TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (1994) 88, 419–421 , n° 4

ISSN = 0035 - 9203

A comparison of parasitological methods for the diagnosis of gambian trypanosomiasis in an area of low endemicity in Côte d'Ivoire

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Abstract

The card agglutination test for trypanosomiasis (CATT) was used to examine 8974 inhabitants in 14 village areas south-west of Daloa, Côte d'Ivoire; 114 (1·3%) were CATT+ or ±, and were further examined by one or more of 6 methods for the direct detection of trypanosomes: lymphatic gland puncture, stained thick blood film (TBF), haematocrit centrifugation technique (HCT), mini-anion exchange column (MAEC), quantitative buffy coat method (QBC®), and kit for *in vitro* isolation of trypanosomes (KIVI). Trypanosomes were seen by at least one method in 16 (14·0%) of the CATT+ group. Blood from 356 of the 8860 CATT− group was inoculated into KIVI; trypanosomes grew from the blood of 1 person. Eleven of the 17 patients with detectable trypanosomes were screened by all 6 methods: 6 were HCT+; 7 were gland+; 10 were MAEC+; 10 were KIVI+; 11 were both TBF+ and QBC®+. One CATT+ patient was KIVI+ but otherwise negative, although TBF was not done. The overall prevalence of trypanosomes was 0·2% rising to 0·8% in one village area. The results support previous evidence that a reappraisal of procedures is required in the customary system of surveillance for gambian sleeping sickness.

Introduction

Recently the quantitative buffy coat (QBC®) and the kit for *in vitro* isolation (KIVI) techniques have been proposed for the parasitological diagnosis of gambian trypanosomiasis (BAILEY & SMITH, 1992; TRUC *et al.*, 1992); low numbers of trypanosomes in the blood are easily detected. QBC® gives a quick result whereas KIVI, although perhaps more sensitive, is limited in value because of the long wait before the organisms multiply adequately in culture.

The initial field trial with the QBC® was in Uganda (BAILEY & SMITH, 1992), where it performed well against several other techniques for detecting trypanosomes in putative patients; these had positive responses to the standard initial screening of whole blood with the card agglutination test for trypanosomiasis (CATT; MAGNUS *et al.*, 1978), and trypanosomes were found by either lymph gland juice aspiration or the haematocrit centrifugation technique (HCT; WOO, 1970). However, the largely neglected examination of stained thick blood films (TBF) also appeared to be efficient; the technique confirmed infection in 95% of the patients parasitaemic by QBC®.

By contrast, according to LUMSDEN *et al.* (1981) TBF missed 37% of the infections found in Côte d'Ivoire by another sensitive detection method, mini-anion-exchange column and centrifugation (MAEC). This may indicate that parasitaemias are lower in Côte d'Ivoire than in Uganda, where the circulating organisms are now known to be genetically different (ENYARU *et al.*, 1993).

Thus, the opportunity was taken to evaluate the methods together during a routine Côte d'Ivoire government survey when CATT was used for the initial screening. At the same time, surveys were carried out on the sympatric animal and tsetse fly populations; the results of these will be described elsewhere.

The prevalence of human sleeping sickness was found to be extremely low, so, unfortunately, decisive conclusions on the diagnostic tests used could not be drawn; however, the indications reported here may be of interest for future trials.

Methods

Study area

The survey area stretched from about 10 to 30 km south-west of the town of Daloa in Côte d'Ivoire. The population of several ethnic groups was, as in many areas of the region (HERVOUËT & LAVEISSIÈRE, 1987), involved mostly in coffee and cocoa production, with subsistence agriculture and limited livestock husbandry. The inhabitants, many of whom dwelt in outlying *campements*, were asked to attend a series of screening centres arranged, over a period of 10 d, in the nearest of 14 large villages (28 October–6 November 1992).

Techniques

The Ivorian surveillance team used CATT (MAGNUS *et al.*, 1978) with finger-prick blood to screen the 8974 people who attended the centres. From most of those who were CATT+ or CATT±, 8·0 mL of venous blood were taken into a syringe containing 0·5 mL of 5% Liquoïde (Roche); 5.0 mL of the mixture was inoculated into a KIVI vial (AERTS *et al.*, 1992; TRUC *et al.*, 1992). The remainder was used for the HCT (WOO, 1970), the MAEC (LUMSDEN *et al.*, 1981), and to provide plasma for a further CATT. Finger-prick blood was taken for one QBC® tube (BAILEY & SMITH, 1992). If a subclavicular lymph node of a patient was palpable, the fluid extruded after puncture was examined for trypanosomes. A thick film of 5 μL of blood was also made from a finger-prick and stained later in the laboratory by Field's method (FIELD *et al.*, 1963). The whole of the film was examined under a ×40 microscope objective, which took about 30 min.

In addition, blood samples for KIVI inoculation were taken from 356 of those attending the survey who were CATT−; during each of the first 9 days of screening, 32–49 individuals were selected without any known bias on our part. All were examined by HCT as well, but field logistics severely limited the number that could be additionally examined by QBC®.

In Côte d'Ivoire, the KIVIs were kept in the shade at the ambient temperature (20–28°C) until arrival in the UK where they were maintained at 27°C; weekly examinations were continued up to a maximum of 8 weeks after inoculation.

Results

Altogether 469 KIVIs were inoculated; 6 (1·3%) were subsequently discarded when found to be contaminated with bacteria or fungi, which inhibit multiplication of trypanosomes *in vitro*.

Blood from one person, who was both CATT− and HCT−, infected a KIVI (Table); since the culture did not become positive until 18 d after inoculation, in the UK, the original blood sample was not tested by any additional method. None of the remaining CATT− people whose blood was also inoculated into KIVI gave rise to a positive culture; all were HCT−, and no trypanosome

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Table. Detection of trypanosomes in patients in an endemic area of Côte d'Ivoire[a]

Survey centre	CATT Blood	CATT Plasma	Lymph glands	HCT	MAEC	TBF	QBC®	KIVI Result	KIVI Day positive
Gboghué	+	+	–	+	+	+	+	+	16
Gokra	±	–	+	ND	+	ND	ND	+	13
Zoboua	–	–	ND	–	ND	ND	ND	+	18
Ziguedia-Goza	+	+	+	–	+	+	+	+	30
Ziguedia-Goza	+	+	+	+	+	+	+	+	12
Ziguedia-Goza	±	+	–	–	– [b]	ND	+	+	16
Ziguedia-Goza	+	+	+	+	+	+	+	+	12
Ziguedia-Goza	+	+	–	+	+	+	+	+	12
Ziguedia-Goza	+	+	+	–	+	+	+	+	12
Guédékiprea	+	+	–	–	– [b]	+	+	+	14
Guédékiprea	+	+	–	–	+	+	+	– [c]	–
Gbieguhé	+	+	+	–	ND	+	+	+	14
Gbieguhé	±	+	–	–	–	ND	–	+	30
Guediboua	+	+	+	+	+	+	+	+	9
Kékegoza	±	+	+	+	+	+	+	+	13
Kékegoza	±	+	+	–	+	+	+	+	18
Kiboua	+	+	+	+	+	ND	ND	+	10

[a]Abbreviations: CATT, card agglutination test for trypanosomes; HCT, haematocrit centrifuge technique; MAEC, mini-anion exchange column; TBF, thick blood film; QBC®, quantitative buffy coat technique; KIVI, kit for *in vitro* isolation of trypanosomes; ND, not done.
[b]Initial result; repeat MAEC was positive.
[c]Fungal contamination may have prevented trypanosome growth.

was seen in any of the 35 who were also examined by QBC®.

One hundred and fourteen persons were CATT+ or CATT± (using whole blood or plasma), and 16 of them had trypanosomes demonstrated by at least one other method (Table). Because of organizational problems in a large, fast moving field survey, only 11 of these candidate patients were examined by all 6 parasitological procedures. Six of them were HCT+; 7 had infected glands, of whom 3 were HCT–. Ten were initially MAEC+, but after the QBC® was reported positive, 1 patient who was MAEC– at first was found to be positive by repeat tests. Discounting this repeat MAEC result, 4 MAEC+ patients were initially HCT–. All 11 of the comprehensively examined patients were QBC®+ and TBF+, while 10 gave rise to positive KIVI cultures; fungal contamination may have inhibited trypanosome proliferation in the twelfth.

One of the 6 incompletely examined patients with demonstrable trypanosomes was HCT– but KIVI+, QBC®+ and TBF+; MAEC was not done. Another was KIVI+, but QBC®–, HCT– and MAEC–; TBF was not done. A third was KIVI+ and QBC®+ but was MAEC+ only when re-tested.

Unexpectedly, the prevalence of infection in the whole area, as demonstrated by the presence of parasites, was only 0·2%. However, this figure was about 4 times higher in one survey centre, Ziguedia-Goza; trypanosomes occurred in 6/782 (0·8%) of those examined at the primary screening.

Discussion

The few infections found meant that an estimate of comparative diagnostic efficiency could not be reliably made. Nevertheless, the study supported previous findings that QBC®, KIVI and TBF are efficient diagnostic techniques. The many apparently uninfected CATT+ people in the population (97/8974) remain of some concern; although, unfortunately, the blood was not examined by TBF, trypanosomes were found only by KIVI in one CATT+ person. It may be that trypanosomes were so sparse in the blood that they were undetectable by any other blood examination method. They may have also been lurking solely in sites away from the bloodstream, such as the cerebrospinal fluid. Since positive lymph nodes were always confirmed by another technique, it seems unlikely that lymph is, at any stage, an exclusive environment for trypanosomes, although again the question of concentration must be considered. Nonetheless, the discovery by KIVI, as before (TRUC *et al.*, 1992), of an otherwise undiagnosed patient poses the question of how many other such people occur in this part of Côte d'Ivoire.

Any estimate must remain largely a matter of conjecture. Extrapolation from the one positive KIVI result among the 356 CATT– people sampled by that method may imply that 25 others among the whole CATT– population of 8860 were also infected. A more extensive investigation is obviously required.

The simplest and cheapest diagnostic technique is TBF; the waiting period is merely the time taken for staining and examining the film in a simple laboratory. KIVI is confirmed as valuable in diagnosis (TRUC *et al.*, 1992), although it was originally designed to overcome the difficulties of isolating *Trypanosoma brucei gambiense*. Its prime disadvantages as a routine tool are high cost and the long period of waiting for positive results, in this survey up to 30 d; moreover, infections may be missed because of microbial contamination inhibiting trypanosome multiplication. However, the sensitivity of KIVI is illustrated by the discovery of infection in, not only a CATT– patient, but also a CATT+ patient in whom trypanosomes were not detected by other sensitive direct techniques.

QBC® has a distinct advantage in giving a clear result in less than 10 min in the field. The initial capital outlay is high, but the equipment should last a considerable time. The running cost for the special capillaries approximates that of the MAEC, which is not an easy method to use in the field. QBC® appears to be at least as efficient as MAEC, which works well in the hands of an expert team, such as the Ivorian unit that conducted the routine part of this survey. A comparable, well organized team could use the QBC® to considerable advantage and increase the numbers of people surveyed.

The success of the TBF is perhaps the most surprising feature of this survey, confirming the earlier observations in Uganda (BAILEY & SMITH, 1992); it appeared better than another widely used technique, HCT. The unfashionable but cheap TBF must be further re-evaluated in the field.

Acknowledgements

We thank the technical staff and many others in Côte D'Ivoire for their assistance in both the field and laboratory, and our colleagues in the UK for their technical help and advice.

Financial support was provided by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases and by the UK Medical Research Council.

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Received 10 August 1993; revised 27 September 1993; accepted for publication 29 September 1993

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (1994) 88, 421–422

Short Report

Serodiagnosis of cysticercosis: specificity of different antigens and enzyme-linked immunosorbent assays

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Cysticercosis is endemic in Hong Kong and various parts of China (KO, 1991). At present, serodiagnosis of this disease remains difficult, mainly due to the fact that the commonly used somatic antigens of larval *Taenia solium* cross-react with a wide range of heterologous antibodies (FLISSER *et al.*, 1982; CHENG & KO, 1991). The lack of animal models also hampers the development of more reliable assays to identify acute or chronic infections.

Since the excretory–secretory (ES) antigens of some helminths are known to be highly specific, the present study was undertaken to evaluate the feasibility of using ES antigens of *Cysticercus cellulosae* in serodiagnosis. The specificity of ES antigens was compared with that of whole cyst (W), membrane (M) and cystic fluid (F) antigens. An attempt was also undertaken to determine the efficacy of 4 recently developed enzyme-linked immunosorbent assays (ELISAs) in detecting immunoglobulin G (IgG) antibodies when different antigens were used.

ES antigens were obtained by maintaining cysts (freshly collected from pork) in medium 199 for 3 d in a CO_2 incubator at 37°C. After ultrafiltration and desalting, the concentrated ES sample was centrifuged for 1 h at 3000 *g* to remove tissue debris. W antigens were obtained by homogenizing intact cysts with a Telflon® homogenizer. M antigens consisted of the cyst wall and protoscolex. F antigens, which were collected by aspiration using a 27 gauge needle, were centrifuged at 15 000 *g* to remove tissue debris.

Sera samples were collected from 39 patients admitted to various hospitals in Hong Kong who were suspected to be suffering from cysticercosis because of the presence of one or more of the following symptoms: meningitis, epileptic fits, presence of cysts in brain or other tissues. Heterologous antisera were collected from patients suffering from other parasitic infections. Negative control sera were obtained from members of our laboratory staff.

The optimal antigenic concentrations for W, M, F and ES used to coat microtitration wells were 10, 10, 1 and 5 μg/mL respectively. Double antibody ELISA was performed according to VOLLER *et al.* (1976). Goat anti-human IgG conjugated with horse-radish peroxidase (HRP) was used as secondary antibody. Triple antibody ELISA was performed according to GAMBLE *et al.* (1983) and KO & YEUNG (1989). Goat anti-human IgG and rabbit anti-goat IgG conjugated with HRP were used as secondary and tertiary antibodies respectively. *o*-Phenylenediamine was used as substrate in both double and triple antibody ELISAs. The FAST-ELISA (Falcon Assay Screening Test ELISA) was modified from the method of HANCOCK & TSANG (1986). For the immunobead ELISA, ferrous metal Bionectic® beads (Organon) served as the solid support. They were coated with antigens following the method of KO & YEUNG (1992). An electromagnetic device designed by one of us (R.C.K.) was used to transfer the beads. Tetramethyl benzidine (TMB) was used as the substrate. Samples were considered positive when the cut-off value of each ELISA was 3 times the mean optical density (OD) of normal serum samples.

The triple antibody ELISA was the best. It yielded the smallest number of false positive reactions with heterologous antisera while giving the largest number of positives with sera from patients suspected of neurocysticercosis (Table). The double antibody ELISA generally yielded lower OD values with positive sera than the triple antibody ELISA. Immunobead ELISA appeared to be the least specific assay as it yielded false positive reactions with sera from patients with hydatidosis, angiostrongyliasis and ascariasis. FAST-ELISA was the least sensitive assay as it yielded a false negative reaction even with serum from a patient with a positive muscle biopsy.

Of the 4 antigens tested, ES antigen was the most specific and yielded the largest number of cases with positive OD. Unlike the other antigens, it did not cross-react with the hydatid serum. A separate study (NG, 1991) has shown that ES antigen of *C. cellulosae* consists of 19 polypeptides. The specificity of W and M antigens was fairly

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