The epidemiological importance of the animal reservoir of Trypanosoma brucei gambiense in the Congo *

2-Characterization of the Trypanosoma brucei complex

F. Noireau, P. Paindavoine[1], J. L. Lemesre, A. Toudic, E. Pays[1], J. P. Gouteux, M. Steinert[1], J. L. Frezil[2]

ORSTOM, Centre de Brazzaville, Brazzaville, Congo; [1]ULB, Departement de Biologie Moleculaire, Rhodes Saint Genese, Belgique; [2]ORSTOM, Centre de Montpellier, Montpellier Cedex, France

Summary

Biological and biochemical characterization of 36 human and 5 animal congolese stocks of *Trypanosoma brucei* were performed. One human and all the animal stocks showed a quick adaptation to rodent host whereas the other 35 human stocks were characterized by a low virulence degree (Group 1 of *T. gambiense*). The virulent stocks showed hybridization patterns specific to the *gambiense* subspecies. Our results confirm the absence of the *T. b. brucei* subspecies in the Congo and the low prevalence of domestic animals infected with *T. b. gambiense* (0.5%). Two cycles of human trypanosomiasis may thus occur in Central Africa: a predominant man-to-man cycle with group 1 trypanosomes and a minor cycle involving an animal reservoir.

Introduction

Studies carried out in the Congo on endemic animal trypanosomiasis within the sleeping sickness foci have revealed a high prevalence of *Nannomonas* infections (16.9%) whereas 0.5% of the domestic animals examined were infected with *Trypanosoma brucei* (Noireau et al., 1986). The results confirm previous observations of Van Hoof (1947) and Kageruka et al. (1977). These observations from Central Africa are different from those in West Africa where the prevalence rate of animal. *T. brucei* infections is high. The characterization of stocks from Ivory Coast and Liberia shows the possible presence of an animal reservoir of *T. b. gambiense*, indicating that the epidemiological cycles of human trypanosomiasis are probably different in West from those in Central Africa (Mehlitz et al., 1982; Zillman et al., 1984).

*This work was supported by the EEC (Contract TSIM 346F) and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR)

Accepted 10 June 1988

Trop. Med. Parasit. 40 (1989) 9–11

In this article, new data on the *T. brucei* complex are analysed from the characterization of *T. brucei* stocks isolated from human and animal hosts in the Congo, and a model applicable to the epidemiology of sleeping sickness in Central Africa is proposed.

Materials and Methods

Trypanosoma brucei stocks

36 stocks were isolated from patients with sleeping sickness between 1984 and 1987. Trypanosomiasis patients were originated from various foci in the country.

Among the seven *T. brucei* stocks isolated from domestic animals (Noireau et al., 1986), five could be analysed for biological and biochemical properties: MOVS/CG/85/ORBZV 503, MOVS/CG/85/ORBZV 504, MSUS/CG/85/ORBZV 505, MOVS/CG/85/ORBZV 506 and MSUS/CG/85/ORBZV 507. The origins of the 41 *T. brucei* stocks are shown in Table 1.

Study of growth rate of T. brucei stocks

The rodent model (Wistar white rat) was used in preference to *Mastomys natalensis* which was found to be less sensitive in our comparative trials. Recent report showed that the rate of proliferation among african trypanosomes was directly related to their virulence in rodent host (Diffley et al., 1987). A stock was considered to be virulent if the following criteria were fulfilled before the third passage:

- incubation time under five days
- exponential increase in the parasitaemia curve, reaching antiLog 8.1 or more before the 8th day, according to the method of Herbert and Lumsden (1976)
- survival of the infected rat under 10 days

DNA hybridization

This biochemical characterization technique was applied to the five animal stocks and one human stock (MHOM/CG/85/ORBZV 113). This last one was clearly differentiated from the other 35 *T. b. gambiense* stocks by its virulence to the rodent.

Preparation of DNA: the lysates of the trypanosome clones were incubated for an hour at 37 °C with 100 µg/ml of RNAase. 100 µg/ml of proteinase K were then added and digestion allowed to proceed for four hours at the same temperature. The lysates were then extracted by an equal volume of phenol saturated with Tris-Hcl 50 mM (pH 8.00), then by an equal quantity of (1 : 1) mixture of phenol saturated with Tris-Hcl 50 mM (pH 8.00) and chloroform.

Table 1 Origin of *Trypanosoma brucei* stocks

Geographic origin	Human host	Animal host: Sheep	Pig
Bouenza	18	3	1
Couloir	8	0	0
Sangha	10	0	0
Pool	0	0	1
Total	36	3	2

Table 2 Biological characterization of *Trypanosoma brucei* stocks

Origin of stocks	Number of isolates	Virulence in the rodent: High	Low
Man	36	1 (2.8%)	35 (97.2%)
Animal	5	5 (100%)	0 (0%)

After addition of sodium acetate (final concentration: 250 mM) to the aqueous phase, the DNA was precipitated by two volumes of ethanol at –20 °C and finally dissolved in Tris-Hcl 10 mM, EDTA 0.1 mM (pH 8.00).

Analysis of DNA: this was carried out by the previously described method (Paindavoine et al., 1986).

Results

Virulence of T. brucei stocks

The results are shown in Table 2. It seems that except for the stock MHOM/CG/85/ORBZV 113 all the human isolates showed a low degree of virulence in the rodent which is an usual characteristic of *T. b. gambiense* stocks in Central Africa. By contrast the five animal stocks were characterized by a very high degree of virulence.

DNA hybridization

The six stocks analysed by three different DNA probes corresponding to the AnTat 1.1, AnTat 1.8 and AnTat 1.13 surface antigens of *T. brucei* presented hybridization patterns specific to the *gambiense* sub-species. They may belong to a group of *T. b. gambiense* found in Central Africa, principally in Cameroon and Congo.

Discussion

Our results confirm the absence of the *T. brucei brucei* subspecies in the Congo. The excellent specificity demonstrated by the indirect immunofluorescent antibody test applied to massscreening in this country may be the consequence of this (Frezil et al., 1977; Noireau et al., 1987). All *Trypanozoon* isolated from sheep and pig are therefore likely to be infective to man although this cannot be formally proven. These epidemiological data are different from those obtained in West Africa where stocks which are pathogenic and non-pathogenic to man are found in domestic animals (Mehlitz et al., 1982). The differences in the prevalence of animals infected with *T. brucei* in West and Central Africa suggest that a *T. b. gambiense* animal reservoir is possible, even probable in West Africa, whereas man seems to be the only effective reservoir in the Congo.

We propose that there are probably two groups of trypanosomes infective to man in the Congo. The first group corresponds most certainly to the group 1 of *T. b. gambiense*, as defined recently by Gibson (1986). The low virulence of the trypanosomes in group 1, which is one of their determining characteristics according to this author, enables most of the Congolese stocks isolated from a human host to be classified in that group. The stock MHOM/CG/85/ORBZV 113 is distinctly differentiated from this group by its high virulence to the rodent. However, the DNA hybridization experiment shows that this stock exhibit VSG patterns specific of *T. b. gambiense* which excludes this stock from the group 2 described by Gibson (1986). In addition, using mechanical transmission procedure, MHOM/CG/85/ORBZV 113 was able to infect pigs while three congolese *T. gambiense* stocks of group 1 did not one (Noireau, unpublished data). The similar characteristics of the five animal stocks of *T. brucei* and MHOM/CG/85/ORBZV 113 suggest that they are closely related. These stocks may be found both in man and domestic animals, unlike those in group 1 which do not seem to have an animal reservoir. Two cycles may thus occur in Central Africa, each characterized by a different group of *T. b. gambiense*: a man-to-man cycle (type 1) predominant in the Congo (*T. b. gambiense* of group 1), and a cycle involving an animal reservoir (type 2), epidemiologically less important and limited to a few foci. The reasons for the low prevalence of *T. b. gambiense* stocks characteristic of the second type of cycle are not known. It is probable that their hosts, whether animal or man, constitute an effective reservoir. Indeed, we have noticed that the animals infected with *T. brucei* seem to tolerate their infection. Similarly, the patient from whom the virulent *T. b. gambiense* stock was isolated, presented at diagnosis a clinical picture of chronic disease without the acute manifestations such as those observed in East Africa.

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Dr. F. Noireau

Centre de Brazzaville, ORSTOM
Boite Postale no. 181
Brazzaville, Republique Populaire du Congo