Exploration of the distribution of *Trypanosoma brucei* ssp. in West Africa, by multilocus enzyme electrophoresis

*Trypanosoma brucei gambiense* and *T. b. rhodesiense* are generally held responsible for the chronic form of human African trypanosomiasis (HAT) seen in West and Central Africa, and the acute form observed in East Africa, respectively. Over the last 25 years, genetic characterization of these parasites has helped in elucidating the epidemiology of HAT. Genetic identification was initially based on the investigation of isoenzyme polymorphism by multilocus enzyme electrophoresis (MLEE). This technique is now considered limited in terms of the level of genetic variability it can be used to demonstrate. However, it remains more robust and cheaper than the newer, DNA-based methods (Gibson *et al.*, 1999), and very useful for basic epidemiological studies, such as the identification of the animal reservoirs of the parasites causing HAT. From a taxonomical point of view, MLEE allows a clear distinction between the homogeneous *T. b. gambiense* group 1 (Gibson, 1986) and the heterogeneous *T. brucei gambiense* group 2 or *T. brucei* 'bouaflé' group (Godfrey *et al.*, 1990; Baker, 1995). The latter group is also suspected to be pathogenic to humans (Truc *et al.*, 1997a). The main objectives of the present study were to use MLEE to identify stocks isolated mainly from humans and to analyse the distribution and persistence of zymodemes in space and time in West Africa, particularly in Côte d'Ivoire, using stocks isolated between 1992 and 1999.

Stocks were isolated, from HAT cases diagnosed during several medical surveys in Côte d'Ivoire (222 cases), Guinea (23) or Equatorial Guinea (six), by inoculation of rodents with infected blood (pre-1991) or, more recently, using the kit for in-vitro isolation (KIVI; Aerts *et al.*, 1992). The positive samples from the KIVI were sub-inoculated in semi-defined medium (Cunningham, 1977) supplemented with fetal calf serum and antibiotic (Truc *et al.*, 1992). The pellets of parasites were conserved in liquid nitrogen until use.

The 251 stocks isolated from humans were compared with 16 reference stocks isolated from humans and other animals between 1977 and 1994 (see Table) and identified as *T. brucei gambiense* group 1, the bouaflé strain-group of *T. brucei*, or *T. congolense* (TSW103; Gashumba *et al.*, 1988).

Thirteen enzyme systems, representing 16 loci, were subjected to electrophoresis on cellulose acetate (Helena® system; Helena Laboratories, Beaumont, TX): alanine aminotransferase (ALAT; EC 2.6.1.2); aspartate aminotransferase [ASAT (=GOT); EC 1.2.1.12]; glucose phosphate dehydrogenase (GPI; EC 5.3.1.9); isocitrate dehydrogenase (IDH; EC 1.1.1.42); malate dehydrogenase (MDH; EC 1.1.1.37); malic enzyme (ME; EC 1.1.1.40); nucleoside hydrolase (EC 3.2.2.1) with inosine substrate (NHI) or deoxyinosine substrate (NHD); peptidase with L-leucyl-L-alanine substrate (PEP2; EC 3.4.11 or EC 3.4.13); 6-phosphogluconate dehydrogenase (PGD; EC 1.1.1.44); phosphoglucononatase (PGM; EC 2.7.5.1); superoxide dismutase (SOD; EC 1.15.1.1); and threonine dehydrogenase (TDH, EC 1.1.1.103). SOD was investigated as described by Stevens *et al.* (1989) and all other enzymes as detailed by Truc *et al.* (1991) and Truc and Tibayrenc (1993). All chemicals were obtained from Sigma. Stocks were grouped by zymodemes and compared with the reference isolates.

A dendrogram of relationships was built, using the method of Jacquard (1973) to calculate the distance (d) between each zymodeme and any other (Serres and Roux, 1986).

Eighteen zymodemes were identified (see Table). The single *T. congolense* stock investigated, TSW103 of zymodeme 27, was clearly distinct (d = 0.9) from the other groups, as Truc *et al.* (1997b) also observed. The two
West African Trypanosoma brucei

6 (T. brucei 'bouaflé') SH017
7 (T. brucei 'bouaflé') SH196
10 (T. brucei 'bouaflé') SH276
11 (T. b. g. group 1) SINF1
12 (T. b. g. group 1) SINF5
12 (T. b. g. group 1) 70/2 GCK, MSY GCK, 15/3 GCK
14 (T. brucei 'bouaflé') TH2
15 (T. brucei 'bouaflé') TSW53 (pig)
27 (T. congolense) TSW103 (pig)
30 (T. brucei 'bouaflé') 132 (kob)
33 (T. brucei 'bouaflé') KK39 (kob)
37 (T. brucei 'bouaflé') AB14 (hartebeest)
38 (T. b. g. group 1) 1972
39 (T. b. g. group 1) D25/41
40 (T. b. g. group 1) Bub6 (hartebeest)
41 (T. b. g. group 1) PT 41, PT 237, PT 265, PT 400, PT 436

Côte d'Ivoire Aboisso 1989
Côte d'Ivoire Daloa 1990
Côte d'Ivoire Daloa 1992
Côte d'Ivoire Sinfra 1992
Guinea Dubreka 1997
Côte d'Ivoire Daloa 1978
Côte d'Ivoire Bouaflé 1982
Liberia Savanah 1977
Côte d'Ivoire Comoé 1993
Côte d'Ivoire Comoé 1980
Côte d'Ivoire Comoé 1980
Côte d'Ivoire Sinfra 1993
Côte d'Ivoire Sinfra 1994
Côte d'Ivoire Sinfra 1994
Côte d'Ivoire Sinfra 1994
Côte d'Ivoire Sinfra 1996
Côte d'Ivoire Sinfra 1997
Côte d'Ivoire Sinfra 1998
Côte d'Ivoire Sinfra 1994
Côte d'Ivoire Sinfra 1995
Côte d'Ivoire Sinfra 1994
Côte d'Ivoire Sinfra 1995
Côte d'Ivoire Sinfra 1995
Côte d'Ivoire Sinfra 1995
Côte d'Ivoire Sinfra 1995

* From humans unless indicated otherwise.
† Reference stock: DAL072, TSW53, AB14 (Stevens et al., 1992); TRAZIE, SIQUE, SH017, SH196, SH276, SINF1, SINF5, 1972, Bab 6 (Truc et al., 1997a); TH2 (Mehlitz et al., 1982); TSW103 (Gashumba et al., 1988); 132 (Truc et al., 1997b); KK39 (Young and Godfrey, 1983).

T. b. g. group 1, Trypanosoma brucei gambiense group 1.

classical groups within T. brucei (T. b. gambiense group 1 and T. brucei 'bouaflé') were also easily separable, also confirming the results of previous studies (Gibson, 1986; Godfrey et al., 1990). Zymodemes 1, 2, 3, 11, 12, 38, 39, 40 and 41 were clustered together as T. b. gambiense group 1 whereas zymodemes 6, 7, 10, 14, 15, 30, 33 and 37 formed the T. brucei 'bouaflé' group. The level of genetic variability within each of these two groups appeared low, with d-values of < 0.2 and < 0.3, respectively (see Fig.).

Within T. b. gambiense group 1, zymodemes 1, 2, 11 and 40 were each represented by a single isolate from Côte d'Ivoire (each a reference stock), whereas three of the 'new' isolates from Guinea were of zymodeme 12, and three of those from Côte d'Ivoire were of zymodeme 39. Zymodemes 38 and 41 were represented by 9 and 11 new stocks from Côte d'Ivoire, respectively. Zymodeme 3 was by far the most common zymodeme, represented by 226 isolates (200 of 237 from Côte d'Ivoire, 20 of 23 from Guinea, and all six isolates from Equatorial Guinea).

All the stocks identified as T. brucei 'bouaflé' were reference stocks isolated, in Côte d'Ivoire, from humans or other animals (see Table). As the two most recent isolates to be identified as 'bouaflé' were collected from a human in 1992 (SH276) and a kob in 1993 (132), all of the 241 stocks isolated since 1992 have been identified as members of T. b. gambiense group 1. In fact, only five zymodemes in T. b. gambiense group 1 (3, 12, 38, 39 and 41) have been isolated since 1992. Although an isolate of zymodeme 38 was collected in Côte d'Ivoire at least once per year between 1993 and 1998, zymodeme 39 (isolated in 1994-1995) has not been recognized.
TABLE
The origin, year of isolation and identification of the 267 stocks investigated

<table>
<thead>
<tr>
<th>Zymodeme</th>
<th>Stocks*</th>
<th>Country</th>
<th>Location</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (T. b. g. group 1)</td>
<td>DAL072†</td>
<td>Côte d'Ivoire</td>
<td>Vavoua</td>
<td>1978</td>
</tr>
<tr>
<td>2 (T. b. g. group 1)</td>
<td>TRAZIE†</td>
<td>Côte d'Ivoire</td>
<td>Sinfra</td>
<td>1991</td>
</tr>
<tr>
<td>3 (T. b. g. group 1)</td>
<td>380, 383, 384, B 120/9, 107/4, 2507, 2508, 2557, 2584, 2595, 2597, 2598, 2600, 2601, 2602, 2603, 2604, 694, B 28/4, T 20/2, B 53/5, B 59/2, B 3/7, CEF, T 9/3, 655, 659, 660, 661, 662, 664, 665, 666, 668, 669, 670, 5033/2, 5038/3, 5061/1, 3513/2</td>
<td>Côte d'Ivoire</td>
<td>Bonon</td>
<td>1999</td>
</tr>
<tr>
<td>603, 623, 635</td>
<td>Côte d'Ivoire</td>
<td>Bonné</td>
<td>1999</td>
<td></td>
</tr>
<tr>
<td>654, 656, 657</td>
<td>Côte d'Ivoire</td>
<td>Doua</td>
<td>1999</td>
<td></td>
</tr>
<tr>
<td>2548, 2549, 2569</td>
<td>Côte d'Ivoire</td>
<td>G. Zathy</td>
<td>1999</td>
<td></td>
</tr>
<tr>
<td>2085, 2084</td>
<td>Côte d'Ivoire</td>
<td>Sinfra</td>
<td>1991</td>
<td></td>
</tr>
<tr>
<td>2067, 1968, 1971</td>
<td>Côte d'Ivoire</td>
<td>Sinfra</td>
<td>1995</td>
<td></td>
</tr>
<tr>
<td>1977, 1978, T76/2, G2/12, F16/1, 07/1, D25/41, N43/6, C5/6, 2107, 2099, 2086, 2090, 2091, 2098, 2101, 2112, 2114, 2120, 2137, 2139, 2155, 2138, 2162, 2167, 2259, C69/4, 20.2, 12.3, 2280, 2240, 1/6, 2226, 23/1, 2265, 2254, 2224, 46/5, 2223, 2218, 2217, C74/7, 2241, 15/3, 28/1, 25/41, 139/10, 53/5/1, Kayib, C5/1, 9/9, C5/1, 7/8, 2145, 2150, 2161</td>
<td>Côte d'Ivoire</td>
<td>Sinfra</td>
<td>1996</td>
<td></td>
</tr>
<tr>
<td>234/8 NX, 234/1, SOUM, L'/F, KMOD, 2548, 2549, 602, 47/5, BO, 2497, 2498, 2499, 2560, 2562, 2570, 2582, 2587, 2588, 600, 602, 610, 611, 612, 613, 614, 616, 619, 622, 625, 634, 636, 638</td>
<td>Côte d'Ivoire</td>
<td>Sinfra</td>
<td>1997</td>
<td></td>
</tr>
<tr>
<td>673, 675, 684, 686, 690, 691, 692, 693, 693, 695, 696, 93/5, 5/7, 648</td>
<td>Côte d'Ivoire</td>
<td>Sinfra</td>
<td>1998</td>
<td></td>
</tr>
<tr>
<td>M 2/4, L 1/7</td>
<td>Côte d'Ivoire</td>
<td>Sinfra</td>
<td>1999</td>
<td></td>
</tr>
<tr>
<td>1965, 1966, 2102</td>
<td>Côte d'Ivoire</td>
<td>Sinfra</td>
<td>1999</td>
<td></td>
</tr>
<tr>
<td>1GE, 4GE, 5GE, 6GE, 7GE, 8GE</td>
<td>Côte d'Ivoire</td>
<td>Sinfra</td>
<td>1999</td>
<td></td>
</tr>
<tr>
<td>9/1 GCN, 4MGC, 9/2 GCN, SAL, 26/16 GCN</td>
<td>Côte d'Ivoire</td>
<td>Sinfra</td>
<td>1999</td>
<td></td>
</tr>
<tr>
<td>F31/4, F10/5, F35/2, B5/2, B4/1, F4/1, F34/1, B18/9, F2/2, F7/6, F55/3, B15/7, B34/2, F12/20, N°1 GC</td>
<td>Côte d'Ivoire</td>
<td>Sinfra</td>
<td>1999</td>
<td></td>
</tr>
</tbody>
</table>

* | Guinea | Doubrak | 1997 |
| Guinea | Doubrak | 1998 |
Fig. A 'UGPMA' dendrogram built from a matrix of Jaccard's distances (not shown), showing how the zymodemes (Z) cluster. Each zymodeme number is followed by the name of a stock representative of that zymodeme. The scale bar indicates a $d$-value of 0.1.
among the isolates collected after 1995. Stocks identified as zymodeme 41 have only been collected around Daloa (in 1992) and Sinfra (in 1995/1996). Zymodeme 12 was isolated in Côte d'Ivoire in 1992 (the isolate becoming reference stock SINFS) but no subsequent isolates from this country have been found to belong to this zymodeme. It appeared in Guinea in 1997 but was not among the 15 Guinean stocks collected in 1998, all of which belonged to zymodeme 3. Zymodeme 3 appears to remain predominant from year to year, particularly in Côte d'Ivoire (where it accounted for 81% of the stocks isolated in 1996 and 96% of those collected in 1998).

The most surprising result of the present study is the observation that zymodeme 3 (Z3) is widely distributed, in both West and Central Africa. In Côte d'Ivoire, Z3 appears to be evenly distributed throughout the country, and the main cause of the current epidemic of HAT, at least since 1991. This zymodeme is common not only in the historical foci of HAT (Aboisso, Bouaflé, Vavoua and Daloa) but also in the 'new' foci around Bonon, Sinfra and Soubré. The spread of Z3 (and therefore of HAT) within Côte d'Ivoire could be the result of the movement of untreated patients from established foci, as observed in Central Africa (Truc and Tibayrenc, 1993). The two other main zymodemes in Côte d'Ivoire (Z38 and Z41) appear to occur only around Daloa and/or Sinfra (even though Sinfra is an area of particularly intense transmission). It is unclear why Z3 is much more widespread than any other zymodeme in Côte d'Ivoire; it may develop better in the local vectors and/or be more infective to humans. The other, minor zymodeme identified in Côte d'Ivoire (Z2, Z11, Z12 and Z39) generally appear restricted to this country (Z12 has also been found in Guinea) and may be responsible for 'endemic' patterns of HAT. That Z3 appears as common in Guinea as Côte d'Ivoire is not surprising as these countries are neighbours, with considerable cross-border travel among their human populations. However, that Z3 is also present and perhaps equally common in Equatorial Guinea, in Central Africa > 1000 km to the east, is more difficult to explain. If, as some suspect (Truc and Tibayrenc, 1993), the historical origin of HAT lies in Central Africa, Z3 may have entered West Africa from Central Africa many years ago, probably in untreated but asymptomatic human cases.

Another surprising result is that, over the last decade, parasites of the *T. brucei* 'bouaflé' group appear to have disappeared in Côte d'Ivoire (at least as human infections). Although such parasites may be responsible for an acute form of HAT (Truc et al., 1997a), the results of a recent, MLEE-based study have indicated that parasites of zymodeme 3 cause a wide variety of clinical patterns, including asymptomatic, 'self-cure', classical, chronic HAT and a similarly acute form of the disease (unpubl. obs.). The variation may reflect differences between individuals in susceptibility to infection, although this needs to be investigated further.

The relevance and usefulness of the present results are to some extent limited because the level of discrimination possible using MLEE is relatively low compared with that achievable using DNA-based methods. For example, multiprimer, RAPD fingerprinting recently revealed that a patient found (by MLEE) to be infected with Z3 was carrying multiple clones of *T. b. gambiense* (P. Truc, V. Jamonneau, V. Lejon, E. Magnus, P. Vincendeau, M. Tibayrenc, and B. Oury, unpubl. obs.). However, MLEE-based characterization remains useful for epidemiological studies of HAT, because of the generally low level of genetic variability within the *T. brucei* subgroups. The use of more sophisticated techniques, such as those based on PCR and analysis of the repetitive sequences of microsatellite DNA analysis, has confirmed such low variability (Biteau et al., 2000). Whatever the technique used for the characterization of African trypanosomes, the parasites still have to be manipulated and cultured, introducing complexity and bias. Use of KIVI for the field isolation of trypanosomes, for example, may select for those clones or zymodemes which multiply best in culture (McNamara et al., 1995). Although the number of parasites needed for DNA-based methods of identification is much smaller than
the number needed for MLEE, a simple, direct method for the identification of the parasites, while they are in samples of blood, lymphatic juice or cerebrospinal fluid, would be very useful.

ACKNOWLEDGEMENTS. We thank the teams of the National Control Programmes of Côte d'Ivoire, Guinea and Equatorial Guinea, and all of the HAT team at the Institut Pierre Richet. This work was supported by a grant from the Fonds d'Aide à la Coopération of the Ministère français des Affaires Etrangères.

V. JAMONNEAU
Institut de Recherche pour le Développement (IRD), Département Sociétés et Santé, B.P. 5045, 34032 Montpellier, France, and Institut Pierre Richet, B.P. 1500, Bouaké 01, Côte d'Ivoire

REFERENCES


WEST AFRICAN Trypanosoma brucei


