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The sheep as a potential reservoir of human trypanosomiasis in the Republic of the Congo

CATHERINE M. SCOTT[1], J-L. FRÉZIL[2], A. TOUDIC[2] AND D. G. GODFREY[1]

[1]*Dept. of Medical Protozoology, London School of Hygiene and Tropical Medicine, Winches Farm Field Station, 395 Hatfield Road, St. Albans, Herts, AL4 0XQ, England;* [2]*Laboratoire d'Entomologie Médicale et Parasitologie, Centre ORSTOM de Brazzaville*

Summary

The identical electrophoretic isoenzyme patterns of a human-plasma-resistant *Trypanozoon* stock from a sheep and of two other stocks from trypanosomiasis patients in the Congo Republic indicated that the sheep stock was probably infective to man. These, and one further human stock from the Congo, closely resembled stocks isolated from man in Liberia and Ivory Coast.

Introduction

The incrimination of animal reservoirs of gambian trypanosomiasis of man should rely on methods that do not involve human volunteers because of insidious and perhaps incurable infection. On one past occasion, in all probability, trypanosomes from a dog in former Fernando Po were experimentally transferred to a man (DENECKE, 1941). A method currently in use to demonstrate potential human infectivity in a trypanosome population is testing resistance to normal human blood, plasma or serum (RICKMAN & ROBSON, 1970; HAWKING, 1976; MEHLITZ, 1978). Another current method is to compare the electrophoretic isoenzyme profiles of trypanosomes isolated from man with those from animals (GODFREY, 1979), a system which has the added advantage of distinguishing between different subspecies or strains. Furthermore, there is evidence that one variant of the enzyme alanine aminotransferase (GODFREY & KILGOUR, 1976), in conjunction with the presence of the West African forms of other enzymes, is characteristic of the aetiologic agents of typical chronic gambian sleeping sickness (GIBSON *et al.*, 1980; MEHLITZ *et al.*, 1982).

Both the human serum sensitivity test and enzyme polymorphism were used to demonstrate the pig and dog as reservoirs of human trypanosomiasis in Liberia and Ivory Coast (MEHLITZ, 1977; GIBSON *et al.*, 1978, 1980). The pig, ox and several game animals are reservoirs of another kind of human trypanosomiasis in Ivory Coast (MEHLITZ *et al.*, 1982). There is, however, circumstantial evidence, reviewed by MOLYNEUX (1980), that many other mammalian species may be reservoirs, and this paper presents evidence that a naturally infected sheep in the People's Republic of the Congo harboured trypanosomes that could cause gambian sleeping sickness in man.

Materials and Methods

The brief histories of the four stocks examined are summarized in Table I, and their geographical origins shown in Fig. 1.

All four stocks were initially slow growing but after many and frequent passages, a high parasitaemia (10^8 trypanosomes ml^{-1}) was achieved in a three to five-day infection in irradiated mice (600 rads 24 hrs before inoculation). A further five human stocks isolated in the Couloir focus in 1980 have not yet reached a sufficiently high parasitaemia.

Trypanosomes were separated from the mouse blood using a DEAE-cellulose column (LANHAM & GODFREY, 1970), and water-lysates prepared and stored in liquid nitrogen (SCOTT, 1981). The samples were subjected to thin-layer starch-gel electrophoresis as described by GODFREY & KILGOUR (1976) and GIBSON *et al.* (1978). 12 enzymes were examined: alanine aminotransferase (ALAT: EC 2.6.1.2.), aspartate aminotransferase (ASAT: EC 2.6.1.1.) malate dehydrogenase (MDH: EC 1.1.1.37), 'malic enzyme' (ME: EC 1.1.1.40), threonine dehydrogenase (TDH: EC 1.1.1.103), isocitrate dehydrogenase (ICD: EC 1.1.1.42), glucose phosphate isomerase (GPI: EC 5.3.1.9), glyceraldehyde 3-phosphate dehydrogenase (GAPDH: EC 1.2.1.12), phosphoglucomutase (PGM: EC 2.7.5.1), purine nucleoside hydrolase (NH: EC 3.2.2.1), two peptidases (PEP1 and PEP2: EC 3.4.11, using L-leucylglycylglycine and L-leucyl-L-alanine respectively as substrates). Infectivity tests for resistance to human plasma (RICKMAN & ROBSON, 1970; MEHLITZ, 1978) were also performed.

Results

The enzyme profiles, labelled according to GIBSON *et al.* (1980) and MEHLITZ *et al.* (1982), are listed in Table I; these two publications are also used for the comparisons with previous *Trypanozoon* results. The new PEP1 X and PEP 2 VII patterns are shown in Fig. 2. Each stock has the isoenzyme pattern for GAPDH which was identical to all other *Trypanozoon* stocks so far examined. The GPI, MDH, TDH and NH patterns were also the most frequent patterns of each enzyme found within the subgenus. In addition, both ICD II and PGM II were the patterns normally seen in West African *Trypanozoon* stocks, while ALAT I and ME II occurred frequently in West African man-infective stocks.

The sheep stock was identical in all 12 enzymes to the human stocks, BB and OK, while the third human stock PA differed in PEP1, PEP2 and ASAT. All four stocks were highly resistant to normal human plasma.

Discussion

The identity of the sheep stock with two human stocks indicates that the sheep is a reservoir host for sleeping sickness, while its high resistance to human plasma provides supporting evidence. Investigations of numerous domestic animal stocks in Liberia and

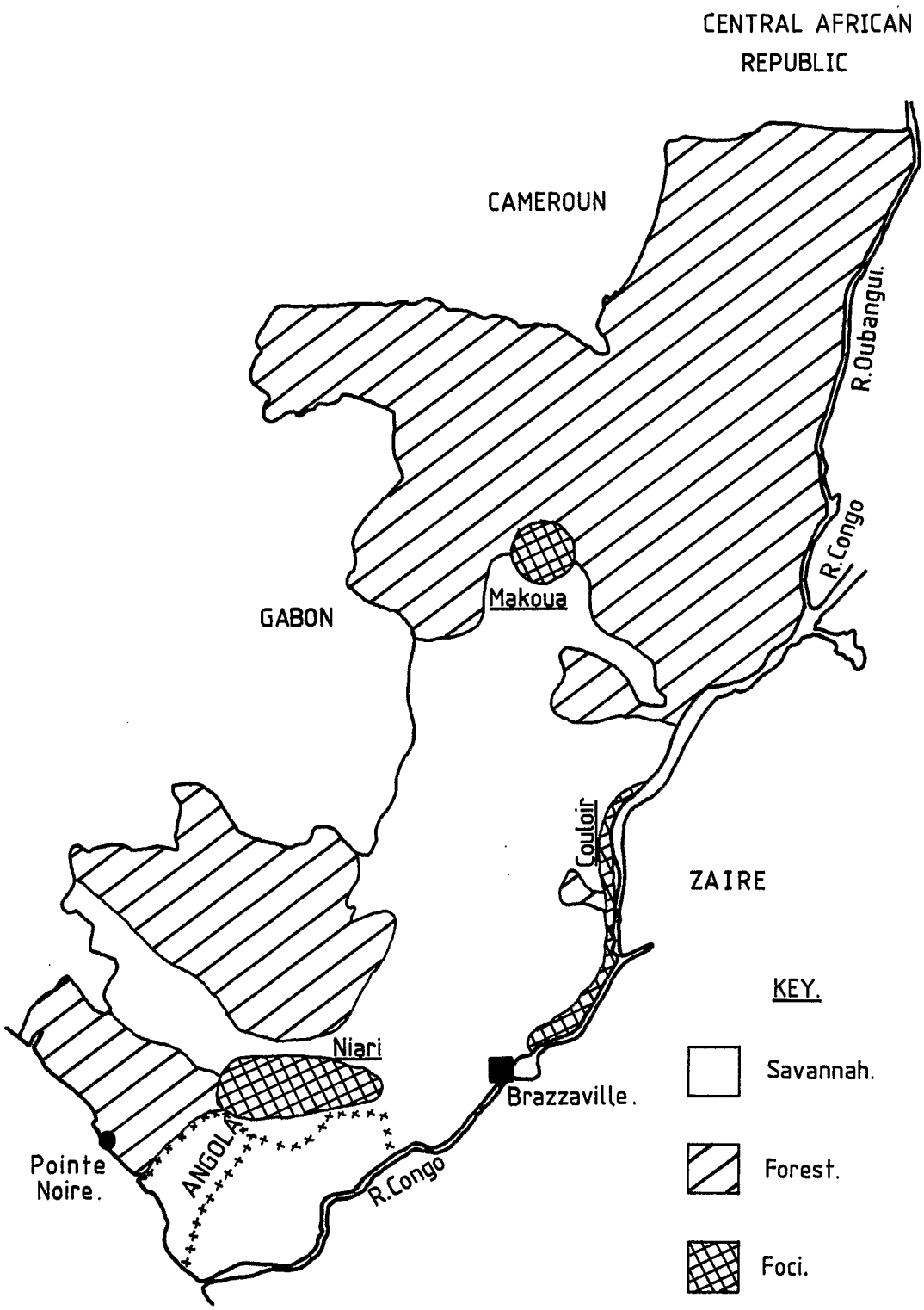

Fig. 1. A map showing the sleeping sickness foci in the People's Republic of Congo where the stocks were isolated.

Table I—Stock histories and enzyme profiles[1]

Stock	Host	Year isolated	Focus & type[2]	Passages before electro-phoresis	ALAT	ASAT	ICD	PEP2	PEP1	PGM	ME	GPI	MDH	TDH	NH	GAPDH
OK	Human ♂ 31 years	1974	Makoua (Forest)	50	I	III	II	VII	X	II	II	I	I	I	I	I
BB	Human ♀ 7 years	1973	Niari (Savannah)	62	I	III	II	VII	X	II	II	I	I	I	I	I
PA	Human ♂ 71 years	1975	Niari (Savannah)	39	I	[illegible]	II	VI	I	II	II	I	I	I	I	I
D12K	Sheep	?	Couloir (Riverine)	29	I	III	II	VII	X	II	II	I	I	I	I	I

[1]Other than newly found PEP1 X and PEP2 VII patterns, the patterns are described by GIBSON *et al.* (1980)
[2]FRÉZIL *et al.* (1979, 1980, 1981).

Fig. 2. Diagram of peptidase patterns seen. The individual isoenzyme bands previously found are coded with lower case letters, while the combinations of bands, i.e. the enzyme pattern, seen in a sample is coded with a roman numeral (GIBSON *et al.*, 1980; MEHLITZ *et al.*, 1982, but where PEP2 VI was incorrectly drawn).

Ivory Coast (GIBSON *et al.*, 1978, 1980; MEHLITZ, 1977, 1978; MEHLITZ *et al.*, 1982) have not revealed the sheep as a reservoir but have implicated other domestic animals, especially pigs. Most of the trypanosomes recovered from sheep during these previous surveys were of the subgenus *Nannomonas* or mixtures of *Nannomonas* and *Trypanozoon* with *Nannomonas* predominating after primary isolation and subsequent subpassaging (Mehlitz, personal communication). Isolates of *T. b. gambiense* from man infected sheep experimentally (KLEINE & FISHER, 1911; DUKE, 1928), and the potential role of the sheep as a reservoir has therefore long been realized; however, man-infective trypanosomes have not previously been recognized in a naturally infected sheep. FISKE (1920) observed sheep to be intolerant of tsetse,

and blood meal analysis has shown that tsetse rarely feed on sheep (WEITZ, 1963) although they are attracted (VALE, 1974). Sheep therefore may not be important as a reservoir host but nevertheless pose a threat, even though rarely fed upon, since the infection may be chronic and long-lasting (EDWARDS *et al.*, 1956; STEPHEN, 1970).

All four stocks examined conform to the wide category of *T. b. gambiense* as defined by GIBSON *et al.* (1980). The zymodeme to which the sheep stock and two identical human stocks belong has not previously been identified; it is, however, closely related to one classed as zymodeme D by MEHLITZ *et al.* (1982) from three patients in Ivory Coast, differing only in the absence of some bands in both peptidases. The third human stock PA was identical in all 12 enzymes to zymodeme A from Ivory Coast, which included trypanosomes from three patients and one stock from a pig (MEHLITZ *et al.*, 1982). The high resistance to normal human plasma confirms the observations of MEHLITZ *et al.* (1982) that this property is constantly associated with the zymodemes with ALAT I, which contain the trypanosomes causing classical gambian sleeping sickness in western Africa.

Even though similar organisms infect man both in Ivory Coast and in the Congo Republic, the epidemiological circumstances must be quite different. The rarity of sheep infection with *Trypanozoon* in the Congo, one in 324 (Frézil, unpublished observation), conforms, as has already been noted, to previous reports, but surprisingly no similar trypanosomes were found in the 295 pigs examined nor in the 108 goats (Frézil, unpublished observation). The absence in the Congolese pigs contrasts markedly with the high prevalence of such animals in other parts of western Africa, for instance in Nigeria (KILLICK-KENDRICK & GODFREY, 1963), in Liberia (MEHLITZ, 1979) and in Ivory Coast (MEHLITZ *et al.*, 1982). In addition, many *Trypanozoon* previously found in the pigs were probably not infective to man (GIBSON *et al.*, 1980; MEHLITZ *et al.*, 1982), corresponding to the broad concept of *T. brucei brucei*, yet no trypanosomes like this appear to occur in the Congo. A further major difference from the Ivory Coast endemic area is the absence in the Congo of another kind of *Trypanozoon* infective to man but enzymically distinct from *'T. b. gambiense'* (MEHLITZ *et al.*, 1982). It may be that Congolese *Trypanozoon* has a low infectivity to rodents and/or a consistently low subpatent parasitaemia in any host, thus making it difficult to detect and isolate. The difficulty may be exacerbated by the frequent presence of *T. congolense*, 26·3% in all domestic animals (Frézil, unpublished observation); this prevalence of *T. congolense* also demonstrates that tsetse are in fact feeding off the animals.

Whatever the reasons, the circumstances contrast markedly in the domestic animal populations closely examined in relation to human gambian trypanosomiasis in three areas: Liberia has a very low incidence of human disease but many pigs infected with various kinds of *Trypanozoon;* Ivory Coast has highly endemic areas with many kinds of *Trypanozoon* circulating in pigs; the Congo Republic has numerous foci of human disease but very few domestic animals are apparently infected with *Trypanozoon*. These Congolese foci are broadly divided into savannah, forest and riverine types, and have been subjected to detailed epidemiological investigation (FRÉZIL *et al.*, 1979, 1980, 1981) but, apart from noting that the same enzymic kind of trypanosome occurred in different types of foci, little can be said about 'strain' differences without classifying a wide selection of stocks.

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