Characterization of *Trypanosoma brucei gambiense* stocks isolated from humans by RAPD fingerprinting in Côte d’Ivoire: another evidence for multiple infections

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Accepted 3 December 2003

*Trypanosoma brucei gambiense* was isolated twice from each of 23 patients in Côte d’Ivoire. Genetic characterization using RAPD (Random Primed Amplified Polymorphic DNA) showed additional variability within a given isoenzyme profile (zymodeme), confirming that this fingerprinting method has a higher discriminative power (faster molecular clock) than isoenzymes. RAPD confirmed also the evidence of multiple infections by different genotypes in the same patient despite a low genetic variability among *Trypanosoma brucei gambiense* stocks. The involvement of this phenomenon in treatment failure is discussed.

**Key words:** Human African Trypanosomiasis, *Trypanosoma brucei gambiense*, RAPD, multiple infections.

**INTRODUCTION**

Human African Trypanosomiasis (HAT) is a serious health problem in the sub-Saharan part of Africa. It is estimated that about 600 000 people are infected in 2003 (W.H.O., personal communication). In West Africa, the causative agent of the chronic form of the disease is *Trypanosoma brucei gambiense*. However, diversified clinical evolutions were recorded in Côte d’Ivoire including a suspicion of an acute form (Jamonneau et al., 2000 a, Truc et al., 1997). The taxonomic status of the subspecies described within the complex *T. brucei* is still under debate, mainly because geographical distribution and pathogenicity of the subspecies are difficult to link with clear genetic categories (Jamonneau et al., 2002). Indeed, the only clear-cut genetic subdivision remains *T. brucei gambiense* group 1 (Gibson, 1986), while it is unclear whether *T. b. brucei* and *T. b. rhodesiense* correspond to distinct phylogenetic subdivisions (Mathieu-Daudé et al., 1994). Trypanosomes were genetically characterized using different methods, including Multilocus Enzyme Electrophoresis (MLEE) and several molecular methods (Gibson et al., 1999), such as...
Table 1. Patient code number, stocks isolated twice from each patient (A and B), corresponding location and focus and year of isolation in Côte d’Ivoire.

| Patient/DNA | Location       | Focus   | Year |
|-------------|----------------|---------|------|
| 611 A and B | Yaokro         | Sinfra  | 1997 |
| 622 A and B | Yaokro         | Sinfra  | 1997 |
| 2499 A and B| Sinfra         | Sinfra  | 1996 |
| 2562 A and B| Dioulabougou   | Sinfra  | 1997 |
| 5/7A and B  | Sinfra         | Sinfra  | 1999 |
| 93/5 A and B| Sinfra         | Sinfra  | 1997 |
| 634 A and B | Konéflu        | Sinfra  | 1997 |
| 614 A and B | Yaokro         | Sinfra  | 1997 |
| 659 A and B | Bonon          | Bonon   | 1998 |
| 51/11 A and B| Sinfra        | Sinfra  | 1997 |
| 664 A and B | Bonon          | Bonon   | 1998 |
| 666 A and B | Bonon          | Bonon   | 1998 |
| 4/5 A and B | Sinfra         | Sinfra  | 1999 |
| 687 A and B | Bouaflé        | Bonon   | 1998 |
| 806/9 A and B| Aboisso       | Aboisso | 1997 |
| B120/9 A and B| Aboisso      | Aboisso | 1997 |
| 654 A and B | Bouaflé        | Bonon   | 1998 |
| 662 A and B | Bonon          | Bonon   | 1998 |
| 668 A and B | Bonon          | Bonon   | 1998 |
| 669 A and B | Bonon          | Bonon   | 1998 |
| 694 A and B | Bouaflé        | Bonon   | 1998 |
| 2508 A and B| Bonon          | Bonon   | 1997 |
| T21/4 A and B| Grand Zathry  | Sinfra  | 1999 |

Random Primed Amplified Polymorphic DNA (RAPD) (Tibayrenc et al., 1993), ribosomal gene 18S sequencing (Stevens, 1999) or microsatellites (Biteau et al., 2000, Truc et al., 2002).

This study has been conducted in Côte d’Ivoire where parasites were isolated twice from each patient at different time, first during the medical survey in the field, and second when arriving at the hospital before treatment (Truc et al., 2002). Isolates were cultivated in vitro, then genetically characterised by RAPD. The aim of this study was first, to record the diversity of *T. brucei* genotypes circulating in this area, and then, to confirm the evidence of multiple infections as previously described by Truc et al. (2002) using another PCR based method.

**MATERIALS AND METHODS**

**Population surveyed**

Twenty three patients were diagnosed according to standard procedures (WHO, 1998) between 1996 and 1999 by the Ivorian National Control Program in three foci of Côte d’Ivoire: Sinfra and Bonon in central-western part and Aboisso in the eastern part of the country, on the border with Ghana (Table 1). Consenting patients were bled twice by venepuncture with a minimum interval of 3 days.

**Parasite collection**

Trypanosomes were isolated using the Kit for In Vitro Isolation of trypanosomes (KIVI, Aerts et al., 1992). Reference stocks were included: JUA and PEYA (*T. b. gambiense* group 1), TSW65 and KP465 (*T. brucei* “bouaflé” group), 058Cl.A3 (*T. b. rhodesiense*), EATRO 1125 (*T. b. brucei) and TRPZ105 (*T. congolense*, savannah group). These reference stocks were previously studied by Truc et al. (1993, 1997) and Mathieu-Daudé et al. (1994). Isolates and reference stocks were cultivated in semi-defined medium (Cunningham, 1977). Parasite pellets were collected, prepared and stored at -20°C according to Truc et al. (1991, 1993, 2002). For each patient, two pellets labelled A and B corresponded respectively to the cultures initiated from each of the two isolates (Truc et al., 2002).

**RAPD (Random Primed Amplified Polymorphic DNA)**

DNAs were extracted according to the method described by Oury et al. (1997). RAPD amplifications were performed according to the protocol described by Welsh and McClelland (1990) and Williams et al. (1990) and modified by Tibayrenc et al. (1993). Genomic DNA samples (20 ng) were amplified in 60 µl of specific buffer (10 mM
This work was supported by a Fonds d'Aide à la Coopération du Ministère François des Affaires...
Figure 1. UPGMA dendrogram built using Jaccard distances for each pair of stocks (A and B) isolated from the same patient (code number). For reference stocks JUA and PEYA (T. b. gambiense group 1), TSW65 and KP465 (T. brucei "bouaflé" group), 058Cl.A3 (T. b. rhodesiense), EATRO 1125 (T. b. brucei) and TRPZ105 (T. congolense, savannah group), see text.
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