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1 ***Trypanosoma brucei gambiense* group 2: the unusual suspect**

2

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10 **Keywords**

11 Human African Trypanosomiasis, *Trypanosoma brucei gambiense* group 2, Elimination,
12 Epidemiology

13

14 **Abstract**

15 *Trypanosoma brucei* causes Human African Trypanosomiasis (HAT). Three
16 subspecies were described: *T. b. gambiense* (*Tbg*) and *T. b. rhodesiense* (*Tbr*) in humans, and
17 *T. b. brucei* (*Tbb*) in animals. Molecular markers subdivided *Tbg* into two groups: *Tbg1* and
18 *Tbg2*, of which the latter is different from *Tbg1* and *Tbr* (absence of the SRA gene), but
19 undistinguishable from *Tbb*. *Tbg2* is considered as a zoonotic form of HAT in West Africa.
20 *Tbg2* was mainly found in Côte d'Ivoire between 1978 and 1992 but the latest description was
21 made in Ghana in 2013. New molecular tools would be welcome to characterize such
22 infections and determine their origins (resistance to human serum or patient
23 immunodeficiency) in the current context of HAT elimination.

24

25

26 ***Trypanosoma brucei*, a multiple pathogen agent**

27 Human African trypanosomiasis (HAT) or sleeping sickness is a **neglected tropical**
28 **disease** (see Glossary) caused by infection with extracellular protozoan parasites transmitted
29 through the bites of infected **tsetse flies** (*Glossina* species) in Sub-Saharan Africa [1]. After
30 devastating epidemics during the 20th century, substantial **control efforts** conducted over the
31 last twenty years enabled a significant reduction of prevalence. With 977 cases reported in
32 2018, we have never been so close to the goals set in up in the World Health Organization
33 (WHO) roadmap in 2012: the **elimination of the disease as a public health problem** in 2020
34 and the interruption of transmission by 2030 [2, 3].

35 The etiological agent is a trypanosome of the species *Trypanosoma brucei*. Within the
36 subgenus *Trypanozoon*, *T. brucei* is **pleomorphic** when compared with *T. evansi* and *T.*
37 *equiperdum*. At the functional level, pleomorphism reflects the ability of *T. brucei* to develop
38 in the tsetse fly [4]. Since human and animal infective African trypanosomes are
39 morphologically identical, *T. brucei* was subdivided into three subspecies based on extrinsic
40 criteria (host range, **pathogenicity** in human, infectivity and **virulence** to laboratory rodents
41 and geographical distribution) [5]. *T. brucei gambiense* (*Tbg*) is human-infective, responsible
42 for a chronic form in West and Central Africa, and is weakly virulent in laboratory rodents. *T.*
43 *brucei rhodesiense* (*Tbr*) is human-infective, responsible of an acute form in East Africa, and
44 is highly virulent in laboratory rodents. *T. brucei brucei* (*Tbb*), a parasite of domestic and wild
45 animals causing nagana throughout the tsetse region of Africa, is non-pathogenic to humans
46 and highly virulent in laboratory rodents. The *gambiense* HAT (g-HAT) is responsible for
47 more than 98% of the current reported cases [3].

48 Further observations using biochemical, genetic and phenotypic characteristics have
49 raised doubts about the validity of these three subspecies. Since the 1980s, two distinct groups
50 of human infective trypanosomes were described within *Tbg* [6, 7]. The most prevalent one
51 was the relatively homogenous *T. b. gambiense* group 1 (*Tbg1*), which is invariably resistant
52 to human serum. The second group has been much less studied and was assigned several
53 names over time due to its genetic and phenotypic diversity: non-*T. b. gambiense* group 1, *T.*
54 *b. gambiense* group 2, bouaflé group, non-*gambiense* or *rhodesiense*-like group. In the present
55 review, we trace the story of these human-infective trypanosomes that do not belong to *Tbg1*
56 or *Tbr* and investigate their current epidemiological significance and relationships with the
57 other *T. brucei* trypanosomes. We argue that to search for an accurate definition of all human
58 infective trypanosomes is of crucial importance in the current elimination context.

59

60 **Characterization of *Trypanosoma brucei* ssp. sub-groups**

61 Before the advent of **molecular tools**, some descriptions of HAT cases and isolated
62 strains did not correspond to the definition of g-HAT based on extrinsic criteria. By the early
63 20th century, HAT was studied and controlled by medical officers, heading large mobile
64 teams. They used to follow up patients for long periods and observed some quasi-
65 asymptomatic forms not consistent with the classical symptoms described for g-HAT (e.g. [8,
66 9]). The most famous case was the patient FEO (Box 1) remaining in first stage of the disease
67 for 25 years despite several treatments [10, 11]. However, the difficult identification of
68 trypanosomes using extrinsic parameters such as morphometric criteria and virulence in
69 rodents [12] encouraged the use of more sophisticated molecular based techniques.

70 Molecular tools allowed the identification of a genetically homogeneous and
71 monophyletic group named *T. b. gambiense* group 1 (*Tbg1*) for the first time in 1986 with the
72 following characteristics: low virulence to experimental animals, human serum resistance
73 (HSR) and limited antigenic repertoire [7]. Further studies then described *Tbg1* as mainly (if
74 not totally) clonal [13, 14], with a common HSR mechanism (see Box 2) involving the *Tbg*-
75 specific glycoprotein (TgsGP) [15-17], a small genome with fewer small chromosomes [18]
76 and transmitted by the *Glossina palpalis* group [19]. Although *Tbg1* was detected in domestic
77 and wild animals, its zoonotic character remains to be clarified [20]. *Tbg1* comprises most of
78 the human-infective trypanosomes diagnosed in West and Central Africa and conforms to the
79 classical concept of *Tbg* that runs a chronic course in humans.

80 Multi-Locus Enzyme Electrophoresis (MLEE) was the first molecular technique that
81 highlighted the existence of stocks isolated from human falling outside the homogeneous
82 *Tbg1*. A group of minor zymodemes from Côte d'Ivoire showed a variable sensitivity to
83 normal human serum (NHS). Some of these zymodemes had their exact counterpart in stocks
84 from domestic and wild animals [21, 22]. This observation was confirmed during the
85 following years using several enzymatic systems [6, 23-37].

86 It was also confirmed over time using various DNA-based analyses, including
87 restriction fragment length polymorphisms (RFLP) [38-41], DNA hybridization [24, 41],
88 repetitive DNA probes [42], arbitrarily Primed-PCR [43], specific DNA probes [16, 39, 44,
89 45], random amplification of polymorphic DNA (RAPD) [32, 46], multiplex-endonuclease
90 analyses [47], microsatellite analyses (MS)[13, 37, 48-54], mobile genetic element (MGE)
91 [36], kinetoplast DNA sequencing [52] and whole genome sequencing (WGS) [14, 55-57].

92 In this review, we identified all human infective *T. brucei* from West and Central
93 Africa falling outside *Tbg1* when using molecular markers. The list (presumably exhaustive)
94 of the 29 stocks registered is given in Table 1. After their first description, most of these
95 stocks were used in several studies as reference. Several names were used: non-*gambiense* or
96 *T. b. non gambiense* [6, 24, 38, 39, 41, 42]; non *Tbg1* [28, 43, 45]; *T. b. rhodesiense*-like [41,
97 58] or bouaflé [25, 27, 30-32, 34, 35, 49]. Since the definition of *Tbg1* vs *T. b. gambiense*
98 group 2 (*Tbg2*) made by Gibson [7], *Tbg2* (group 2 or type 2) was progressively more widely
99 used in the literature to designate the human-infective trypanosomes genetically different
100 from *Tbg1* and *Tbr* [14, 17, 29, 33, 36, 37, 40, 44, 47-57, 59].

101 The term “bouaflé” was first used because of the geographical origin of stocks:
102 Bouaflé is a town located in the Western-Center part of Côte d’Ivoire (Figure 1). These stocks
103 were grouped thanks to MLEE specific patterns [25]. Eventually, “bouaflé” was used to
104 designate all the West African *T. brucei* stocks sharing the same MLEE patterns [27, 30, 60]
105 including stocks isolated from domestic and wild animals in Côte d’Ivoire and Burkina-Faso,
106 and also stocks isolated from human in the Western-Center part of Côte d’Ivoire. In further
107 MLEE based analyses, such human and animal stocks were still named “bouaflé” [31, 32, 34,
108 35]. In studies using other tools than MLEE, “bouaflé” has been replaced by the term *Tbg2*
109 for human stocks, but some pig stocks from Côte d’Ivoire previously named “bouaflé” were
110 wrongly named *Tbg2*: the TSW33 and TSW 65 stock wrongly reported as human ones in
111 [14]] and [36] respectively; the TSW53 and TSW65 stocks [37, 50]; the TSW187 stocks [44];
112 the 2171 and 2178 stocks [49] and all the TSW stocks in [53]. Stocks isolated from domestic
113 pigs in Cameroon, initially considered as *Tbg2*, because of a non-*Tbg1* MLEE profile [33],
114 were finally considered as classical *Tbb* [36, 52, 61].

115

116 **Characteristics of *Tbg2* and relationships with other *T. brucei* ssp.**

117 In the present review, we propose to use and restrict the label *Tbg2* to all human-
118 infective *T. brucei* trypanosomes from West and Central Africa that do not fit into *Tbg1* using
119 molecular methods (Table 2). Although most of the stocks appear in several studies, some of
120 them have been cited only once and/or have been poorly studied. For instance, STIB 368 was
121 only characterized by MS and kinetoplast DNA [52], TH1 78E (032) by MLEE [22], TH922
122 83E Kou by RFLP and DNA hybridization [38, 41] and SH136 by MLEE [30]. Stocks
123 SH196, SH276 and SH017 were cited in several studies only using MLEE. The two *Tbg2*
124 stocks observed in Ghana were each studied once [50, 56].

125 For some other stocks, discordant results were observed regarding different molecular
126 markers. KOBIR was labelled *Tbg2* using repetitive DNA probes [42] and WGS [14], but
127 identified *Tbg1* by RFLP and DNA-hybridization [38]. LIGO was characterized as *Tbg2* by
128 RFLP and DNA-hybridization [38], repetitive DNA probes [42], MLEE [37] and MS [37, 48,
129 50]. However, it fitted into *Tbg1* when using WGS [14]. IPR128-6 was characterized as *Tbg1*
130 by MLEE [27] but as *Tbg2* by the same method [29]. PT 312 was characterized as *Tbg1* by
131 RFLP and RAPD [39, 46] and MLEE [30] but identified as *Tbg2* by Multiplex-endonuclease
132 analysis [47]. Another surprising result concerns BIYAMINA. This stock displaying low
133 virulence in rodents was first identified as *Tbg1* by MLEE [23]. This stock was then cloned
134 and BIYAMINA Clone B was found to be *Tbg2* by MLEE [37, 45], hybridization with a
135 kinetoplast DNA probe [45], arbitrarily Primed-PCR [43] and MS [37, 50]. Discordant results
136 were also observed for LIGO and KOBIR with the TgsGP PCR (see below). Finally, the story
137 of the patient FEO and isolated stock(s) is so special that it is described in Box 1.

138 For old and poorly studied stocks, authors assumed a mislabeling or a laboratory error.
139 This is the case for STIB 368 considered as anomalous and old isolate, which may well have
140 been mixed up during prolonged maintenance in the lab [52]. For KWANG 9A and GBOKO,
141 identified as *Tbg2* by MLEE, and for whom a high infectivity in rats was observed, authors
142 presumed a laboratory error [58, 62]. GBOKO was electrophoretically more similar to stocks
143 isolated from Nigerian animals than from man, and KWANG 9A had greater affinity with
144 human stocks from East Africa than from West Africa [62].

145 For BIYAMINA Clone B and FEO-S, a mislabeling was also hypothesized. Such
146 hypothesis was also formulated regarding some discordances described above. This is indeed
147 likely due to the prolonged maintenance and repeated passage in various rodents for most of
148 the stocks. However, the hypothesis of mixed infections due to co-infections in the
149 corresponding patients cannot be excluded. Regarding the stock KOBIR, an explanation for
150 the observed discrepancy is that the original isolate was a mixture of a *gambiense* and '*non-*
151 *gambiense*' trypanosomes, and that by chance during the passaging of this strain, two different
152 lines, described by the same name, were obtained [42]. It is possible that a patient first
153 infected by *Tbg1* could develop an immunodeficiency allowing a subsequent *Tbb* infection.
154 Such pattern had already been suspected in long-term asymptomatic patients in Côte d'Ivoire
155 [63]. In case of *Tbg1* and *Tbb* co-infections, *Tbg1* is usually preponderant in human, but an
156 **isolation** in rodent would be favorable to *Tbb* by hiding or removing *Tbg1*. This could explain
157 the characterization of *Tbb* or *Tbg1* from the same stocks depending on the method used. This
158 could also explain that some stocks isolated from humans are sensitive to NHS (see below and

159 Table 2). Regarding such a hypothesis, *Tbg2* would be a *Tbb* that infected human due to some
160 immunodeficiency mechanism. Likewise, a constitutive immunodeficiency was suspected to
161 explain the *T. brucei* infections of the two Ghanaian patients [50, 56]. Constitutive
162 immunodeficiency can result in atypical infections due to trypanosomes other than *T. brucei*
163 [64].

164 Human serum resistance (HSR) has been used for a long time for trypanosome
165 characterisation with several methods (Box 2). Although *Tbg1* stocks are invariably resistant
166 using the blood incubation infectivity test (BIIT, [65]), HSR is variable in the 13 *Tbg2* stocks
167 tested by this method with 7 resistant or partially resistant stocks and 6 sensitive ones (Table
168 2). The serum resistance associated (SRA) gene PCR was described as a reliable molecular
169 diagnostic tool specific for *Tbr* [66, 67]. The eight *Tbg2* stocks tested with this PCR were
170 negative (Table 2), whatever the method used [50, 52, 57, 67-69].

171 TgsGP PCR was then proposed for the specific diagnostic of *Tbg* [16, 17]. Out of 10
172 *Tbg2* stocks tested (Table 2), 6 were invariably negative [14, 16, 17, 53, 57, 69]. For the four
173 others (TH2 78E, KOBIR, OUSOU and LIGO) discordances were observed depending on the
174 primers/method used. Using PCR targeting the 3' end of the gene encoding for TgsGP, LIGO
175 and TH2 78E were negative while KOBIR and OUSOU were positive [16]. On the opposite,
176 LIGO was positive while KOBIR and OUSOU were negative when using a different forward
177 primer [14]. When using primers targeting to the 5' half of the gene, the stock TH2 78E was
178 PCR positive [17]. The discrepancy in the PCR results obtained for this stock is based on the
179 target that differs between the two PCRs. One targeted the 3' end of the TgsGP gene, well
180 conserved in *Tbg1* while the other targeted genes similar to TgsGP [17]. The TgsGP-like
181 genes in some isolates of *Tbb*, *Tbr* or *Tbg2*, which can be amplified using primers targeting
182 the 5' end of the TgsGP gene, are closely similar to VSG Tb10.v4.0178, and it is likely that
183 this is the ancestral gene of TgsGP [70].

184 *Tbg2* is then characterized by the absence of the SRA gene and a complex mechanism
185 for HSR illustrated by the fact that some stocks isolated from human are sensitive to NHS
186 when tested by the BIIT method and that these mechanisms do not necessarily involve TgsGP
187 as illustrated by the results obtained regarding STIB 386 (also named TH114) stock [69].
188 Regarding the different mechanisms of trypanosome resistance to human serum, it can be
189 hypothesized that *Tbg2* might arise by a zoonotic episode involving trypanosomes infecting
190 animals (probably *Tbb*) under particular circumstances.

191 *Tbg1* causes a chronic infection [1]. Very little information is available about the
192 clinical evolution of *Tbg2*-infected patients. *Tbg2* was defined as a virulent form of *Tbg* in

193 foci of gambian sleeping sickness [60], based on the only well-documented study that
194 concerned the four SH stocks (see Table 1), for which three are suspected to cause an acute
195 form with a rapid turn into the neurological stage [30]. However, no neurological symptom
196 was described in the two Ghanaian patients (Patient03 and GHANA) and a spontaneous cure
197 was observed for Patient03 [50, 56].

198 There is no specific genetic marker for *Tbg2*. Most of the time, only *Tbg1* appears
199 homogeneous (i.e. monophyletic) and separated from the other stocks, while *Tbg2*, *Tbb* and
200 *Tbr* appear as extremely heterogeneous without any clear population structure. Nevertheless,
201 some published studies found more ordered structuration. One study using MS but only with
202 three *Tbg2* stocks (STIB 386, TH113 and TH2 78E) found a Bayesian cluster containing the
203 three *Tbg2* and six *Tbb* from various part of Africa (Cameroon, DRC and Burkina Faso) [52].
204 A principal component analysis confirmed this clustering and positioned the three *Tbg2*
205 stocks half way between all *Tbg1* and a group of *Tbr* and *Tbb* stocks [52]. Three *Tbg2* stocks
206 (STIB 386, KOBIR and OUSOU) also appeared halfway between *Tbg1* stocks and other
207 stocks of *Tbb* and *Tbr* with genome wide SNPs analysis [14]. Other genome wide SNPs
208 analyses were undertaken [56, 57] where *Tbg2* stocks (STIB 386, TH126 and ABBA) tended
209 to group together with some other West African *Tbb* stocks, but quite remotely as compared
210 to *Tbg1*. Interestingly, the new deviant GHANA branched half way between *Tbg1* and the
211 *Tbb* TREU927 [56].

212 This suggests that human infection by non-*gambiense* trypanosomes can occur either
213 from *Tbb* that are genetically the closest to *Tbg1* or from rare hybridization events between
214 *Tbg1* and *Tbb* strains, which seems supported by other data [40, 52]. The hybrid origin of
215 *Tbg1* itself, between two ancestral *Tbb* like strains, cannot be excluded, as it would explain
216 the strictly clonal propagation since then [14]. Hybridization is perhaps the most common
217 route to parthenogenesis [71]. Whatever the origins of these unusual strains, *Tbg2* bears the
218 same relationship to West African *Tbb* as *Tbr* does to East African *Tbb* [42]. The reason why
219 *Tbg2* phenomenon apparently only occurred in West Africa and mainly in Côte d'Ivoire is
220 unclear. It seems that, contrarily to the strictly or almost strictly clonal *Tbg1*, *Tbg2*, *Tbb* and
221 *Tbr* seem to extensively recombine within and between each other [53, 72]. Nonetheless, *Tbr*
222 also appears mainly or totally clonal, depending on the focus and the study [73, 74]. In the
223 dendrogram of Figure 2 (Key Figure), the generally longer leaves with shorter nodes that
224 seems to characterize trypanosome stocks, especially non-*gambiense* ones, suggest long time
225 evolving clones with rare sexual recombination events. *Tbg2*, *Tbb* and *Tbr* are obviously

226 highly diverse lineages that remain to be investigated more thoroughly in order to clarify the
227 taxonomy of these entities, the relationships they share and their reproductive strategies [75].

228

229 **Geographical distribution of *Tbg2***

230 Figure 1 shows the localization of the 29 *Tbg2* stocks mentioned in this review. We
231 decided to distinguish the stocks for which a mislabeling has been suspected or a discordance
232 across different studies was noticed above. It is obvious that *Tbg2* mainly involves
233 specifically the old HAT foci of Daloa, Bouaflé and Vavoua in Western Center Côte d'Ivoire
234 with 21 stocks isolated in these foci. Eight stocks were isolated in 1978 and 0-2 stocks were
235 isolated each year between 1979 and 1992. The last stock identified as *Tbg2* in Côte d'Ivoire
236 is SH276 isolated in 1992. One *Tbg2* stock was also isolated in 1989 in the Aboisso focus in
237 southeastern Côte d'Ivoire, probably due to large movements of populations between foci for
238 cash crop cultivation [76]. No *Tbg2* could be observed among 253 stocks isolated by the Kit
239 for In Vitro Isolation of trypanosomes (KIVI, [77]) between 1994 and 2000 in Côte d'Ivoire
240 and characterized by MLEE, RAPD and MS [31, 32, 34]. The exclusive use of KIVI (i.e.
241 selection bias) since 1992 was suspected as being responsible for this observation [34].
242 Nevertheless, no *Tbg2* could be detected with MLEE and/or MS within the 118 stocks
243 isolated from patients in the Bonon focus between 2000 and 2004, whether KIVI, rodent
244 inoculation or direct blood amplified MS were used [13, 35, 37, 48]. No other stocks could be
245 isolated since 2004 in Côte d'Ivoire and, thanks to the control efforts conducted until present,
246 only few HAT cases are still being diagnosed each year [78, 79].

247 The five putative *Tbg2* stocks isolated in South Sudan, Uganda, Nigeria and Togo
248 represent anecdotal phenomena. However, a special attention should be paid to the two found
249 in the historical focus of Takoradi in Ghana where no new HAT cases have been reported
250 since the end of the 1980s [50]. For Patient03 diagnosed in 2003 and who spontaneously
251 recovered, a transient infection with *Tbb* due to a transitory ApoL1 deficiency was
252 suspected [50]. For GHANA patient diagnosed in 2013, an infection with an atypical *Tbg*
253 strain lacking the TgsGP defense mechanism against ApoL1 was suspected in a patient with a
254 probable altered ApoL1 trypanolytic activity [56]. Interestingly, Takoradi is close to the
255 historical foci of Aboisso and Agni in Côte d'Ivoire [80] and it cannot be excluded that the
256 two cases from Ghana are linked to the Côte d'Ivoire *Tbg2* phenomenon.

257

258

259 **Concluding remarks**

260 The present review clearly confirms the existence of human infective *T. brucei* from
261 West and Central Africa that do not fit into *Tbg1*, although we also identified some stocks that
262 were wrongly named *Tbg2* in the literature and probably in this review due to previous
263 mislabeling. It is difficult to clearly define and characterize *Tbg2* mainly because it is not
264 possible to differentiate it from *Tbb* based on both phenotypic and genotypic criteria. This is
265 complicated by the few data available and by the low number of stocks used in the different
266 studies.

267 *Tbg2* might be a sub group of *Tbb* that shares a more recent common ancestor with
268 *Tbg1*. This may predispose some of its representatives to occasionally infect humans. Some
269 might represent rare hybrids between *Tbb* and *Tbg1* strains. Finally, some other strains may
270 take the opportunity of immune compromising of some *Tbg1* infected human individuals to
271 co-infect these patients. These different hypotheses are not exclusive. The *Tbg1* clade
272 probably originated in West Africa less than 10000 years ago [14]. This may relate to the fact
273 that *Tbg2* almost only arose in this geographic area.

274 A logical perspective of this review paper would be to conduct a global study on all
275 the presumably *Tbg2* stocks still available using the most efficient and informative molecular
276 methods (see Outstanding Questions). Table 1 lists the institutions/lab where stabilates of the
277 *Tbg2* stocks are still cryo-conserved. Although 13 seem no longer available, the others are
278 still accessible under institution conditions. To this respect, development of adapted molecular
279 markers to identify the biological, genetic and systematic characteristics of the different
280 members of the *Trypanosoma brucei* species complex is timely. A simple and rapid molecular
281 tool to identify and distinguish the different *T. brucei* subspecies directly from biological
282 fluids (without uncertain and laborious isolation and culture steps) would be most welcome.
283 Waiting to find out more, we confirm that to us as proposed above, the best definition we can
284 propose is to restrict the term *Tbg2* to all human-infective *T. brucei* trypanosomes from West
285 and Central Africa that do not fit into *Tbg1* using the available molecular markers.

286 In term of public health, it is obvious that *Tbg2* does not represent a major concern
287 even if the frequency of this phenomenon is probably under evaluated partly due to miss
288 diagnosis. However, in the current elimination context, the number of g-HAT patients
289 decreasing may increase the susceptibility of “exposed population” by a reduction of infected
290 bites by tsetse flies (and therefore the acquired immunity), and also could allow the
291 emergence of *Tbg2* as an atypical zoonotic disease. It is then crucial to be able to detect such

292 infections using adapted effective diagnosis, especially in the frame of the passive
293 surveillance that is more and more used, and to determine if they are due to HSR
294 trypanosomes or patient immunodeficiency (constitutive or transient) in order to implement
295 adapted control strategies including effective treatment. The study of the HSR mechanisms
296 from the available *Tbg2* stocks would provide essential elements to anticipate the appearance
297 of new mechanisms.

298

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306

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Table 1 List of *Tbg2* mentioned in the literature

Patients	Country	Focus	Locality ^a	Isolation year	Isolation method ^a	First citation	Lab ^b
STIB 368 or EATRO 210	Uganda	West Nile	NI	1959	NI	[52]	NA
FEO-S	Togo	Pagouda	NI	1961	Rodent	[6]	1
KWANG 9A	Nigeria	Kwang	NI	1967	Rodent	[58]	NA
GBOKO	Nigeria	Gboko	NI	1968	Rodent	[58]	NA
TH2 78E (020)	Côte d'Ivoire	Bouaflé	Koudougou	1978	Mastomys	[22]	1, 2, 3
TH113 78E (020)	Côte d'Ivoire	Bouaflé	Koudougou	1978	Mastomys	[22]	NA
TH1 78E (032)	Côte d'Ivoire	Bouaflé	NI	1978	Mastomys	[22]	NA
TH1 78E (037)	Côte d'Ivoire	Bouaflé	Kouassi-Perita	1978	Mastomys	[22]	2
TH114 or STIB 386	Côte d'Ivoire	Bouaflé	Koudougou	1978	Mastomys	[22]	2, 4
TH112 78E (020)	Côte d'Ivoire	Bouaflé	Koudougou	1978	Mastomys	[22]	1, 2
TH126 78E (020)	Côte d'Ivoire	Bouaflé	Koudougou	1978	Mastomys	[22]	1, 2
TH162 78E (021)	Côte d'Ivoire	Bouaflé	Sietinfla	1978	Mastomys	[22]	2
MURAZ03	Côte d'Ivoire	Vavoua	Koetinga	1979	Rodent	[23]	1, 3
KOBIR or DAL 503	Côte d'Ivoire	Daloa	NI	1982	Mice	[42]	3, 4
OUSOU or DAL 494	Côte d'Ivoire	Daloa	NI	1982	Mice	[42]	3, 4
BIYAMINA CIB	Sudan	Yambio	NI	1982	Rodent	[23]	1, 3
ABBA or DAL 626	Côte d'Ivoire	Daloa	NI	1983	Mice	[42]	1, 3, 4
TH922 83E Kou	Côte d'Ivoire	Bouaflé	Koudougou	1983	Mastomys	[41]	NA

LIGO or DAL 655	Côte d'Ivoire	Daloa	NI	1984	Mice	[42]	1, 3, 4
IPR15-5 or HTAG15-5	Côte d'Ivoire	Daloa	Tagoura	1985	Mastomys	[27]	NA
IPR128-6 or HTAG128/6	Côte d'Ivoire	Daloa	Tagoura	1985	Mastomys	[27]	NA
IPR107-1 or HTAG107-1	Côte d'Ivoire	Daloa	Tagoura	1986	Mastomys	[27]	1, 2
SH136	Côte d'Ivoire	Vavoua	Ketro-Bassam	1988	Rodent	[30]	NA
SH017	Côte d'Ivoire	Aboisso	NI	1989	Rodent	[30]	NA
SH196	Côte d'Ivoire	Daloa	Gueguigbeu	1990	Rodent	[30]	NA
PT312	Côte d'Ivoire	Daloa	Gbiebuhe	1992	KIVI	[39]	2
SH276	Côte d'Ivoire	Daloa	Zoukougbeu	1992	Rodent	[30]	NA
Patient03	Ghana	Takoradi	NI	2003	DNA ext	[50]	NA
GHANA	Ghana	Takoradi	NI	2013	DNA ext	[56]	NA

^a NI = No information. ^b Lab = laboratory where stabilates are still available at the time of writing; NA = not available ; 1 = Institut de Recherche pour le Développement (IRD), UMR INTERTRYP IRD-CIRAD, TA A-17/G, Campus International de Baillarguet, F-34398 Montpellier, France. Contact vincent.jamonneau@ird.fr; 2 = University of Bristol, School of Biological Sciences, Life Sciences Building, 24 Tyndall Avenue Bristol BS8 1TQ, United Kingdom. Contact W.Gibson@bristol.ac.uk; 3 = Department of Biomedical Sciences, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium. Contact pbuscher@itg.be; 4 = Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Henry Wellcome Building, 464 Bearsden Road, Glasgow, United Kingdom. Contact Annette.Macleod@glasgow.ac.uk.

Table 2 Molecular characterization of *Tbg2*

Patients	HSR	MM^e results in favour of <i>Tbg2</i>	MM results in favour of <i>Tbg1</i>	PCR TgsGP	PCR SRA
STIB 368 or EATRO 210	NA ^a	MS [52]	NRF ^f	NA	neg [52]
FEO-S	No ^b	MLEE [6, 37]; DNA-h [41]; RFLP [38]; MS [37, 50, 54]	NRF	NA	NA
KWANG 9A	NA	MLEE [58, 62]	NRF	NA	NA
GBOKO	NA	MLEE [58, 62]	NRF	NA	NA
TH2 78E (020)	Mid ^c	MLEE[22, 28, 30-35, 37]; PCR [43, 44]; kDNA [45, 52]; RFLP [40]; MGE [36]; MS [37, 48, 50, 52, 54, 81]	NRF	neg ^g [16] neg [17] pos ^h [17]	NA
TH113 78E (020)	No	MLEE [22, 28]; PCR [43]; MGE [36]; kDNA [52]; MS [37, 50, 52]	NRF	NA	NA
TH1 78E (032)	No	MLEE [22]	NRF	NA	NA
TH1 78E (037)	No	MLEE [22, 28]; DNA-h [41]; MS [53]	NRF	neg [53]	NA

TH114 ou STIB 386	Yes ^d	MLEE [22, 24, 27]; DNA-h [24, 41]; RFLP [38]; rDNAseq [42]; MS [51-53, 82]; WGS [14, 56, 57]	NRF	neg [69] neg [53] neg [14] neg [57]	neg [69] neg [57]
TH112 78E (020)	No	MLEE [22, 27, 28]; kDNA [45, 52]; PCR [43]; MS [49, 53]	NRF	neg [53]	NA
TH126 78E (020)	Mid	MLEE [22, 27]; MS [49, 53]; WGS [55-57]	NRF	neg [53] neg [57]	neg [57]
TH162 78E (021)	No	MLEE [22, 24, 27]; DNA-h [24]	NRF	NA	NA
MURAZ03	NA	MLEE [23, 28]; PCR [43]; MGE [36]	NRF	NA	NA
KOBIR or DAL 503	Yes	rDNAseq [42] WGS [14]	RFLP [38]	pos [16] neg [14]	neg [67]
OUSOU or DAL 494	Yes	rDNAseq [42] WGS [14]	NRF	pos [16] neg [14]	neg [67]
BIYAMINA CIB	NA	MLEE [28, 37]; kDNA [45]; PCR [43]; MS [37, 50]	MLEE [23]	NA	NA
ABBA or DAL 626	NA	RFLP [38]; rDNAseq [42]; MLEE [37]; MS [37, 48, 50]; WGS [57]	NRF	neg [16] neg [57]	neg [68] neg [67] neg [57]
TH922 83E Kou	Mid	DNA-h [41]; RFLP [38]	NRF	NA	NA

LIGO or DAL 655	Yes	RFLP [38]; rDNAseq [42]; MLEE [37]; MS [37, 48, 50]	WGS [14]	neg [16] pos [14]	neg [67]
IPR15-5 or HTAG15-5	NA	MLEE [27, 29]; PCR [44]; MS [49]	NRF	NA	NA
IPR128-6 or HTAG128/6	NA	MLEE [29]	MLEE [27]	NA	NA
IPR107-1 or HTAG107-1	NA	MLEE [27, 29]; PCR [44]; MS [49, 54, 81]	NRF	NA	NA
SH136	NA	MLEE [30]	NRF	NA	NA
SH017	NA	MLEE [30-32, 34, 35]	NRF	NA	NA
SH196	NA	MLEE [30-32, 34, 35]	NRF	NA	NA
PT312	NA	Mea [47]	RAPD [46]; MLEE [30]	NA	NA
SH276	NA	MLEE [30-32, 34, 35]	No	NA	NA
Patient03	NA	MS [50]	No	neg [50]	Neg [50]
GHANA	NA	WGS [56]	No	neg [56]	NA

^a NA = Data Not Available in the literature. ^b No = No HSR. ^c Mid = Partially HSR. ^d Yes = HSR. ^e MM = Molecular Methods; MS = microsatellite analyses; MLEE = multi-locus enzyme electrophoresis; DNA-h = DNA hybridization; RFLP = restriction fragment length polymorphisms; PCR = arbitrarily of specific PCR primers; kDNA = kinetoplast DNA markers; MGE = mobile genetic element; rDNAseq = repetitive DNA-sequences; WGS = whole genome sequencing; Mea = multiplex-endonuclease analyses; RAPD = random amplification of polymorphic DNA. ^f NRF = No Reference Found in the literature. ^g neg = PCR negative. ^h pos = PCR positive.

Glossary

Control efforts: As a vector-borne disease, g-HAT control classically relies on case finding and treatment, and vector control.

Elimination of HAT as a public health problem: It is monitored through several indicators. The primary global indicators are fewer than 2000 annually reported cases and a 90% reduction of the area at risk reporting ≥ 1 case/10,000 people/year (calculated over a 5-year period) compared to 2000–2004. The indicator at country level is < 1 case/10,000 people/year, in each health district of the country averaged over the previous 5-year period.

Isolation: For *Trypanosoma brucei*, this involves inoculating a host biological fluid containing trypanosomes in laboratory rodents (mice or rats) or *Mastomys* (*in vivo* isolation) or an axenic medium (*in vitro* isolation). The isolated stocks (or strain) can be preserved in nitrogen liquid (stabilates), thawed and multiplied as much as needed at any time for further studied.

Molecular tools: Methods involving DNA, RNA, proteins and other macromolecules used to study and characterize cells, organisms and/or populations.

Neglected tropical diseases: A diverse group of tropical infections caused by pathogens, including parasitic worms, protozoa, bacteria and viruses that are common in low-income populations in developing countries. By definition, they (together) cause a huge burden and yet almost no money is spent on research (either basic or applied).

Pathogenicity: The ability for a pathogenic agent to induce weight loss, anemia or mortality to its host. Pathogenicity in human and laboratory rodents is a phenotypical trait widely used to distinguish *gambiense* and *rhodesiense*-HAT.

Pleomorphic: The ability of some microbes to alter their shape or size in response to environmental conditions.

Tsetse flies: Only present in Sub-Saharan Africa, they belong to the genus *Glossina* and constitute the biological vector of several trypanosome species. Regarding HAT, *G. palpalis* and *G. fuscipes (palpalis riverine group)* are the main vectors of *Tbg* in West and Central Africa, while *G. morsitans* and *G. pallidipes (morsitans savannah group)* are the main vectors of *Tbr* in East Africa.

Virulence: The capacity for a pathogenic agent to multiply inside a host. Virulence in laboratory rodents is a phenotypical trait widely used to distinguish between trypanosomes subspecies.

Box 1. FEO, an atypical HAT patient

The patient FEO was diagnosed in 1938 in Togo. She was 4 years old. Despite several treatments, she harbored trypanosomes for 25 years in blood and/or lymphatic juice [10]. FEO remained healthy and never reached the neurological stage [11]. She was hospitalized during the first nine years of infection, avoiding any re-infection by tsetse. Trypanosomes observed were monomorphic without flagellum, leading to the suspicion of *Trypanosoma congolense*. The first attempt to isolate trypanosomes by inoculation of infected blood in rat failed in 1949. Only in 1961, a rat inoculation was successful, and subsequent passages allowed isolation of the strain [83]. This latter resulted into a chronic infection with a moderate parasitaemia and a death of the rats happening after at least three months. The trypanosomes in rodents were pleomorphic, with a free flagellum, indicating *T. brucei* ssp. and *Tbg* in particular as the original strain was isolated from human. The strains were grown *in vivo* in Burkina Faso and France [83]. In the early 1980's, the appearance of new biological techniques and genetic markers, such as evaluation of the resistance of trypanosomes to NHS and MLEE, allowed a taxonomic identification of the "FEO strain". In fact, two FEO strains were described, FEO-R resistant to NHS was classified *Tbg1* [6] while FEO-S, sensitive to NHS, was associated and used as a reference stock of *Tbg2* (e.g. [54]).

It could be postulated that FEO has been infected in 1938 by two different strains of trypanosomes and accidentally "cloned" during rodent inoculation. A strain sensitive to NHS is coherent with the initial monomorphic trypanosomes, but not for such a long survival in a human. Although the *Tbg2* isoenzymatic profile appears to make this hypothesis realistic, *Tbg2* is usually virulent in rodents [7] and suspected to cause an acute form of HAT [30], two parameters that were not observed for FEO. The taxonomic identification of FEO strains stays very doubtful as mistakes during labelling and/or *in vivo* multiplication of trypanosomes cannot be ruled out. Therefore, FEO was an atypical chronic and asymptomatic HAT case, which original taxonomic identification remains unsolved.

Box 2. *Trypanosoma brucei* and human serum resistance

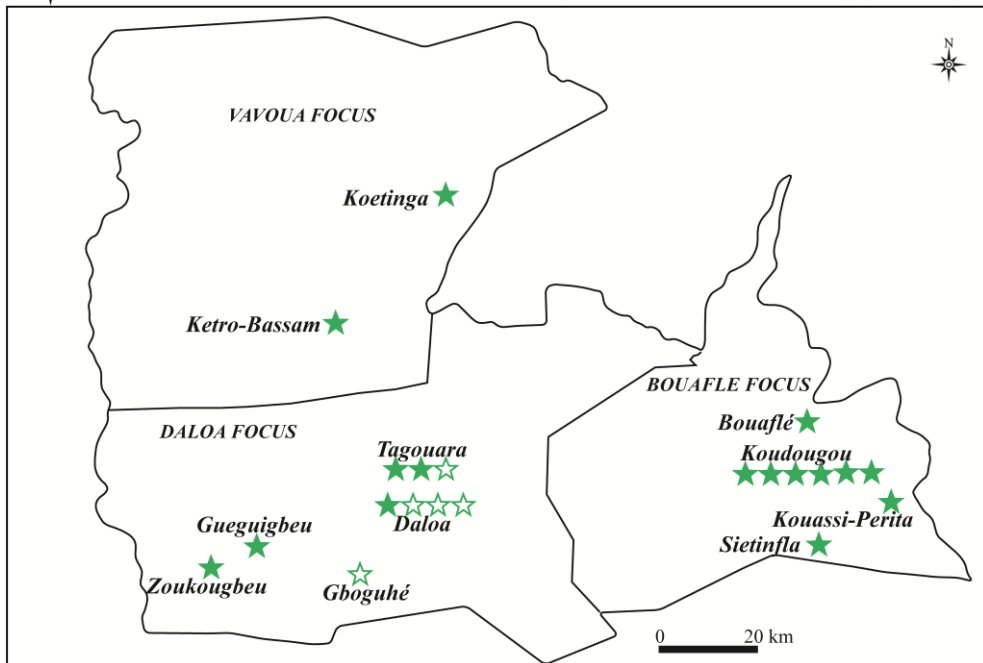
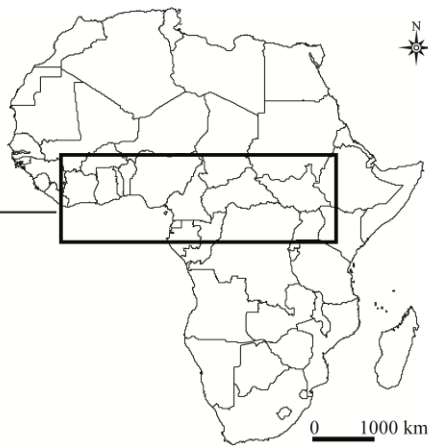
Human serum resistance was first assessed using phenotypic assays such as the blood incubation infectivity test (BIIT) [65] only applicable on isolated strains and not fully gambiense-specific [84]. Research then focused on the HSR mechanisms. *Tbb* is unable to infect human thanks to the Trypanolytic factor (TLF). This TLF is constituted by two serum complexes (TLF 1 & 2), which both contain haptoglobin-related protein (HPR) and apolipoprotein LI (ApoL1) that provide innate protection against several trypanosome species. TLF-1 binds to the parasite through an interaction between the haptoglobin-related protein and the haptoglobin haemoglobin receptor (HpHbR) in the flagellar pocket of the trypanosome. Lysis by APOL1 occurs when the protein penetrates the lysosomal membrane and forms pores leading to the death of the parasite [53]. Similarly, TLF-2 enters trypanosomes via HpHbR, through a different route and contributes to the lysis of the trypanosome. *Tbg* and *Tbr* differ in the HSR mechanism, the former being constitutively resistant to serum whereas in the latter, this mechanism is reversible. In *Tbr*, human infectivity seems to be conferred by a unique gene, the serum resistance associated (SRA) gene related to a multigene family for variant surface glycoproteins (VSG) [85]. It is absent in all other trypanosomes of the subgenus *Trypanozoon*. Similarly to SRA, a shortened VSG gene was searched in *Tbg* and a specific VSG was identified, the *T. gambiense* specific glycoprotein (TgsGP). TgsGP is specific to *Tbg1* and cannot alone confer HSR [69]. *Tbg1* resists to TLFs thanks to TgsGP, which prevents ApoL1 action by stiffening of membranes upon interaction with lipids [86]. Two additional features also contribute to resistance to lysis: reduction of sensitivity to ApoL1 and HpHbR inactivation. The mechanism that allows specifically *Tbg2* to resist to NHS are largely unknown. Nevertheless, similar genes (VSG) of TgsGP have been identified in most isolates of *Tbb*, *Tbr* and *Tbg2* [15].

Figure legends

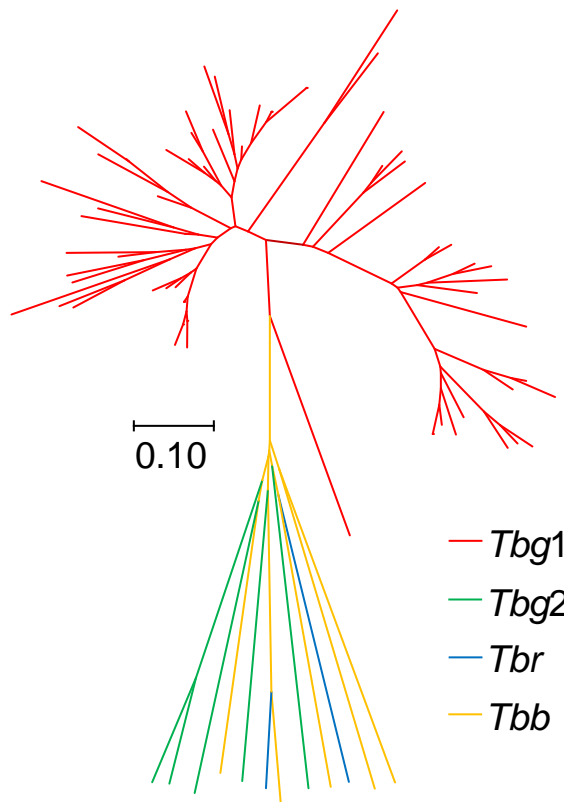
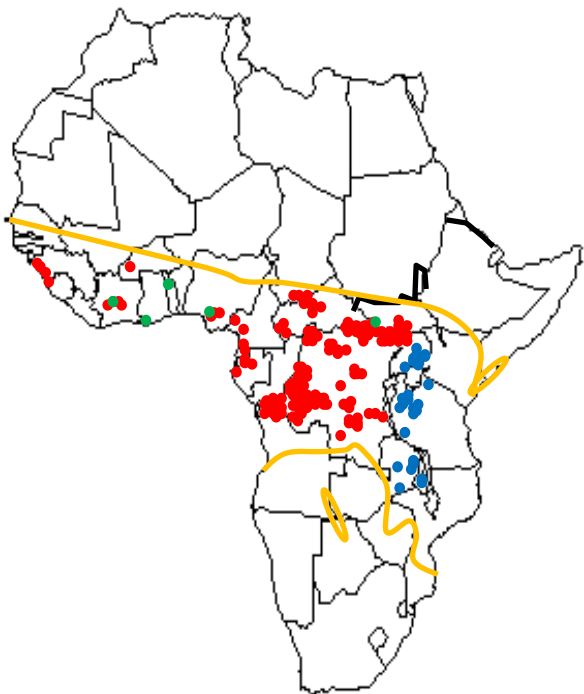
Figure 1: Geographical distribution of the 29 *Tbg2* stocks listed in the literature. The empty green stars identify the possible *Tbg2* stocks for which a mislabeling was suspected or a discordance was described. The plain green stars identify the ascertained *Tbg2* stocks. This figure was specifically created for this manuscript by the mapping service of our team based in the Institut Pierre Richet (Bouaké, Côte d'Ivoire).

Figure 2 (Key Figure): Distribution of human and animal African trypanosomosis cases in sub-Sahara Africa and phylogenetic relationships between stocks. The distribution was inspired from [87] and [88]. The tree was built with the Neighbor joining method (NJTree) [89] from a chord distance matrix [90] based on the microsatellite data of [13]. Color codes are the same for the map and the NJTree.

- ★ Possible *Tbg2* stock
- ☆ Ascertained *Tbg2* stock



- *Trypanosoma brucei gambiense* group 1 (*Tbg1*)
- *Trypanosoma brucei gambiense* group 2 (*Tbg2*)
- *Trypanosoma brucei rhodesiense* (*Tbr*)
- Tsetse belts with probable presence of *Trypanosoma brucei brucei* (*Tbb*)



- *Tbg1*
- *Tbg2*
- *Tbr*
- *Tbb*