antigène les taux avaient cependant atteint 25%. Par ailleurs, on note au début des investigations une fréquence plus élevée de *T. congolense* (55,6% des infestations) par rapport à *T. vivax* (44,4%),contrairement aux résultats de 1992. Mais cette situation s'est finalement renversée en faveur de *T. vivax,* avec 23 cas contre 22 à *T. congolense* (Tableau II).

### 3.3. Résultats sérologiques

Le nombre total de sérums testés est de 1373, soit 1181 prélevés sur des bovins de Sokone, zone infestée, et 192 sérums de la banque du "Panafrican Rinderpest Campaign (PARC) récoltés sur des bovins de Podor, zone située dans le Nord du Sénégal indemne de glossines. Les résultats de la zone indemne sont les suivants:

- *T. brucei*: 3 séropositifs sur 192 analyses, soit 1,56% , ce qui correspond à une spécificité supérieure à 98%.
- *T. congolense*: 2 cas sur 192, ou 1,04%, soit une spécificité de 99%
- *T. vivax*: 1 séropositif, soit 0,52%, soit une spécificité supérieure à 99%.

Les observations liées à les résultats de sérologie varient selon le type d'anticorps monoclonal considéré et aussi selon qu'il s'agit de la spécificité ou de la sensibilité de ces anticorps. Les résultats de la zone indemne semblent indiquer une spécificité satisfaisante pour chacun des trois tests: (98% pour *T. brucei*, 99% pour *T. congolense* et *T. vivax*).

Pour ce qui est de la sensibilité, le test à *T. brucei* semble plus performant que les autres (Tableau III).

TABLEAU III. RESULTATS DU DIAGNOSTIC SEROLOGIQUE DES TRYPANOSOMOSES DANS LA ZONE DE SOKONE

| Visite | Nombre d'analyses | Nombre de bovins positifs en ELISA-Ag avec trypanosomes | | | | | |
|---|---|---|---|---|---|---|---|
| | | *T. b.* | *T. c.* | *T. v.* | *T.b./Tc* | *T.b./Tv* | Total |
| 1-2 | 523 | 31 | 1 | 6 | 1 | 1 | 40 (7,65%) |
| 3 | 236 | 12 | - | - | - | - | 12 (5,51%) |
| 4 | 149 | 7 | 1 | - | - | - | 8 (5,36%) |
| 5 | 147 | 7 | - | 2 | - | - | 9 (6,12%) |
| 6 | 126 | 5 | - | 2 | - | - | 7 (5,55%) |
| TOTAL | 1181 | 62 (5,25%) | 2 (0,17%) | 10 (0,85%) | 1 (0,08%) | 1 (0,08%) | 76 (6,43%) |

*T. b.* = *Trypanosoma brucei.*
*T. c.* = *T. congolense.*
*T. v.* = *T. vivax.*

Cependant, il faut signaler qu'aucun cas de trypanosomose à *T. brucei* n'a été observé aux examens microscopiques, alors qu'en ELISA-Antigène, 62 cas monospécifiques et 2 cas d'infection mixte impliquant cette espèce de trypanosome ont été trouvés. Quant aux tests à *T. congolense* et à

*T. vivax*, ils ont décelé moins de cas que la technique BCT, et aucun des bovins positifs au BCT n'a été confirmé en sérologie. La sensibilité de ces deux anticorps semble encore insuffisante, du moins pour les souches de trypanosomes de la zone centre-Sud du Sénégal.

### 3.4. Résultats de l'hématocrite

Les bovins porteurs de trypanosomes offrent des moyennes de l'hématocrite sensiblement inférieures à celles des animaux trouvés négatifs (Tableau IV). En outre, les animaux qui hébergent *T. congolense* semblent plus anémiés que ceux positifs à *T. brucei* ou *T. vivax*. Ce sont là des réalités classiques chez les bovins. On note également des différences entre l'hématocrite moyen des visites 1 et 2 d'une part et celui des visites 3 et 4 d'autre part, même lorsqu'il s'agit des bovins négatifs. L'explication est qu'après le dépistage effectué lors des deux premières visites, seuls les animaux trouvés porteurs de trypanosomes et ceux à faible hématocrite ont été prélevés. D'une manière générale, l'hématocrite de la période post thérapeutique a connu une remontée régulière. Et à la dernière visite, les moyennes ont atteint ou dépassé leur niveau initial, dans tous les groupes d'animaux.

TABLEAU IV. MOYENNES POURCENTAGES DE L' HEMATOCRITE (PCV) ± INTERVALLE DE CONFIANCE A 5%

| Visite | Négatifs | | Séropositifs *T. brucei** | | Positifs *T. congolense* | | | | Positifs *T. vivax* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | B.C.T. | | ELISA-Ag | | B.C.T. | | ELISA-Ag | |
| | No. | PCV | No. | PCV | No. | PCV | No. | PCV | No. | PCV | No. | PCV |
| 1-2 | 466 | 31,7 ± 0,6 | 31 | 28,5 ± 2,2 | 15 | 23,3 ± 2,7 | 1 | 24 | 12 | 25,5 ± 4,2 | 6 | 31,3 ± 6,9 |
| 3 | 193 | 27,9 ± 0,7 | 12 | 25,4 ± 1,8 | 2 | 20 et 28 | 0 | - | 3 | 23,5 ± 5,7 | 0 | - |
| 4 | 186 | 27,9 ± 0,7 | 7 | 28,4 ± 3,4 | 0 | - | 1 | 24 | 2 | 20 - 31 | 0 | - |
| 5 | 138 | 32,4 ± 0,8 | 7 | 29,5 ± 2,9 | 3 | 29 - 31 - 33 | 0 | - | 5 | 31,4 ± 5,6 | 2 | 33 - 35 |
| 6 | 116 | 32,0 ± 0,8 | 5 | 28,4 ± 1,2 | 2 | 30 et 40 | 0 | - | 1 | 38 | 2 | 33 - 35 |

* Il n'y a pas eu de cas de *T. brucei* au Buffy Coat Technique (B.C.T).
P.C.V. = packed red cell volume.

### 3.5. Traitements trypanocides

#### *3.5.1. Traitements initiaux des bovins suivis*

On observe une baisse significative de la fréquence des trypanosomoses après les traitements au Samorin effectués en visites 2 et 3. Les souches de trypanosomes concernées semblent en majorité sensibles à ce trypanocide lorsqu'il est administré selon les normes. Cependant, deux parmi les cinq bovins encore positifs au BCT en visite 3 (Tableau II) ont montré une parasitémie post thérapeutique persistante et ont gardé leurs trypanosomes deux mois au moins après le traitement. Il s'agit de souches de *T. vivax*, que nous avons isolées et passé sur chèvres. Le comportement de ces deux souches en thérapeutique expérimentale est décrit ci-dessous (3.5.2.).

*3.5.2. Thérapeutique expérimentale sur chèvres au laboratoire*

Un total de 20 chèvres ont été inoculées avec 5 souches de trypanosomes prélevées sur des bovins à parasitémie persistante, soit 2 souches de *Trypanosoma congolense* et 3 souches de *T. vivax*, 4 chèvres par souche. Ces chèvres ont ensuite été réparties en lots de traitement trypanocide comme indiqué plus haut. Les examens (B.C.T.) ont eu lieu tous les deux jours.

Les 2 souches de *T. congolense* passées sur chèvres se sont montrées sensibles à la dose standard des trois trypanocides. Les parasites ont disparu du sang dans les 48 heures qui ont suivi le traitement, et les examens parasitologiques (B.C.T.) effectués tous les deux jours sont restés négatifs pendant deux mois au moins, ou jusqu'à la mort de l'animal.

Quant aux souches de *T. vivax*, deux d'entre elles ont montré une sensibilité normale aux trois trypanocides (Tableau V).

TABLEAU V. RÉSULTATS DE THÉRAPEUTIQUE EXPÉRIMENTALE SUR CHÈVRES INFECTÉS AVEC UNE SOUCHE DE *TRYPANOSOMA VIVAX*

| Periode (en jours) | Chèvre no. 964 témoin non traité | Chèvre no. 704 | Chèvre no. 705 | Chèvre no. 706 |
|---|---|---|---|---|
| | BCT | BCT | BCT | BCT |
| 0 (Inoculation*) | - | - | - | - |
| 3 | - | - | - | - |
| 5 | T.v. + | T.v. + | T.v. + | T.v. + |
| 7 | ++ | ++ Traitement avec Berenil® (7.0 mg/kg) | ++ Traitement avec Samorin® (0.5 mg/kg) | +++ Traitement avec Ethidium® (1.0 mg/kg) |
| 8 | ++ | + | + | +++ |
| 9 | +++ | - | - | ++ |
| 12 | +++ | - | - | - |
| 14 | Morte | - | - | - |
| 16 | | - | - | - |
| 19 | | - | - | - |
| 21 | | - | - | - |
| 23 | | - | - | - |
| 26 | | - | - | - |
| 28 | | - | - | - |
| 30 | | - | - | - |
| 33 | | - | - | - |
| 35 | | - | - | - |
| 37 | | - | - | - |
| 40 | | Morte | - | - |
| 42 | | | - | - |
| 44 | | | - | - |
| 49 | | | Morte | - |
| 51 | | | | Morte |

* = chèvres inoculées avec 5 ml du sang avec *Trypanosoma vivax* souche Sokone 001/ LNERV/1994.
BCT = bffy coat technique; T.v. = *Trypanosoma vivax*.

La troisième souche (SOKONE 002/LNERV/1994) provient d'un bovin du village de Karang, localité où des cas de parasitémie et d'antigénémie post thérapeutiques persistantes avaient déjà été notés en 1989-92. Les résultats sont les suivants (Tableau VI):

- chèvre N° 714 traitée au Bérénil®: parasitémie persistante pendant plus de 60 jours après traitement avec cependant une période aparasitémique de 18 jours;
- chèvre N° 715 traitée au Samorin®: elle est devenue négative 2 jours après le traitement et est restée aparasitémique jusqu'à sa mort survenue 21 jours après traitement, suite à une entérite coccidienne.
- chèvre N° 716 traitée à l'Ethidium®: examens négatifs du 5ème au 21ème jours, puis retour et persistance de la parasitémie jusqu'au 42ème jour qui suit le traitement. Ensuite mort de l'animal;

TABLEAU VI. RÉSULTATS DE THÉRAPEUTIQUE EXPÉRIMENTALE SUR CHÈVRES INFECTÉS AVEC UNE SOUCHE DE *TRYPANOSOMA VIVAX*

| Periode (en jours) | Chèvre no. 962 témoin non traité | Chèvre no. 714 | Chèvre no. 715 | Chèvre no. 716 |
|---|---|---|---|---|
| | BCT | BCT | BCT | BCT |
| 0 (Inoculation*) | - | - | - | - |
| 2 | T.v. + | - | - | - |
| 5 | ++ | T.v. ++ | T.v. ++ | T.v. ++ |
| 7 | ++ | +++ Traitement avec Berenil® (7.0 mg/kg) | ++ Traitement avec Samorin® (0.5 mg/kg) | ++++ Traitement avec Ethidium® (1.0 mg/kg) |
| 8 | ++ | + | + | ++ |
| 9 | +++ | - | - | + |
| 12 | +++ | - | - | - |
| 14 | + | - | - | - |
| 16 | + | - | - | - |
| 19 | + | - | - | - |
| 21 | + | - | - | - |
| 23 | + | - | - | ++ |
| 26 | Morte | - | - | + |
| 28 | | ++ | Morte | + |
| 30 | | + | | + |
| 33 | | +++ | | + |
| 35 | | ++ | | + |
| 37 | | ++ | | + |
| 40 | | ++ | | + |
| 44 | | + | | + |
| 49 | | + | | Morte |
| 54 | | + | | |
| 56 | | + | | |
| 61 | | ++ | | |
| 65 | | ++ | | |
| 68 | | ++ | | |
| 70 | | + | | |
| 71 | | Morte | | |

* = chèvres inoculées avec 5 ml du sang avec *Trypanosoma vivax* souche Sokone 002/ LNERV/1994.
BCT = buffy coat technique.
T.v. = *Trypanosoma vivax*.

Signalons enfin que dans tous les cas, les chèvres témoins non traités sont restées parasitémiques jusqu'à la mort.

*3.5.3. Traitements "sanatifs" sur le terrain*

Au cours de la visite 5 qui a suivi les essais thérapeutiques sur chèvres, les résultats de laboratoire ont été testés sur les bovins du terrain. Dans chaque troupeau visité, les bovins numérotés présents ont été répartis en deux lots: un lot traité au Samorin en solution à 1%, 0,5 mg/kg, I.M., et un deuxième lot non traité, qui sert de témoin. Les examens effectués en visite 6 ont permis de mesurer l'incidence des trypanosomoses sur les deux lots un mois après le traitement.

Les résultats confirment ceux des essais sur les chèvres et ceux des traitements initiaux effectués sur ces mêmes bovins: un mois après traitement, aucun cas de trypanosomose n'a été relevé dans le lot traité, alors que la prévalence a augmenté dans le lot témoin. Il apparaît clairement que le Samorin®, lorsqu'il est bien utilisé, est apte à limiter significativement l'incidence des trypanosomoses dans cette zone (Tableau VII).

TABLEAU VII. NOMBRE DE CAS DE TRYPANOSOMOSE DANS LES DEUX LOTS DES TRAITEMENTS "SANITIFS" SUR BOVINS DE SOKONE

| Visite | Lot traité au Samorin® en visite 5 | | | Lot témoin non traité | | |
|---|---|---|---|---|---|---|
| | No. | T.c. | T.v. | No. | T.c. | T.v. |
| 5 | 89 | 3 | 4 | 58 | 0 | 1 |
| 6 | 72 | 0 | 0 | 54 | 2 | 1 |

No. = Nombre de bovins.
T.c. = *Trypanosoma congolense*; T.v. = *Trypanosoma vivax*.

## 4. CONCLUSIONS

En conclusion, on peut affirmer l'existence effective de souches chimiorésistantes de *Trypanosoma vivax* dans la région de Sokone. Cela confirme les soupçons nés des recherches préliminaires menées dans cette région en 1992. Pour ce qui est du médicament sanatif, il ressort des résultats actuels qu'aucune souche testée au laboratoire n'a montré de résistance à la dose usuelle de Samorin®. Une utilisation adéquate de ce trypanocide chez les bovins de cette région donnerait sans doute satisfaction aux éleveurs et aux vétérinaires locaux.

S'agissant de la technique ELISA de détection des antigènes sériques trypanosomiens, d'importantes modifications ont été apportées à la technique de base utilisée en 1991-92: nature des plaques, température et temps d'incubation, nature et mode de préparation du chromogène, titre et milieu de dilution des différents réactifs, conditions d'acceptation des plaques de travail en fonction des résultats des témoins de reférence. Ces modifications exigent une rigueur constante, depuis la collecte et la conservation des sérums jusqu'aux analyses. A cela s'ajoute l'expression des résultats sous forme de pourcentage de positivité (et non de densité optique uniquement). Ces innovations devraient déboucher sur une précision accrue et une plus grande fiabilité des résultats. Il semble cependant nécessaire d'améliorer la sensibilité des tests à *T. congolense* et à *T. vivax*. Notamment en poursuivant les investigations concernant les titres de dilution des sérums, des anticorps monoclonaux de sensibilisation, des conjugués. Rappelons à ce sujet qu'avec le protocole de 1989-92, la concentration sérique de 10% utilisée dans les tests à *T. congolense* et *T. vivax* était double de celle retenue pour *T. brucei*, alors qu'ici les sérums sont uniformément dilués à 5% dans les trois tests. Une fois ces paramètres mieux maîtrisés, il est certain que cette technique rendra de précieux services dans le diagnostic des trypanosomoses bovines et le suivi des programmes de lutte contre leurs vecteurs.

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# MONITORING OF TSETSE AND TRYPANOSOMOSIS CONTROL PROGRAMMES IN SOUTH EASTERN UGANDA



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**Abstract**

MONITORING OF TSETSE AND TRYPANOSOMOSIS CONTROL PROGRAMMES IN SOUTH EASTERN UGANDA.

A total of 3035 cattle, 2733 from a tsetse infested area and 302 from a tsetse free area, were screened for trypanosomosis by the buffy coat technique (BCT). In addition, samples from the tsetse free area were analysed for the presence of trypanosome antigens by antigen ELISA (Ag-ELISA). Using the BCT, trypanosomes were detected in 64 cattle from the tsetse infested area and none were detected in the animals from the tsetse free area. However, using the Ag-ELISA, 17 (5.6%) of the cattle from the tsetse free area were found positive for *T. brucei*, one (0.3%) for *T. vivax* and none for *T. congolense*. The results indicate that the tsetse and animal trypanosomosis situation has improved markedly since the control programme started in 1990. However, both tsetse and animal trypanosomosis still occur particularly in the southern zones of the control area.

## 1. INTRODUCTION

Animal trypanosomosis is the single most important disease constraint to livestock production in Uganda [1]. In southeastern Uganda, where sleeping sickness caused by *Trypanosoma brucei rhodesiense* has been endemic since 1976 [2], cattle have been reported to be reservoirs of the disease [3, 4]. A bilateral programme to control tsetse, animal trypanosomosis and sleeping sickness was initiated in 1990 along the Kenya/Uganda border. To avoid tsetse migration across the common border between Kenya and Uganda, the control programme started at the same time on both sides of the border and operations in both countries were harmonized.

## 2. MATERIALS AND METHODS

### 2.1. Study Area

The project area is situated along the Kenya/Uganda border. It is about 90 km long and 15 km wide. The project area was divided into 4 zones A, B, C and D, adjacent to similar zones in the Kenyan project area. Zone A was the most northern part of the project area near the foot hills of Mount Elgon and zone D was the most southern part, consisting of the islands in Lake Victoria. The area has approximately 50 000 Zebu cattle, 40 000 goats, 15 000 sheep, 2000 pigs and hundreds of dogs.

The predominant vegetation is savanna type with rivers, swamps and remnants of tropical rain forests. Mixed farming is widely practized and the area has a medium to high infestation of *Glossina fuscipes fuscipes*. Initial surveys showed the tsetse density in the area to vary from 2 to >50 flies/trap/day (F/T/D). The prevalence of animal trypanosomosis using the buffy coat technique (BCT) was found to vary between 10 and 50%. During the year 1990, when the control programme started, 150 cases of human sleeping sickness were diagnosed in the study area out of a total population of 280 000. The negative control sera were obtained from Kapchorwa which is at an altitude of 4500 masl and is presumed to be tsetse free.

### 2.2. Control measures

Human sleeping sickness was controlled by the Ministry of Health through passive and active surveillance. The Department of Tsetse Control implemented tsetse control by deploying pyramidal traps impregnated with Deltamethrin®. In addition, Spot-on® was applied on cattle in areas with active foci of human sleeping sickness. The Department of Veterinary Services suppressed animal

trypanosomosis through passive surveillance, i.e. treatments of clinical cases. Block treatment with trypanocidal drugs was attempted in areas with high infection rates. The research institute, LIRI, was responsible for monitoring the efficacy of the control operations. Monitoring was done by a multi-disciplinary team of entomologists, medical and veterinary doctors.

### 2.3. Collection of samples

The veterinary team sampled every month animals in 3 or 4 randomly selected administrative areas, in such a way that each herd was screeded once every 3 months (Fig. 1). The cattle being screened in each administrative area, were randomly selected. Cattle were monitored for trypanosomosis by BCT and packed red cell volume (PCV) values were recorded. Thick and thin blood smears were prepared from positive animals to facilitate species identification. Serum samples were collected and kept at -20°C until analysis by Antigen ELISA. Negative control sera for use in the Ag-ELISA were collected from Kapchorwa, a tsetse free area on the slopes of Mount Elgon.

## 3. RESULTS

No trypanosomes were detected in zone A and the average PCV value of the 243 cattle was 31.3%. In zone B, two trypanosome species, *T. brucei* and *T. vivax* were diagnosed. The overall prevalence of trypanosomosis in 915 cattle was 1.9% and the average PCV value was 30.2%. All three trypanosome species pathogenic to cattle, *T. brucei, T. congolense* and *T. vivax*, were detected in zone C. The overall prevalence of trypanosomosis in the 531 cattle was 3.8% and the average PCV value was 27.5%. In zone D, no control measures have been implemented. The three important trypanosome species were diagnosed with an overall prevalence of 14.2% and an average PCV level of 24.7% (Table I).

TABLE I. PREVALENCE OF INFECTION WITH DIFFERENT TRYPANOSOME SPECIES AND PACKED RED CELL VOLUME VALUES IN CATTLE IN THE PROJECT AREA

| Zone | Visit | No. cattle | No. sera | T.b. | T.c. | T.v. | T.b./ T.c. | T.b./ T.v. | T.c./ T.v. | Prev. (%) | Mean PCV |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 1 | 76 | 76 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33.3 |
| | 2 | 77 | 76 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 29.9 |
| | 3 | 90 | 85 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30.7 |
| | Total | 243 | 237 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 31.3 |
| B | 1 | 355 | 97 | 0 | 0 | 3 | 0 | 0 | 0 | 0.8 | 31.4 |
| | 2 | 354 | 240 | 2 | 0 | 11 | 0 | 1 | 0 | 4.0 | 28.2 |
| | 3 | 206 | 206 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 31.0 |
| | Total | 915 | 543 | 2 | 0 | 14 | 0 | 1 | 0 | 1.9 | 30.2 |
| C | 1 | 212 | 128 | 0 | 0 | 4 | 0 | 0 | 0 | 1.9 | 30.3 |
| | 2 | 271 | 203 | 2 | 4 | 5 | 0 | 0 | 2 | 4.8 | 24.7 |
| | 3 | 48 | 48 | 0 | 1 | 1 | 0 | 1 | 0 | 6.3 | 29.1 |
| | Total | 531 | 379 | 2 | 5 | 10 | 0 | 1 | 2 | 3.8 | 27.5 |
| D | 1 | 352 | 130 | 7 | 12 | 26 | 1 | 1 | 3 | 14.2 | 24.7 |
| Total | 2041 | 1159 | | | | | | | | | |

No. = number of animals sampled.
T. b. = *Trypanosoma brucei*.
T. c. = *Trypanosoma congolense*.
T. v. = *Trypanosoma vivax*.
Prev. = prevalence.
PCV = packed red cell volume.

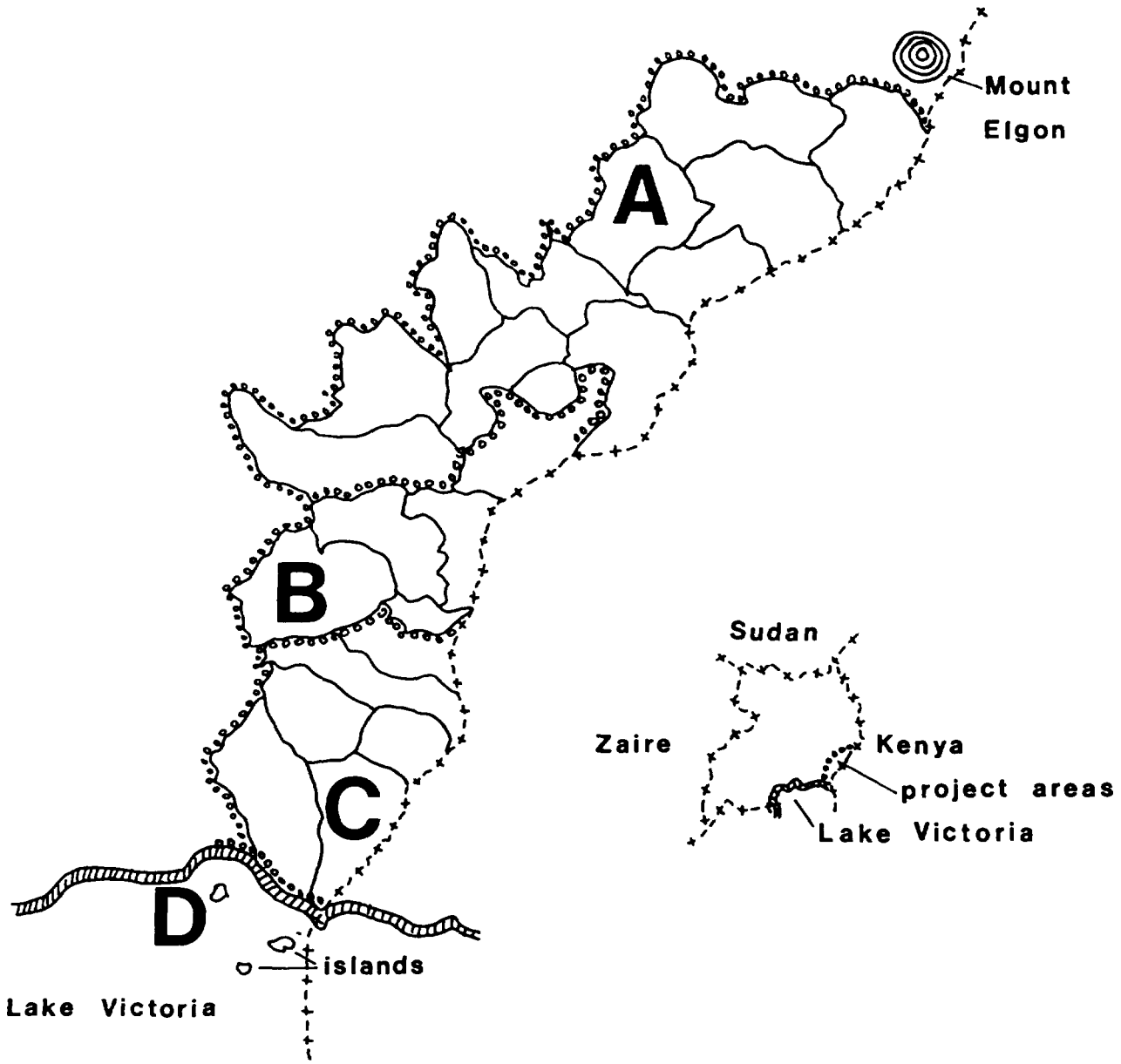


*FIG. 1. Map showing the tsetse and trypanosomosis control area along the Uganda/Kenya international border (-+-+-+) and indicating the boundaries (ooooo) of the four project areas A, B, C and D as well as the boundaries of the governmental administrative zones (----).*

In the tsetse free area of Kapchorwa none of the 302 cattle was found positive by buffy coat technique. The mean PCV level was 34.3%. Using the Ag-ELISA and a cut-off value of 10% (PP >10), none of the cattle was positive for *T. congolense* (Table II). However, the test detected 14 cattle (4.6%) as positive for *T. brucei* (Table III) and 1 animal (0.3%) positive for *T. vivax* (Table IV). It was not possible to calculate the specificity of the Ag-ELISA since the presumably negative population contained some positive cases.

TABLE II. FREQUENCY DISTRIBUTION OF PERCENT POSITIVITY (PP) OF *T. CONGOLENSE* ANTIGENS IN CATTLE SERA FROM A TSETSE FREE AREA

| PP | Frequency | Cumulative frequency | % Specificity |
|---|---|---|---|
| <2 | 0 | 289 | 95.7 |
| 2 | 0 | 289 | 95.7 |
| 3 | 11 | 300 | 99.3 |
| 4 | 1 | 301 | 99.7 |
| 5 | 1 | 302 | 100 |

TABLE III. FREQUENCY DISTRIBUTION OF PERCENT POSITIVITY (PP) OF *T. BRUCEI* ANTIGENS IN CATTLE SERA FROM A TSETSE FREE AREA

| PP | Frequency | Cumulative frequency | % Specificity |
|---|---|---|---|
| <3 | 0 | 142 | 47.0 |
| 3 | 37 | 179 | 59.3 |
| 4 | 41 | 220 | 72.8 |
| 5 | 26 | 246 | 81.5 |
| 6 | 12 | 258 | 85.4 |
| 7 | 15 | 273 | 90.4 |
| 8 | 8 | 281 | 93.0 |
| 9 | 3 | 284 | 94.0 |
| 10 | 4 | 288 | 95.4 |
| 11 | 2 | 290 | 96.0 |
| 12 | 3 | 293 | 97.0 |
| 13 | 4 | 297 | 98.3 |
| 14 | 1 | 298 | 98.7 |
| 15 | 2 | 300 | 99.3 |
| 16 | 0 | 300 | 99.3 |
| 19 | 1 | 301 | 99.7 |
| 52 | 1 | 302 | 100 |

## 4. DISCUSSION

The prevalence of animal trypanosomosis in the project area has been reduced markedly in zones A and B. However, the control of animal trypanosomosis in zone C has not made much progress since 1992 [5]. These findings are corroborated by the average PCV values of the cattle in the respective zones.

The Ag-ELISA proved to be a more sensitive method than the BCT as it was able to detect positive cases in Kapchorwa where the BCT was unable to detect any. It is anticipated to screen the remaining 1,497 bovine serum samples collected since May 1994 in the project area for the presence of trypanosomal antigens using the Ag-ELISA.

TABLE IV. FREQUENCY DISTRIBUTION OF PERCENT POSITIVITY (PP) OF *T. VIVAX* ANTIGENS IN CATTLE SERA FROM A TSETSE FREE AREA

| PP | Frequency | Cumulative frequency | % Specificity |
|---|---|---|---|
| <2 | 0 | 286 | 94.3 |
| 3 | 9 | 295 | 97.7 |
| 4 | 3 | 298 | 98.7 |
| 5 | 2 | 300 | 99.3 |
| 9 | 1 | 301 | 99.7 |
| 13 | 1 | 302 | 100 |


## ACKNOWLEDGEMENTS

The authors wish to thank the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture for financial and technical support. The technical assistance of Mr. J.S.P. Mayende and Mr. M. Oretum is thankfully acknowledged.


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# TRYPANOSOMOSIS SURVEILLANCE ON ZANZIBAR ISLAND, USING THE TRYPANOSOMAL ANTIGEN DETECTION ELISA (ENZYME-LINKED IMMUNOSORBENT ASSAY) TECHNIQUE



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**Abstract**

TRYPANOSOMOSIS SURVEILLANCE ON ZANZIBAR ISLAND, USING THE TRYPANOSOMAL ANTIGEN DETECTION ELISA (ENZYME-LINKED IMMUNOSORBENT ASSAY) TECHNIQUE.

The effectiveness of trypanosomosis control programs depends greatly on prior knowledge of basic data of the epidemiological situation of the disease, which in turn depends, among others, on the use of techniques that give a fairly quick and accurate diagnosis. An antigen-detection (Ag) ELISA was first introduced into Tanzania and validated at the Animal Disease Research Institute (ADRI) through the FAO/IAEA Research Contract (RC) No. 5030/NL. Incorporation of the Ag-ELISA technique into a FAO animal disease control project (1986 - 1993) on Unguja island, in 1992, revealed useful information of high trypanosomosis prevalence in an area previously declared free of the disease using just stained blood smears and buffy coat examinations. This triggered further efforts into more intensive surveys of the tsetse and trypanosomosis situation on Unguja island. The present study is a continuation of previous work in an effort to confirm the practical application of Ag-ELISA in trypanosomosis control operations. Results obtained from a known tsetse and trypanosomosis-free area, on Pemba island, showed a high specificity of the test for *Trypanosoma congolense*, *T. vivax* and *T. brucei*. A preliminary cut-off value of 10% (Percent Positivity = PP) was used. When the PP of 10 was used on sera of trypanosomosis-endemic areas (Mangapwani, Ndijani, Dunga and Kikungwi) on Unguja island, the results reflected the 'real' trypanosomosis situation in the affected areas.This was most strongly felt in the Mangapwani area, where tsetse and trypanosomosis were considered under control by 1994 (no tsetse flies were caught and no samples were encountered positive by the buffy coat technique). However, it should be stressed that the buffy coat technique and the Ag-ELISA complement each another and should be used in conjunction.

## 1. INTRODUCTION

Bovine trypanosomosis in Tanzania and indeed in Sub-Saharan Africa is a disease of great economic importance. In Tanzania it is caused by three species of Salivarian trypanosomes (*Trypanosoma congolense*, *T. vivax* or *T. brucei*) transmitted cyclically by several species of tsetse flies, such as *Glossina morsitans, G. swynnertoni, G. pallidipes, G. brevipalpis* and *G. austeni* [1]. Although exact data on the purchase and use of trypanocidal drugs are not available for Tanzania, it is believed that the figures are fairly high. Although no widespread drug resistance has been reported in Tanzania, sporadic cases of Berenil-resistant strains of *T. congolense* have been described [1, 2]. Effective management of animal trypanosomosis would depend on strategic control of the vector, as well as control of the disease in cattle. However, control of the disease relies on accurate diagnosis. Parasite detection has been used routinely as the method of choice for proper diagnosis. Nevertheless, the sensitivity is comparatively low, particularly in chronic infections, which show low and fluctuating parasitaemias. Antibody-detection techniques are superior to parasite detection methods, but can not distinguish between current and previous infections and, moreover, are not species specific [3]. On the other hand, antigen-detection ELISA (enzyme-linked immunosorbent assay) detects species-specific antigens [4], providing evidence of infection with a particular trypanosome species. A combination of Ag-ELISA and the buffy coat technique (BCT) offers an even more reliable diagnosis.

During the first phase of the project, which terminated in 1992, the antigen-detection ELISA (Ag-ELISA) was successfully validated and established at the Animal Disease Research Institute, Temeke, Dar-es-Salaam [5]. In the present study the Ag-ELISA was used to assess the effectiveness of a Tsetse and Trypanosomosis Eradication Project (URT/5/016 - a 'Model' Project aimed at reduction and finally eradication of *G. austeni*, the single tsetse species present on Zanzibar). Zanzibar is part of the United Republic of Tanzania and constitutes two major islands, Unguja and Pemba, situated about 22 aeronautical miles off the Tanzanian Coast in the Indian Ocean.

## 2. MATERIALS AND METHODS

### 2.1 Sera from a tsetse-free area

For many years no case of animal trypanosomosis has been reported on the island of Pemba. In order to determine a reliable cut-off value for the Ag-ELISA, it was necessary to compare the values of a known positive population with that of a known negative population. Therefore, 480 cattle sera were collected from different parts of Pemba island and analysed for the presence/absence of trypanosome antigen(s). For logistical reasons examination of packed red cell volume (PCV) and buffy coat were not done. Thick and thin blood smears were examined. A preliminary cut-off value of 10% (percent positivity = PP) was used to assess the specificity of the antigen ELISA for *T. brucei*, *T. congolense* and *T. vivax*. Doubtful results were not included in the determination of the specificity of the test.

### 2.2. Sera from a tsetse infested area

Blood and serum samples were obtained from cattle in Blocks 9 (Mangapwani), 25 (Dunga), 27 (Ndijani) and 28 (Kikungwi) on Unguja island (Fig. 1). Blood samples were examined by buffy coat technique (BCT) as described by Murray *et al.* [6], whereas sera were tested for the presence/absence of trypanosomal antigen(s) using the technique described by Nantulya *et al.* [4].

### 2.3. Reagents and equipment

Reagents were suppled as an "Antigen ELISA Kit" by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria. Optical density values were read using an ELISA reader (Multiskan Plus MK II) linked to a computer (286 PC) and calculated using a computer software programme supplied by IAEA (TREIA 1.01). Since the specificity of the test with the sera collected on the trypanosomosis-free island of Pemba was good, a cut-off value of 10 PP (percent positivity) was used for the three species of trypanosomes (*T. brucei, T. congolense* and *T. vivax*).

## 3. RESULTS

### 3.1. Sera from a tsetse-free area

Out of 480 sera tested, 13 were positive to *T. brucei*, 3 to *T. vivax* and none to *T. congolense* (Table I). Nine dubious sera were retested and were subsequently negative, but were not used in determining the specificity of the test. Thus, a trypanosome antigen point prevalence of 3.4% was recorded for the sera from Pemba (considered to be 'false' positives).

TABLE I. SPECIFICITY OF ANTIGEN ELISA FOR *TRYPANOSOMA BRUCEI, T. CONGOLENSE* AND *T. VIVAX* OF SERA FROM PEMBA, A TRYPANOSOMOSIS-FREE AREA

| Trypanosome species | Total number of sera tested | Total -ve | Total +ve | Prevalence (%) | Specificity* (%) |
|---|---|---|---|---|---|
| *T. brucei* | 471 | 458 | 13 | 2.8 | 97.2 |
| *T. congolense* | 471 | 471 | 0 | 0 | 100.0 |
| *T. vivax* | 471 | 468 | 3 | 0.6 | 99.4 |

- ve = negative.
+ ve = positive.
* = $\frac{\text{Total negative} \times 100}{\text{Total tested}}$.

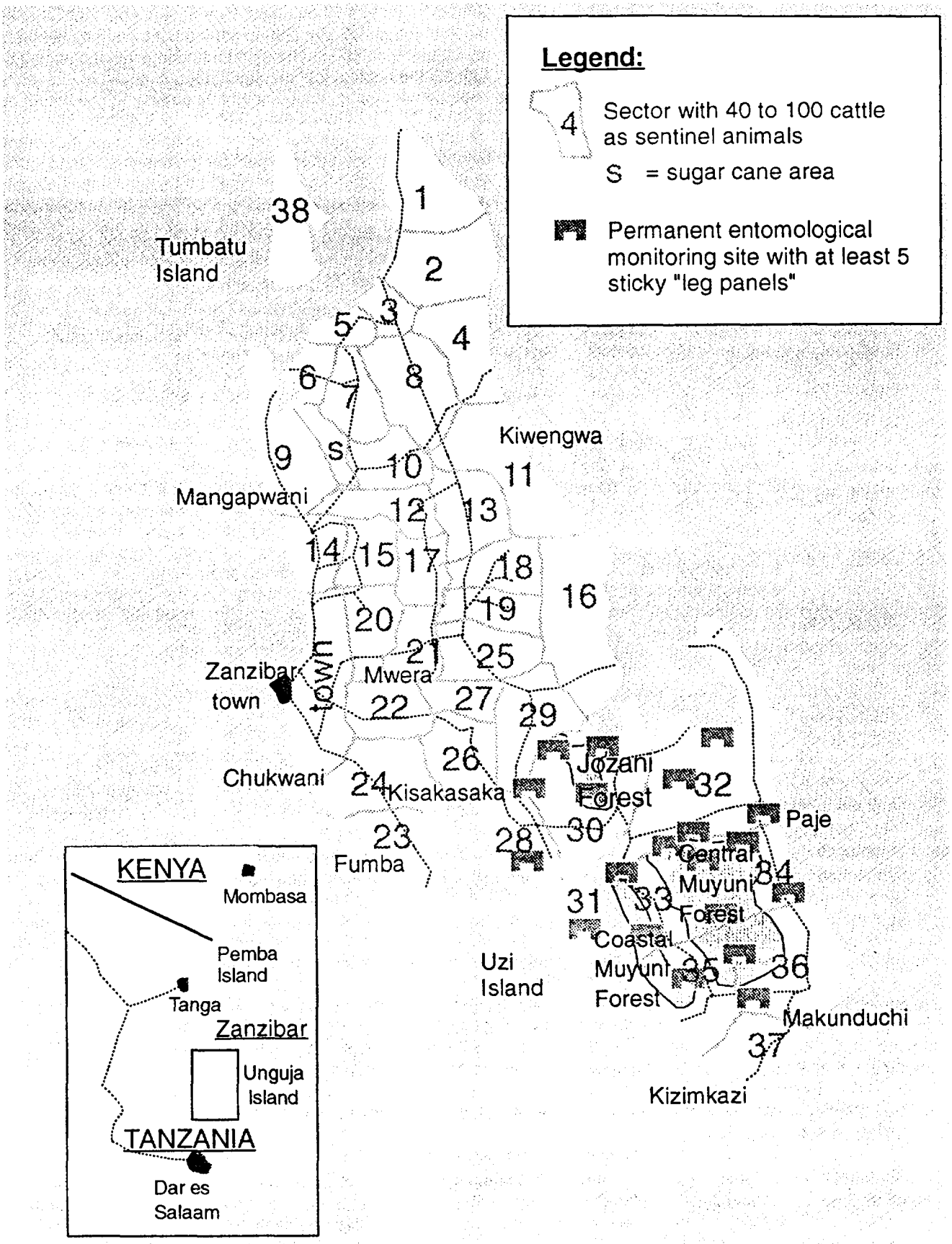


*FIG. 1. Map of Unguja island, Zanzibar, showing the division in blocks. In each block 40 to 100 cattle were being monitored on a regular basis for parasite levels by buffy coat technique and antigen levels by antigen-detection ELISA.*

### 3.2. Sera from a tsetse infested area

A total of 587 sera were collected from cattle in trypanosome infested areas on Unguja island, namely Block 9 (327 sera), Block 25 (36 sera), Block 27 (98 sera) and Block 28 (126 sera).

*3.2.1. Mangapwani (block 9)*

Of the 327 sera tested for trypanosome antigens, two animals were positive for *T. brucei* during the 1st bleeding; two (one *T. brucei*, one *T. vivax*) during the 2nd bleeding; two *T. brucei* during the 3rd bleeding; and three (one *T. brucei* and two *T. vivax*) during the 4th bleeding. One mixed infection of *T. congolense* and *T. vivax* was detected by BCT (Table II).

TABLE II. CHANGE IN TRYPANOSOMOSIS SITUATION ON MANGAPWANI (BLOCK 9), UNGUJA ISLAND, FOLLOWING INTEGRATED CONTROL STRATEGY*

| | Sampling visit** | | | | |
|---|---|---|---|---|---|
| | First | Second | Third | Fourth | Total |
| Total no. of cattle bled | 48 | 78 | 104 | 97 | 327 |
| BCT | 1 T.c./ T.v. | 0 | 0 | 0 | 1 |
| Ag-ELISA | 2 T.b. | T.b. 1 T.v. | 2T.b. | 3 T.b. 2 T.v. | 11*** |
| Incidence (%) Ag-ELISA | 4.2 | 2.6 | 1.9 | 5.2 | |

* = Use of deltamethrin® (pour-on) to control tsetse on cattle, artificial attractants (sticky targets- blue screens, blue cloth impregnated with insecticide) and treatment of trypanosome-infected cattle with trypanocide (diminazene aceturate®).
** = At two-month-intervals.
*** = Cut-off value of 10.0% based on the specificity achieved assaying the negative population of Pemba island (Table I).
BCT = buffy coat technique.
Ag-ELISA = antigen-capture enzyme-linked immunosorbent assay.
T.b. = *Trypanosoma brucei*; T.c. = *T. congolense*; T.v. = *T. vivax*.

*3.2.2. Dunga (block 25)*

Of the 36 sera tested none was positive by BCT and one was positive for *T. brucei* using the Ag-ELISA (Table III). One hundred seven sera still need to be tested by Ag-ELISA.

TABLE III. RESULTS OF BLOOD AND SERA TESTED FROM DUNGA (BLOCK 25), UNGUJA ISLAND OF ZANZIBAR

| Site | Sampling visit | Total No. bled | Total No. tested | BCT positive | Ag-ELISA positive | Total sera not tested |
|---|---|---|---|---|---|---|
| Dunga | Second | 143 | 36 | Nil | T.b. | 107 |

BCT = buffy coat technique.
Ag-ELISA = antigen-capture enzyme-linked immunosorbent assay.
T.b. = *Trypanosoma brucei*.

*3.2.3. Ndijani (block 27)*

Of the 98 cattle screened for parasites in Block 27, 15 were positive by BCT and 6 were antigenaemic (Table IV). *T. vivax* was most prevalent in the blood samples (86.7%), whereas *T. brucei* was the species encountered most frequently by Ag-ELISA (83.3%).

TABLE IV. RESULTS OF BLOOD AND SERA TESTED FROM NDIJANI (BLOCK 27), UNGUJA ISLAND OF ZANZIBAR

| | Sampling visit* | | | |
|---|---|---|---|---|
| | First | Second | Third | Total |
| Total no. of cattle bled | 28 | 68 | 2 | 98 |
| BCT | 5 T.v. | 6 T.v.; 2 T.c. | 1 T.v.; 1 T.c./T.v. | 15 |
| Ag-ELISA | 0 | 3 T.b.; 1 T.v.; 1 T.b./T.c. | T.b. | |

BCT = buffy coat technique.
Ag-ELISA = antigen-capture enzyme-linked immunosorbent assay.
T.b. = *Trypanosoma brucei*; T.c. = *T. congolense*; T.v. = *T. vivax*.
* = At two-month-intervals.

*3.2.4. Kikungwi (block 28)*

A total of 126 sera were tested in Block 28. Nine were positive by BCT and 2 sera were antigenaemic for *T. brucei*. *T. vivax* was the most prevalent species in the BCT (88.9%), while no *T. brucei* was detected by BCT (Table V).

TABLE V. RESULTS OF BLOOD AND SERUM SAMPLES TESTED FROM KIKUNGWI (BLOCK 28), UNGUJA ISLAND OF ZANZIBAR

| | Sampling visit* | | |
|---|---|---|---|
| | First | Second | Total |
| Total no. of cattle bled | 34 | 92 | 126 |
| BCT | 4 T.v. | 3 T.v.; 1T.c.; 1T.c./T.v. | 9 |
| Ag-ELISA | 1 T.b.** | T.b. | 2 |

BCT = buffy coat technique.
Ag-ELISA = antigen-capture enzyme-linked immunosorbent assay.
T.b. = *Trypanosoma brucei*; T.c. = *T. congolense*; T.v. = *T. vivax*.
* = At two-month-intervals.
** = Also positive for *T. vivax* by BCT.
No. = number.

## 4. DISCUSSION

Pemba island of Zanzibar has been considered free of tsetse and trypanosomosis for a long time (I. Shambwana, Assistant Commissioner Livestock Development Zanzibar, personal communication). Although Pemba samples were not examined by BCT, thick smears did not reveal

any trypanosomes and serological screening by trypanosomal-antigen-detection ELISA showed a low antigen prevalence (3.4%) of which *T. brucei* accounted for 2.8% and *T. vivax* for 0.6% (Table I). The high specificity of the Ag-ELISA test for *T. congolense* (100%), *T. vivax* (99.4%) and *T. brucei* (97.2%) does confirm the reported absence of trypanosomosis on Pemba island; the detected prevalence of 3.4% of the samples positive for trypanosomal antigens may be regarded as 'false' positives. Apart from the presence of 'false' positives, the preliminary threshold of percent positivity (PP) of 10 seems to offer results reflecting the 'actual' trypanosomosis situation on Pemba island.

Mangapwani area on Unguja island, is a well known focus of bovine trypanosomosis. Following a series of tsetse and trypanosomosis control operations supported by various FAO/IAEA projects, the tsetse fly (*G. austeni*) population was reduced to very low numbers and only sporadic cases of disease were reported. This situation is reflected by the results of a series of sampling visits (Table II), whereby during the last (4th) visit in 1994, no trypanosomes were detected by BCT and a low level of antigenaemia was observed by Ag-ELISA. Thus, antigen-detection ELISA was shown to be more sensitive than the buffy coat technique. Masake and Nantulya reported a four-fold higher sensitivity of the Ag-ELISA over the BCT [7]. The Ag-ELISA has in particular a high sensitivity in chronic infections.

Following a satisfactory reduction of the tsetse fly population in the Mangapwani area, the control operation was extended southwards to Jozani forest, an area with the highest tsetse fly density on Unguja island. Bovine blood samples collected in this forested area (Blocks 25, 27 and 28) showed persistent parasitaemias, indicating active infections (Tables IV and V ). Trypanosomal antigens were recorded in serum samples but at low levels. The failure of the Ag-ELISA to detect some patent infections has been reported previously by Nantulya and Lindqvist [8]. The authors attributed the failure of detection to low levels of circulating antigens in the early stages of infection. Detectable amounts of antigens become available following destruction of a significant number of parasites by the host's immune response and the subsequent release of internal antigens. However, it is interesting to note that antigen detection (ELISA) and parasite detection (BCT) are complementary. Hence, the antigen ELISA was shown to be a suitable tool in monitoring the effectiveness of tsetse and trypanosomosis control programs.

## ACKNOWLEDGEMENTS

The support by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria, providing reagents, equipment and funds, is greatly appreciated. The Assistant Commissioner, Livestock Development, Zanzibar, is acknowledged for permission to execute the work on the islands, and for providing the support at all stages of the work by departmental staff. The support by the Director of Animal Disease Research Institute (ADRI), Temeke, Dar-es-Salaam is greatly appreciated. I am thankful to the staff of the Protozoology Section, ADRI, for technical assistance.

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# APPLICATION OF THE ANTIGEN ELISA FOR MONITORING THE EFFECTIVENESS OF THE TSETSE AND TRYPANOSOMOSIS CONTROL CAMPAIGN IN ZAMBIA

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**Abstract**

APPLICATION OF THE ANTIGEN ELISA FOR MONITORING THE EFFECTIVENESS OF THE TSETSE AND TRYPANOSOMOSIS CONTROL CAMPAIGN IN ZAMBIA.

Antibody and antigen-detection ELISA were used to screen sera originating from a tsetse free area. In addition, bovine samples from tsetse infested areas were analysed using the Buffy Coat Technique (BCT) and the antigen ELISA. Few samples from a negative control population were detected positive resulting in a very good specificity for both tests. When screening a trypanosome infected population the BCT detected more acute and the Ag-ELISA more chronic infections. In conclusion, the antigen ELISA should be used in combination with the BCT, especially in geographical areas where the BCT does not detect any patent infections.

## 1. INTRODUCTION

Bovine trypanosomosis is still a serious constraint to livestock production in large parts of Zambia. Consequently, a number of tsetse and trypanosomosis control programmes are being carried out to reduce this constraint. The Regional Tsetse and Trypanosomosis Control Programme (RTTCP) in Zambia has been carrying out a control campaign within the Common Fly Belt, which is a discrete fly belt common to Malawi, Mozambique, Zambia and Zimbabwe. For any disease control programme to be effective, the availability of a simple, rapid and accurate diagnostic test is essential. Due to the limitations of the current parasitological methods for disease diagnosis, additional tests such as immunodiagnostic assays have been applied. Thus, the use of antibody assays would enable an overall assessment of the population exposed to infection, while antigen assays would enable identification of individuals with active infections, species differentiation and the detection of drug resistance. The objective of the present study was fourfold:

(1) To establish the specificity of the antigen ELISA (Ag-ELISA) using cattle sera from an area where there is no history of tsetse or trypanosomosis.
(2) To establish the sensitivity of the Ag-ELISA using sera from cattle with active trypanosome infections.
(3) To use the Ag-ELISA to monitor the effectiveness of the RTTCP campaign in Lusitu area.
(4) To use the Ag-ELISA to estimate the trypanosomosis prevalence before and after implementation of a community based programme in Eastern Province.

## 2. MATERIALS AND METHODS

### 2.1. Tsetse free area

Animals belonging to closed herds in tsetse-free areas around Lusaka were randomly selected. A total of 100 cattle were sampled.

### 2.2. Tsetse infested area

Animals with a parasitologically detected trypanosome infection and from a tsetse-fly infested area were selected. A total of 202 cattle were sampled.

### 2.3. Lusitu area

Lusitu area is a well established target control area, where tsetse flies are believed to have been eradicated by the RTTCP. A total of 199 animals gathered at three crush pens were sampled.

### 2.4. Msanzara area, Eastern province

Animals were sampled in a trypanosomosis endemic area, Msanzara, where RTTCP and a Belgian Project are intending to carry out a community based control campaign. A total of 220 pre-control sera were collected from animals gathered at three crush pens.

All serum samples were stored at -20°C until further analysis.

## 3. RESULTS

### 3.1. Antibody ELISA

The results from the antibody ELISA for the negative and positive population are presented in figure 2. At a chosen cut-off point of 0.204, the assay had a specificity of 93.8% and a sensitivity of 88.6%. Analysis of sera from Lusitu area produced results similar to the negative group, although there was an indication that antibody titers were low for *T. brucei* (Fig. 3) and *T. congolense* (Fig. 4).

### 3.2. Antigen ELISA

All the samples from Lusitu area were negative by buffy coat technique (BCT), and only 4 (2%) of 199 sera were positive for *T. brucei* using the Ag-ELISA (Table I). From a total of 141 positive sera, the (BCT) detected 132 (93.6%) *T. congolense*, 9 (6,4%) *T. vivax* and no *T. brucei* infections (Tables I and II).

TABLE I. DETECTION OF TRYPANOSOME INFECTIONS USING THE BUFFY COAT TECHNIQUE AND THE ANTIGEN ELISA

| Origin of samples | Total number examined | Positive by BCT | Positive by Ag-ELISA |
|---|---|---|---|
| Known negative | 99 | 0 | 0 |
| Known positive | 141 | 141 | 56 |
| Lusitu | 199 | 0 | 4 |
| Eastern Province | 190 | 79 | 116 |
| Total | 629 | 220 | 176 |

BCT= buffy coat technique.
Ag-ELISA = antigen-detection enzyme-linked immunosorbent assay.

On the other hand, the antigen ELISA detected 49 (34.8%) cases of *T. brucei* and 7 (4.9%) of *T. congolense* (Table II). No samples have been tested yet for the presence of *T. vivax*.

TABLE II. COMPARISON OF THE SENSITIVITY OF THE BUFFY COAT TECHNIQUE AND THE ANTIGEN ELISA USING KNOWN POSITIVE SAMPLES

| Total number examined | BCT positive samples | | | | Ag-ELISA positive samples | | | |
|---|---|---|---|---|---|---|---|---|
| | T.b. | T.c. | T.v. | Total | T.b. | T.c. | T.v. | Total |
| 141 | 0 | 132 | 9 | 141 | 49 | 7 | NT | 56 |

BCT= Buffy Coat Technique; NT = not tested.
Ag-ELISA = antigen-detection enzyme-linked immunosorbent assay.
T.b.= *Trypanosoma brucei*; T.c. = *Trypanosoma congolense*; T.v. = *Trypanosoma vivax*.

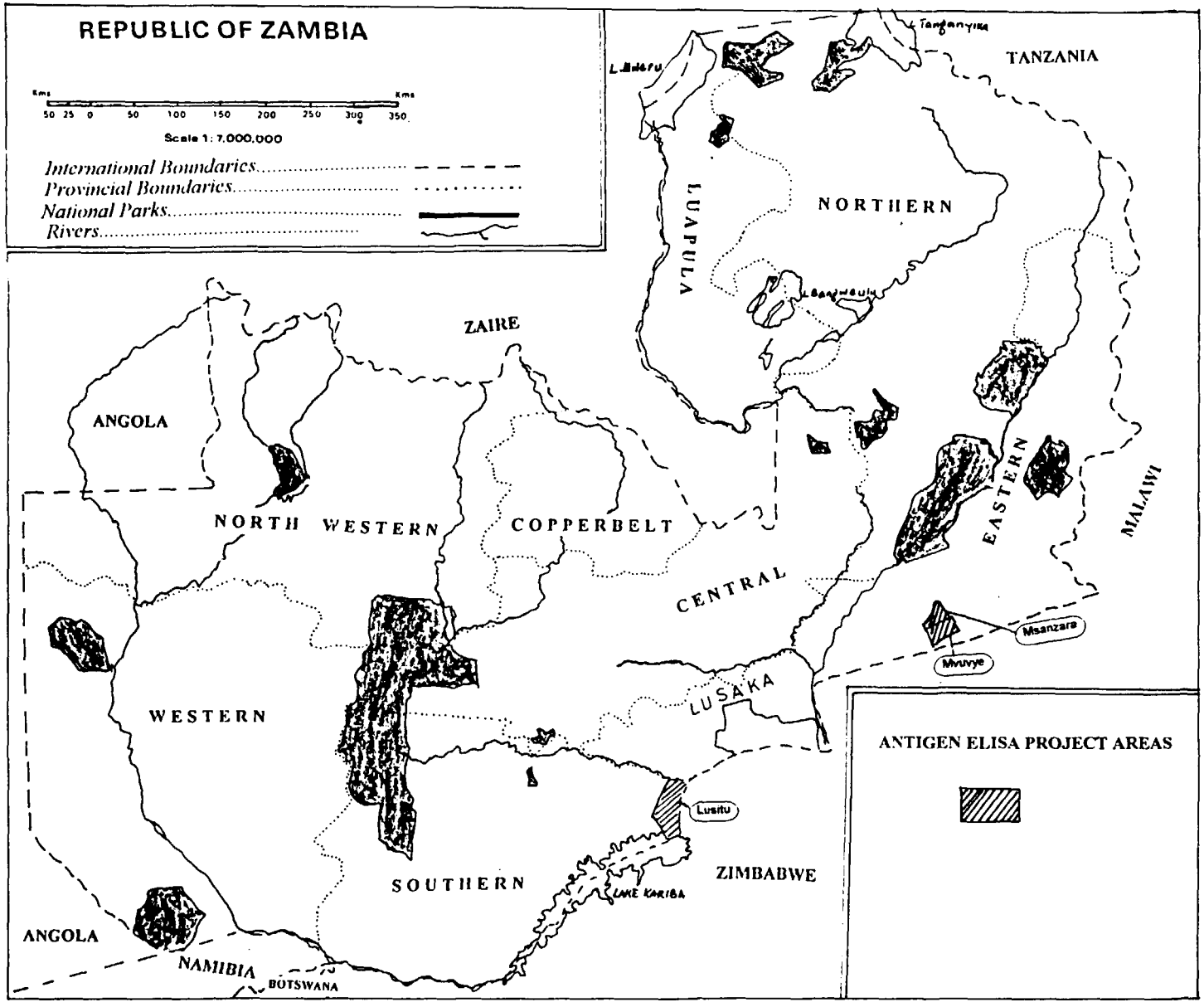


*FIG. 1. Map of Zambia showing the National Parks (solid black) and study areas (hatched).*

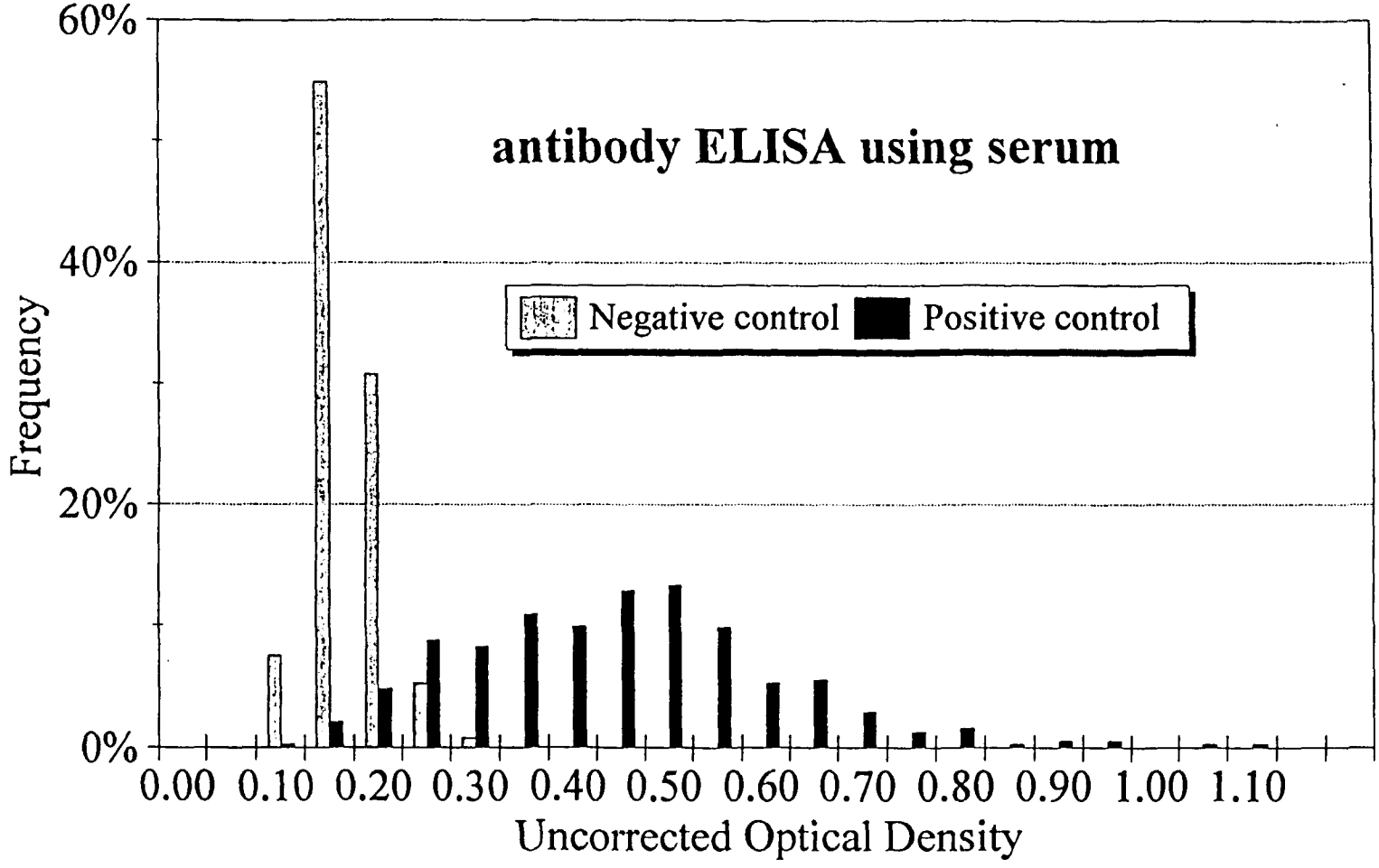


*FIG. 2. Antibody ELISA results for negative and positive cattle populations.*

Similarly, from the 190 pre-control samples collected in the Msanzara area, Eastern Province, the BCT detected 75 (39.5%) *T. congolense*, 2 (1.0%) *T. vivax*, no *T. brucei* and 2 mixed infections of *T. congolense/T. brucei* and *T. congolense/T. vivax* (Table III). On the other hand, the antigen ELISA detected 95 (50%) *T. brucei* and 21 (11%) *T. congolense* infections (Table III).

TABLE III. COMPARISON OF THE SENSITIVITY OF THE BUFFY COAT TECHNIQUE AND THE ANTIGEN ELISA USING SAMPLES FROM EASTERN PROVINCE (MSANZARA)

| Total number examined | BCT positive samples | | | | | | Ag-ELISA positive samples | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | T.b. | T.c. | T.v. | T.b./ T.c. | T.c./ T.v. | Total | T.b. | T.c. | T.v. | Total |
| 190 | 0 | 75 | 2 | 1 | 1 | 79 | 95 | 21 | NT | 116 |

BCT= buffy coat technique.
NT = not tested.
Ag-ELISA = antigen-detection enzyme-linked immunosorbent assay.
T.b.= *Trypanosoma brucei*; T.c. = *Trypanosoma congolense*; T.v. = *Trypanosoma vivax*.

The results of the number of positive cases detected by BCT as compared to the Ag-ELISA is presented in Table IV for each of the three trypanosome species and for mixed infections. At a cut-off point of 10 percent positivity (PP) the apparent specificity of the antigen ELISA was 100% and the apparent sensitivity 23.3%.

TABLE IV. DISTRIBUTION OF TRYPANOSOME SPECIES DETECTED IN INFECTED ANIMALS

| Test | No. samples | No. pos. samples | T.b. | T.c. | T.v. | T.b./T.c. | T.c./T.v. | T.b./T.v. |
|---|---|---|---|---|---|---|---|---|
| BCT | 614 | 220 | 0 | 208 | 9 | 0 | 8 | 0 |
| Ag-ELISA | 614 | 176 | 150 | 25 | NT | 24 | NT | NT |

BCT= buffy coat technique.
NT = not tested.
Ag-ELISA = antigen-detection enzyme-linked immunosorbent assay.
T.b.= *Trypanosoma brucei*; T.c. = *Trypanosoma congolense*; T.v. = *Trypanosoma vivax*.

## 4. DISCUSSION

Analysis of sera from the Lusitu area, where trypanosomosis has not been diagnosed in any of the sentinel herds for the past few years, showed a low titre of antibodies using the antibody ELISA and 2% *T. brucei* infections using the antigen ELISA. The 2% positive cases of *T. brucei* are likely to be false positives due to unspecific reactions inherent to any serological test. When comparing the BCT and antigen ELISA test results, it appears that the BCT detects more of the patent infections which are acute cases, while the antigen ELISA detects more chronic cases. Moreover, the antigen ELISA seems to detect more *T.brucei* cases not detected by BCT. Thus, the antigen ELISA seems to be very well suited to detect chronic cases as well as *T. brucei* infections. In conclusion, the antigen ELISA should be used in combination with the BCT, especially in geographical areas where the BCT does not detect any patent infections.

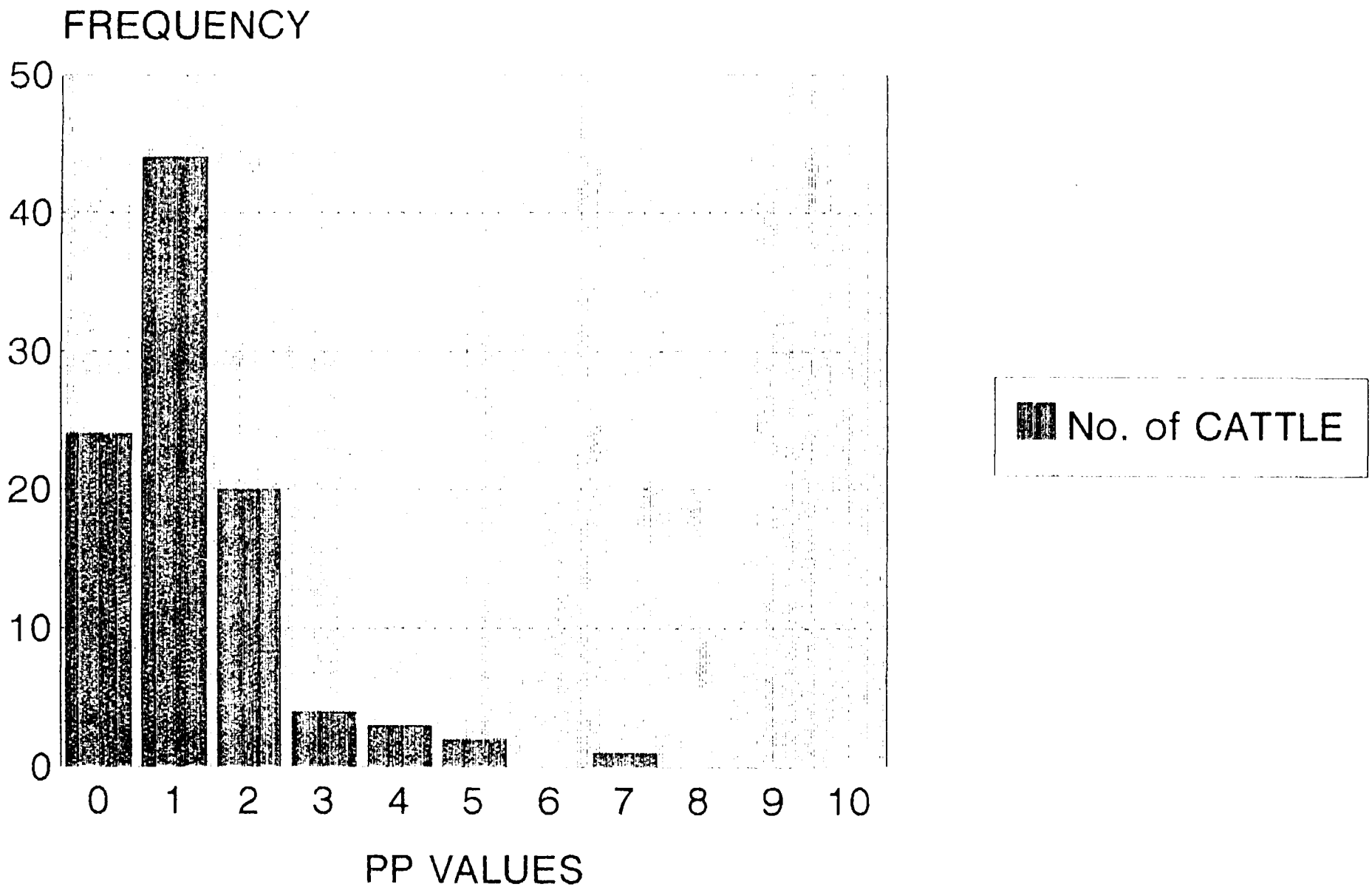


*FIG. 3. Results of an ELISA detecting antibodies against* Trypanosoma brucei *in serum samples from Lusitu area, where tsetse have been eradicated.*

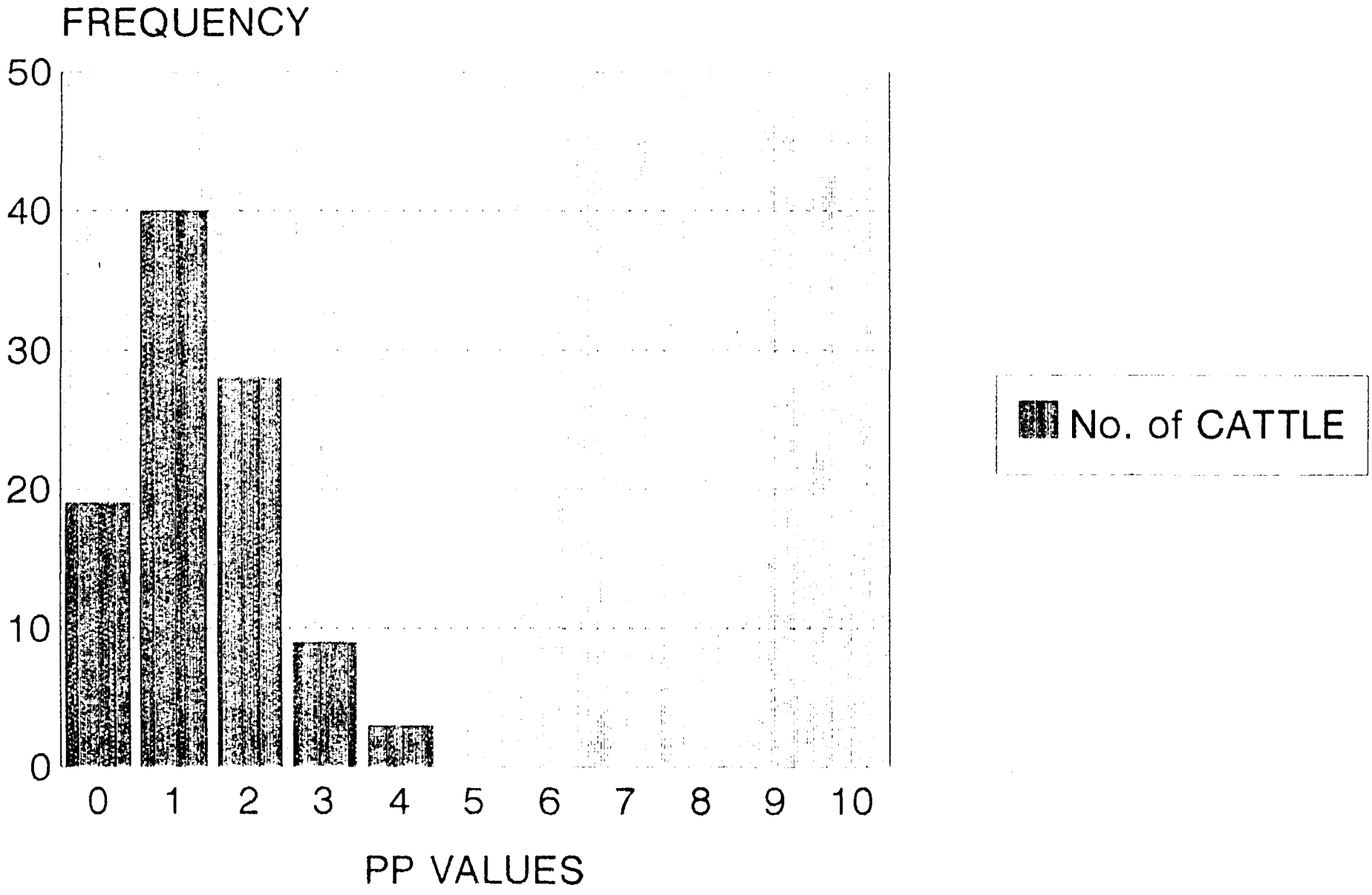


*FIG. 4. Results of an ELISA detecting antibodies against* Trypanosoma congolense *in serum samples from Lusitu area, where tsetse have been eradicated.*

## ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance of the Regional Trypanosomosis and Tsetse Control Programme (Lusaka) and the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture for assistance and funding. We are thankful to Dr. Julian Hopkins for providing antibody ELISA results.

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[1] NANTULYA, V.M., LINDQUIST, K.J., Antigen-detection enzyme immunoassays for the diagnosis of *Trypanosoma vivax*, *T. congolense* and *T. brucei* infections in cattle, Trop. Med. Parasit. **40** (1989) 267-272.

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# THE USE OF THE ANTIGEN ELISA FOR MONITORING TSETSE AND TRYPANOSOMOSIS CONTROL PROGRAMMES IN ZIMBABWE

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**Abstract**

THE USE OF THE ANTIGEN ELISA FOR MONITORING TSETSE AND TRYPANOSOMOSIS CONTROL PROGRAMMES IN ZIMBABWE.

Blood and serum samples from cattle originating from tsetse free and tstse infested areas were analysed using the Buffy Coat technique and an ELISA to detect trypanosomes and trypanosomal antigens, respectively. The results of the two tests were compared and apparent sensitivity and trypanosome prevalence were calculated.The BCT seemed to be the most suitable test to detect acute infections, while the antigen capture ELISA (Ag-ELISA) was able to detect more chronic infections. The specificity of the Ag-ELISA was found to be very good, but the sensitivity of the test should be improved. One way to detect more *T. congolense* and *T. vivax* infections was to lower the cut-off point of percent positivity from 10 to 5%.

## 1. INTRODUCTION

The number of samples analysed was small due to a lack of biological reagents and due to an outbreak of Newcastle disease in July 1994 in Zimbabwan poultry farms. As a consequence of this disease emergency the priority of the Field Branch of the Department of Veterinary Services was to vaccinate 10,000,000 chickens in the communal and small scale sectors. Due to the vaccination commitments the Field Branch could not establish a sampling routine as part of the surveillance for trypanosomosis.

## 2. MATERIALS AND METHODS

Serum samples were tested using the antigen detection enzyme-linked immunosorbent assay (Ag-ELISA) to determine the sensitivity and specificity of the assay. Hundred samples were collected from cattle in a commercial farming area near Harare which is a tsetse free area. Using the Ag-ELISA it was possible to test 38 sera of this negative population.

From a previous study 18 serum samples were selected of animals detected positive for trypanosomes by the buffy coat technique (BCT). The animals in this study had been moved from a tsetse free area to an area of medium to high tsetse challenge and had been monitored every two weeks by BCT. Since the animals were treated with Berenil® whenever they were detected positive by BCT, the infections can be considered of an acute nature.

A third group of serum samples was analysed from animals suspected to have chronic infections. The samples originated from the Mudzi district and the Dande Area. The Mudzi district has a persistent low tsetse challenge, which does not often result in overt, acute infections. Since animals were treated only when overt infections were detected, it seems likely that the majority of infections were of a chronic nature. Animals in sub-standard health were examined and blood was collected for monitoring packed red cell volume (PCV) and BCT and for serological purposes. A total of 39 sera was selected for testing in the Ag-ELISA, of which 13 were BCT positive and 26 were from animals with low PCV values. The Dande area is a communal area in which there is an on-going tsetse control programme using Deltamethrin® dipping and targets impregnated with insecticides. Tsetse flies were no longer caught in this area but the occasional case of trypanosomosis was detected. Therefore, it can be assumed that the infections detected were most likely chronic. Thirty-six sera were available for testing by Ag- ELISA.

The results of the BCT and the ELISA were analysed and the apparent sensitivity for each test was calculated as (ELISA positive/BCT positive) × 100. In addition, trypanosome prevalence was determined using BCT and ELISA results.

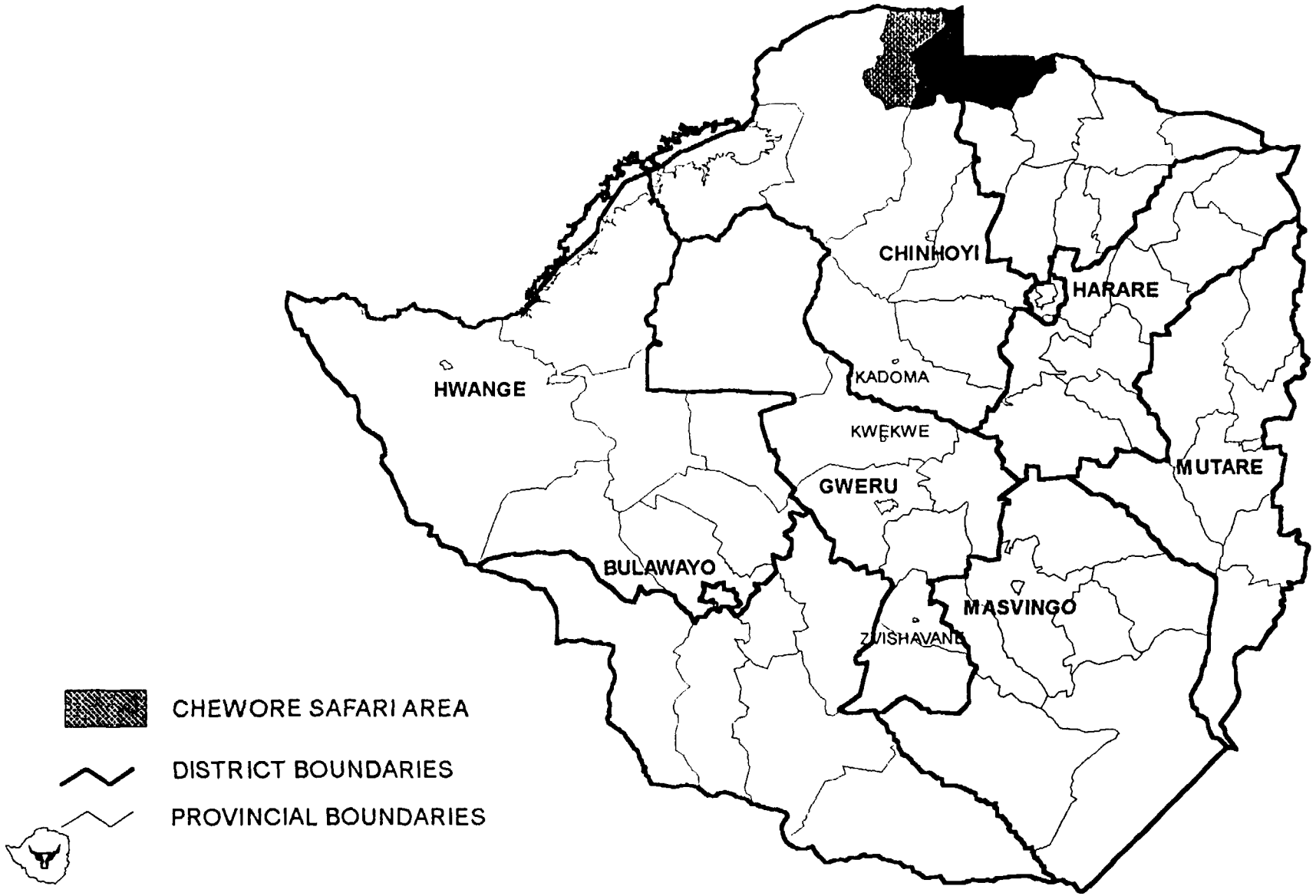


*FIG. 1. Map of Zimbabwe showing provincial bounderies and the study area of Dande communal area (dark shading) with the adjoining Chewore safari area (light shading).*

## 3. RESULTS

The ELISA results are based on a cut-off point of 10 % positivity (PP) for each of the three trypanosome species. The specificity of the test to detect *T. brucei*, *T. congolense* and *T. vivax* was 94.7%, 100% and 100%, respectively (Table I).

TABLE I. ANTIGEN ELISA RESULTS FOR THE NEGATIVE POPULATION

| Number of serum samples | Trypanosome species | | |
|---|---|---|---|
| | *T. brucei* | *T. congolense* | *T. vivax* |
| Tested | 38 | 38 | 38 |
| Positive | 2 | 0 | 0 |

In Table II the BCT results are compared with Ag-ELISA results of samples obtained from animals with acute trypanosome infections. In Table III the apparent sensitivity of the ELISA test and the trypanosome prevalence have been calculated for these infections.

TABLE II. COMPARISON OF RESULTS OBTAINED USING THE BUFFY COAT TECHNIQUE AND ANTIGEN ELISA OF ACUTE INFECTIONS

| | | Diagnosis by BCT | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | -ve | T.b. | T.c. | T.v. | T.b./ T.c. | T.b./ T.v. | T.c./ T.v. | Total |
| Diagnosis by Ag-ELISA | Negative | 0 | 1 | 5 | 2 | 3 | 1 | 1 | 13 |
| | Positive *T. brucei* | 0 | 1 | 2 | 0 | 0 | 1 | 1 | 5 |
| | Total | 0 | 2 | 7 | 2 | 3 | 2 | 2 | 18 |

BCT = buffy coat technique; -ve = negative.
Ag-ELISA = antigen-detection enzyme-linked immunosorbent assay.
T.b. = *Trypanosoma brucei*; T.c. = *Trypanosoma congolense;* T.v. = *Trypanosoma vivax.*

TABLE III. APPARENT SENSITIVITY OF THE ANTIGEN ELISA AND TRYPANOSOME PREVALENCE AS DETECTED BY BCT AND ELISA

| | Trypanosome species | | |
|---|---|---|---|
| | *T. brucei* | *T. congolense* | *T. vivax* |
| Apparent sensitivity | 28.6 % | 0 % | 0 % |
| Trypanosome prevalence as detected by BCT | 38.9 % | 66.6 % | 33.3 % |
| Trypanosome prevalence as detected by ELISA | 27.8 % | 0 % | 0 % |

BCT = buffy coat technique; ELISA = enzyme-linked immunosorbent assay.

The results of the analysis of serum samples from animals with chronic infections collected in Mudzi district and the Dande Area are presented in Tables IV and V.

TABLE IV. COMPARISON OF RESULTS OBTAINED USING THE BUFFY COAT TECHNIQUE AND ANTIGEN ELISA OF ACUTE INFECTIONS

| | | Diagnosis by BCT | | | | |
|---|---|---|---|---|---|---|
| | | -ve | T.b. | T.c. | T.v. | Total |
| | Negative | 42 | 3 | 7 | 0 | 52 |
| | T. b. | 15 | 1 | 0 | 0 | 16 |
| Diagnosis by | T. c. | 1 | 0 | 0 | 0 | 1 |
| | T. v. | 0 | 0 | 0 | 1 | 1 |
| Ag-ELISA | T. b./T. c. | 2 | 0 | 0 | 0 | 2 |
| | T. b./T. v. | 2 | 0 | 1 | 0 | 3 |
| | Total | 62 | 4 | 8 | 1 | 75 |

BCT = buffy coat technique.
-ve = negative.
Ag-ELISA = antigen-detection enzyme-linked immunosorbent assay.
T.b. = *Trypanosoma brucei.*
T.c. = *Trypanosoma congolense.*
T.v. = *Trypanosoma vivax.*

TABLE V. APPARENT SENSITIVITY OF THE ANTIGEN ELISA AND TRYPANOSOME PREVALENCE AS DETECTED BY BCT AND ELISA

| | Trypanosome species | | |
|---|---|---|---|
| | *T. brucei* | *T. congolense* | *T. vivax* |
| Apparent sensitivity | 25.0 % | 0 % | 50 % |
| Trypanosome prevalence as detected by BCT | 5.3 % | 10.7 % | 2.7 % |
| Trypanosome prevalence as detected by ELISA | 26.7 % | 4.0 % | 5.3 % |

BCT = buffy coat technique.
ELISA = enzyme-linked immunosorbent assay.

However, in 4 out of the 37 BCT positive samples (from acute and chronic infections combined), the ELISA detected antigens to trypanosomes which were not detected by the BCT (Table VI). Of the 62 BCT negative samples, 20 were positive for antigens, predominantly *T. brucei* consisting of 15 single infections; 2 mixed infections with *T. vivax* and 2 mixed infections with *T. congolense*. *T. congolense* was found once as a single infection.

## 4. DISCUSSION

The specificity of the Ag-ELISA as determined by the testing of a negative cattle population was good, being 94.7 % for *T. brucei*, 100 % for *T. congolense* and 100 % for *T. vivax*. However, the sensitivity of the test using field samples from tsetse infested areas was not optimal. It appeared that in acute infections, characterized by patent parasitaemia as detected by the BCT, the Ag-ELISA did not detect trypanosomal antigens in the majority of cases.

TABLE VI. COMPARISON OF SAMPLES POSITIVE FOR TRYPANOSOMES IN THE ANTIGEN ELISA AND BUFFY COAT TECHNIQUE

| Trypanosome species | Number of samples testing positive | |
|---|---|---|
| | using the ELISA | using the BCT |
| *T. brucei* | 3 | 11 |
| *T. congolense* | 0 | 20 |
| *T. vivax* | 1 | 7 |

BCT = buffy coat technique.
ELISA = enzyme-linked immunosorbent assay.

Following a reduction of the cut-off point in the ELISA to 5 PP for *T. congolense* and *T. vivax*, we assessed the effect on specificity, apparent sensitivity and prevalence of infection (Table VII). The reduction of the cut-off point lowered the specificity for *T. vivax* by 3%, but increased the detection of both acute and chronic infections. On the other hand, lowering the cut-off point will increase the apparent sensitivity for *T. congolense* system as well as the detection of both acute and chronic infections (Table VII).

TABLE VII. EFFECT OF REDUCING THE CUT-OFF POINT OF PERCENT POSITIVITY WHEN DETECTING *T. CONGOLENSE* AND *T. VIVAX* BY ANTIGEN ELISA

| | Cut-off point for *T. congolense* | | Cut-off point for *T. vivax* | |
|---|---|---|---|---|
| | 10 PP | 5 PP | 10 PP | 5 PP |
| ELISA specificity | 100% | 100% | 100% | 97% |
| Apparent ELISA sensitivity (of acute cases) | 0% | 8% | 0% | 0% |
| Trypanosome prevalence (of acute cases) | 0% | 8% | 0% | 16% |
| Trypanosome prevalence (of chronic cases) | 4% | 6.7% | 5.3% | 6.7% |

PP = percent positivity in the Ag-ELISA
ELISA = enzyme-linked immunosorbent assay

The monoclonal antibodies in the antigen ELISA are directed against an internal trypanosomal antigen and, therefore, destruction of trypanosomes by the host must take place before antigens are released and can be detected. This characteristic of the test is reflected by the low apparent sensitivity for detecting acute infections of each of the three trypanosome species. Consequently, acute infections can more reliably be detected by the BCT, which is 100% specific. However, the antigen ELISA does detect antigens of trypanosomes not detected by the buffy coat technique resulting in a higher prevalence of mixed infections than detected by conventional parasitological techniques.

The higher prevalence of *T. brucei* infections is understandable considering the pathogenesis of the three trypanosome species. Both *T. congolense* and *T. vivax* replicate more rapidly in cattle than *T. brucei* and therefore in a mixed infection the *T. congolense* or *T. vivax* will predominantly stimulate the host's immune system. While the immune system is responding to the *T. congolense* or *T. vivax* infection, the *T. brucei*, which replicates more slowly and is also tissue invasive, may remain undetected by conventional techniques and may result in causing a chronic infection.

The low prevalence of *T. congolense* infections as detected by Ag-ELISA could be due to the fact that this trypanosome is highly pathogenic, causing acute infections and is therefore rarely implicated in chronic infections.

## 5. CONCLUSIONS

As the results were obtained from a limited sample size, the conclusions should be corroborated for a larger number of samples. However, the above results indicate that the antigen ELISA detected more mixed and chronic trypanosome infections than the standard parasitological techniques [1] and should, therefore, be used in combination with the latter tests. The major application of the antigen ELISA is likely to be in areas where standard parasitological techniques are failing to detect infections i.e. in tsetse control areas where the prevalence of trypanosomosis has been changed to a low level.

## ACKNOWLEDGEMENTS

We wish to acknowledge the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture for financial assistance.

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## CONCLUSIONS AND RECOMMENDATIONS

As a result of investigations in fourteen research institutes in Africa during the years 1993/1995 using a combination of standard parasitological techniques and the trypanosomal antigen detection ELISA to monitor tsetse and trypanosomosis control programmes, the following set of conclusions were reached.

### 1. CONCLUSIONS

#### 1.1. Diagnostic techniques

The antigen detection ELISA and parasitological techniques such as the buffy coat technique (BCT) and the micro haematocrit centrifugation technique (MHCT) are when used in combination valuable tools in the monitoring of tsetse and trypanosomosis control programmes.

#### 1.2. Antigen detection ELISA

The FAO/IAEA trypanosomal antigen detection ELISA has been established in all laboratories through participation in the FAO/IAEA Co-ordinated Research Programme on the "Use of Immunoassay Methods for Improved Diagnosis of Trypanosomosis and Monitoring of Tsetse and Trypanosomosis Control Programmes".

#### 1.3. Improved diagnosis

The ELISA and the parasitological techniques proved to be complementary techniques and it is essential that the two techniques be used together. By combining the two techniques it was possible to detect many more animals infected with trypanosomes and to increase the sensitivity of each individual test.

#### 1.4. Specificity of the antigen detection ELISA

Based on negative cattle populations from tsetse free areas, the specificity of the ELISA using a cut-off value of 10 percent positivity was shown to be:

96.0 $\pm$ 2% in the case of *T. brucei*,
99.5 $\pm$ 1% in the case of *T. congolense*,
99.0 $\pm$ 1% in the case of *T. vivax*.

#### 1.5. False positives

Since the specificity of the antigen detection ELISA ranges from 94% to 100% it means that between 0 and 6 false positive cases for each species could be detected for every 100 samples examined.

#### 1.6. Variation in the detection of parasites according to stage of infection

It seems that the ELISA and parasitological techniques detect trypanosome infections at different stages of the disease. The ELISA seemed more suited to detect chronic infections, while parasitological techniques tend not to detect low parasitaemias, which are typical manifestations of a chronic infection. On the other hand, the ELISA was not sensitive enough to detect patent infections in contrast to standard trypanosome detection methods, such as the buffy coat technique.

#### 1.7. Variation in the detection of parasites according to trypanosome species

There is a discrepancy in the prevalences detected for the different trypanosome species between parasitological techniques and ELISA. In the majority of cases *T. brucei* was not detected by the parasitological techniques used. In these cases the ELISA detected a relatively higher number of cases. Therefore, the ELISA seems to be more sensitive than parasitological techniques for the detection of *T. brucei*, whereas for the detection of *T. congolense* the parasitological techniques appear to be more sensitive.

### 1.8. Variation in the detection of mixed infections

The antigen detection ELISA was able to diagnose many more mixed infections than the parasitological techniques. As a result of investigations in different African Research Institutes using the antigen detection ELISA, it appears that mixed infections occur much more frequently than previously thought.

### 1.9. Use of other diagnostic techniques

The choice of the most appropriate test or combination of tests (buffy coat technique, antibody-ELISA, antigen-ELISA, polymerase chain reaction) is dependent on the objectives of the testing programme (e.g. disease mapping, monitoring of control programmes or treatment stategy).

Based on the previous results and conclusions a set of recommendations was prepared during round-table discussions involving all participants. These recommendations will be used for guiding future research activities which will be executed during 1995-1999 as part of a new Co-ordinated Research Programme financially supported by the Government of the Netherlands.

## 2. RECOMMENDATIONS

### 2.1. Proper execution of the buffy coat technique (BCT)

The recommended parasitological technique (BCT) should be properly executed accordng to a standardized methodology using a dark ground/phase contrast microscope. Parasitaemia should be scored using the scoring system previously published by Paris *et al.* in 1982.

### 2.2. Standardized data analysis

A database should be set up using a predefined format in a computer software program to facilitate standardized recording and analysing of results. Such a standardized methodology should be internationally accepted and the Research Contract holders should be encouraged to use the standardised data management software programme to analyse ELISA data, parasitological data and production parameters. The analysis should be supported by technical backstopping.

### 2.3. Collection of additional data

Additional data on environment, socio-economic factors and farming systems should be assembled for analysis together with the parasitological, entomological and serological results. Instructions and guidelines should be developed for this purpose and distributed to all participants. Prior to distribution, participants should be encouraged to collect detailed information (topographical and vegetation maps, cattle and tsetse densities, etc.) of the study areas.

### 2.4. Evaluation of ELISA data

The results for *T. congolense* and *T. vivax* should be re-evaluated using a lower cut-off value which should be determined after analysis of the data collected so far. This is likely to lead to an increase in the sensitivity of the antigen detection ELISA.

### 2.5. Technical adjustments to the ELISA kit

Various technical adjustments should be made to the antigen detection ELISA kit as provided by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. The stability and the dilution of the reagents, in particular the conjugate, should be checked, the amount of reagents provided as part of the kit content (PBS for washing, dilution buffer) should be increased, the upper and lower control limits of the internal quality controls should be revised.

### 2.6. External quality assurance programme

An external quality assurance programme (EQAP) for the FAO/IAEA trypanosomosis ELISA kit should be established on a twice yearly basis. As part of this programme sera of known infection status should be submitted to the participating laboratories. The laboratories should perform assays on these sera without knowledge of the infection status and submit the results for comparative purposes to the FAO/IAEA Agriculture and Biotechnology Laboratory. Following analysis of the results laboratories will be informed.

## 2.7. Establishment of upper and lower control limits and internal control charts

Upper and lower internal quality control limits should be established using optic density (OD) and percentage positivity (PP) values from various laboratories using standard deviations, or percentiles. In addition, it is recommended to instruct the Research Contract holders in constructing internal quality control (IQC) charts.

## 2.8. Calibration of equipment

The equipment, in particular the ELISA readers and the pipettes as used in the participating laboratories, needs to be quality controlled. The FAO/IAEA Agriculture and Biotechnology Laboratory should provide a system to verify and assure the quality of equipment. In addition, Good Laboratory Practice (GLP) and Standard Operation Procedures (SOP) shoud be introduced in the participating laboratories at a later stage.

## 2.9. Use of other diagnostic techniques

A combination of the use of antibody- and antigen-ELISA should be assessed for its usefulness in the diagnosis and treatment of individual animals. It is recommended to improve the performance of the antibody-ELISA by the incorporation of recombinant antigens and to use the test in the final verification phase of tsetse and trypanosomosis eradication programmes. In addition, it is recommended to assess the polymerase chain reaction (PCR) technique for its suitability to diagnose mixed infections, to characterize the parasite, both in the host and in the vector, and to serve as a "gold standard" for diagnostic purposes.

## ABBREVIATIONS

| | |
|---|---|
| Ag | antigen |
| BCT | Buffy-Coat Technique |
| CRP | co-ordinated research programme |
| DG/PC | dark-ground/phase contrast (microscopy) |
| DGIS | Directorate General of Development Co-operation of the Netherlands |
| ELISA | enzyme-linked immunosorbent assay |
| EPI INFO | A word processing, database and statistics system for epidemiology on microcomputers |
| EQC | external quality control |
| FAO | Food and Agriculture Organization of the United Nations |
| ILRAD (ILRI) | International Laboratory for Research on Animal Diseases, now called International Livestock Research Institute |
| KETRI | Kenya Trypanosomiasis Research Institute |
| MHCT | microhaematocrit centrifugation technique |
| masl | meters above sea level |
| NARS | National Agricultural Research Systems |
| NITSE | Nigerian tsetse trap, a monoconical trap |
| ODA | Overseas Development Agency of the United Kingdom |
| PARC | Pan African Rinderpest Campaign |
| PBS | phosphate buffered saline |
| PCV | packed red cell volume |
| PP | percent positivity |
| RBC | red blood cells |
| RTTCP | Regional Tsetse and Trypanosomosis Control Programme |
| SIT | sterile insect technique |
| STDM | standard trypanosome detection methods |
| TCP | Technical Co-operation Project |

## LIST OF PARTICIPANTS


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