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Review

Trypanosoma brucei gambiense Group 2: The Unusual Suspect

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***Trypanosoma brucei* causes human African trypanosomiasis (HAT). Three subspecies were described: *T. b. gambiense* (*Tbg*) and *T. b. rhodesiense* (*Tbr*) in humans, and *T. b. brucei* (*Tbb*) in animals. Molecular markers subdivided *Tbg* into two groups: *Tbg*1 and *Tbg*2, of which the latter is different from *Tbg*1 and *Tbr* (absence of the SRA gene), but indistinguishable from *Tbb*. *Tbg*2 is considered to be a zoonotic form of HAT in West Africa. *Tbg*2 was found mainly in Côte d'Ivoire between 1978 and 1992, but the latest description was made in Ghana in 2013. New molecular tools would be welcome to characterize such infections and determine their origins (resistance to human serum or patient immunodeficiency) in the current context of HAT elimination.**

T. brucei, a Multiple-Pathogen Agent

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

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

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

Highlights

*Tbg*2 was defined as all human-infective *T. brucei* trypanosomes from West and Central Africa that do not fit into the category *Tbg*1. *Tbg*2 is genetically heterogeneous, and differs from *Tbg*1 when using various molecular markers.

*Tbg*2 is also genetically different from *Tbr*, with a consistent lack of the serum-resistance-associated gene in those strains that were tested. *Tbg*2, *Tbb*, and *Tbr* are highly diverse lineages that remain to be investigated more thoroughly.

*Tbg*2 was found mainly in Côte d'Ivoire between 1978 and 1992, but the latest descriptions of *Tbg*2 were made in Ghana in 2003 and 2013. No other record could be found between 1992 and 2003.

*Tbg*2 represents a zoonotic form of HAT. Human infectivity probably arose multiple times and so could do so again. In the elimination context, it is crucial to detect such infections and determine their origins (resistance to human serum or patient immunodeficiency).

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Characterization of *T. brucei* ssp. Subgroups

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

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

Multilocus enzyme electrophoresis (MLEE) was the first molecular technique that highlighted the existence of stocks, isolated from humans, that fell outside the homogeneous *Tbg*1. A group of minor zymodemes from Côte d'Ivoire showed a variable sensitivity to normal human serum (NHS). Some of these zymodemes had their exact counterpart in stocks from domestic and wild animals [21,22]. This observation was confirmed during the following years using several enzymatic systems [6,23–37].

It was also confirmed over time using various DNA-based analyses, including restriction fragment length polymorphism (RFLP) [38–41], DNA hybridization [24,41], repetitive DNA probes [42], arbitrarily-primed PCR [43], specific DNA probes [16,39,44,45], random amplification of polymorphic DNA (RAPD) [32,46], multiplex-endonuclease analyses [47], microsatellite (MS) analyses [13,37,48–54],

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 9 years of infection, avoiding any reinfection 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 was a rat inoculation successful, and subsequent passages allowed isolation of the strain [83]. This latter resulted in a chronic infection with moderate parasitemia and the death of the rats after at least 3 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 a human. The strains were grown *in vivo* in Burkina Faso and France [83]. In the early 1980s, 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 as *Tbg*1 [6], while FEO-S, sensitive to NHS, was associated and used as a reference stock of *Tbg*2 (e.g., [54]).

It could be postulated that FEO was infected in 1938 by two different strains of trypanosomes and that were accidently '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 *Tbg*2 isoenzymatic profile appears to make this hypothesis realistic, *Tbg*2 is usually virulent in rodents [7] and is suspected of causing an acute form of HAT [30] – two parameters that were not observed for FEO. The taxonomic identification of the FEO strains remains very doubtful as mistakes during labeling and/or *in vivo* multiplication of trypanosomes cannot be ruled out. Therefore, FEO was an atypical chronic and asymptomatic HAT case, in which the original taxonomic identification remains undetermined.

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 with 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 into 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 studies.
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: (as discussed in this review) the ability of a pathogenic agent to induce weight loss, anemia, or mortality in its host. Pathogenicity in human and laboratory rodents is a phenotypic 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, these flies belong to the genus *Glossina* and are the biologic vectors of several trypanosome species. Regarding HAT, *G. palpalis* and *G. fuscipes*

Box 2. *T. brucei* and Human Serum Resistance

Human serum resistance (HSR) was first assessed using phenotypic assays such as the blood incubation infectivity test (BIIT) [65] that is applicable only to isolated strains [84]. Research then focused on HSR mechanisms. *Tbb* is unable to infect humans thanks to the trypanolytic factor (TLF). The TLF is constituted by two serum complexes (TLF-1 and TLF-2) which both contain haptoglobin-related protein (HPR) and apolipoprotein L1 (ApoL1) that provide innate protection against several trypanosome species. TLF-1 binds to the parasite through an interaction between HPR and the haptoglobin hemoglobin 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 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 (VSGs) [85]. It is absent in all other trypanosomes of the subgenus *Trypanozoon*. Similarly to SRA, a shortened VSG gene was searched for in *Tbg* and a specific VSG was identified, the *T. gambiense*-specific glycoprotein (TgsGP). TgsGP is specific to *Tbg*1 and cannot alone confer HSR [69]. *Tbg*1 resists 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 *Tbg*2 to resist NHS is largely unknown. Nevertheless, similar genes (VSGs) of TgsGP have been identified in most isolates of *Tbb*, *Tbr*, and *Tbg*2 [15].

(*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: (as discussed in this review) the capacity of a pathogenic agent to multiply inside a host. Virulence in laboratory rodents is a phenotypic trait widely used to distinguish between trypanosome subspecies.

mobile genetic element (MGE) [36], kinetoplast DNA sequencing [52], and whole-genome sequencing (WGS) [14,55–57].

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

The term 'bouaflé' was first used because of the geographic origin of stocks: Bouaflé is a town located in the Western-Center part of Côte d'Ivoire (Figure 1). These stocks were grouped thanks to MLEE-specific patterns [25]. Eventually, 'bouaflé' was used to designate all the West African *T. brucei* stocks sharing the same MLEE patterns [27,30,60], including stocks isolated from domestic and wild animals in Côte d'Ivoire and Burkina Faso, and also stocks isolated from humans in the Western-Center part of Côte d'Ivoire. In further MLEE-based analyses, such human and animal stocks were still named 'bouaflé' [31,32,34,35]. In studies using tools other than MLEE, 'bouaflé' has been replaced by the term *Tbg*2 for human stocks, but some pig stocks from Côte d'Ivoire previously named 'bouaflé' were wrongly named *Tbg*2: the TSW33 and TSW 65 stock wrongly reported as human ones in [14] and [36] respectively; the TSW53 and TSW65 stocks [37,50]; the TSW187 stocks [44]; the 2171 and 2178 stocks [49], and all the TSW stocks in [53]. Stocks isolated from domestic pigs in Cameroon, initially considered as *Tbg*2, because of a non-*Tbg*1 MLEE profile [33], were finally considered as classical *Tbb* [36,52,61].

Characteristics of *Tbg*2 and Relationships with Other *T. brucei* ssp

In the present review we propose to use and restrict the label *Tbg*2 to all human-infective *T. brucei* trypanosomes from West and Central Africa that do not fit into the category *Tbg*1 when using molecular methods (Table 2). Although most of the stocks appear in several studies, some of them have been cited only once and/or have been poorly studied. For instance, STIB 368 was only characterized by MS and kinetoplast DNA [52], TH1 78E (032) by MLEE [22], TH922 83E Kou by RFLP and DNA hybridization [38,41], and SH136 by MLEE [30]. Stocks SH196, SH276, and SH017 were cited in several studies using only MLEE. The two *Tbg*2 stocks observed in Ghana were each studied once [50,56].

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 ClB	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, UK. 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, UK. Contact Annette.Macleod@glasgow.ac.uk.

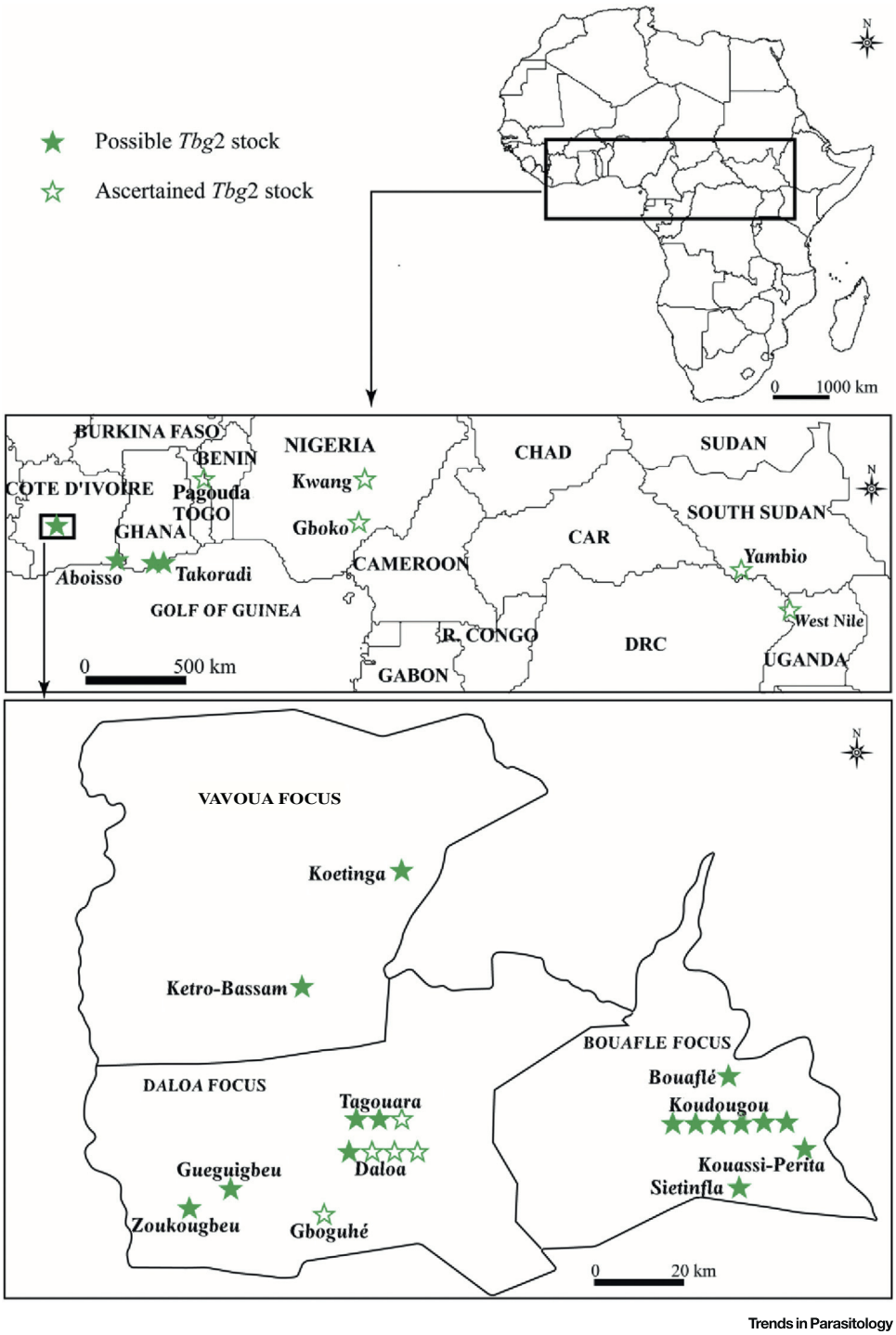

Figure 1. Geographic Distribution of the 29 *Tbg2* Stocks Listed in the Literature.
The empty green stars identify the possible *Tbg2* stocks for which mislabeling was suspected or a discordance was described. The plain green stars identify the ascertained *Tbg2* stocks. This figure was specifically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

Table 2. Molecular Characterization of *Tbg2*

Patients	HSR	MM[e] results in favor of *Tbg2*	MM results in favor of *Tbg1*	PCR TgsGP	PCR SRA gene
STIB 368 or EATRO 210	NA[a]	MS [52]	NRF[f]	NA	Neg[g] [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 ClB	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

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Table 2. *Continued*

Patients	HSR	MM[e] results in favor of *Tbg2*	MM results in favor of *Tbg1*	PCR TgsGP	PCR SRA gene
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 human serum resistance (HSR).
[c]Mid = Partially HSR.
[d]Yes = HSR.
[e]MM = molecular methods; MS = microsatellite analyses; MLEE = multilocus enzyme electrophoresis; DNA-h = DNA hybridization; RFLP = restriction fragment length polymorphism; 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.

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

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

For BIYAMINA clone B and FEO-S, a mislabeling was also hypothesized. Such a hypothesis was also formulated regarding some discordances described above. This is indeed likely due to the prolonged maintenance and repeated passage in various rodents for most of the stocks. However, the hypothesis of mixed infections due to coinfections in the corresponding patients cannot be excluded. Regarding the stock KOBIR, an explanation for the observed discrepancy is that the original isolate was a mixture of a *gambiense* and '*non-gambiense*' trypanosomes, and that, by chance, during the passaging of this strain, two different lines, described by the same name, were obtained [42]. It is possible that a patient first infected by *Tbg1* could develop an immunodeficiency allowing a subsequent *Tbb* infection. Such a pattern had already been suspected in long-term asymptomatic patients

in Côte d'Ivoire [63]. In case of *Tbg*1 and *Tbb* coinfections, *Tbg*1 is usually preponderant in humans, but an **isolation** in rodents would be favorable to *Tbb* by hiding or removing *Tbg*1. This could explain the characterization of *Tbb* or *Tbg*1 from the same stocks depending on the method used. This could also explain that some stocks isolated from humans are sensitive to NHS (see below and Table 2). Regarding such a hypothesis, *Tbg*2 would be a *Tbb* that infected humans due to some immunodeficiency mechanism. Likewise, a constitutive immunodeficiency was suspected to explain the *T. brucei* infections of the two Ghanaian patients [50,56]. Constitutive immunodeficiency can result in atypical infections due to trypanosomes other than *T. brucei* [64].

HSR has been used for a long time for trypanosome characterization with several methods (Box 2). Although *Tbg*1 stocks are invariably resistant using the blood incubation infectivity test (BIIT, [65]), HSR is variable in the 13 *Tbg*2 stocks tested by this method, with 7 resistant or partially resistant stocks and 6 sensitive ones (Table 2). The serum-resistance-associated (SRA) gene PCR was described as a reliable molecular diagnostic tool specific for *Tbr* [66,67]. The eight *Tbg*2 stocks tested with this PCR were negative (Table 2), whatever the method used [50,52,57,67–69].

TgsGP PCR was then proposed for the specific diagnosis of *Tbg* [16,17]. Out of 10 *Tbg*2 stocks tested (Table 2), six were invariably negative [14,16,17,53,57,69]. For the four others (TH2 78E, KOBIR, OUSOU, and LIGO), discordances were observed depending on the primers/method used. Using PCR targeting the 3′ end of the gene encoding TgsGP, LIGO and TH2 78E were negative while KOBIR and OUSOU were positive [16]. On the opposite, LIGO was positive while KOBIR and OUSOU were negative when using a different forward primer [14]. When using primers targeting the 5′ half of the gene, the stock TH2 78E was PCR-positive [17]. The discrepancy in the PCR results obtained for this stock is based on the target that differs between the two PCRs. One targeted the 3′ end of the TgsGP gene, well conserved in *Tbg*1, while the other targeted genes similar to TgsGP [17]. The TgsGP-like genes in some isolates of *Tbb*, *Tbr*, or *Tbg*2, which can be amplified using primers targeting the 5′ end of the TgsGP gene, are closely similar to VSG Tb10.v4.0178, and it is likely that this is the ancestral gene of TgsGP [70].

*Tbg*2 is then characterized by the absence of the SRA gene and a complex mechanism for HSR illustrated by the fact that some stocks isolated from humans are sensitive to NHS when tested by the BIIT method and that these mechanisms do not necessarily involve TgsGP as illustrated by the results obtained regarding STIB 386 (also named TH114) stock [69]. Regarding the different mechanisms of trypanosome resistance to human serum, it can be hypothesized that *Tbg*2 might arise by a zoonotic episode involving trypanosomes infecting animals (probably *Tbb*) under particular circumstances.

*Tbg*1 causes a chronic infection [1]. Very little information is available about the clinical evolution of *Tbg*2-infected patients. *Tbg*2 was defined as a virulent form of *Tbg* in foci of Gambian sleeping sickness [60], based on the only well-documented study that concerned the four SH stocks (Table 1), for which three are suspected of causing an acute form with a rapid turn into the neurologic stage [30]. However, no neurologic symptom was described in the two Ghanaian patients (Patient03 and GHANA), and a spontaneous cure was observed for Patient03 [50,56].

There is no specific genetic marker for *Tbg*2. Most of the time, only *Tbg*1 appears homogeneous (i.e., monophyletic) and separated from the other stocks, while *Tbg*2, *Tbb*, and *Tbr* appear as extremely heterogeneous without any clear population structure. Nevertheless, some published studies found a more ordered structure. One study – using MS, but with only three *Tbg*2 stocks (STIB 386, TH113, and TH2 78E) – found a Bayesian cluster containing the three *Tbg*2 and six *Tbb* from various parts of Africa (Cameroon, DRC, and Burkina Faso) [52]. A principal component analysis confirmed this clustering and positioned the three *Tbg*2 stocks half way between all *Tbg*1 and a group of *Tbr* and *Tbb* stocks [52]. Three *Tbg*2 stocks (STIB 386, KOBIR, and OUSOU) also appeared half way between *Tbg*1 stocks and other stocks of *Tbb* and *Tbr* with genome-wide SNP analysis [14]. Other genome-wide SNP analyses were undertaken [56,57] in which *Tbg*2 stocks (STIB 386, TH126, and ABBA) tended to group together with some other West African *Tbb* stocks, but quite remotely as compared with *Tbg*1. Interestingly, the new deviant GHANA branched half way between *Tbg*1 and the *Tbb* TREU927 [56].

Key Figure

Distribution of Human and Animal African Trypanosomiasis Cases in Sub-Saharan Africa, and the Phylogenetic Relationships between Stocks

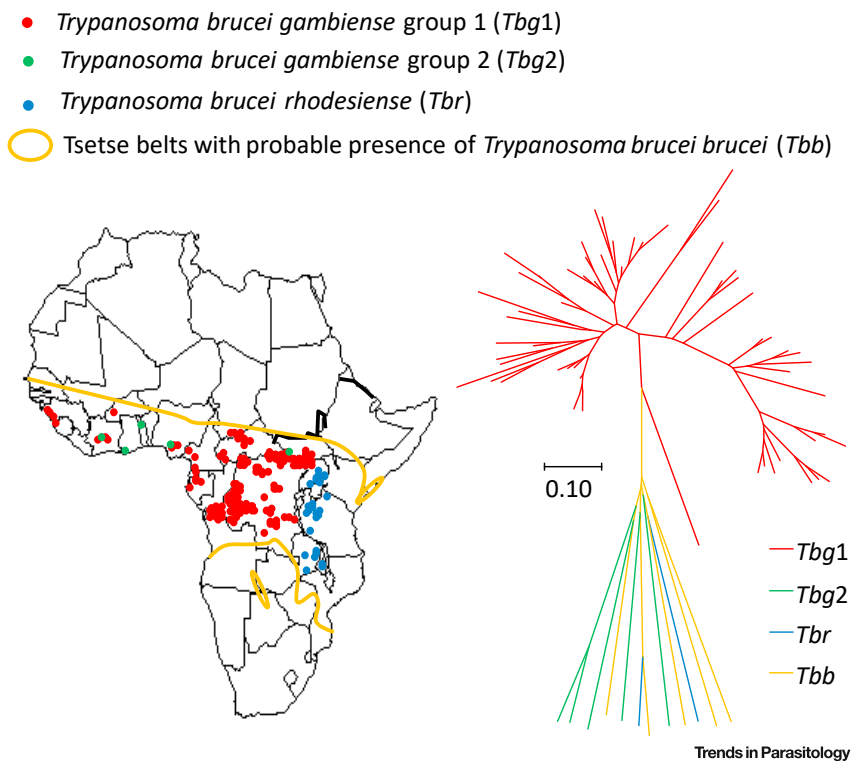

Figure 2. The distribution was inspired by [87] and [88]. The tree was built with the neighbor-joining method (NJTree) [89] from a chord distance matrix [90] based on the microsatellite data of [13]. Color codes are the same for the map and the NJTree.

This suggests that human infection by non-*gambiense* trypanosomes can occur either from *Tbb* that are genetically the closest to *Tbg*1 or from rare hybridization events between *Tbg*1 and *Tbb* strains, which seems supported by other data [40,52]. The hybrid origin of *Tbg*1 itself, between two ancestral *Tbb*-like strains, cannot be excluded, as it would explain the strictly clonal propagation since then [14]. Hybridization is perhaps the most common route to parthenogenesis [71]. Whatever the origins of these unusual strains, *Tbg*2 bears the same relationship to West African *Tbb* as *Tbr* does to East African *Tbb* [42]. The reason why the *Tbg*2 phenomenon apparently occurred only in West Africa, and mainly in Côte d'Ivoire, is unclear. It seems that, contrarily to the strictly or almost strictly clonal *Tbg*1, *Tbg*2, *Tbb*, and *Tbr* seem to extensively recombine within and between each other [53,72]. Nonetheless, *Tbr* also appears mainly or totally clonal, depending on the focus and the study [73,74]. In the dendrogram of Figure 2, Key Figure, the generally longer leaves with shorter nodes that seems to characterize trypanosome stocks, especially non-*gambiense* ones, suggest long time evolving clones with rare sexual recombination events. *Tbg*2, *Tbb*, and *Tbr* are obviously highly diverse lineages that remain to be investigated more thoroughly in order to clarify the taxonomy of these entities, the relationships they share, and their reproductive strategies [75].

Geographic Distribution of *Tbg2*

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

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

Concluding Remarks

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

Tbg2 might be a subgroup of *Tbb* that shares a more recent common ancestor with *Tbg1*. This may predispose some of its representatives to occasionally infect humans. Some might represent rare hybrids between *Tbb* and *Tbg1* strains. Finally, some other strains may take the opportunity of immune compromising of some *Tbg1*-infected human individuals to coinfect these patients. These different hypotheses are not exclusive. The *Tbg1* clade probably originated in West Africa less than 10 000 years ago [14]. This may relate to the fact that *Tbg2* mainly arose in this geographic area.

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

In terms of public health, it is obvious that *Tbg2* does not represent a major concern, even if the frequency of this phenomenon is probably underevaluated, partly due to misdiagnosis. However, in the current elimination context, the number of g-HAT patients decreasing may increase the susceptibility of the 'exposed population' by a reduction in the acquired immunity, and could thus allow the emergence of *Tbg2* as an atypical zoonotic disease. It is then crucial to be able to detect such infections using adapted effective diagnosis, especially in the era of passive surveillance that is more and more used, and to determine if they are due to HSR trypanosomes or patient immunodeficiency (constitutive or transient) in order to implement adapted control strategies including effective treatment. The study of the HSR mechanisms from the available *Tbg2* stocks would provide essential elements to anticipate the appearance of new mechanisms.

Outstanding Questions

How can we differentiate *Tbg2* from the other *T. brucei* in both isolated stocks and biologic samples?

What are the HSR mechanisms of *Tbg2*?

What sort of patient immunodeficiency allows a *Tbg2* infection?

Why has *Tbg2* not been found recently?

What strategies could be used to detect *Tbg2* in non-human hosts?

Are current diagnostic tools effective for detecting *Tbg2*?

Are current treatments effective against *Tbg2*?

What is the real taxonomic status and population structure of each *T. brucei* entity?

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