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Field evaluation of Cellognost indirect haemagglutination test in the diagnosis of human trypanosomiasis (sleeping sickness) in Kura Local Government Area of Kano State, Nigeria

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Summary

The Cellognost indirect haemagglutination (IHA) test was compared with conventional techniques, namely, wet blood film examination, stained thin and thick films, animal inoculation and the microhaematocrit centrifugation, for detecting sleeping sickness cases. Sixty-four out of 245 serum samples collected from suspected and some diagnosed cases of the disease were positive by the Cellognost screening test, while only three cases were detected by stained thin film or microhaematocrit centrifugation. Of the 64 cases 45 strongly positive samples were subjected to quantitative assay. Twenty-five of these had anti-*Trypanosoma gambiense* antibody titres above 1:20 representing active infection in the population. This represented 10.2% of the total sample, and strongly suggested the persistence of the disease in the population.

Key words: *Trypanosoma brucei gambiense;* Cellognost; indirect haemagglutination; sleeping sickness (human trypanosomiasis); serological diagnosis.

Introduction

The disease (sleeping sickness) caused by *Trypanosoma brucei gambiense* in humans results in economic losses, which if quantified could be enormous. In order to control or eradicate this disease, particularly through chemotherapy it is of paramount importance to detect the infection in inhabitants within a community. Diagnosis is therefore an essential tool in epidemiological surveys.

There are several serological and parasitological techniques for the diagno-

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sis of human trypanosomiasis. These include card-agglutination test with stained trypanosomes (CATT) (Magnus et al., 1978), enzyme-linked immunosorbent assay (ELISA) (Vervoort et al., 1978), haematocrit centrifugation (Woo, 1970, 1971), and the miniature anion-exchange centrifugation technique of Lumsden et al. (1979).

The very low level of parasitaemia during infections by *T. brucei gambiense* makes most parasitological techniques inadequate for effective diagnosis of the disease. Serological techniques on the other hand are not always easy to carry out in the field and where possible some time must elapse between obtaining the blood samples and availability of the results.

A good diagnostic technique should be efficient, reliable, cheap and easily performed in the field by rural health workers, with minimum equipments and skill and the results available on the "spot" (WHO, 1976).

In this paper, the Cellognost indirect haemagglutination test is compared with existing parasitological techniques, such as wet, thick and thin blood films, animal inoculation and the haematocrit centrifugation in the diagnosis of human trypanosomiasis.

Materials and Methods

The Cellognost[1] trypanosomiasis kit consists of trypanosomiasis IHA-reagent, trypanosomiasis control sera (positive and negative) and Tris-buffer solution. The IHA-reagent consists of stabilized erythrocytes sensitized with a soluble *Trypanosoma gambiense* antigen, obtained from the trypomastigote form from the blood of experimentally infected rats. It comes in lyophilized form and is reconstituted with distilled water for use. Positive control serum was prepared from goat infected with *T. gambiense* and has a titre of 1:512 to 1:1024, under recommended experimental conditions. The negative serum was produced from pooled liquid human serum.

Rats for animal inoculation were obtained from a breeding colony at NITR, Vom. Other requirements included microhaematocrit capillary tubes, sterile lancets, vials, needles and syringes.

Study area

An Epidemiological Division of NITR based in Kano city conducts surveillance work throughout Kano State and the northern parts of Kaduna and some areas of Sokoto States of Nigeria. Two hamlets were selected on the recommendation of the Epidemiology field staff, viz., Gamada and Tofa. Both are in Kura Local Government Area (LGA) of Kano State. Kura, the LGA headquarters, is 34 kilometres from Kano while Gamada and Tofa are 32 and 43 kilometres from Kano, respectively. Both hamlets consist principally of rural population of subsistence farmers. The patients were made up of 184 males and 61 females ranging from 4 to 70 years of age.

Procedure

In the screening exercise, patients were bled to collect about 10 ml of blood for serum preparation and other diagnostic studies. Wet blood films, thin and thick smears were made immediately and either examined or dried for staining accordingly. About 0.5 ml of blood from each patient was injected into a marked rat to induce infection by the animal inoculation method. One capillary tube

[1] Cellognost is a trade name for the IHA reagent marketed by Hoechst Behringwerke, W. Germany. The use here follows recommendation by WHO that the method of diagnosis meets necessary requirements for field use.

Table 1. Results of diagnostic methods employed in survey of 245 patients in Kura LGA

Method	Positive result *or* trypanosome present	% positive
Wet film	0	0
Stained thin and thick film	1	0.41
Microhaematocrit centrifugation	2	0.82
Animal inoculation	0	0
Cellognost IHA test	64	26.1

Table 2. Interpretation of Cellognost IHA qualitative test

Reaction	Result	Total
Complete agglutination of cells (4+ or ++++)	positive	2
Agglutination with slight button formation (+++, ++, +)	weakly positive	62
Sedimented cells (button formation)	negative	181
	Total sampled	245

was filled with anticoagulated blood for the microhaematocrit centrifugation. After spinning the buffy coat was examined at 40× for presence of trypanosomes. The remaining blood was allowed to clot and serum separated in a protable bench centrifuge at the NITR field station in Kano. These constituted the test sera for both screening and quantitative Cellognost IHA test.

Screening and quantitative IHA test

Cellognost trypanosomiasis reagents serve for the qualitative and quantitative determination of antibodies to *T. b. gambiense* in human sera. A more highly diluted erythrocyte suspension is used for qualitative than for quantitative assay. Both methods can be completed in U-bottomed microtitre plates.

For the screening test the reconstituted IHA reagent was dispensed at 100 microlitre (μl) per well. 5 μl of positive control serum were added to the twelve wells of row A and reapeated in row B for negative serum. Three wells in all other rows were serially assigned to each test sample and 5 μl of test serum added to each well. The reagents and serum were mixed thoroughly and allowed to stand undisturbed at room temperature for 2 h. The results were read for presence or absence of haemagglutination.

Positive sera were further subjected to a quantitative test to determine the antibody titres. Serial doubling dilutions of positive and negative control sera and all test samples were made in the microplates. Row A received the positive control serum, row B the negative while test specimens were diluted in all subsequent rows. 50 μl of serum were used in each case. All wells had earlier been filled with 50 μl of Tris buffer, pH 8.0. Finally 25 μl of IHA reagent was added to each well, mixed thoroughly and allowed to stand undisturbed for 2–3 h at room temperature.

Results

245 cases were sampled by the six methods. No infection was detected by examination of wet film and stained thick film or by animal inoculation. In one

Table 3. Quantitative Cellognost assay

	Titre											Total
	0	8	16	32	64	128	256	512	1024	2048	4096	
No. positive at titre given	7	5	8	7	3	4	7	1	1	1	1	45

A total of 20 sera had titres less than 1:32 although by qualitative analysis they appeared to have positive agglutination. The 45 samples were selected from the 64 positive cases based on the intensity of the agglutination reaction (+ + + + to +). The 20 negative results were sera showing little agglutination.

case, parasitaemia was detected by examination of stained thin blood film, while in two cases parasites were detected by the microhaematocrit centrifugation method (Table 1). The Cellognost qualitative IHA test detected 64 (26.1%) possible cases of infection in the population examined. These were cases with different degrees of haemagglutination interpreted as in Table 2.

45 out of 64 positive sera showing strong agglutination (+ + + +, + + +, or + +) were included in the quantitative assay. The results (Table 3) showed that 25 samples, representing 10.2% of the total surveyed, had anti-*T. b. gambiense* titres equal or greater than 1:32 which is highly suggestive of active infection. Three cases had titres of 1:1024, 1:2048, and 1:4096, respectively. Of these three, one (1:4096) was the patient in whose blood parasites were detected by both stained film and by the microhaematocrit methods while the other two were patients who had been treated in an earlier survey but had subsequently gone into relapse.

Five serum samples of non-human origin were included in the quantitative assay, namely: serum from rabbit infected with *T. b. gambiense* for over 4 months (titre 1:4096); serum from a rat with 8-week-old *T. b. gambiense* infection (titre 1:256); rabbit serum from an animal immunized with homogenized whole *T. b. brucei* strain 8/18 (titre 1:2048); serum from a ram with 12-week infection with *T. b. brucei* 58/35 (titre 1:4096) and rat antiserum to *T. vivax* Y58 which gave a negative reaction. Microfilaria were occasionally observed in wet and stained film preparations. This does not seem to cross-react with the reagents as one patient with a microfilaria density of 30 per microscope field in wet blood film was negative in the qualitative Cellognost screening test for trypanosomiasis.

Discussion

The diagnostic techniques currently in use can be divided into the protozoological and serological methods (Lumsden, 1977). Wet film examination, stained thin and thick films, the microhaematocrit technique, animal inocula-

tion, miniature anion-exchange centrifugation, all emphasize the actual presence of the parasite. Serological methods including capillary-tube agglutination (CA) test (Aiyedun and Amodu, 1973), CATT, ELISA, complement fixation, mercuric chloride test, and currently the IHA (Cellognost) described here, measure the host reaction to the presence of the organism even though the latter might have been eliminated by an active immunological process. Problems in establishing the actual presence of parasites in a host due to low parasitaemia are now leading to a shift to immunological tests, originally aimed at supplementing the latter, as a primary tool for diagnosis. The main handicap of most of such methods is the need for optimum laboratory conditions for precision and dependability.

The Cellognost IHA test unlike most other methods mentioned can however be completed in an open tropical condition such as a mobile clinic or on any flat bench, for that matter, on the same day serum specimens are collected. Quantitative analysis of sera reduces the chances of making a false positive diagnosis if only titres above 1:20 are regarded as significant, indicating an infection by *T. b. gambiense.*

The differences in the rates of positive results obtained by the parasitological and serological methods are highly significant. Whereas only two cases, representing 0.82% of total surveyed, were identified by a combination of the microhaematocrit and stained film methods, 64 cases or 26.1% were detected by serodiagnosis. This is related to the very low parasitaemia observed with *T. b. gambiense* (Baltz et al., 1981), such that the chances of identifying the parasite in one examination of blood film during infection are really limited. Thus the efficiency of wet film, stained thick and thin blood film and microhaematocrit centrifugation methods is very low.

T. b. gambiense usually has a low infectivity to rats and mice especially when freshly isolated from humans (Mulligan, 1953; Gray, 1967). Infectivity can be enhanced by rapid passage in nursling rats, hedgehogs and immunosuppressed mice, a procedure which takes several weeks and hence is unsuitable for rapid diagnosis. This explains why it was not possible to infect rats even with blood from two patients showing positive parasitaemia.

On the other hand the serological test employed here measured host response to infection. Antibodies are produced against invading parasites as a result of activation of lymphocytes and these may continue to produce antibodies after the parasites have been destroyed. In trypanosomiasis such antibody production occurs early in infection (Mansfield and Bagasra, 1978) and thus becomes available for the diagnosis of human infection.

Cross-reactivity observed with *T. b. brucei* was not unexpected because *T. b. brucei, T. b. rhodesiense* and *T. b. gambiense* have a very similar antigenic structure as far as their stable antigens are concerned (Le Ray, 1975). These stable antigens are included in the Cellognost reagent. The group-specificity of the reagent is reflected by the negative result obtained with the rat antiserum

against *T. vivax*. It is thus apparent that the Cellognost test is dependable for rapid diagnosis of Gambian sleeping sickness.

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