Direct isolation *in vitro* of *Trypanosoma brucei* from man and other animals, and its potential value for the diagnosis of gambian trypanosomiasis

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Abstract

A recently described simple kit for isolating African trypansomes *in vitro* (KIVI) was tested further with blood samples from man and other animals in Côte d'Ivoire and République du Congo. A high rate of success was achieved, with positive cultures being found 5–36 d after inoculation. The method was also of value in diagnosis. Parasitaemia was initially detected by the haematocrit method; in addition, the mini-anion exchange column was used for human blood and lymph fluid from patients with swollen glands was examined. The card agglutination test (CATT) was applied to the human blood samples. In Côte d'Ivoire, all 5 parasitaemic patients, who were also positive by CATT, yielded positive KIVI cultures. Of 15 animals, 2 parasitaemic and 10 apparently aparasitaemic individuals gave positive cultures. In the Congo, none of the 22 animals was parasitaemic and none gave a positive culture. Of 647 human subjects initially screened, 61, mostly with a positive CATT, were examined by KIVI; 20 gave positive cultures. Seven of these cultures originated from patients in whom no trypanosome had been seen in blood or lymph fluid, although blood from 2 parasitaemic patients failed to yield positive KIVI cultures. Some patients with CATT-negative whole blood and/or serum were positive by KIVI.

Introduction

Since the work of BRUCE (1895), *Trypanosoma brucei* has been isolated for laboratory studies mainly by inoculating infected blood to laboratory animals. *T. brucei gambiense,* the causative agent of the gambian form of human sleeping sickness, is of low virulence to rodents, which has led to poor sampling in the field and insufficient amounts of material for modern genetic studies. Although laboratory tsetse flies can be used for efficient isolation (DUKES *et al.*, 1989), the method is not generally convenient.

It is necessary to isolate numerous samples of trypanosomes in order to improve our knowledge of both the parasite itself and the epidemiology of gambian sleeping sickness. Isolation *in vitro* appears to be an obvious solution to the problem, but success has been irregular over many years (e.g., see LAVERAN & MESNIL, 1912; BRUTSAERT & HENRARD, 1938; WEINMAN, 1960). A kit for *in vitro* isolation (KIVI) has proved promising in trials in the Congo (AERTS *et al.*, 1992) and has provided sufficient material for genetic identification (TRUC, 1991). Of the 10 samples taken from patients with low parasitaemias, 7 yielded positive cultures while only 3 gave rise to infections in rats.

Following a further small trial in Côte d'Ivoire, a more extensive one has now been carried out in République du Congo. The trials were extended to domestic animals because of their possible rôle as reservoir hosts of *T. b. gambiense* (DENECKE, 1941; MELHITZ *et al.*, 1982). Besides confirming the efficiency of KIVI for isolating and amplifying *T. b. gambiense,* these trials also suggested that the method may be a useful supplementary diagnostic test.

Materials and Methods

Serological and parasitological diagnosis in man

Currently a card agglutination test (Testryp® CATT; MAGNUS *et al.*, 1978) is used for large scale diagnosis of gambian trypanosomiasis. In the standard procedure, subjects with positive CATT tests on whole blood also have their sera tested; those with both positive blood and serum are then subjected to detailed examination before treatment. Parasitological examination of blood is carried out by the microhaematocrit centrifugation technique (HCT; WOO, 1970) or by the mini-anion exchange column (MAEC; LUMSDEN *et al.*, 1977). In addition, the subclavicular and jugular lymph nodes are examined and, if palpable, are punctured; the extruded fluid is examined microscopically for trypanosomes.

This approach was applied to 5 people in the neighbourhood of Daloa in Côte d'Ivoire; blood samples from all were inoculated into the KIVI. In République du Congo, 647 people were similarly examined in the most active focus at Bouenza (J. Jannin, personal communication); 61 were bled and blood was inoculated into the KIVI.

Parasitological examination of animals

In Côte d'Ivoire, in the areas of Daloa and Kouassi-Périta, 12 pigs were bled from the vena cava, and 3 goats from the jugular vein. 10 ml of blood were taken into 0·5 ml of 5% Liquoïde® (sodium polyanetholsulphonate). The samples were examined for trypanosomes by the HCT, and then inoculated into the KIVI. In the Bouenza area of République du Congo, 7 sheep, 5 goats and 10 pigs were similarly bled and examined.

Culture

The inoculated KIVI vials were transported to the Tsetse Research Laboratory in Bristol, UK. Subpassages of positive cultures were made from the seventh day onwards in a semi-defined medium (CUNNINGHAM, 1977). The original vials were examined for up to 60 d after inoculation.

Results

Patients from Côte d'Ivoire

The KIVI cultures from all 5 patients became positive (Table 1), which permitted cryopreservation of the stocks. All the patients were positive by CATT on both

Table 1. Isolation of *Trypanosoma brucei* from patients by KIVI

	Field examinations				KIVI[a]	
No. of	CATT[b]		Trypanosomes		No.	Day first
patients	Blood	Serum	Blood	Lymph nodes	positive	positive
Côte d'Ivoire						
1	+	+	+	+	1	14
4	+	+	+	−	4	9–19
Congo						
3	+	+	+	+	2	13–17
7	+	+	+	−	6	7–36
4	+	+	−	+	4	7–36
18	+	+	−	−	3	14–17
5	+	−	−	−	1	13
1	+	−	+	−	1	8
19[c]	−	+	−	−	2	15,19
3[d]	−	−	−	−	0	−
1	−	−	−	−	1	7

[a]Kit for *in vitro* isolation.
[b]Card agglutination test for trypanosomiasis (MAGNUS *et al.*, 1978).
[c]One had swollen lymph nodes.
[d]All had swollen lymph nodes.

blood and serum, and trypanosomes were found in the blood; one had trypanosomes in the lymph gland fluid. The average time for the KIVI to become positive was 14·4 d.

Patients from République du Congo

The KIVI successfully isolated trypanosomes from 87% of the patients (13/15) in whom trypanosomes had been detected in the blood or gland exudate (Table 1). Moreover, 7 patients, in whom trypanosomes had not been detected microscopically, also gave positive cultures. Almost all stocks were cryopreserved; the exception was one from a patient whose culture was positive on the seventh day after inoculation but which became negative by the tenth day.

The mean time for the cultures to become positive was 16·4 d.

Table 2. Isolation of *Trypanosoma brucei* from domestic animals in Côte d'Ivoire by KIVI

No. and species of animal	Trypanosomes in original blood	KIVI[a] No. positive	KIVI[a] Day first positive
1 goat	+	1	6
2 goats	–	1	6
1 pig	+	1	5
11 pigs	–	9	5–13

[a]Kit for *in vitro* isolation.

Animals in Côte d'Ivoire

The results are summarized in Table 2. Most stocks were cryopreserved; two KIVI cultures became contaminated with fungi and were not stabilated.

The mean time before KIVI cultures became positive was 6·1 d.

Animals from the Congo

Twenty-two animals (7 sheep, 5 goats, 10 pigs) were bled. None showed trypanosomes in the blood and all the KIVI cultures remained negative.

Discussion

The collection and subsequent amplification of trypanosomes from patients with positive blood in West and Central Africa no longer appears to present any problem, and the inefficiency and inconvenience of using rodents are eliminated (see DUKES *et al.*, 1991). The KIVI also removes the requirements of isolation via tsetse, deep-freezing blood samples in the field and maintenance of the flies in the laboratory (DUKES *et al.*, 1989).

An unexpected advantage was the value of the KIVI in diagnosis; patients in whom trypanosomes could not be microscopically detected were shown to be infected. However, the lower limit of detection of parasitaemia by KIVI remains to be determined. The delay in identifying positive cultures is, of course, a disadvantage, and another is that strict aseptic procedures must be maintained whilst obtaining blood and inoculating the medium.

KIVI has several advantages for diagnosis over other methods. An important one is that, in effect, 5·0 ml of blood are examined, in contrast to a maximum of 0·2 ml used in the concentration and microscopy methods. Furthermore, no electrical equipment or microscope is required in the field. The trypanosomes grow in KIVI at normal ambient temperatures and the inoculated vial can remain unopened for over a week, the organisms remaining alive for more than 4 weeks.

The obtaining of positive cultures from Congolese patients with negative CATT results merits further investigation. It is of course possible that errors in reading the CATT occurred.

One patient, who was completely negative by the CATT, was also negative by indirect immunofluorescence, enzyme-linked immunosorbent assay, and trypanolysis (E. Magnus & N. Van Meirvenne, unpublished results). However, the KIVI was positive 7 d after inoculation, but became negative by the tenth day. It is possible that this patient had been very recently infected, or perhaps the infection was a transient one with so-called *T. b. brucei* (see VAN HOOF, 1947).

The KIVI was also useful for isolating trypanosomes from animals in Côte d'Ivoire; the seeming failure in the Congo accorded with previous findings that few infections have been found there in domestic animals (NOIREAU *et al.*, 1986). Only 2 animals from Côte d'Ivoire had microscopically patent parasitaemias, yet 12 others yielded cultures. Thus, again, the value of the KIVI for diagnosis was demonstrated. The MHT is obviously inadequate for efficient screening, confirming earlier observations obtained by inoculating blood into *Mastomys natalensis* (MEHLITZ, 1986). Although KIVI will permit a reappraisal of the rôle of animal reservoirs of gambian sleeping sickness, the isolates acquired from animals may well contain mixtures of not only different subgenera and species of trypanosome, but of genetically separate stocks of *T. brucei* as well (GODFREY *et al.*, 1990).

Overall, KIVI has considerably improved the collection and laboratory amplification of *T. b. gambiense* from man and possibly from other animals; the technique may also be of value in diagnosis, but perhaps mostly for evaluating new quick serological methods. The time is reduced for obtaining adequate amounts of material in the laboratory. Collection of a large number of stocks will permit comprehensive studies on population genetics, the evaluation of the significance of sexual versus clonal reproduction, the medical significance of any particular genetic variant (TIBAYRENC *et al.*, 1990) and the possibility of asymptomatic human reservoirs (VAN HOOF, 1947; WOODRUFF *et al.*, 1982).

Investigations are under way regarding sensitivity and the possible improvement of the practical application of KIVI.

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