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Wild chimpanzees are infected by *Trypanosoma brucei*

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ABSTRACT

Although wild chimpanzees and other African great apes live in regions endemic for African sleeping sickness, very little is known about their trypanosome infections, mainly due to major difficulties in obtaining their blood samples. In present work, we established a diagnostic ITS1-based PCR assay that allows detection of the DNA of all four *Trypanosoma brucei* subspecies (*Trypanosoma brucei brucei*, *Trypanosoma brucei rhodesiense*, *Trypanosoma brucei gambiense*, and *Trypanosoma brucei evansi*) in feces of experimentally infected mice. Next, using this assay we revealed the presence of trypanosomes in the fecal samples of wild chimpanzees and this finding was further supported by results obtained using a set of primate tissue samples. Phylogenetic analysis of the ITS1 region showed that the majority of obtained sequences fell into the robust *T. brucei* group, providing strong evidence that these infections were caused by *T. b. rhodesiense* and/or *T. b. gambiense*. The optimized technique of trypanosome detection in feces will improve our knowledge about the epidemiology of trypanosomes in primates and possibly also other endangered mammals, from which blood and tissue samples cannot be obtained.

Finally, we demonstrated that the mandrill serum was able to efficiently lyse *T. b. brucei* and *T. b. rhodesiense*, and to some extent *T. b. gambiense*, while the chimpanzee serum failed to lyse any of these subspecies.

1. Introduction

African trypanosomes of the *Trypanosoma brucei* group are the causative agents of sleeping sickness and nagana (Simarro et al., 2011). *T. brucei* has been found throughout sub-Saharan Africa and comprises three morphologically identical but genetically different subspecies, specifically *Trypanosoma brucei brucei*, *Trypanosoma brucei rhodesiense*, and *Trypanosoma brucei gambiense* (Tait et al., 2011), and *Trypanosoma brucei evansi* (Lai et al., 2008). Except the last one, these subspecies are transmitted by tsetse flies of the genus *Glossina* (Franco et al., 2014), with *T. b. gambiense* and *T. b. rhodesiense*, causing the West-African and East-African human sleeping sickness, respectively, both being lethal, yet having distinct clinical syndromes (Brun et al., 2010).

T. b. brucei is limited to animals and non-infective to humans due to trypanolytic factors found in human serum, whereas other two subspecies responsible for human trypanosomiasis, *T. b. gambiense* and *T. b. rhodesiense*, have developed mechanisms for escaping from lysis mediated by the trypanosome lytic factor (TLF) (Lugli et al., 2004; Wheeler, 2010), which is primarily composed of Apolipoprotein L1 (ApoL1) and a haptoglobin-related protein (Raper and Friedman, 2013). Both trypanosomes able to infect humans exhibit different ways of developing the TLF-mediated lysis. In the case of *T. b. gambiense*, the resistance is achieved by the interplay among a unique modification of the TLF receptor, expression of a specific TgSGP glycoprotein, and changes in lysosomal physiology (Uzureau et al., 2013). *T. b. rhodesiense* developed the serum-resistance-associated protein, which is alone sufficient to confer complete resistance to the human TLF (Xong et al., 1998; Vanhollebeke and Pays, 2010; Stephens et al., 2012). The sera of some non-human primates (baboons, sooty mangabeys, mandrills and gorillas) were shown to be capable of ApoL1-mediated killing of the flagellates, while the serum of chimpanzees showed no trypanolytic activity due to secondary loss of the ApoL1 gene (Lugli et al., 2004; Poelvoorde et al., 2004; Thomson et al., 2009, 2014). Moreover, early studies showed that experimental infections with *T. b. rhodesiense* and *T. b. brucei* mostly caused the death of untreated chimpanzees, while infections with *T. b. gambiense* were mild and did not result in apparent clinical symptoms (Baker, 1962, 1968; Baker and Taylor, 1971; Godfrey and Killick-Kendrick, 1967; Hoare, 1972).

Within recent years, with the advent of genetic characterization, molecular markers have been developed to study the genetic diversity of trypanosomes (Tait et al., 2011). These methods, such as determination of microsatellite markers for determination of allelic variations, demonstrated great diversity of the *T. brucei* group in humans due to frequent genetic exchange (Capewell et al., 2013a; Duffy et al., 2014). Recently, Echodu et al. (2015) showed the emergence of the human infective strains from the non-infective *T. b. brucei* strains of different genetic backgrounds and highlighted the importance of cattle as possible reservoir of sleeping sickness. Moreover, Capewell et al. (2013a) revealed the existence of hybrids of *T. b. gambiense* and *T. b. brucei* with novel resistance mechanism, which are able to infect humans. These facts showed the importance of research on the trypanosomatids in other mammalian host, especially free-ranging non-human primates.

Obtaining blood samples from wild African apes is complicated and in most cases ethically unacceptable (Leendertz et al., 2006). As a result, the blood and tissue samples have been collected mostly from chimpanzees and other primates that died of anthrax or respiratory diseases in the Taï National Park (NP), Ivory Coast (Leendertz et al., 2004; Kondgen et al., 2010) have been screened for the presence of *Trypanosoma* spp. Being aware of the breakthroughs in our understanding of the evolution of human *Plasmodium* species precipitated by their detection in feces of African apes (Kaiser et al., 2010), we designed an assay capable of amplifying trypanosome DNA from this material.

2. Methods

2.1. Ethics statement

General permission for sample collection from deceased wild primates was obtained from the authorities in charge of Côte d'Ivoire, Guinea, Uganda, and Democratic Republic of Congo. Deceased animals were found during the course of a long term project focused on the behavior and infectious diseases in wild primates (Leendertz et al., 2006). No animal was anaesthetized or handled for the sole purpose of sample collection. Fecal samples were collected without disturbing the animals within 2–3 min after defecation.

All samples from African sanctuary-living wild-born great apes were collected during routine health checks by the sanctuary on-site veterinarians. Since no animal was sampled specifically for this study, approval from the relevant institutional committee was not needed. All samples were collected according to the guidelines - Chimpanzee sanctuaries: guidelines and management workshop report (available at http://pages.ucsd.edu/~jmoore/courses/methp rimconsweb08/chimpsanct.pdf; date of access to website in 2004).

All sera from animals living in Czech zoological gardens were collected during preventive veterinary health checks. No animal was sampled specifically for this study. All samples were collected in accordance with legal requirements of the Czech Republic (Act no. 161/1992) and with the rules of the respective zoos. Samples collected during necropsies of primates that died from various causes in zoos and primate facilities were also included.

Imports of samples from free-ranging chimpanzees proceeded according to German veterinary regulations for import of organic materials. Tissue and blood samples were exported with the appropriate CITES permissions from the respective country and Germany. No permit was needed for transport between laboratories in Germany and Czech Republic as both are members of European Union. The experimental infection of mice was approved by the Ethical Committees of the Czech Ministry of Education and Biology Centre (no. 90/2013).

2.2. Detection of trypanosomes in feces of experimentally infected mice

Four laboratory mice were infected with *T. b. brucei* (STIB 920), *T. b. rhodesiense* (Etat 1.2 R variant), *T. b. gambiense* (LiTat 1.3) and *T. b. evansi* (STIB805) by intraperitoneal inoculation of 10^5 bloodstream stages (trypomastigotes), and the course of infection was controlled by searching for the flagellates in blood smears obtained as a drop from the tail. First search for the presence of parasites was performed 72 h post-infection and continued until trypomastigotes appeared in the blood every 8 h. At that time, fecal and blood samples were collected and used for isolation of total DNA using protocols described below.

Blood samples were placed on a filter paper for ethanol evaporation and total DNA was isolated by incubation in a 200 μL volume with Chelex 100 (Sigma–Aldrich, St. Louis, MO, USA) (final concentration 5%) at 56 °C for 1 h, followed by boiling for 10 min. Before use, samples were centrifuged for 1 min at 15,000 rpm, and 2.5 μL of supernatant was used for PCR. DNA from fecal samples was isolated by a commercial stool kit (Qiagen, Venlo, Limburg, Netherlands). PCR and sequencing were performed for each sample at least in triplicate and, to avoid contamination, in a laboratory that does not work with trypanosomes, always with positive and negative controls included.

A nested approach was used to amplify a region of the Internal Transcribed Spacer 1 (ITS-1) of *Trypanosoma* spp. (~180-640 bp). Primers Tryp_3 (5′- TGCAATTATTGGTCGCGC -3′) and Tryp_4 (5′- CTTTGCTGCGTTCTT -3′) were used for the first round of PCR, while internal primers Tryp_1 (5′- AAGCCAAGTCATCCATCG -3′) and Tryp_2 (5′- TAGAGGAAGCAAAAG -3′) were used for the second round of PCR (Adams et al., 2006). PCR was performed with Taq-polymerase (TopBio, Prague, Czech Republic) using the following program: 1 min at 95 °C, 35 cycles of 1 min at 94 °C, 1 min at 54 °C, 30 s at 72 °C, and 5 min at 72 °C, and PCR products were resolved in ethidium-bromide stained agarose gels.

2.3. Collection of primate samples

Tissue samples were obtained from carcasses found mainly in Taï NP, Côte d'Ivoire. Furthermore, necropsy samples were collected

from 93 wild primates from the Democratic Republic of Congo and Uganda. Additional 16 tissue samples were obtained from sanctuaries from Gambia, Guinea, Sierra Leone, Cameroon, Republic of Congo, Uganda, and two German zoos (Table 1). Finally, 13 fecal samples were collected without disturbing the animals within 2–3 min after defecation and fixed in 96% ethanol.

DNA extractions and PCR were performed in physically isolated facilities. DNA from tissue samples and feces was extracted using the DNAeasy tissue kits (Qiagen, Venlo, Limburg, Netherlands) and the EURx Gene Matrix Stool Kit (Roboklon), respectively. All amplicons were gel-purified using the Gel Extraction Kit (Qiagen, Venlo, Limburg, Netherlands) and cloned in TOPO TA Cloning (Invitrogen, Carlsbad, CA, USA) as specified by the manufacturer.

Samples positive for ITS1 in nested PCR reactions were further analyzed for the presence of the ~900 bp-long *T. b. gambiense* glycoprotein (TgSGP) gene, again via nested PCR, using outer primers 65-F (5′-GTGGCAATTACTAGCAATAGCG-3′) and 66-R (5′-GCCATCGTGCTTGCCGCTC-3′), and inner primers 61-F (5′-TCACGGCCATCAGACGGAGA-3′) and 62-R (5′-GGGCTCCTGCCTCAATTGCTGCA-3). Conditions were the same for both rounds: 2 min at 95 °C, 24 cycles of 1 min at 95 °C, 1 min at 55 °C, 2 min at 72 °C, and 2 min at 72 °C. As a positive control, DNA from blood of mouse experimentally infected with *T. b. gambiense* (LiTat 1.3) was used.

2.4. Phylogenetic analysis

Highly polymorphic ITS1 sequences obtained by nested PCR from primate fecal and tissue samples were aligned with Clustal-X (ver. 2.0; gap opening penalty 12; gap extension penalty 5) and neighbor joining clustering with K2P distances was performed on the unmodified alignment using PAUP (4.0, beta version). The final alignment included 452 characters. The accession numbers of sequences retrieved from the GenBank (*Trypanosoma* sp. ex Wildebeest JN673403, for *Trypanosoma theileri* JX178185, HQ664848, and HQ664849) and used in phylogenetic reconstructions and ITS1 alignment are available from the authors upon request.
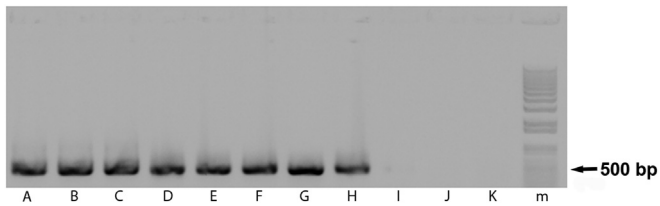
2.5. Trypanosome lysis in primate sera

We used fresh sera from five non-human primate species (barbary macaque [*Macaca sylvanus*], mandrill [*Mandrillus sphinx*], spider monkey [*Ateles geoffroyi*], chimpanzee [*Pan troglodytes*], and Sumatran orangutan [*Pongo abelii*]) from the Plzeň, Liberec and Prague zoos, Czech Republic. At final concentration of 10% the sera were added to trypanosomatids cultivated in either HMI-9 or M199 medium with 10% fetal bovine serum (FBS), when concentration reached 5×10^5 cells/ml. The following isolates were used: *T. b. brucei* (STIB 920), *T. b. rhodesiense* (Etat 1.2 R variant), and *T. b. gambiense* (LiTat 1.3). Fresh normal human serum (NHS) obtained from a Central European individual and FBS were used as positive and negative controls, respectively. Trypanosomatids were cultivated in triplicate with the various primate sera for 24 h at 37 °C. After this period, resazurin (Sigma–Aldrich, St. Louis, MO, USA) was added to 1 ml of each culture (final concentration 58 pM) and cell viability was measured after 24 h (i.e. 48 hrs after the cells were mixed with primate sera) in triplicate using Tecan Infinite M200PRO under emission at 590 nm and excitation at 560 nm, and the results were analyzed using Anova. The relative survival was calculated as a ratio in a given serum compared to FBS.
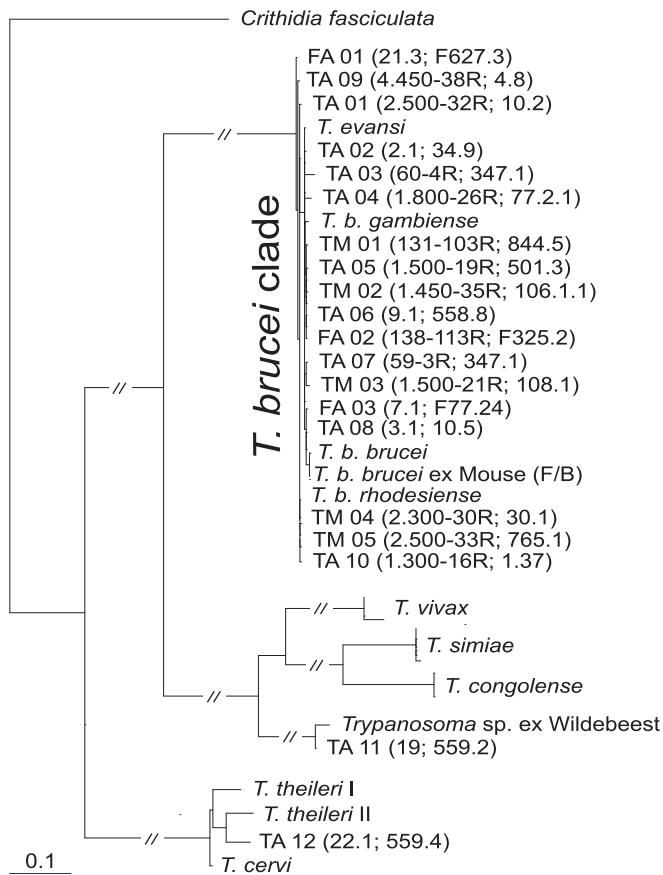
3. Results

3.1. Detection of trypanosomes in feces of experimentally infected mice

Using the nested PCR protocol, the ITS1 region of *T. b. brucei*, *T. b. gambiense*, *T. b. rhodesiense* and *T. b. evansi* was successfully amplified from DNA isolated from fecal samples of infected mice, with relevant controls being negative (Fig. 1). Single abundant amplicons were of expected size (~450 bp) and sequence (Fig. 2). This experiment proved that under our experimental conditions, the flagellate DNA can be reliably detected in feces of laboratory mice using a 35 cycle PCR protocol.

Table 1
Summary of primate samples investigated for the presence of trypanosomes.

Site (country)	Primate species	Origin	Examined/T-pos. Individuals (%)	Examined/T-pos. samples (%)	DNA source of T-pos. samples
Taï NP (Côte d'Ivoire)	Western chimpanzee	Wild	16/8	28/11 (39)	Spleen (4×), liver (3×), lymph node (1×); feces (3×)[a]
	Sooty mangabey	Wild	10/1	10/1 (10)	Liver
	Western red colobus	Wild	18/2	18/2 (11)	Blood
	Black-and-white colobus	Wild	5/0	5/0	—
River Gambia NP (Gambia)	Chimpanzee	Semi-captive	1/1	2/2 (100)	Spleen, liver[a]
Chimpanzee Sanctuary (Guinea)	Western chimpanzee	Captive	1/1	2/1 (50)	Liver
Budongo NP (Uganda)	Eastern chimpanzee	Wild	9/1	9/1 (11)	Liver
Kasyoha-Kitomi FR (Uganda)	Eastern chimpanzee	Wild	1/0	1/0	—
Lui Kotale (DRC)	Western red colobus	Wild	2/2	5/2 (40)	Spleen
ND	Pennant's red colobus	Wild	3/0	3/0	—
Limbe Wildlife Centre (Cameroon)	Western lowland gorilla	Semi-captive	3/0	5/0	—
ND (Kongo)	Western lowland gorilla	Captive	1/0	1/0	—
ND (Kongo)	Chimpanzee	Captive	1/0	1/0	—
Ngamba (Uganda)	Chimpanzee	Semi-captive	27/0	27/0	—
Tacugama (Sierra Leone)	Chimpanzee	Semi-captive	6/0	8/0	—
Limbe Wildlife Centre (Cameroon)	Chimpanzee	Captivity	1/0	1/0	—
Munster Zoo (Germany)	Colobus monkey	Captivity	3/0	4/0	—
Frankfurt Zoo (Germany)	Western lowland gorilla	Captivity	1/0	2/0	—
Suma			109/16 (15%)	132/20 (15%)	

T-pos. — *Trypanosome*-positive; NP — National Park; FR — Forest Reserve; % — prevalence; sanctuary ND — not determined (from sanctuary); DRC — Democratic Republic of Congo. Western chimpanzee — *Pan troglodytes verus*, Eastern chimpanzee — *Pan t. schweinfurthii*, Chimpanzee — *Pan troglodytes*, Western lowland gorilla — *Gorilla gorilla gorilla*, Western red colobus — *Piliocolobus badius*, Pennant's red colobus — *P. pennantii*, Black-and-white colobus — *Colobus polykomos*, Sooty mangabey — *Cercocebus atys*.
[a] Several samples were collected from one individual.

Fig. 1. ITS1-based detection of trypanosomes in blood and feces of experimentally infected mice. (A–D, I) detection in blood; (E–H, J) detection in feces. (A, E) *Trypanosoma b. brucei*; (B, F) *T. b. gambiense*; (C, G) *T. b. rhodesiense*; (D, H) *T. b. evansi*; (I) blood from a non-infected mouse; (J) feces from a non-infected mouse; (K) negative control; (m) marker.

Fig. 2. ITS1-based dendrogram of trypanosomes from primate tissue and fecal samples. Sequences generated in this study are marked as follows: T – tissue samples of apes (TA) and monkeys (TM); F – fecal samples of apes (FA); sequences retrieved from GenBank are labeled with Latin names (*Trypanosoma* sp. ex Wildebeest JN673403, for *T. theileri* JX178185, HQ664848, and HQ664849).

3.2. Detection of trypanosomes in primates and phylogenetic analysis

Overall, 132 samples composed of 119 various tissues and 13 feces, collected from 109 individual primates belonging to six species were subjected to diagnostic PCR with trypanosome-specific primers (Tables 1 and 2). Nested PCR protocol established to amplify ITS1 from the fecal-derived DNA of laboratory-infected mice (Fig. 1) was applied to DNA isolated from the tissue and fecal samples. The sequences of amplicons obtained from 17 primate tissue samples were subjected to BLAST analysis, aligned and grouped in a dendrogram, which showed that our sequences belong to the *T. brucei* group (Fig. 2, see below).

Since in the experimentally infected mice, the ITS1-specific primers were able to amplify the target region of trypanosomes from the fecal samples, we have performed the same PCR on DNA isolated from fecal samples of wild chimpanzees. Out of 13 freshly collected feces, three were positive for trypanosome DNA (Fig. 2), as confirmed by sequencing. DNA isolated from the same samples and analyzed independently in another laboratory by the same method lead to identical results.

Next, we have generated an alignment from all ITS1 sequences (for detail see Table 2) amplified from the tissue samples of chimpanzees (number of samples = 12), sooty mangabeys (n = 1) and Western red colobus monkeys (n = 4), and also from the feces of chimpanzees (n = 3) (Table 2). The dataset was complemented with relevant sequences available in GenBank, with *Crithidia fasciculata* used as outgroup. With two exceptions (TA11 and TA12, see Fig. 2), all newly obtained sequences fell into the *T. brucei* clade, as shown by a neighbor joining dendrogram (Fig. 2). The *T. brucei* clade contains sequences from all known subspecies and is characterized by extremely short branches, which is not surprising given the known minimal differences among individual subspecies. A total of 15 sequences obtained from the tissues of chimpanzees, Western red colobus monkeys and a sooty mangabey fell into this clade, as well as three sequences obtained from the feces of three chimpanzees, providing strong evidence for the infections by the *T. brucei* group parasites (Table 2; Fig. 2).

All our attempts to amplify the TgSGP gene specific for *T. b. gambiense* (Capewell et al., 2013b) from the tissues and feces of apes positive for *Trypanosoma* ITS1 PCR assay invariably failed, although this gene can be easily amplified from feces and blood of mice infected with this *T. brucei* subspecies (Supplementary data 1). Out of 18 positive samples belonging to the *T. brucei* clade, two samples, one from a spleen and second one from a lymph node of two post-mortem dissected wild Western chimpanzees originating from Taï NP, Côte d'Ivoire, fell outside of the *T. brucei* clade (Table 2; Fig. 2). While TA 11 is almost identical with an unnamed *Trypanosoma* sp. (Access. No. JN673403) from a wildebeest (*Connochaetes*) captured in Serengeti NP, Tanzania, TA 12 from an adult female chimpanzee from Taï NP is strongly affiliated with *T. theileri* (Fig. 2).

Due to the limited information residing in the obtained ITS1 region, multiple attempts to amplify (fragments of) the SSU rRNA gene of the above-described pathogens were performed seeking to establish more precisely their species status. Unfortunately, these attempts almost invariably failed. However, in the case of the TA 12 tissue sample, a fragment of the SSU rRNA gene was successfully amplified which allowed us to assign the respective parasite with high confidence to *T. theileri*.

3.3. Trypanolytic capacity of primate sera

Only mandrill and human sera (NHS) efficiently lysed *T. b. brucei*, with relative survival 0.101 ± 0.005 (mean ± SD; p < 0.001) and 0.068 ± 0.074 (p < 0.001), respectively (Supplementary data 2). When the cultured *T. b. gambiense* cells were subjected to the panel of different sera, the lytic factor allowing their limited killing was present only in the mandrill's serum (0.488 ± 0.087; p < 0.001). As anticipated, NHS failed to lyse *T. b. gambiense*, with a relative survival of 0.886 ± 0.170 (p > 0.05) (Supplementary data 2), while this pathogen also prospered in the other sera. The cultured *T. b. rhodesiense* cells were very efficiently lysed by the mandrill serum (0.138 ± 0.001; p < 0.001), with the sera of spider monkey and orangutan showing a moderate lysis (0.837 ± 0.054, p < 0.01 and 0.848 ± 0.067, p < 0.01 respectively). Under our conditions, NHS caused a very minor yet significant lysis as well (0.874 ± 0.075; p < 0.05).

Table 2
List of primate samples positive for *Trypanosoma* spp. DNA.

Primate species	DNA source	Country/origin	Label of ITS1 sequence	GenBank no.
Western chimpanzee[a]	Spleen	Côte d'Ivoire/wild	TA 01 (2.500-32R; 10.2)	KR092349
Western chimpanzee	Spleen	Côte d'Ivoire/wild	TA 06 (9.1; 558.8)	KR092354
Western chimpanzee	Spleen	Côte d'Ivoire/wild	TA 10 (1.300-16R; 1.37)	KR092358
Western chimpanzee	Spleen	Côte d'Ivoire/wild	TA 12 (22.1; 559.2)	KR092365
Western chimpanzee	Liver	Côte d'Ivoire/wild	TA 04 (1.800-26R; 77.2.1)	KR092352
Western chimpanzee[a]	Liver	Côte d'Ivoire/wild	TA 08 (3.1; 10.5)	KR092356
Western chimpanzee	Liver	Côte d'Ivoire/wild	TA 09 (4.450-38R; 4.7)	KR092357
Western chimpanzee	Liver	Republic of Guinea/captive	TA 02 (2.1; 34.9)	KR092350
Western chimpanzee	Lymph node	Côte d'Ivoire/wild	TA 11 (19; 559.4)	KR092364
Western chimpanzee	Feces	Côte d'Ivoire/wild	FA 01 (21.3; F627.3)	KR092346
Western chimpanzee	Feces	Côte d'Ivoire/wild	FA 02 (138-113R; F325.2)	KR092347
Western chimpanzee	Feces	Côte d'Ivoire/wild	FA 03 (7.1; F77.24)	KR092348
Eastern chimpanzee	Liver	Uganda (Budongo NP)/wild	TA 05 (1.500-19R; 501.3)	KR092353
Chimpanzee[a]	Spleen	Gambia/semi-captive	TA 03 (60-4R; 347.2)	KR092351
Chimpanzee[a]	Liver	Gambia/semi-captive	TA 07 (59-3R; 347.1)	KR092355
Sooty mangabey	Liver	Côte d'Ivoire/wild	TM 01 (131-103R;844.5)	KR092359
Red colobus	Spleen	DRC/wild	TM 02 (1.450-35R; 106.1.1)	KR092360
Red colobus	Spleen	DRC/wild	TM 03 (1.500-21R; 108.1.)	KR092361
Western red colobus	Blood	Côte d'Ivoire/wild	TM 04 (2.300-30R; 30.1)	KR092362
Western red colobus	Blood	Côte d'Ivoire/wild	TM 05 (2.500-33R; 765.1)	KR092363

[a] The same individual.

4. Discussion

Within the last decade it has become abundantly clear that African great apes play a significant role in evolution, emergence, and transmission of a range of infectious diseases important for human populations. Simian immunodeficiency viruses and apicomplexan parasites of the genus *Plasmodium* are among the best known examples (Liu et al., 2010; Calvignac-Spencer et al., 2012; Liu et al., 2014). Although the wild chimpanzees and other African great apes live in regions endemic for African sleeping sickness, the research on their trypanosome infections has been neglected. This situation is mostly caused by the virtually insurmountable difficulties in obtaining blood samples from free-ranging animals and ethical unacceptability of experimental infections on captive animals.

Interestingly, African primates exhibit a widely different degree of resistance against parasites of the *T. brucei* group (Wheeler, 2010). In the literature, there are only very few notes on flagellates found in the blood of dead free-ranging chimpanzees, without any evidence that their death was a consequence of trypanosomiasis (Hoare, 1972). Early experimental infections of chimpanzees with different subspecies of *T. brucei* led to controversial results: while *T. b. brucei* and *T. b. rhodesiense* resulted in fatal parasitemia (Baker, 1962, 1968; Godfrey and Killick-Kendrick, 1967; Baker and Taylor, 1971), *T. b. gambiense* did not cause any obvious pathology despite the presence of trypomastigotes in the cerebro-spinal fluid (Godfrey and Killick-Kendrick, 1967; Hoare, 1972). This suggests that chimpanzees might serve as reservoir hosts for *T. b. gambiense*. Moreover, recent results demonstrated the existence of trypanosomatid hybrids among some subspecies of the *T. brucei* group, which have various animal reservoirs and are capable of infecting humans (Tait et al., 2011; Capewell et al., 2013a; Duffy et al., 2014; Echodu et al., 2015). Hence, addressing the question whether the free-ranging non-human primates may serve as a host or reservoir for some of these trypanosomes is relevant.

Inspired by recent advances in our understanding of mechanisms of resistance to trypanosomes mediated by the TLF (Raper and Friedman, 2013), we performed the experiments, in the frame of which three subspecies of the *T. brucei* group were incubated with sera from mandrill, barbary macaque, spider monkey, orangutan and chimpanzee. The obtained results on *T. b. brucei* and *T. b. gambiense* are in concordance with previous experiments of Lugli et al. (2004), though we also demonstrated that the serum from a mandrill is able to also lyse *T. b. gambiense*. Furthermore, the chimpanzee sera failed to lyse *T. b. brucei*, *T. b. rhodesiense* and also *T. b. gambiense*. However, results obtained with the latter subspecies are in contrast with the early infection experiments (Baker, 1962, 1968; Godfrey and Killick-Kendrick, 1967; Baker and Taylor, 1971), perhaps due to the fact that those have been conducted on animals originating from nature. This indicates that wild chimpanzees could have a (partial) resistance to *T. b. gambiense*, especially in the endemic regions. Our experiment confirmed the notion that chimpanzees are fully susceptible to *T. b. brucei*, against which humans and other primates developed efficient resistance (Xong et al., 1998; Raper and Friedman, 2013; Uzureau et al., 2013; Thomson et al., 2014). These results suggest that the transmission of any of the *T. brucei* group flagellates into the blood of chimpanzees shall result into an infection.

Inspired by successful detection of the *Plasmodium* DNA in primate feces (Liu et al., 2010; Jirků et al., 2012), we proved the ability of ITS1-based PCR assay to amplify the DNA of all four *T. brucei* subspecies in feces of experimentally infected mice. PCR-based screening of the available set of fecal samples from wild chimpanzees revealed the presence of trypanosomes, and this finding was further supported by positive results from a number of tissue samples taken from dead chimpanzees. By allowing the detection of trypanosomes in feces, this assay overcomes the inaccessibility of blood and tissue samples from animals that are endangered in the wild. This detection method provides new opportunities for studies of trypanosomatid parasites in the African great apes, and potentially in other rare or highly endangered mammals.

Our dendogram of the ITS1 region showed that absolute majority of the obtained sequences fell into the robust *T. brucei* clade, providing strong evidence that these infections were caused by one of its subspecies. However, since all attempts to amplify *T. b. rhodesiense* and *T. b. gambiense*-specific DNA regions failed, the subspecies status could not be established. In any case, the introduction of a PCR assay, capable of amplifying trypanosome DNA from the feces of great apes and potentially other infected mammals, will allow non-invasive large-scale screening for these important parasites. In summary, via feces- and tissue-based PCR assays, we have demonstrated that chimpanzees are frequently infected with trypanosomes from the *T. brucei* group and rarely also by other species. Future research might shed light on how

chimpanzees control trypanosomiasis, and whether transmission occurs among them, other primates and humans.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijppaw.2015.05.001.

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