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Short communication

# Multiple-strain infections of *Trypanosoma brucei* across Africa

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ABSTRACT

It is becoming increasingly clear that parasitic infections frequently contain multiple strains of the same parasite species. This may have important consequences for the parasite dynamics in the host and thus alter disease and transmission dynamics. In *Trypanosoma brucei*, the causal agent of human African trypanosomiasis (sleeping sickness), multiple-strain infections have previously been demonstrated to occur. Here, we analyzed field isolates of *T. b. gambiense*, *T. b. rhodesiense*, and *T. b. brucei*, isolated throughout Africa to assess the commonness of multiple-strain infections across the natural range of this parasite. Using eight highly variable microsatellite loci, we found multiple strains in 8.8% of our isolates. Due to the technical challenges of detecting multiple infections this number represents a minimum estimate and the true frequency of multiple-strain infections is likely to be higher. Multiple-strain infections occurred across the entire East–West range of the parasite. Together with previous results, these findings strongly suggest that multiple-strain infections are common for this parasite and that their consequences for epidemiology and parasite evolution should be investigated in detail.



## 1. Introduction

Parasitic infections commonly consist of heterogeneous mixes of genetically distinct parasites. Concomitant infections with multiple parasite species are thought to be the norm rather than the exception, at least in the developing world (Utzinger and de Savigny, 2006). However, it is becoming increasingly clear that infections also often contain multiple strains of the same parasite species, a phenomenon that may have important epidemiological and evolutionary implications. For the purpose of this paper, we define the term 'strain' as meaning all parasite individuals that are indistinguishable by genetic markers. This is in contrast to the term 'isolate', which we use to refer to a sample taken from an infected host or vector, cultured or not, that may contain parasites from several strains.

We investigated multiple-strain infections in *Trypanosoma brucei*, the causative agent of African sleeping sickness (human African trypanosomosis), a vector-borne disease that is fatal if untreated (Barrett et al., 2003) and ranks second among parasitic diseases in sub-Saharan Africa only to malaria in terms of mortality (WHO, 2002). Previous work has shown that multiple-strain infections occur in *T. brucei* in hosts and vectors (Scott, 1981; Letch, 1984; Stevens et al., 1994; MacLeod et al., 1999, 2000; Truc et al., 2002; Koffi et al., 2007). However, these studies were restricted to three countries (Ivory Coast, Uganda, Kenya) and some had very low sample sizes, making inferences about the frequency and distribution of multiple-strain infections problematic. Only one study (Godfrey et al., 1990) investigated multiple-strain infections across Africa. But this study employed isoenzymes, which are known to have lower resolution than modern markers, and reported surprisingly low levels of multiple-strain infections well below those reported in the other, geographically restricted, studies. It is therefore still unclear how common multiple-strain infections are in general in this parasite and if they are restricted to certain locations or host species. We therefore analyzed available cryo-preserved *T. brucei* isolates from throughout Africa to re-evaluate the frequency of multiple infections among infected human and non-human vertebrate hosts and the tsetse fly (*Glossina* sp.) vector. We employed modern microsatellite markers to increase resolution and sampled

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previously unsampled areas to cover almost the entire range of the parasite.

## 2. Materials and methods

### 2.1. Isolates

We investigated 137 cryo-preserved isolates and laboratory strains of *T. brucei* isolated throughout Africa between 1959 and 2003 (Table 1). These included 63 *T. b. gambiense*, which cause chronic sleeping sickness in West and Central Africa, 22 *T. b. rhodesiense*, which cause acute sleeping sickness in East Africa, and 41 non-human infective *T. b. brucei* from vertebrate hosts; and 10 *T. b. brucei* and 1 *T. b. rhodesiense* from tsetse flies. The isolates were initially isolated and (in parts) subsequentially cultivated by a range of methods. We were unable to establish the exact history for a large portion of them and so cannot take isolation and cultivation history into account in our analyses. However, no isolates were included in this study that have been cloned in the past because they cannot contain multiple strains anymore. The isolates were kindly provided by four different laboratories (Serap Aksoy, Yale University; Reto Brun, Swiss Tropical Institute; Wendy Gibson, University of Bristol; and Pascal Grébaut and Anne-Clarisse Lékané Likeufack, CIRAD-IRD/LRCT, Montpellier) either as extracted DNA or as cryo-preserved blood samples from the original host or from a rodent used to culture the original isolate.

### 2.2. Molecular analysis

Parasite DNA of isolates not received as extracted DNA already was extracted using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's protocol and resuspended in 50 μl of distilled water. Eight microsatellite markers (TB1/8, TB2/19, TB5/2, TB6/7, TB8/11, TB9/6, TB10/5, and TB11/13), each amplifying a single polymorphic locus on a different chromosome (Balmer et al., 2006), were amplified for every isolate using the following PCR profile: 1 cycle of 4 min 94 °C; 35 cycles of 45 s 94 °C, 30 s 53 °C and 45 s 72 °C; 1 cycle of 7 min 72 °C. Reactions contained 1× PCR buffer II with $MgCl_2$ (Applied Biosystems), 0.8 mM of each dNTP (Promega), 0.2 μM of each primer, 0.25 U AmpliTaq polymerase (Applied Biosystems). Allele sizes were determined using an ABI3100 Genetic Analyzer (Applied Biosystems) and GeneMapper 3.5 software (Applied Biosystems, 2003).

The microsatellite loci amplified are diploid. Therefore, the presence of more than two alleles at any locus in an isolate was used as indication for a multiple-strain infection. The proportion of detected multiple-strain infections was used as minimum estimate of the true multiple-strain infections rate. In isolates where more than two alleles were found at any locus, PCR and allele size determination were repeated for that locus at least once to confirm the presence of multiple strains. For *T. b. gambiense* and *T. b. rhodesiense*, humans and other vertebrate hosts were treated together as 'vertebrate hosts'.

## 3. Results

Multiple strains were detected in 12 (8.8%) of the 137 analyzed isolates. The majority (126) of the isolates were from vertebrate hosts (including humans). Of those, 12 (9.5%) contained multiple-strain infections: 5 of 63 (7.9%) *T. b. gambiense*, 6 of 41 (14.6%) *T. b. brucei*, and 1 of 22 (4.5%) *T. b. rhodesiense* isolates (Table 2).

The frequency of multiple-strain infections in *T. b. brucei* and *T. b. rhodesiense* did not differ significantly (Fisher's exact test, $p = 0.41$), so our results provide no evidence that these two taxa, which differ only by the presence of the SRA gene conferring human infectivity to *T. b. rhodesiense* (Xong et al., 1998; Gibson, 2005), differ in terms of multiplicity of infection. Multiple-strain infections were found in isolates from vertebrate hosts from the entire East–West

**Table 1**
Names and origin of *Trypanosoma brucei* isolates from humans, other vertebrates or tsetse flies screened for multiple-strain infections

| Taxon | Origin[a] | Isolates[b] |
|---|---|---|
| *T. b. brucei* | Burkina Faso | GAOUA89 |
| | Kenya | KETRI1738, KETRI1814[c], KETRI1902[c], KETRI2090, KETRI2108, LF1, LUMP266(MRC241flyK4), LUMP1342(LUMP450), LVBG118N, M249, RB67, RUMP503 |
| | Somalia | STIB794A |
| | Tanzania | RUMP501, STIB056, STIB201, STIB202, STIB204, STIB205, STIB206[c], STIB207, STIB209, STIB210, STIB211[c], STIB213, STIB214[c], STIB215[c], STIB216, STIB217, STIB218, STIB219, STIB221, STIB247, STIB286, STIB316, STIB337 |
| | Uganda | EATRO1296, Katerema41, STIB340, STIB390, STIB776, STIB783, STIB795 |
| | Zambia | H3, J10, TRPZ239, TRPZ260, TRPZ286(pop1), TRPZ320(pop1), TRPZ323 |
| *T. b. rhodesiense* | Botswana | **STIB338** |
| | Ethiopia | STIB707, **STIB809** |
| | Kenya | **EATRO0237**, **STIB365**, **STIB706** |
| | Mozambique | **KETRI2538** |
| | Tanzania | STIB236, STIB241-A, STIB243, STIB250, STIB262, STIB263, STIB324, **STIB389**[c], **STIB704** |
| | Uganda | **EATRO0240**, STIB391, **STIB799**, **STIB848**, **STIB849**, **STIB851**, **STIB854** |
| *T. b. gambiense* | Angola | **001K1Angola**, **003K1**[c] |
| | Cameroon | **BIP04**, **BIP08**, **BIP09**, **BIP40**, **BIP42**, **C3359**, **DOUME1**, **JUA**[c], P7F, P8F, P16F, **P26F**, **SEMI**, **SOMABc**, **TSEMESO** |
| | CAR | **BAT10**, **BAT31**, **BAT37**, **BAT39**, **BAT40**, **BAT42**, **BAT45**, **BAT51**, **BAT58**, **BAT60**, **BIBIANA**, **MBADI** |
| | Chad | **NATONDJI** |
| | Congo | **DEMBA**, **MALOUNDA** |
| | DRC | **ITMAP020578**, **ITMAP141267**, **ITMAP160986**, **ITMAP1780**, **ITMAP210879**, **ITMAP211290**, **ITMAS060401** |
| | Eq. Guinea | **13.97D**, **14.97D**, **15.97D** |
| | Ivory Coast | **DAL069**, **DAL1086**, **DAL1086R**, **DAL1402**, **STIB386**, **STIB733**, **STIB739**[c], **STIB754**, **STIB755** |
| | Liberia | **STIB756** |
| | Sudan | **K00014JD**, **K0303028**[c], **K0303030**, **K0303043**, **K0303045**, **K0303048** |
| | Uganda | **F43UG**, **R56UG**, **R60UG**, **STIB368**, **STIB887**[c] |

[a] CAR: Central African Republic; DRC: Democratic Republic of Congo.
[b] Isolate names are coded by host species: bold, human; plain, other vertebrate; underlined, tsetse fly. *Sources*: Serap Aksoy, Yale University; Reto Brun, Swiss Tropical Institute; Wendy Gibson, University of Bristol; Pascal Grébaut and Anne-Clarisse Lékané Likeufack, CIRAD-IRD/LRCT, Montpellier.
[c] Isolates containing multiple strains.