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Prevalence of *Troglodytella abrassarti* Brumpt and Joyeux, 1912 in Wild Chimpanzees (*Pan troglodytes schweinfurthii*) at Mahale Mountains National Park in Western Tanzania

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ABSTRACT: We examined stool samples for trophozoites of the entodiniomorphid ciliate *Troglodytella abrassarti* Brumpt and Joyeux, 1912, from a habituated group of chimpanzees (*Pan troglodytes schweinfurthii*) at Mahale Mountains National Park in western Tanzania. In our study, fresh fecal samples from identified individuals were collected immediately after defecation and fixed in 10% formalin. In total, 52 samples from 38 chimpanzees (61% of 62 chimpanzees in the group) were examined using a direct smear method. A stool sample from an individual collection date from an individual chimpanzee was examined up to 3 separate times before it was called negative. Forty-eight (92%) of the 52 samples were positive, and stools from 37 (97%) of the 38 chimpanzees were positive for trophozoites of *T. abrassarti*. The high prevalence of *T. abrassarti* in these chimpanzees is consistent with previous reports of this organism in chimpanzees.

Troglodytella abrassarti Brumpt and Joyeux, 1912 is an entodiniomorphid ciliate often observed in the feces of primates (Goussard et al., 1983; O'Donoghue et al., 1993). It lives in the large intestine of its host and has been suggested to be beneficial in digesting fibrous material (Irbis et al., 2008). Recent studies using the small subunit rRNA gene and internal transcribed spacer rDNA spacer region have demonstrated that the organisms from gorillas (*Gorilla gorilla gorilla*) and chimpanzees are the same (Modry et al., 2009). The organism is usually considered a commensal, but it has been associated with diarrhea in siamangs (*Hylobates syndactylus*) from a zoo in Australia (O'Donoghue et al., 1993).

Our study subjects were chimpanzees, *Pan troglodytes schweinfurthii*, at the Mahale Mountains National Park in western Tanzania. Beginning in March 1966, habituation efforts were initiated by researchers from Japan to attract chimpanzees from 1 unit-group, referred to as the M-Group, allowing human observers to approach them in their natural habitat (Nishida, 1990). The purpose of the present study was to examine stools from chimpanzees in this population for the prevalence of trophozoites of *T. abrassarti*.

We examined 52 stool samples from 38 individual chimpanzees (61%) from M-Group (n = 62 chimpanzees). Males (14) and female (24) chimpanzees were sampled from 4 age groups, i.e., 2 infants (0–3 yr), 6 juveniles (4–8 yr), 9 adolescents (9–14 yr), and 21 adults (≥15 yr). A single sample was examined from 26 of the chimpanzees, samples from 10 chimpanzees were examined from 2 different collection days, and samples from 2 chimpanzees were examined from 3 different collection days. Samples were collected from June and August 2005, and fresh stool samples from identified individuals were collected immediately after defecation. Stool consistencies (loose = soft or not well formed; firm = well formed) were recorded. Only that part of fecal material not in contact with the forest floor was collected. A portion of the sample was used to examine for the presence of occult blood (Hemoccult® Fecal Occult Blood Test; Beckman Coulter, Fullerton, California). Another portion of the stool sample was placed in a weigh boat and thoroughly mixed with a clean wooden applicator stick. Approximately, 10 g of stool were placed in a 50-ml sterile tube containing 10 ml of 10% buffered formalin solution and mixed vigorously by shaking. Samples were stored at 30 C until shipped to the Department of Biomedical Sciences and Pathobiology (DBSP), Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia. An approximate 8-ml aliquot of the sample was placed in a 15-ml plastic-screw-capped centrifuge tube and kept at 4 C until examined.

The stool solution was vigorously shaken by hand, and a drop was placed on a glass microscope slide and covered with a 22-mm^2 coverslip. The entire coverslip was scanned at ×10 using an Olympus BX60 microscope (Olympus America Inc., Center Valley, Pennsylvania) by the same observer (DSL). The observer was blind to the identity of each stool sample. Identification of *T. abrassarti* trophozoites was confirmed by using the ×40 objective of the microscope to see structural features consistent with this organism (O'Donoghue et al., 1993). The length of *T. abrassarti* trophozoites was determined using a calibrated ocular micrometer and a ×40 objective. Additional drops of stool suspension were examined up to a maximum of 3 times on a sample from an individual subject before a subject was considered negative.

Forty-eight (92%) of the 52 stool samples contained trophozoites (Figs. 1–3) of *T. abrassarti*. Formalin-fixed trophozoites (N = 47) of *T. abrassarti* measured 150 ± 24 μm (range = 93 to 214 μm). The adoral ciliature, somatic ciliature (Fig. 1), and L-shaped macronucleus (Fig. 2) were not always readily visible, but trophozoites were easily recognized because of their large size and the posterior protuberance (Fig. 3). Twenty-five (96%) of the 26 stool samples from chimpanzees examined only on 1 occasion were positive for trophozoites of *T. abrassarti*. The single sample negative for trophozoites of *T. abrassarti* was from an adult female. Stool samples from 8 (80%) of 10 chimpanzees examined on 2 occasions were positive for trophozoites of *T. abrassarti* on both examination dates. Samples from 1 chimpanzee were negative on the first examination date but positive on the second examination date, and samples from another chimpanzee were positive on the first examination date and negative on the second. Trophozoites of *T. abrassarti* were detected in both chimpanzees that were examined on 3 separate occasions. Stool samples from 1 of these chimpanzees were positive on the first examination, negative on the second, and positive on the third, whereas all samples from the second chimpanzee that was examined on 3 occasions were consistently positive.

Stool consistency was reported on 22 of the 52 samples. Of the 22 samples, 21 contained trophozoites of *T. abrassarti*, of which 3 of the 21 were loose; 1 of these 3 stool samples was positive for occult blood. The other 18 of the 21 positive samples (86%) were firm, of which 8 (of the 18, or 44%) were positive for occult blood. The 1 stool sample that was negative for trophozoites of *T. abrassarti* was firm and occult blood negative.

The trophozoites of *T. abrassarti* in the present study (Figs. 1–3) ranged from 93 to 214 μm in size and averaged 150 μm in length. This is larger than the average length of 134 μm (range 100 to 180 μm; N = 50) for trophozoites of *T. abrassarti* reported by O'Donoghue et al. (1993) from captive siamangs (*H. syndactylus*) in an Australian zoo. It is possible that these differences in length are because of the different methods used to examine samples. O'Donoghue et al. (1993) examined samples that had been processed by protargol impregnation, and we examined formalin-fixed samples. It is also possible that the 2 different hosts (*P. t. schweinfurthii* vs. *H. syndactylus*) have different species of *Troglodytella*. Additionally, the wide range in measurements of trophozoites (93 to 214 μm) of *T. abrassarti* suggests that more than 1 species of *Troglodytella* may be present in chimpanzees. Further genetic analysis is needed to clarify the taxonomy of this group of entodiniomorphid ciliates.

There have been a few studies examining the prevalence of *T. abrassarti* in chimpanzees in their natural habitat (File et al., 1976; Ashford et al., 2000; Murray et al., 2000; Krief et al., 2005; Muehlenbein, 2005). Trophozoites of *T. abrassarti* were observed in 24 (75%) of 32 wild chimpanzees from Gombe National Park, Tanzania, using the formalin-ether concentration technique (File et al., 1976). *Troglodytella abrassarti* trophozoites were observed in chimpanzees from all age groups (infant, juvenile, adolescent, and adult) examined (File et al., 1976). In another study of chimpanzees and baboons, *Papio cynocephalus anubis*, from Gombe National Park, Tanzania, trophozoites of *T. abrassarti* were

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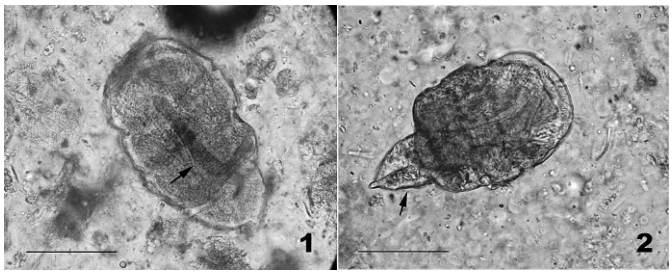

FIGURE 1. Trophozoite of *Troglodytella abrassarti* in nonstained fecal smears. Trophozoite demonstrates an L-shaped macronucleus (arrow). Note the somatic ciliature is not readily visible. Bar = 50 μm.

FIGURE 2. Trophozoite of *Troglodytella abrassarti* in nonstained fecal smears. The trophozoite demonstrates a prominent posterior protuberance (arrow). Bar = 50 μm.

observed in 3 (14%) of 20 samples from chimpanzees and none of 35 samples from baboons scrutinized using the direct smear (Murray et al., 2000). Ashford et al. (2000) examined 123 stool samples from 45 chimpanzees from the Kanyawara area of the Kibale National Park, Uganda, and found that 98% of the 45 stool specimens from individual chimpanzees sampled were positive for trophozoites of *T. abrassarti*. They reported that 91% of the 123 total samples were positive for trophozoites of *T. abrassarti* (Ashford et al., 2000). In another study of chimpanzees from the Kanyawara area of the Kibale National Park, Uganda, Krief et al. (2005) found that 111 (61%) of stool samples from 38 chimpanzees were positive for trophozoites of *T. abrassarti* during the dry season, and 50 (77%) of 65 stool samples collected during the rainy season were positive for trophozoites of *T. abrassarti*. They based their results on examination of 200 μl of formalin-fixed stool sample from each animal. Muehlenbein (2005) conducted a study on 121 stool samples from 37 male chimpanzees from the Ngogo area of Kibale National Park, Uganda, using the formalin-ethyl acetate sedimentation technique. They found that 97% of the 37 chimpanzees were positive for trophozoites of *T. abrassarti*, while 84% of the 121 total samples from these chimpanzees were positive for trophozoites of *T. abrassarti*. Our study is in agreement with the findings of Ashford et al. (2000) and Muehlenbein (2005), indicating that chimpanzees are frequently hosts for trophozoites of *T. abrassarti*. It should be noted that Murray et al. (2000) were not focusing on the prevalence of *T. abrassarti* in their study and used only a direct smear to detect infections; this may explain the lower prevalence of 20% (3 of 20) of positive chimpanzees that they observed.

Methods that rely on fecal flotation are unlikely to detect trophozoites of *T. abrassarti* as demonstrated by Krief et al. (2005). They examined 239 fecal samples from 38 chimpanzees from the Kibale National Park, Uganda, by the McMaster method using $MgSO_4$, and found no positive samples for trophozoites of *T. abrassarti* (Krief et al., 2005). In the present study, the only chimpanzee that had no detectable trophozoites of *T. abrassarti* in its stool sample was examined just once. We showed that stool samples from different collection days revealed 100% prevalence of trophozoites of *T. abrassarti* in the 12 chimpanzees that were examined on multiple occasions. Because of these findings, we recommend that 3 stool samples should be found to be negative from an individual chimpanzee before it can be considered truly negative for trophozoites of *T. abrassarti*.

Because we did not examine stool samples for other pathogens, we can make no definitive comments on the association of *T. abrassarti* infection and the presence of blood in the stool or loose stool in the chimpanzees examined. However, our results do suggest that the presence of trophozoites of *T. abrassarti* is not associated with blood in the stool or loose stools.

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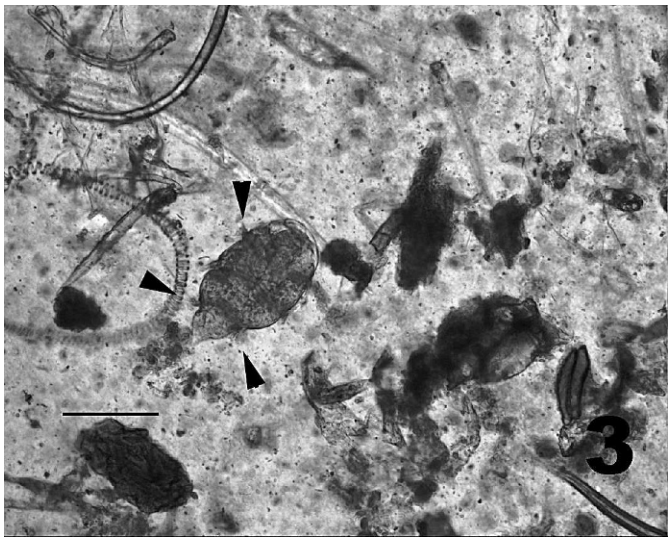

FIGURE 3. Trophozoite of *Troglodytella abrassarti* in nonstained fecal smear. The arrowheads point to somatic ciliature that is visible on this specimen. Bar = 100 μm.

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