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 microscopic 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).

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 trypansomiasis.
(5) 154 other trypanosomiasis patients from the same origin as group 4.
(6) 245 individuals free of trypansomiasis 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. (1979). The test was carried out according to the modifications of Frezil 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., 1979).

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 (quantitative method). This screening system used 5 µl of serum.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of patients</th>
<th>Geographical origin</th>
<th>Parasitology(^1)</th>
<th>Sample</th>
<th>Tests used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95</td>
<td>Bouenza &amp; Plateaux</td>
<td>T</td>
<td>serum</td>
<td>IFAT &amp; CATT</td>
</tr>
<tr>
<td>2</td>
<td>105</td>
<td>Pool</td>
<td>o</td>
<td>serum</td>
<td>IFAT &amp; CATT</td>
</tr>
<tr>
<td>3</td>
<td>195</td>
<td>Lekoumou, Pool &amp; Bouenza</td>
<td>o</td>
<td>serum</td>
<td>IFAT &amp; CATT</td>
</tr>
<tr>
<td>4</td>
<td>87</td>
<td>Bouenza</td>
<td>T</td>
<td>blood</td>
<td>IFAT</td>
</tr>
<tr>
<td>5</td>
<td>154</td>
<td>Bouenza</td>
<td>T</td>
<td>blood</td>
<td>CATT</td>
</tr>
<tr>
<td>6</td>
<td>255</td>
<td>Pool</td>
<td>o</td>
<td>blood</td>
<td>IFAT</td>
</tr>
<tr>
<td>7</td>
<td>141</td>
<td>Lekoumou</td>
<td>o</td>
<td>blood</td>
<td>CATT</td>
</tr>
</tbody>
</table>

\(^1\) T = trypanosomiasis patients; o = patients free of trypanosomiasis.

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 threshold dilution, as observed in diseases (group 2).

As shown 91.6% were used. Although the 1/5 dilution preferential samples no when tested performed that observed its lack in type of serum dilu

Discussion

The data shows that the method is suitable for large-scale...
Table 2—Specificity of the CATT on serum: numbers of samples giving negative results

<table>
<thead>
<tr>
<th>Parasitic infection</th>
<th>Qualitative screening test</th>
<th>Serum 1/5</th>
<th>dilution 1/10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filariasis</td>
<td>No. (%)</td>
<td>81/115 (70.4)</td>
<td>104/115 (90.4)</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>No. (%)</td>
<td>46/52 (88.5)</td>
<td>48/52 (92.3)</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>No. (%)</td>
<td>18/28 (64.3)</td>
<td>26/28 (92.8)</td>
</tr>
<tr>
<td>Healthy patients</td>
<td>No. (%)</td>
<td>105/105 (100)</td>
<td>105/105 (100)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Qualitative screening test</th>
<th>Serum dilution 1/5</th>
<th>dilution 1/10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%)</td>
<td>92/95 (96.3)</td>
<td>87/95 (91.6)</td>
</tr>
</tbody>
</table>

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 DUVALAR 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.

Acknowledgements

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Book Review


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.

C. J. Leake