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ANTHROPOZOONOTIC *GIARDIA DUODENALIS* GENOTYPE (ASSEMBLAGE) A INFECTIONS IN HABITATS OF FREE-RANGING HUMAN-HABITUATED GORILLAS, UGANDA

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ABSTRACT: To facilitate ecotourism and research, free-ranging mountain gorillas of Uganda have been habituated to humans. Testing of fecal samples of gorillas (n = 100), people sharing gorilla habitats (n = 62), and local pre- and postweaned cattle (n = 50) having access to these habitats with fluorescein isothiocyanate–conjugated monoclonal antibodies revealed *Giardia duodenalis* cysts at prevalences of 2, 5, and 10%, respectively. The identification of *G. duodenalis* was confirmed by fluorescent in situ hybridization with 2 species-specific 18-bp oligonucleotide probes conjugated to hexachlorinated 6-carboxyfluorescein. The mean pathogen concentration was 2.5, 2.8, and 0.2 × 10^4 cysts/g of the gorilla, people, and cattle feces, respectively. All cyst isolates aligned with genotype (assemblage) A, as confirmed by polymerase chain reaction amplification and sequencing of a 130-bp region near the 5′ end of the small subunit–ribosomal RNA gene. A single genotype (assemblage) A recovered from 3 genetically distant but geographically united host groups indicates anthropozoonotic transmission of *G. duodenalis*. A large percentage of the local community does not follow park regulations regarding the disposal of their fecal waste, as self-reported in a questionnaire. This genotype may have been introduced into gorilla populations through habituation activities and may have then been sustained in their habitats by anthropozoonotic transmission.

Giardia spp. are protozoan enteric parasites of vertebrates with an infectious stage, the cyst, transmitted via the fecal–oral route and feces-associated contamination, e.g., via food and water (Thompson et al., 1993). There are 5 morphologically distinct species: *G. duodenalis* (syn. *G. lamblia*, *G. intestinalis*) (mammals), *G. muris* (rodents), *G. agilis* (amphibians) (Filice, 1952), *G. ardea* (birds), and *G. psittaci* (birds) (Erlandsen et al., 1990; McRoberts et al., 1996). Molecular techniques have revealed genetic diversity in *G. duodenalis*, and mammalian isolates with zoonotic potential appear to belong to 1 out of 2 genotypic groupings (assemblages) (Thompson, Hopkins, and Homan, 2000). These genotypes are distributed worldwide and are referred to in Europe as "Polish" and "Belgian" (Homan et al., 1992), in North America as Groups 1–2 and 3 (Nash, 1995), and in Australia as assemblages A and B (Mayrhofer et al., 1995; Monis et al., 1996; Ey et al., 1997; Thompson, Hopkins, and Homan, 2000). Genotype (assemblage) A, which most commonly infects humans, can also be found in mammals including livestock. People and other mammals (excluding cattle) can be infected with genotype (assemblage) B (Ey et al., 1997; Karanis and Ey, 1998; Thompson, Morgan et al., 2000). The genotypes of *G. duodenalis* with a limited host range (indicative of higher host specificity) include those from domestic cats, rats, dogs, and hoofed livestock (Ey et al., 1997; Thompson, Morgan et al., 2000). Genotypes identified in cats and hoofed livestock are closely related to genotype A, whereas those from dogs, rats, and voles/muskrats, are genetically quite distinct from both genotypes A and B (Thompson, Hopkins, and Homan, 2000).

Although numerous studies have demonstrated genetic similarities among mammalian isolates of *Giardia* (Hopkins et al., 1997), cross-transmission experiments that tested their zoonotic potential remain inconclusive (Thompson et al., 1990). Molecular identification of the genotype directly from the cyst stage (as opposed to in vitro–amplified trophozoites because not all isolates of *Giardia* can be grown in vitro) is the most reliable means to demonstrate zoonotic transmission (Hopkins et al., 1997; Issac-Renton et al., 1997). For example, the genotype of *G. duodenalis* cysts from a waterborne outbreak in Canada were identical to the genotype from a beaver colony living in the endemic area (Issac-Renton et al., 1997). To investigate zoonotic transmission and the molecular epidemiology of giardiasis, a nested polymerase chain reaction (PCR) for genotyping *Giardia* cysts has been developed and successfully used to differentiate between genotypes of *Giardia* infecting people and dogs in endemic communities in Australia (Hopkins et al., 1997). Also, recently, a fluorescent in situ hybridization (FISH) technique has been developed for specific identification of *G. duodenalis* cysts (Dorsch and Veal, 2001). FISH employs fluorescently labeled 18-bp oligonucleotide probes targeted to species-specific sequences of 18S ribosomal RNA (rRNA) of *G. duodenalis* (Dorsch and Veal, 2001).

Some families of mountain gorillas (*Gorilla gorilla beringei*) that free range within the Bwindi Impenetrable National Park, Uganda, have been habituated to humans (Butynski and Kalina, 1993). Information gathered to date indicates that intensified reciprocal human–gorilla interactions may have enhanced the transmission of anthropozoonotic pathogens (Ashford et al., 1990; Graczyk et al., 1999; Graczyk, DaSilva et al., 2001; Graczyk, Mudakikwa et al., 2001; Nizeyi et al., 1999, 2001; Nizeyi, Cranfield et al., 2002; Nizeyi, Sebunya et al., 2002). Although no information is available on *Giardia* sp. in people sharing gorilla habitats, *Giardia* sp. cysts have been recovered from gorilla feces (Nizeyi et al., 1999; Sleeman et al., 2000) and from

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local cattle grazing in the park vicinity and illegally within the park (Mwebe, 1998; Nizeyi, Cranfield et al., 2002). As bovine giardiasis is common and has a worldwide distribution (Olson et al., 1997; O'Handley et al., 2000), the access of cattle to gorilla habitats (Mwebe, 1998) raised additional concerns for pathogen transmission.

The purpose of the present study was to determine the species and genotype of *Giardia* cysts recovered from gorillas, cattle, and people sharing gorilla habitats. The greatest zoonotic risk derives from *G. duodenalis* genotypes in assemblage A (Ey et al., 1997; Karanis and Ey, 1998; Thompson, Morgan et al., 2000); thus, finding this genotype would indicate potential anthropozootic transmission.

MATERIALS AND METHODS

Study site

The Bwindi Impenetrable National Park, approximately 330 km^2, is situated in southwestern Uganda and supports the existence of approximately 300 free-ranging mountain gorillas (Mwebe, 1998). Human activity within the park includes gorilla tracking for tourism and research, antipoaching and military patrols, and a limited scale license-based forest harvesting, pit sawing, and sawmilling (Mwebe, 1998). The main illegal activities within the park include grazing of local cattle and unlicensed harvesting of the forest (Mwebe, 1998).

Sample collection and processing

The human population (n = 62) sharing gorilla habitats has been divided into 4 groups on the basis of activity: Group 1, n = 19, park staff, i.e., guides, trackers, workers, guards, patrols, and clerks; Group 2, n = 33, non–park staff, i.e., local communities of the Bakiga, Bafumbira, and Banyarwanda; Group 3, n = 7, soldiers; and Group 4, n = 3, tourists. All categories (except clerks) entered the park on a daily basis and tourists only once. Fecal samples were collected in plastic cups and refrigerated at 4 C until processed. Each specimen was accompanied by a questionnaire for the individual from whom the sample originated.

Gorillas were visualized from a trail and their age and sex determined (Mwebe, 1998). The fecal samples were collected as soon as possible after defecation in a plastic vial and transported to the laboratory in a cooler. If defecation was not observed, the gorilla age was determined on the basis of the fecal lobe diameter or the presence of silver hairs (Mwebe, 1998). A total of 100 samples were collected from 3 human-habituated families (43 gorillas) and 2 wild nonhabituated groups of unknown number of gorillas. In the laboratory, samples were sorted into 6 age classes, as described previously (Nizeyi et al., 1999).

Cattle rectal fecal samples (n = 50), approximately 0.2-kg weight, were collected using disposable plastic gloves at the 5 farms that were in closest proximity to the park and stored at 4 C until processed. At 2 farms, samples originated from postweaned cattle, n = 13 and 7, respectively, and from preweaned animals at the 3 remaining farms n = 16, 10, and 4, respectively. Two farms, 1 with postweaned and 1 with preweaned animals, were not fenced and were located at the park's edge. Cattle at these farms had access to the park. The 3 remaining farms, located 5 km from the park, were fenced.

In the laboratory, samples were individually processed as described previously (Graczyk et al., 2000); the final pellets were restored in 300 μl of 95% ethanol (Vesey et al., 1998).

Detection of *Giardia* sp. cysts

Aliquots (10 μl) of feces-recovered material were incubated (1:1 v/v; 30 min, room temperature) with fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies (mAb) against the cell wall antigens of *Giardia* from the MER*IFLUOR*™ test kit (Meridian Diagnostic, Inc., Cincinnati, Ohio). Five-microliter portions of the solution were each deposited on glass slide wells (11-mm diameter). The slide was covered with a 24- × 50-mm coverslip, and the entire well area was examined with an Olympus BH2-RFL epifluorescent microscope, dry 60× objective, and BP-490 exciter filter. *Giardia* cysts were counted, and their concentration was calculated using previously described formulas (Nizeyi et al., 1999; O'Handley et al., 2000). The size of *Giardia* cysts recovered from cattle, gorillas, and people (n = 30 from each group) was measured using a calibrated ocular.

DNA isolation

Approximately 100–200 μl of the *Giardia* cyst suspension was added to a 1.5-ml eppendorf tube with 80 μl of tissue lysis buffer (QIAgen, Hilden, Germany) and boiled for 10 min; 180 μl of AL buffer (QIAgen) followed by 10 μl of glassmilk (Bio-Rad Laboratories, Hercules, California) were added to the cyst suspension that was vortexed well and incubated at 72 C for 10 min. Samples were spun at 14,000 rpm for 1 min in an Eppendorf Centrifuge 5417C and the supernatant discarded. The pellet was washed with 700 μl of AW wash buffer (QIAgen). The suspension was spun again at 14,000 rpm for 1 min and the supernatant discarded. The wash step was repeated and the pellet dried in a vacuum desiccator for 5–10 min. The pellet was resuspended in 20 μl of TE buffer and incubated at 72 C for 5 min. Samples were spun at 14,000 rpm for 2 min, and the supernatant containing the DNA was transferred to a clean tube.

PCR amplification, sequencing, and genotyping of *Giardia* sp. cysts

The molecular characterization of *Giardia* cyst isolates was carried out using nested PCR amplification and sequencing of a 130-bp region near the 5′ end of the small subunit–rRNA gene, as described previously (Hopkins et al., 1997; Read et al., 2002).

FISH and FITC-conjugated mAb

Two probes specific to *G. duodenalis* (as tested against *G. microti* [rodents], *G. ardea*, and *G. muris*) were used, Giar-4 (5′-CGG CGG GGG GCC AAC TAC-3′) and Giar-6 (5′-CGG GGC TGC CGC GGC GCG-3′) (Dorsch and Veal, 2001). The probes were synthesized by the DNA Analysis Facility of the Johns Hopkins University, Baltimore, Maryland, in a 1.0-mM scale, purified by high-performance liquid chromatography, and labeled with a single molecule of a fluorochrome, hexachlorinated 6-carboxyfluorescein. FISH and FITC-conjugated mAb testing were carried out in eppendorf tubes in a total volume of 100 μl of the hybridization buffer (Deere et al., 1998) at 48 C for 1 hr (Dorsch and Veal, 2001). The concentration of each oligonucleotide probe was 1 mmol L^{-1} (Dorsch and Veal, 2001), and the mAb was 1:1 v/v diluted. After incubation, the tubes were centrifuged twice at 4 C (8,000 *g*, 2 min), and the pellets were resuspended in 100 μl of phosphate-buffered saline. Five 20-μl samples were transferred onto 5 lysine-coated wells (5-mm diameter) on a glass slide (Carlson Scientific, Inc., Peotone, Illinois) and air-dried. The cysts were counted.

RESULTS

Detection of *Giardia* sp. cysts

The prevalence of positive fecal samples and the concentration of *Giardia* cysts are presented in Table I. *Giardia*-positive cattle were located at farms that were not fenced, located at the park's edge, and the animals had access to the park.

On the basis of a self-administered questionnaire, 3 people who were tested positive had experienced at least a single diarrheal condition (lasting for at least 5 days) within 1 mo before the sample collection. Also, 15 of the 19 park staff members (79%) reported having frequent contact, i.e., handling gorilla dung within a month before the testing. Of these 15 people, 10 washed their hands and cleaned their shoes of the dung, 3 wiped the dung off their shoes, and 2 did nothing. Fourteen of the 33 local community members (42%) reported of never burying their solid waste after defecating in the bushes in the park. Three of the 19 park staff members (16%) reported not complying with the park regulations regarding fecal disposal, i.e., burying their fecal waste within the park. Of 62 participants in

TABLE I. The results of testing for *Giardia* sp. in fecal samples from free-ranging human-habituated mountain gorillas (*Gorilla gorilla beringei*) of the Bwindi Impenetrable National Park, Uganda, the local community members that share gorilla habitats, and cattle grazing within the park and its vicinity.

Sampled group	Number of samples			Concentration (10^4 cysts/g)		Species, assemblage	Comments for positive samples
	Collected	Positive	Percentage	Range	Mean		
Humans, n = 62	62	3	5	2.3–3.1	2.8	*G. duodenalis*, A	Park staff members 19, 27, and 39 yr old, all experienced diarrhea
Gorillas, n = 100	100	2	2	2.0–2.5	2.5	*G. duodenalis*, A	Nonhabituated, unknown age and sex, stools with blood and mucous
Cattle, n = 50	50	5	10	0.1–0.3	0.2	*G. duodenalis*, A	4 preweaned, 1 postweaned, diarrhetic stools

the study, 49 (79%) reported drinking water directly from streams while in the park. *Giardia* cysts from human samples were within the length range of 12.1–14.9 μm (x = 13.3 ± 0.8 μm) by width of 7.1–11.8 μm (x = 9.1 ± 1.0 μm). *Giardia* cysts from gorillas were 11.7–14.7 μm (x = 12.9 ± 0.7 μm) by 6.7–9.9 μm (x = 8.1 ± 0.7 μm) and from cattle 11.9–14.1 μm (x = 12.7 ± 0.8 μm) by 6.9–9.6 μm (x = 7.9 ± 0.7 μm).

Molecular characterization and genotyping of *Giardia* sp. cysts

Molecular analysis of 5 cattle, 3 human, and 2 gorilla isolates revealed that all isolates were genetically identical over the 130-bp sequence to *G. duodenalis* genotype (assemblage) A.

FISH and FITC-conjugated mAb

All isolates of *G. duodenalis* cysts produced positive FISH and mAb reactions, and the fluorescence intensity of all isolates from humans, gorillas, and cattle was similar. The vast majority of cysts, over 95%, were intact (Fig. 1). Nonviable cysts were represented by: (1) shells with apparently structurally damaged walls, and (2) intact cysts with a very small amount of internal structures with a diffused appearance (Fig. 1). Intact cysts were filled out completely with cytoplasm, or sometimes the cytoplasm was condensed and there was a gap between the internal structures and the wall (Fig. 1). Neither FISH-negative and mAb-negative nor FISH-positive and mAb-negative cysts were observed. No fluorescence of other organisms was observed except for autofluorescence of the nonstructural debris.

DISCUSSION

The current taxonomy of *Giardia* sp. recognizes the existence of only a limited number of species in the genus (Thompson et al., 1990, 1993). Of these, *G. duodenalis* exhibits considerable intraspecific variability with some genotypes not rigidly host-specific and thus of potential zoonotic significance (Thompson et al., 1990, 1993; Ey et al., 1997). Genotypic variation among *G. duodenalis* isolates determines the epidemiology of giardiasis, and the greatest zoonotic risk derives from genotypes in assemblage A (Ey et al., 1997; Karanis and Ey, 1998; Thompson, 2000; Thompson, Hopkins, and Homan, 2000). Of concern in the present study was the finding that all isolates of *G. duodenalis* from people, gorillas, and cattle were identical to genotype A, the most common genotypic group infecting people (Thompson, Hopkins, and Homan, 2000). The fact that 3 genetically distant but geographically localized host groups were found to harbor *Giardia* sp. belonging to the same genotypic grouping suggests a common source of infection in the 3 species of host. In this respect, it seems likely that humans introduced *Giardia* sp. infection into this localized endemic focus of transmission (Fig. 2). Cattle are unlikely to have been the source of infection because molecular epidemiological studies in Canada and Australia have shown that although cattle are susceptible to infection with genotype A, the majority of animals in a herd are infected with the "hoofed livestock" genotype of *G. duodenalis* (O'Handley et al., 2000; Thompson, 2000).

To our knowledge, the present study constitutes the first published report on *G. guodenalis* genotype (assemblage) A in gorillas. The interesting result of the present study is the identification of only genotype (assemblage) A in cattle that were expected to harbor the hoofed livestock (syn. "artiodactyl-specific") genotype (Ey et al., 1997; O'Handley et al., 2000; Thompson, Hopkins, and Homan, 2000). As demonstrated by axenic culture or growth in mice and subsequent molecular analysis, 15 out of 31 isolates of *G. duodenalis* from hoofed farm animals in Australia represented artiodactyl-specific genotype, 10 (including isolates from cattle) were identical with genotype (assemblage) A, and 5 had close affinity with both genotypes (assemblages) A and B (Ey et al., 1997). It has been concluded by this study that isolates indistinguishable from human genotype (assemblage) A had the greatest zoonotic potential (Ey et al., 1997). Extensive surveys with the genotypic characterization of *G. duodenalis* from dairy calves in Canada and Australia demonstrated that the hoofed livestock genotype dominated over genotype (assemblage) A (O'Handley et al., 2000; Thompson, 2000). Four out of 5 and 8 out of 10 isolates aligned with the hoofed livestock genotype in Australia and Canada, respectively, and the remaining isolates aligned with genotype (assemblage) A (O'Handley et al., 2000).

The question remains as to how such an anthropozoonotic cycle of transmission of *G. duodenalis* has been achieved in the area of the Bwindi Impenetrable National Park. Genotype A of *G. duodenalis* is the most common genotype found in people (Thompson, Morgan et al., 2000). It may have been introduced into gorilla populations through habituation activities or ecotourism, or both, and may have then been sustained in their habitats by anthropozoonotic transmission and enhanced by promiscuous defecation (Fig. 2). Only park staff members were found to be infected with *G. duodenalis*. The question-

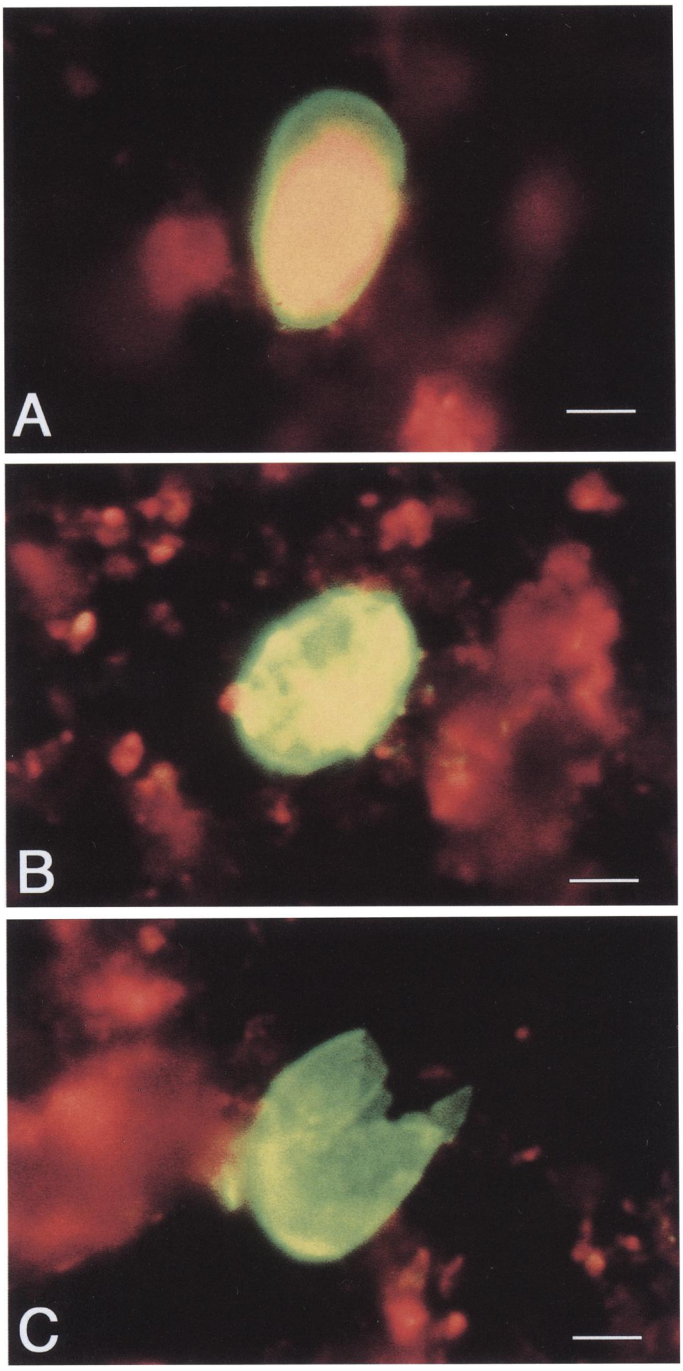

FIGURE 1. FISH and FITC-conjugated mAb images of *Giardia duodenalis* genotype (assemblage) A cysts recovered from fecal specimens of free-ranging human-habituated mountain gorillas (*Gorilla gorilla beringei*) of the Bwindi Impenetrable National Park, Uganda, the local community members that share gorilla habitats, and cattle grazing within the park and its vicinity. Viable *G. duodenalis* cyst with a gap between the internal structures and the wall (panel A), nonviable cyst (panel B), and cyst shell (= nonviable cysts) with structural wall damage (panel C). Bar = 4 μm.

naire survey revealed that not all staff members buried their own fecal waste while in the park and that over one-third of the staff members who came in contact with gorilla dung did not use appropriate precautions. Furthermore, almost half of the

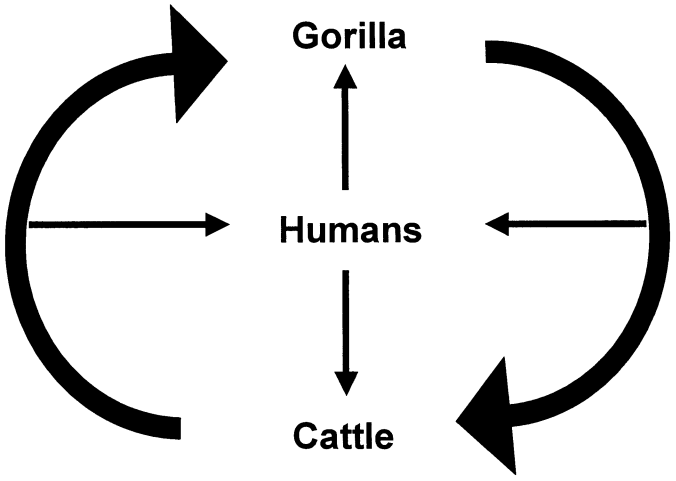

FIGURE 2. Anthropozoonotic transmission cycles of *Giardia duodenalis* genotype (assemblage) A in habitats of free-ranging human-habituated mountain gorillas (*Gorilla gorilla beringei*) of the Bwindi Impenetrable National Park, Uganda.

local community did not bury their fecal waste while promiscuously defecating in the park. Over three-quarters of the study participants drink water directly from streams, and *Giardia* sp. can be efficiently transmitted via water (DeRegnier et al., 1989; Erlandsen and Bemrick, 1998). This means that on a daily basis, a large percentage of people who frequently enter the park do not follow the park regulations regarding the disposal of fecal waste. Also, allo- and autocoprophagy practiced by gorillas can greatly facilitate the establishment of this pathogen in gorilla populations (Graczyk and Cranfield, 2001).

Genotypic characterization of *Giardia* was complemented in the present study by the application of the FISH and immunofluorescent mAb (IFA) techniques. As discussed by Dorsch and Veal (2001), the FISH technique has multiple diagnostic advantages over PCR or IFA techniques used alone. Incorporation of IFA into the FISH allows (irrespective of the FISH outcome) the observation of the external morphology of cysts and the assessment of any structural damage to their walls, as demonstrated in Figure 1, panel C.

This study has reinforced the epidemiological and public health value of the genotypic identification of *Giardia* sp. on the basis of the cystic stages in environmental samples, particularly in determining the source of environmental contamination (Thompson, Morgan et al., 2000). It was shown that poor husbandry and sanitary practices can lead to the maintenance of an artificially introduced pathogen in a cycle of transmission involving domestic animals and wildlife (Fig. 2). The potential clinical impact of *Giardia* infection in gorillas remains to be determined and will require further investigation. These results emphasize the need for stronger enforcement of park regulations and mandatory implementation of farmer education into the management of gorillas and the risks of *Giardia* sp. transmission in this confined area.

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LITERATURE CITED

ASHFORD, R. W., G. D. F. REID, AND T. M. BUTYNSKI. 1990. The intestinal faunas of man and mountain gorillas in a shared habitat. Annals of Tropical Medicine and Parasitology **84:** 337–340.

BUTYNSKI, T., AND J. KALINA. 1993. Three new mountain gorilla national parks for Uganda. Oryx **27:** 212–224.

DEERE, D., G. VESEY, M. MILNER, K. WILLIAMS, N. ASHBOLT, AND D. A. VEAL. 1998. Rapid method for fluorescent in situ ribosomal RNA labelling of *Cryptosporidium parvum*. Journal of Applied Microbiology **85:** 807–818.

DEREGNIER, D. P., L. COLE, D. G. SCHUPP, AND S. L. ERLANDSEN. 1989. Viability of *Giardia* cysts suspended in lake, river, and tap water. Applied and Environmental Microbiology **55:** 1223–1229.

DORSCH, M. R., AND D. A. VEAL. 2001. Oligonucleotide probes for specific detection of *Giardia lamblia* cysts by fluorescent in situ hybridization. Journal of Applied Microbiology **90:** 836–842.

ERLANDSEN, S. L., AND W. J. BEMRICK. 1988. Waterborne giardiasis: Sources of *Giardia* cysts and evidence pertaining to their implications in human infection. *In* Advances in *Giardia* research, P. M. Wallis, and B. R. Hammond (eds.). University of Calgary Press, Calgary, Canada, p. 227–236.

———, ———, C. L. WELLS, D. E. FREELY, L. KNUDSON, R. S. CAMPBELL, H. VANKEULEN, AND E. L. JARROLL. 1990. Axenic culture and characterization of *Giardia ardeae* from the Great blue heron (*Ardea herodias*). Journal of Parasitology **76:** 717–724.

EY, P. L., M. MANSOURI, J. KULDA, E. NOHYNKOVA, P. T. MONIS, R. H. ANDREWS, AND G. MAYRHOFER. 1997. Genetic analysis of *Giardia* from hoofed farm animals reveals artiodactyl-specific and potentially zoonotic genotypes. Journal of Eukaryotic Microbiology **44:** 626–635.

FILICE, F. P. 1952. Studies on the cytology and life history of a *Giardia* from the laboratory rat. University of California Publications in Zoology **57:** 53–143.

GRACZYK, T. K., AND M. R. CRANFIELD. 2001. Coprophagy and intestinal parasites: Implications to human-habituated mountain gorillas (*Gorilla gorilla beringei*). Recent Research Developments in Microbiology **5:** 283–293.

———, A. J. DASILVA, M. R. CRANFIELD, J. B. NIZEYI, G. R. N. N. KALEMA, AND N. J. PIENIAZEK. 2001. *Cryptosporidium parvum* genotype 2 infections in free-ranging mountain gorillas (*Gorilla gorilla beringei*) of the Bwindi Impenetrable National Park, Uganda. Parasitology Research **87:** 368–370.

———, B. M. EVANS, C. J. SHIFF, H. J. KARREMAN, AND J. A. PATZ. 2000. Environmental and geographical factors contributing to watershed contamination with *Cryptosporidium parvum* oocysts. Environmental Research **82:** 263–271.

———, L. J. LOWENSTINE, AND M. R. CRANFIELD. 1999. *Capillaria hepatica* (Nematoda) infections in human-habituated mountain gorillas (*Gorilla gorilla beringei*) of the Parc National de Volcans, Rwanda. Journal of Parasitology **85:** 1168–1170.

———, A. B. MUDAKIKWA, M. R. CRANFIELD, AND U. EILENBERGER. 2001. Hyperkeratotic mange caused by *Sarcoptes scabiei* (Acariformes; Sarcoptidae) in juvenile human-habituated mountain gorillas (*Gorilla gorilla beringei*). Parasitology Research **87:** 1024–1028.

HOMAN, W. L., F. H. VAN ENCKEVORT, L. LIMPER, G. S. VAN EYS, G. J. SCHOONE, W. KASPRZAK, A. C. MAJEWSKA, AND F. VAN KNAPEN. 1992. Comparison of *Giardia* isolates from different laboratories by isoenzyme analysis and recombinant DNA probes. Parasitology Research **78:** 316–323.

HOPKINS, R. M., B. P. MELONI, D. M. GROTH, J. D. WETHERALL, J. A. REYNOLDSON, AND R. C. A. THOMPSON. 1997. Ribosomal RNA sequencing reveals differences between the genotypes of *Giardia* isolates recovered from humans and dogs living in the same locality. Journal of Parasitology **83:** 44–51.

ISSAC-RENTON, J. L., C. CORDEIRO, K. SARAFIS, AND H. SHAHRIARI. 1997. Characterization of *Giardia duodenalis* isolates from a waterborne outbreak. Journal of Infectious Diseases **167:** 431–440.

KARANIS, P., AND P. L. EY. 1998. Characterization of axenic isolates of *Giardia intestinalis* established from humans and animals in Germany. Parasitology Research **84:** 442–449.

MAYRHOFER, G., R. H. ANDREWS, P. L. EY, AND N. B. CHILTON. 1995. Division of *Giardia* isolates from humans into two genetically distinct assemblages by electrophoretic analysis of enzymes encoded at 27 loci and comparison with *Giardia muris*. Parasitology **111:** 11–17.

MCROBERTS, K. M., B. P. MELONI, U. M. MORGAN, R. MARANO, Z. BINZ, S. L. ERLANDSEN, S. A. HALSE, AND R. C. A. THOMPSON. 1996. Morphological and molecular characterization of *Giardia* isolates from the straw-necked ibis (*Threskiornis spinicollis*) in Western Australia. Journal of Parasitology **82:** 711–718.

MONIS, P. T., G. MAYRHOFER, R. H. ANDREWS, W. L. HOMAN, L. LIMPER, AND P. L. EY. 1996. Molecular genetic analysis of *Giardia intestinalis* isolates at the glutamate dehydrogenase gene. Parasitology **112:** 1–12.

MWEBE, R. A. 1998. Survey on *Cryptosporidium* prevalence within the mountain gorilla population (*Gorilla gorilla beringei*) of the Bwindi Impenetrable National Park in South-western Uganda. B.V.M. Thesis. Makerere University, Kampala, Uganda, 231 p.

NASH, T. E. 1995. Antigenic variation in *Giardia lamblia*. *In* Molecular approaches to parasitology, J. C. Boothroyd, and R. Komuniecki (eds.). Wiley-Liss, New York, New York, p. 31–42.

NIZEYI, J. B., M. R. CRANFIELD, AND T. K. GRACZYK. 2002. Cattle near the Bwindi Impenetrable National Park, Uganda, as a reservoir of *Cryptosporidium parvum* and *Giardia duodenalis* for local community and free-ranging gorillas. Parasitology Research **88:** 380–385.

———, R. B. INNOCENT, J. ERUME, G. R. N. N. KALEMA, M. R. CRANFIELD, AND T. K. GRACZYK. 2001. Campylobacteriosis, salmonellosis, and shigellosis infections in free-ranging human-habituated mountain gorillas of Uganda. Journal of Wildlife Diseases **37:** 239–244.

———, R. MWEBE, A. NANTEZA, M. R. CRANFIELD, G. R. N. N. KALEMA, AND T. K. GRACZYK. 1999. *Cryptosporidium* sp. and *Giardia* sp. infections in mountain gorillas (*Gorilla gorilla beringei*) of the Bwindi Impenetrable National Park, Uganda. Journal of Parasitology **85:** 1084–1088.

———, D. SEBUNYA, A. J. DASILVA, M. R. CRANFIELD, N. J. PIENIAZEK, AND T. K. GRACZYK. 2002. Cryptosporidiosis in people sharing habitats with free-ranging mountain gorillas (*Gorilla gorilla beringei*). American Journal of Tropical Medicine and Hygiene. [In press.]

O'HANDLEY, R. M., M. E. OLSON, D. FRASER, P. ADAMS, AND R. C. A. THOMPSON. 2000. Prevalence and genotypic characterization of *Giardia* in dairy calves from Western Australia and Western Canada. Veterinary Parasitology **27:** 193–200.

OLSON, M. E., N. J. GUSELLE, R. M. O'HANDLEY, M. L. SWIFT, T. A. MCALLISTER, M. D. JELINSKI, AND D. W. MORCK. 1997. *Giardia* and *Cryptosporidium* in dairy calves in British Columbia. Canadian Veterinary Journal **38:** 703–706.

READ, C., J. WALTERS, I. D. ROBERTSON, AND R. C. A. THOMPSON. 2002. Correlation between genotype of *Giardia duodenalis* and diarrhoea. International Journal for Parasitology. [In press.]

SLEEMAN, J. M., L. L. MEADER, A. B. MUDAKIKWA, J. W. FOSTER, AND S. PATTON. 2000. Gastrointestinal parasites of mountain gorillas (*Gorilla gorilla beringei*) in the Park National des Volcans, Rwanda. Journal of Zoo and Wildlife Medicine **31:** 322–328.

THOMPSON, R. C. A. 2000. Giardiasis as a re-emerging infectious disease and its zoonotic potential. International Journal for Parasitology **30:** 1259–1267.

———, R. M. HOPKINS, AND W. L. HOMAN. 2000. Nomenclature and genetic groupings of *Giardia* infecting mammals. Parasitology Today **16:** 210–213.

———, A. J. LYMBERY, AND B. P. MELONI. 1990. Genetic variation in *Giardia* Kunstler, 1882: Taxonomic and epidemiological significance. Protozoological Abstracts **14:** 1–28.

———, U. M. MORGAN, R. M. HOPKINS, AND L. J. PALLANT. 2000. Enteric protozoans infections. *In* Molecular epidemiology of infectious diseases, R. C. A. Thompson (ed.). Arnold, London, U.K., p. 194–209.

———, J. A. REYNOLDSON, AND A. H. W. MENDIS. 1993. *Giardia* and giardiasis. Advances in Parasitology **32:** 72–160.

VESEY, G., N. ASHBOLT, E. J. FRICKER, D. DEERE, K. L. WILLIAM, D. A. VEAL, AND M. DORSCH. 1998. The use of a ribosomal RNA targeted oligonucleotide probe for fluorescent labelling of viable *Cryptosporidium parvum* oocysts. Journal of Applied Microbiology **85:** 429–440.