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Trypanosoma equiperdum: master of disguise or historical mistake?

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After 100 years of research, only a small number of laboratory strains of *Trypanosoma equiperdum* exists, and the history of most of the strains is unknown. No definitive diagnosis of dourine can be made at the serological or molecular level. Only clinical signs are pathognomonic and international screening relies on an outdated cross-reactive serological test (the complement-fixation test) from 1915, resulting in serious consequences at the practical level. Despite many characterization attempts, no clear picture has emerged of the position of *T. equiperdum* within the *Trypanozoon* group. In this article, we highlight the controversies that exist regarding *T. equiperdum*, and the overlap that occurs with *Trypanosoma evansi* and *Trypanosoma brucei brucei*. By revisiting the published data, from the early decades of discovery to the recent serological- and molecular-characterization studies, a new hypothesis arises in which *T. equiperdum* no longer exists as a separate species and in which current strains can be divided into *T. evansi* (the historical mistake) and *Trypanosoma brucei equiperdum* (the master of disguise). Hence, dourine is a disease caused by specific host immune responses to a *T. b. equiperdum* or *T. evansi* infection.

What is dourine?

According to the World Organization for Animal Health (OIE; http://www.oie.int/eng/en_index.htm), 'dourine is a chronic or acute contagious disease of breeding solipeds that is transmitted directly from animal to animal during coitus. The causal organism is *Trypanosoma equiperdum*. It is the only trypanosome that is not transmitted by an invertebrate vector and it differs from other trypanosomes in that it is primarily a tissue parasite that rarely invades the blood. There is no known natural reservoir of the parasite other than infected equids. The clinical signs are marked by periodic exacerbation and relapse, ending in death or, possibly, recovery. Fever, local oedema of the genitalia and mammary glands, cutaneous eruptions, incoordination, facial paralysis, ocular lesions, anaemia, and emaciation may all be observed. Oedematous cutaneous plaques, 5–8 cm in diameter and 1 cm thick, are pathognomonic.' Because dourine is considered to be incurable, seropositive horses should be slaughtered. The latest official reports from the OIE list the occurrence of dourine in Botswana, Ethiopia, Germany, Kyrgyzstan, Namibia, Pakistan, Russia, South Africa and Uzbekistan.

The history of *Trypanosoma equiperdum*

A dourine-like disease was mentioned in early Arab texts but the first recognized description of dourine in Europe was by Ammon and Dirkhausen who, in 1796, observed cases in a Prussian stud [1]. However, it was only in 1894 that Rouget demonstrated the presence of *T. equiperdum* in the blood of an infected Algerian horse. However, this parasite was lost before Rouget could reproduce the disease in horses [2]. It was several years later that Buffard and Schneider reproduced dourine in a horse after the subcutaneous inoculation of a parasite – isolated from a naturally infected Algerian horse – that was maintained through several passages in experimentally infected dogs [3]. After confirmation of these results in 1900 [4], this trypanosome was considered to be the causative organism of dourine; the name *T. equiperdum* was postulated by Doflein in 1901 [5]. Stabilates of this original strain are no longer available. Because of the apparent difficulties detecting the parasite in some cases of dourine in Algeria, Chauvrat and Busy expressed their doubt about *T. equiperdum* being the causative organism [6]. Buffard and Schneider suggested in 1902 that the parasite might cause surra or nagana, but not dourine. However, trypanosomes had been isolated from other cases of dourine in France, Hungary, Germany and Canada [5]. Other sources mention the possibility of symptomless carriers of *T. equiperdum* in Canada and Russia [7,8].

Since the 19th century, dourine has occurred sporadically in Europe. Around 1918, the disease was reported only in Russia, Turkey, Hungary and Spain. During World War II, *T. equiperdum* was reintroduced into Western Europe by Russian and Algerian horses, which were used in the German army and in France, respectively [9]. After the war, the disease was eradicated from Western Europe by systematic screening and control: clinical examination, confirmatory diagnosis by the complement-fixation test (CFT) and enforcement of sanitary measures, including stamping out and treatment with high dosages of neoarsphenamine in some cases.

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Horses suffering from the clinical symptoms of dourine have been reported recently in Ethiopia [10] and Mongolia [11] but, unfortunately, without successful parasite isolation. Several other attempts to isolate *T. equiperdum* in Eastern Europe were also unsuccessful [12]. Thus, although this parasite has been present for more than 100 years, most of its history has been lost; during the past three decades, nobody has isolated a new strain that could be used as reference material.

The availability of laboratory strains of *Trypanosoma equiperdum*

Table 1 shows a list of *T. equiperdum* strains that are maintained in laboratory collections and available for research. The Bordeaux *Trypanosoma* antigen type (BoTat) 1 clone is derived from the *T. equiperdum* strain Institut Pasteur Paris. This strain arrived in Bordeaux in 1961 and was maintained for ten years through serial passages in mice; in 1971, it was cloned and kept in liquid nitrogen [13]. This strain was probably isolated from a horse in Morocco in 1924. In 1976, the *T. equiperdum* Onderstepoort Veterinary Institute (OVI) strain was isolated in South Africa from a horse showing clinical signs of dourine, *in casu* progressive emaciation and posterior paresis [14]. The Swiss Tropical Institute Basel strain (STIB) 818 was isolated in China in 1979 from a horse [15]; the strain was obtained after six months of serial passages, in rabbits initially and then in mice. Unfortunately, no information was provided about the clinical signs of the original host. From the other strains, the exact history is unknown or doubtful. The American and Canadian strains were maintained by serial passage in rats at the National Veterinary Service Laboratories (http://www.aphis.usda.gov/vs/nvsl/) from 1923 to 1977 and then kept in liquid nitrogen [16]. There are no records of their origin or whether they were isolated from horses suffering from dourine. The names suggest that they originated in the USA and Canada, respectively. No references are available for the Alfort, Hamburg and Staatliches Veterinärmedizinisches Prüfungsinstitut (SVP) strains. Nonetheless, they are thought to be strains of *T. equiperdum* in that they are used as reference strains for dourine diagnosis in Germany (P.H. Clausen, personal communication). Antwerp *Trypanosoma* antigen type (AnTat) 4 was cloned from a wild-type *T. equiperdum* at the Institute of Tropical Medicine in Antwerp (Filip Claes, PhD thesis, Katholieke Universiteit Leuven, 2003). Within the American Type Culture Collection (ATCC; http://www.atcc.org), the dyskinetoplastic *T. equiperdum* strain ATCC 30023 is derived from *T. equiperdum* strain ATCC 30019 after a long *in vitro* laboratory cultivation [17]. As stated by Hoare and repeated by the OIE, the origin and identity of some laboratory strains of *T. equiperdum* from dourine horses is so uncertain that fresh isolates are desperately needed [18].

Table 1. Available laboratory strains of *Trypanosoma equiperdum*

Code[a]	Country	Host	Year of isolation	Refs
BoTat 1.1	Morocco	Horse	1924	[13,40,42,48]
STIB 818	China	Horse	1979	[15,36,40,42,48]
OVI	South Africa	Horse	1977	[14,35,42,48]
ATCC 30019	France	Horse	1903?	[17,35,37]
ATCC 30023	France	Horse	1903?	[17,35,37]
Am. stabilate	America?	Horse	Unknown	[16,48]
Can. stabilate	Canada?	Horse	unknown	[16,48]
AnTat 4.1	Unknown	Unknown	Unknown	[35,48]
Alfort	Unknown	Horse	1949?	[35,48]
Hamburg	Unknown	Unknown	Unknown	[35]
SVP	Unknown	Unknown	Unknown	[35,48]
TREU 2259	Unknown	Unknown	Unknown	[49,50]

[a]Abbreviations: Am., American; Can., Canadian; TREU, trypanosome research Edinburgh University.

Clinical signs of dourine

Equines are considered to be the only natural host of *T. equiperdum*. The disease in horses is chronic, persists for one or two years and is generally divided into three phases, although the clinical course can vary considerably under different conditions. The first period is characterized by oedema, tumefaction and damage to the genitalia, and begins one to two weeks after infection. The second stage of disease is pathognomonic for dourine. In this period, typical cutaneous plaques or skin thicknesses can occur, with sizes ranging from extremely small to hand sized. Interestingly, these plaques have also been observed sporadically in animals infected with *Trypanosoma evansi* [19]. The third phase of dourine is characterized by progressive anaemia, disorders of the nervous system – mainly paralysis of the hind legs and paraplegia – and, finally, death [20].

Experimental infections in horses through infusion into the urogenital tract have been performed in South Africa using the OVI strain [14], in the USA using the American and Canadian strains [16] and in Kazakhstan* using a wild-type strain. In the US study, none of the 20 infected horses developed typical clinical signs; they showed only general signs of trypanosomiasis. In South Africa and Kazakhstan, however, the animals showed typical signs of dourine, such as scrotal oedema, emaciation and posterior paresis, but the presence of the pathognomonic dourine plaques were not reported by the authors. Apparently, differences in pathology are observed between animals in these experimental infections but it remains unclear whether the differences are related to the *T. equiperdum* strain that is used or whether they are caused by differences in the host immune response.

Under laboratory conditions, dogs can develop dourine [21]. Different routes of infection (e.g. subcutaneous, intraperitoneal, intravenous, intraurethral and intravaginal transmission) were tested and all gave rise to obvious clinical signs of dourine [5]. Early experiments with rabbits reported specific clinical signs of dourine [3,22]. By contrast, in recent experimental infections with *T. equiperdum*, rabbits developed clinical signs that could not be distinguished from those developed by rabbits infected with *T. evansi* [23].

Mice and rats can be infected with *T. equiperdum* but do not develop the 'normal' form of dourine, although all available laboratory strains of the parasite grow easily in

* G.D. Ilgekbayeva *et al*. Second Symposium of the New World on Trypanosomes and other Haemoparasites, Venezuela, October 1999.

these animals (Théo Baltz, PhD thesis, University of Bordeaux 2, 1982). Ruminants seem to be refractory to infection with *T. equiperdum* [5], but Wang produced clinical manifestations of dourine in sheep and goats after inoculation with a murine-adapted strain of *T. equiperdum* [24]. Thus, although *T. equiperdum* occurs naturally only in equines, it seems that other animals can also be susceptible, which suggests that these parasites are strains of *T. evansi* or *Trypanosoma brucei brucei* and that dourine pathology is strain related or host related.

Transmission and diagnosis

Generally, it is believed that natural transmission of *T. equiperdum* parasites occurs only during copulation [20]. However, intravenous or intraperitoneal experimental infections indicate that mechanical transmission by bloodfeeding flies cannot be excluded as a possible route of infection, even if the number of parasites in the blood is extremely low. Indeed, in recent experiments with cattle infected with *T. b. brucei*, in the chronic phase – when blood examinations and PCR of blood samples were negative – tsetse flies could still be infected by feeding on these animals [25]. A similar phenomenon might also occur with *T. equiperdum* that is transmitted by blood-sucking flies.

Clinical signs of dourine can provide a strong indication of the presence of the disease, as can its chronic evolution, but confirmatory diagnosis is needed. It is extremely difficult to detect the parasite in the body fluids of infected horses; therefore, diagnosis of *T. equiperdum* infection is based on serological evidence. Despite the development of antibody and antigen enzyme-linked immunosorbent assays for *T. equiperdum* [26], CFT remains the only internationally recommended test [27], although it does not distinguish clearly among *T. equiperdum*, *T. evansi* and *T. b. brucei* [28]. Indeed, because possible cross-reactions with *T. evansi* and *T. b. brucei* might occur, these parasites cannot be distinguished from *T. equiperdum* unless the test samples originate from regions that are free from *T. evansi* and *T. b. brucei*. Unfortunately, apart from South Africa and parts of Russia, countries in which dourine is currently reported often lie within the distribution area of *T. evansi*. Therefore, it is essential to develop tests that accurately differentiate dourine from surra infections in *Equidae*. To develop such tests, the available laboratory strains should first be characterized properly using different serological and molecular techniques.

Treatment

There are no officially approved drugs to treat horses suffering from dourine, although some older publications mention experimental treatment of horses with naganol and neoarsphenamine [29], or quinapyramine sulfate [30].

International regulations currently impose the slaughtering of CFT-positive horses. Nevertheless, *in vitro* sensitivity of different *T. equiperdum* strains to suramin, diminazene, quinapyramine and melarsomine has been reported [31]. Taking these results into account, it seems that it would be worthwhile to perform efficacy trials on the treatment of *T. equiperdum* infections with the drugs currently used for *T. evansi* treatment in horses (suramin and melarsomine) or camels (cymelarsan). However, without reliable diagnostic tests, it will remain difficult, if not impossible, to assess cure rates *in vivo* in such efficacy trials. Therefore, it might be useful to evaluate the *in vitro* sensitivity of the available *T. equiperdum* strains first.

Serological- and molecular-characterization studies

In the 1970s, research of the variable antigenic repertoire of one *T. equiperdum* strain (BoTat 1) defined some preferentially expressed variable antigenic types (VATs) of *T. equiperdum* [13]. The occurrence of these BoTat VATs has not yet been examined in other strains of *T. equiperdum* or trypanosome taxa (e.g. *T. evansi*). However, it has been shown that Rhode trypanosome antigenic type (RoTat) 1.2 is a predominant VAT of *T. evansi* against which antibodies can be detected in the serum of infected animals [32]. This has enabled the development of different serodiagnostic tests –based on the RoTat 1.2 variable surface glycoprotein (VSG) – that have been applied to different host species worldwide [33,34]. Experimental infection of rabbits indicates that anti-RoTat-1.2 antibodies are also generated during *T. equiperdum* infection [35]. Of 11 strains examined, only two (OVI and BoTat) did not express the RoTat 1.2 VSG during a 35-day infection of rabbits. This is an early indication that there are at least two groups within the existing *T. equiperdum* collection and that the largest group shares characteristics with *T. evansi*. Of the strains examined, only OVI had been shown to cause clinical symptoms of dourine in horses. Clinical and pathological data are lacking for all other strains. Isoenzyme analysis has been used to demonstrate differences among 12 *T. evansi* strains and one *T. equiperdum* strain (STIB 818). Of the 16 enzymes tested, only malate dehydrogenase and alanine aminotransferase were different for some *T. evansi* strains. *T. equiperdum*, however, could not be distinguished from some *T. evansi* strains [36].

Some authors suggest that the absence of maxicircles from the kinetoplast DNA of *T. evansi* is a major difference between this parasite and *T. equiperdum* [37,38]. However, because dyskinetoplastic strains exist in both *T. evansi* and *T. equiperdum*, the validity of this characteristic is questionable. Moreover, Borst *et al.* stated in 1987 that 'there is no theoretical reason why *T. evansi* strains with a defective maxicircle could not exist' [39]. Zhang *et al.* used Southern blot analysis to demonstrate differences among *T. equiperdum* (STIB 818, BoTat 1.1 and the OVI strain), *T. b. brucei* (one strain) and *T. evansi* stocks (15 strains). BoTat 1.1 and the South African OVI strain clustered out from the *T. evansi* group. Moreover, this cluster shared more similarity with *T. b. brucei* than with the *T. evansi* cluster. Another *T. equiperdum* (STIB 818) was found within the *T. evansi* group. The authors stated that this outlier STIB 818 could reflect the limit of sensitivity of Southern blot on restriction fragment length polymorphism or that it could be due to the misclassification of this strain [40]. Using the same DNA probes, a dissimilarity index of 56% was observed between one *T. equiperdum* and one

T. evansi strain. However, this cluster analysis was based on only one strain of each species and, unfortunately, the identity of the strains used was not revealed in the article [41]. Microsatellite markers did not reveal general differences between *T. equiperdum* and *T. evansi*. Of the three *T. equiperdum* stocks tested (BoTat 1.1, STIB 818 and the South African OVI strain), no species-specific alleles were found. The Chinese *T. equiperdum* (STIB 818), however, shared common alleles with the Chinese *T. evansi* tested, and the BoTat 1.1 clone had identical genotypes with four loci of the KETRI 2480 *T. evansi* strain. This study highlights the heterogeneity within the strains classified as *T. equiperdum* [42]. Internal transcribed spacer 1 analysis did not lead to species identification within the *Trypanozoon* group [43].

The variability of transferrin receptor genes (*ESAG6/7*) in *T. evansi, T. equiperdum* and *T. b. brucei* was studied by comparing several *T. b. brucei* or *T. evansi* strains with one *T. equiperdum* strain (STIB 818) [44,45]. Both studies attest that the transferrin gene sequences obtained from STIB 818 are a subcluster within the sequences of *T. b. brucei* or *T. evansi*. These studies merely confirm that multiple transferrin receptor genes are present in all genomes and that these genes are relatively conserved in the three *Trypanosoma* species. Both articles conclude that the variability of the transferrin receptor is more limited in *T. equiperdum* and that this might be related to the limited host specificity of this parasite. However, this conclusion has been contested recently by Salmon *et al.*, who proved that transferrin receptors are not host-species specific [46]. The examples that have been mentioned illustrate the problems related to the differentiation of the three species and the limited statistical power of some published results because only a few strains were compared. Because of these scattered data and the use of only a few, mostly different strains of *T. equiperdum* in the analyses, it is difficult (if not impossible) to draw meaningful conclusions about *T. equiperdum* from these observations.

Recently, a putative PCR that is specific to *T. evansi* was developed based on the RoTat 1.2 VSG. Nine of the 11 so-called *T. equiperdum* strains tested positive in this PCR [47]. Moreover, the two *T. equiperdum* strains that lacked the RoTat 1.2 *VSG* gene (BoTat and OVI) also seemed to be different from the other tested populations in random amplified polymorphic DNA (RAPD) and in multiple endonuclease genotyping approach (MEGA) (Figure 1). Indeed, they resembled *T. b. brucei* more

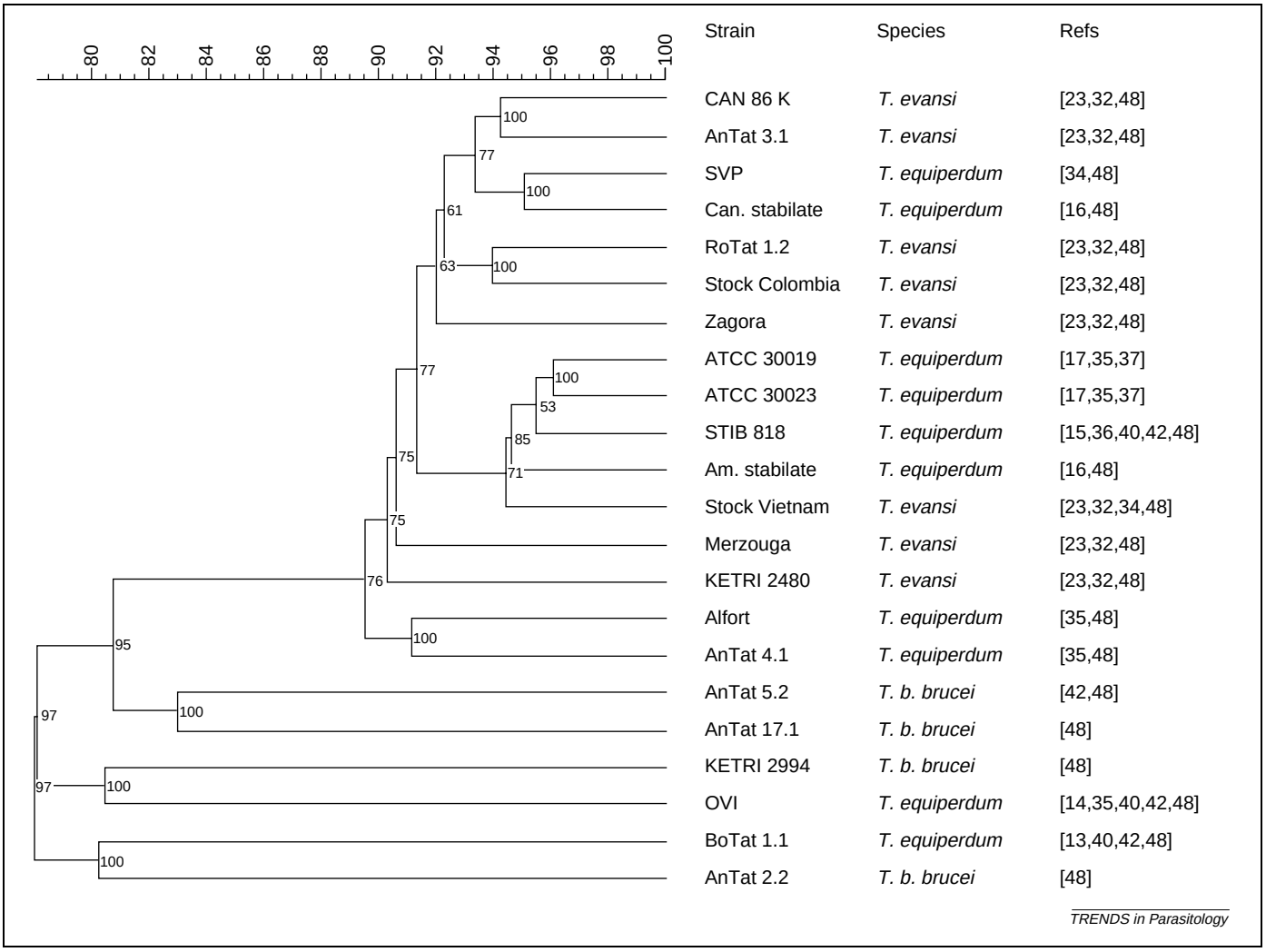

Figure 1. Unweighted pair group method with arithmetic mean homology tree. The tree (based on percentage similarity) was obtained by combining the data from RAPD 606, RAPD ILO 525 and MEGA [48]. Numbers at nodes are the percentages of 1000 bootstrap replicates in which these nodes appeared.

closely, whereas all other *T. equiperdum* strains clustered in a homogenous group of *T. evansi* [48]. This finding is in accordance with those of Zhang *et al.* [40], who also observed a different cluster for BoTat 1.1 and the OVI strain compared with the STIB 818 strain, which clustered within the *T. evansi* group. Moreover, also in functional genome analysis (microarrays), the expression profiles for *T. equiperdum* BoTat and OVI resemble those of *T. b. brucei* rather than those of *T. evansi* (F. Claes, unpublished).

From these new insights, it seems that dourine can be caused by particular strains of *T. equiperdum* that are closely related to *T. b. brucei*, and that most so-called *T. equiperdum* strains cluster serologically and molecularly within *T. evansi*. Nonetheless, one must keep in mind that, irrespective of the strain used, the clinical outcome of an infection might depend entirely on the immunological response of the host.

Concluding remarks

Published data that have been gathered using different serological and molecular methods do not enable consistent discrimination between *T. evansi* and *T. equiperdum*, and several questions remain. Recent data indicate that the *T. equiperdum* collection is not homogenous and that more attention should be paid to the differences between the so-called *T. equiperdum* strains.

Furthermore, we hypothesize that some *T. equiperdum* strains are actually *T. b. brucei* or members of a subspecies of *T. brucei (Trypanosoma brucei equiperdum)* and that all other *T. equiperdum* strains are misidentified and are, in fact, *T. evansi*. Consequently, we propose an alternative definition of dourine: a chronic contagious disease of solipeds caused by *T. b. brucei* or *T. evansi* that is transmitted directly from animal to animal during coitus or by insect vector.

This hypothesis can be investigated by performing experimental infections of horses with *T. equiperdum* and by comparing clinical signs with the pathology of confirmed *T. evansi* and *T. b. brucei* strains. Controlled experimental infections of horses, preferably with new isolates, might lead to a better insight into the pathology of dourine. Therefore, obtaining new isolates is an essential step for a better understanding of the parasite that causes this disease. The close relationship between *T. evansi* and *T. b. brucei* makes it necessary to continue the characterization of both species, in particular using molecular or serological markers for the different groups of *T. equiperdum* and *T. b. brucei*. Nevertheless, for such characterization to be successful, studies should be performed on a large collection of well-documented *T. equiperdum* strains. When a reliable diagnostic test becomes available, it will be possible to screen trypanocidal drugs for efficacy against this parasite in experimental infections.

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