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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).

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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/methprimconsweb08/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