Serodiagnosis of sleeping sickness in the Republic of the Congo: comparison of indirect immunofluorescent antibody test and card agglutination test

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Abstract

The card agglutination test for trypanosomiasis (CATT) was evaluated and compared to the classical immunofluorescent antibody test (IFAT) in the immunological diagnosis of Gambian trypanosomiasis. Tests were performed on serum and whole blood. Cross-reactions were found in the CATT with sera from patients suffering from parasitic infections other than sleeping sickness, but could be largely overcome by selecting 1/10 as the specific threshold dilution. At 1/40 dilution no false positive result was observed in the IFAT. At the specific threshold dilution, the sensitivity of IFAT was 94·7%, compared with 91·6% for the CATT. On whole blood, a more convenient sample in the field, IFAT specificity (100%) was greater than that of the CATT (94·3%), as was its sensitivity (92% compared with 82·5%). In view of its simplicity and rapidity of execution, the CATT is an efficient serological test to detect new foci. When greater sensitivity is required, IFAT should be preferred to CATT.

Introduction

At least 45 million people in Africa are estimated to be exposed to trypanosomiasis. In the People's Republic of the Congo, more than 600 new cases are reported annually. The causative organism, *Trypanosoma brucei gambiense*, can usually be detected in the circulating blood, lymph nodes or cerebrospinal fluid by repeated microscopical examination. However, because of the relatively low sensitivity of parasitological techniques, serological tests are indispensable for large scale screening of the population at risk. Only a few comparative evaluations of serological tests have been carried out (WHO, 1976; KNOBLOCK *et al.*, 1984); and their usefulness is still disputed. 2 immunoassays are currently used in the Congo: the indirect immunofluorescent antibody test (IFAT), considered as the reference method, and more recently the card agglutination test for trypanosomiasis (CATT), described by MAGNUS *et al.* (1978). We present here an evaluation and comparison of these serological techniques in the diagnosis of Gambian sleeping sickness.

Materials and Methods

Serum samples

Sera were collected from 366 Congolese persons of the following 3 groups.

(1) 95 patients with parasitologically confirmed trypanosomiasis; their mean age was 28·9±16·1 years (range 4-74); the male/female ratio was 1·17.

(2) 105 healthy Congolese living in Brazzaville, an area free of human trypanosomiasis.

(3) 195 patients with one or more of the 3 following parasitic infections. (a) Filariasis (*Loa loa* and/or *Mansonella perstans*); 115 patients belonging to two ethnic groups—Bantu (81) and Pygmy (34). The diagnosis of filariasis was based upon clinical (Calabar swelling, worm migration under the conjunctiva) or parasitological data. (b) Schistosomiasis: 52 patients with parasitologically confirmed *Schistosoma haematobium* infection. (c) Toxoplasmosis: 28 patients with positive serological diagnosis (high titre obtained in passive agglutination test).

Except for sub-group (3b), these patients had not been exposed to the risk of *Trypanosoma* infection. The mean age of the subjects of groups (2) and (3) was 37·7±16·5 years (range 6-80) and the male/female ratio was 1·57.

Serum samples were stored frozen at −20°C until used.

Dried blood samples

These were collected from persons in the following 4 groups.

(4) 87 patients from Bouenza focus with parasitologically confirmed trypanosomiasis.

(5) 154 other trypanosomiasis patients from the same origin as group 4.

(6) 255 individuals free of trypanosomiasis but exposed to *Onchocerca volvulus* infection.

(7) 141 patients exposed to *Loa loa* and *Mansonella perstans* infection.

Test systems

IFAT on serum. Details of this test have been described by WÉRY *et al.* (1970). The test was carried out according to the modifications of FRÉZIL *et al.* (1974). A Congolese *T. b. gambiense* stock (MHOM/CG/ORBZV/113) was maintained in rats for antigen production. Sera were tested at twofold dilutions ranging from 1/20 to 1/160.

IFAT on dried blood. Dried blood samples were collected on Whatman No. 1 filter paper as described by BAILEY *et al.* (1967). Discs of 5 mm diameter were punched out of each sample. The resulting dilution of blood in phosphate-buffered saline (pH 7·2) was approximately equal to a 1/10 serum dilution (WÉRY *et al.*, 1970).

CATT on whole blood. This qualitative screening test, and the other CATT procedures, were carried out according to the method of the manufacturer (Smith Kline-RIT, lot number 47/7). Agglutination patterns of 1+ or more were considered as positive.

CATT on serum (qualitative method). This screening system used 5 µl of serum.

CATT on serum (quantitative method). Sera with

Table 1—Tests used for each group of samples of various geographical origins

Group	Number of patients	Geographical origin	Parasitology[1]	Sample	Tests used
1	95	Bouenza & Plateaux	T	serum	IFAT & CATT
2	105	Pool	o	serum	IFAT & CATT
3	195	Lekoumou, Pool & Bouenza	o	serum	IFAT & CATT
4	87	Bouenza	T	blood	IFAT
5	154	Bouenza	T	blood	CATT
6	255	Pool	o	blood	IFAT
7	141	Lekoumou	o	blood	CATT

[1]T = trypanosomiasis patients; o = patients free of trypanosomiasis.

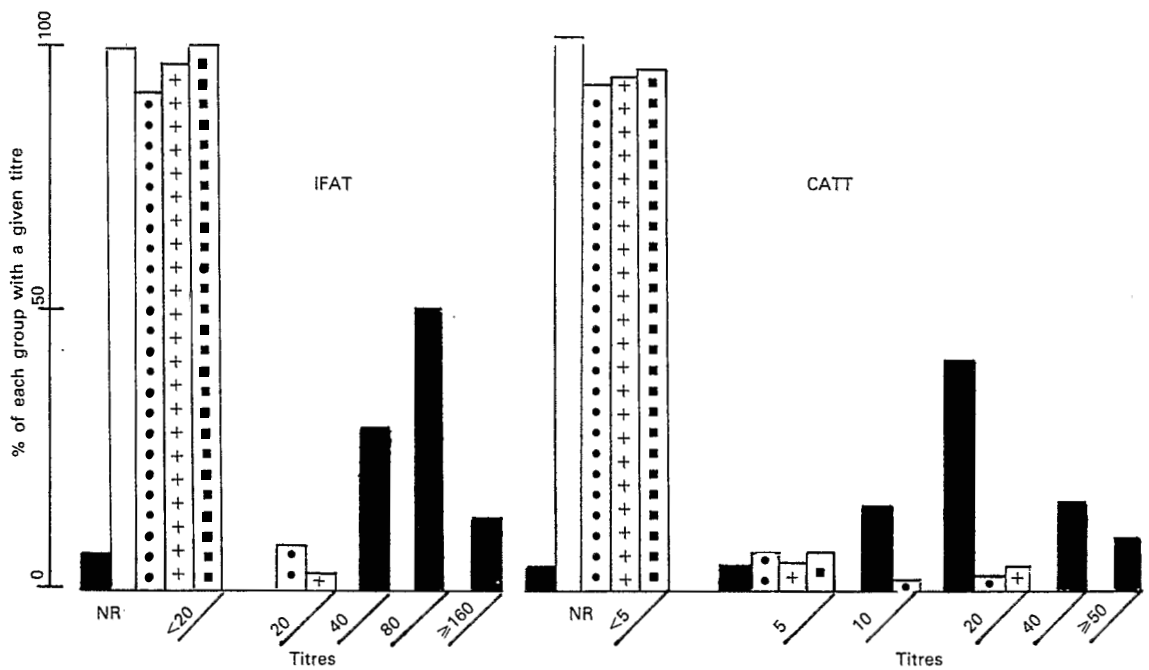

Figure. Distribution of end-titres in sera from: 95 trypanosomiasis cases ■, 105 healthy patients (□), 115 patients with filariasis (▣), 52 patients with schistosomiasis (⊞) and 28 patients with toxoplasmosis (◙).
NR = no reaction.

positive qualitative tests were titrated at twofold dilutions from 1/5 to 1/80.

Sample origins and ranges of tests applied to each group of samples are summarized in Table 1.

Results

IFAT results

The Figure shows the distribution of the IFAT titres obtained with sera from the different groups. Analyzing individuals free of trypanosomiasis (groups 2 and 3), no false positive reaction was observed at a serum dilution of 1/40, so this titre was selected as the specific threshold dilution. Using samples collected from *T. b. gambiense* patients, IFAT sensitivity was 94·7%, and its specificity was 100%. Only 5 patients reacted negatively. Among them, 2 did not present any cerebrospinal fluid alteration. Whatever the type of sample used (serum or dried blood), there was no significant difference in the IFAT sensitivities obtained using serum and dried blood (94·7% and 92% respectively). The specificity of IFAT performed on dried blood samples was 100% (group 6).

CATT results

Titres given by CATT on sera collected from patients free of trypanosomiasis are shown in the Figure; they formed a unimodal distribution around 1/20. The 1/10 dilution was considered as the specific

Table 2—Specificity of the CATT on serum: numbers of samples giving negative results

		Qualitative screening test	Serum dilution 1/5	1/10
Parasitic infection				
Filariasis	No.	81/115	104/115	113/115
	(%)	(70·4)	(90·4)	(98·3)
Schistosomiasis	No.	46/52	48/52	51/52
	(%)	(88·5)	(92·3)	(98·1)
Toxoplasmosis	No.	18/28	26/28	28/28
	(%)	(64·3)	(92·8)	(100)
Healthy patients	No.	105/105	105/105	105/105
	(%)	(100)	(100)	(100)

Table 3—Sensitivity of the CATT on serum: numbers of samples from trypanosomiasis patients giving positive results

	Qualitative screening test	Serum dilution 1/5	1/10
No.	92/95	92/95	87/95
(%)	(96·8)	(96·8)	(91·6)

threshold dilution for the CATT (Table 2). At lower dilutions, false positive reactions were frequently observed in patients suffering from other parasitic diseases (group 3), but never in healthy patients (group 2).

The qualitative CATT gave many non-specific reactions when samples of group 3 were analysed (Table 2). In filariasis patients, the presence of microfilariae seemed to have no influence on the test. 33·5% of patients with microfilariae were positive by the qualitative test, compared with 27·7% of those without microfilaraemia. The CATT appeared to be more specific in the Bantu group than in the Pygmies (75·3% compared with 58·8% $P<0·1$).

As shown in Table 3, the CATT had a sensitivity of 91·6% when the specific threshold dilution (1/10) was used. Although we observed greater sensitivity with the 1/5 dilution, we considered that 1/10 dilution was preferable because it was more specific. Among the 3 samples non-reactive to CATT, 2 were also negative when tested by IFAT. When the qualitative test was performed on sera, the sensitivity was comparable to that observed when 1/5 dilution was used. However, its lack of specificity precludes adoption of this method. The sensitivity of CATT performed on whole blood was lower (127/154; 82·5%). With this type of sample (group 7), the test specificity was high (94·3%), and was similar to that obtained with 1/5 serum dilution.

Discussion

The diagnosis of Gambian trypanosomiasis under field conditions presents some difficulties. The lack of sensitivity of the classical parasitological methods, which are moreover time-consuming, precludes their large-scale application. An initial serological screening test is therefore required. The easiest type of sample to use for mass surveys in the field is blood collected by finger prick. For testing whole blood, IFAT, because of its great specificity and sensitivity, is preferable to CATT. But, due to its simplicity and rapidity of execution, CATT must be preferred to IFAT for detecting new foci. The high specificity of the CATT on whole blood confirms the results of BAFORT *et al.* (1986), but better specificity was obtained by testing serum at the 1/10 dilution.

In our experience the fine agglutination observed with some sera tested at 1/5 or lower dilutions is a non-specific reaction, possibly caused by other parasitic infections. This particular phenomenon may be masked by red cells when the test is performed on whole blood.

Our results confirm the statements of FREZIL *et al.* (1977) and DUVALLET *et al.* (1979) that a positive IFAT is fully reliable evidence for the diagnosis of Gambian sleeping sickness. In the Congo, the very high specificity of this test might be the consequence of the absence of trypanosomes of the subgenus *Trypanozoon* other than *T. b. gambiense* (see NOIREAU *et al.*, 1986). In conclusion, even if the value of serological tests for individual diagnosis of trypanosomiasis remains controversial, we are convinced that their value for mass surveys is beyond doubt.

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Book Review

Vector control in primary health care. WHO Technical Report Series, No. 755. Geneva: World Health Organization, 1987. 61 pp.

A WHO Scientific Group met late in 1986 to review the technical aspects of vector control appropriate to primary health care, the available resources, the training needs of personnel involved in vector control, and the role of the professional core group and the district health management team as a technical resource for the control of vector-borne disease. The magnitude of disease vector problems was outlined and the potential for control through primary health care examined. On a regional basis the present status of programmes was described and the target species for which control is feasible were identified. Subsequent sections dealt with delivery of vector control through primary health care, the requirement for communication in planning and management of control activities and epidemiological support, the suitability of various vector control measures, human resource needs and development, and research requirements. The twelve recommendations of the group cover different aspects of these topics.

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