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CRYPTOSPORIDIOSIS IN PEOPLE SHARING HABITATS WITH FREE-RANGING MOUNTAIN GORILLAS (*GORILLA GORILLA BERINGEI*), UGANDA

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Abstract. Cryptosporidiosis, a zoonotic diarrheal disease, significantly contributes to the mortality of people with impaired immune systems worldwide. Infections with an animal-adapted genotype (Genotype 2) of *Cryptosporidium parvum* were found in a human population in Uganda that shares habitats with free-ranging gorillas, from which the same genotype of *C. parvum* had been recovered previously. A high prevalence of disease was found in park staff members (21%) who frequently contact gorillas versus 3% disease prevalence in the local community. This indicates a zoonotic transmission cycle of this pathogen against which no effective prophylaxis or therapy exists. The results of the study questionnaire demonstrated a high percentage of people not undertaking appropriate precautions to prevent fecal-oral transmission of *C. parvum* in the Bwindi Impenetrable National Park, Uganda. This human population will benefit from stronger compliance with park regulations regarding disposal of their fecal waste within the park boundaries.

INTRODUCTION

Cryptosporidiosis, long considered to be a veterinary disease, has emerged as a serious human health problem,[1] being the most frequent secondary diagnosis in people with AIDS and significantly contributing to their mortality.[2] Human *Cryptosporidium parvum*-associated disease is the result of zoonotic or anthroponotic transmission of the parasite's infectious stages, the oocysts.[3–5] The parasite is transmitted via a fecal-oral route and very frequently via contaminated water and food.[6] Molecular studies showed that the oocysts causing zoonotic cryptosporidiosis and those originating from anthroponotic sources are genetically and immunologically distinct[3,4,7] although they are morphologically and morphometrically indistinguishable.[8] Genotype 2 of *C. parvum* is cross-transmissible between humans and a variety of mammalian species (predominantly cattle), whereas the Genotype 1 is thought to cycle exclusively within the human population.[3,4,7]

Cryptosporidium species infections have been identified in free-ranging mountain gorillas (*Gorilla gorilla beringei*) of the Bwindi Impenetrable National Park, Uganda.[9,10] These gorillas have been habituated to humans for conservation purposes and ecotourism, and it is believed that this habituation has enhanced transmission of anthropozoonotic parasites,[11,12] including *Cryptosporidium.*[10,13] Molecular analysis of *Cryptosporidium* species isolates originating from free-ranging gorillas of Uganda yielded positive identification of *C. parvum* Genotype 2.[13] Because this genotype is cross-transmissible between humans and animals, it has been postulated that *C. parvum* can be propagated in the habitats of gorillas through both zoonotic or anthroponotic transmission cycles.[13] However, cryptosporidiosis in people living in the vicinity of the park who frequently enter gorilla habitats and interact with gorillas has never been investigated. The purpose of the current study was to determine whether *C. parvum* infections are present in people sharing habitats with free-ranging mountain gorillas and, if so, to identify the genotype of *C. parvum* causing infections in humans.

MATERIALS AND METHODS

The Bwindi Impenetrable National Park, approximately 330 km^2, is situated in southwestern Uganda and supports the existence of approximately 300 mountain gorillas that free range within the park boundaries.[9] Human activity within the park includes tracking gorillas for tourism and research purposes, antipoaching and military patrols, and a limited-scale license-based harvesting of the forest, pitsawing, and sawmilling.[9] The main illegal human activities within the park boundaries include cattle grazing and unlicensed harvesting of the forest.[9]

The human population (N = 62) that shares habitats with mountain gorillas has been divided into four groups based on activity: Group 1 (n = 19), park staff (i.e., guides, trackers, workers, guards, patrols, and clerks); Group 2, (n = 33), nonpark staff (i.e., local community of the Bakiga, Bafumbira, and Batwa tribes); Group 3 (n = 7), soldiers stationed within the park; and Group 4 (n = 3), tourists. All these categories (except clerks) entered the park on a daily basis during the study. Fecal samples collected in plastic cups were preserved in buffered formalin and refrigerated at 4°C. Each of the fecal specimens was accompanied by a questionnaire completed by the person from whom the fecal sample originated. Fecal specimens were processed by ethyl acetate sedimentation.[14] Direct wet smears prepared from each of the specimens in quadruplicate were air dried and then processed separately with acid-fast stain (AFS)[14] and immunofluorescent antibody (IFA) of the MERIFLUOR™ test kit (Meridian Diagnostic, Cincinnati, OH).[10] Slides were examined for *C. parvum* oocysts by light microscopy as described previously.[10]

The DNA from *C. parvum* oocysts from specimens determined to be positive by AFS and IFA was extracted as described previously with minor modifications.[15] Samples were

centrifuged at 14,000g for 5 minutes and washed once in phosphate-buffered saline (0.01 Mole, pH 7.2) containing 1 mmol ethylenediaminetetraacetic acid (EDTA) by centrifugation at 14,000g for 5 minutes. The pellets were resuspended in 100 μl of a digestion buffer containing 50 mmol tris-hydrochloride (pH 8.5), 1 mmol EDTA, and 1% lauryl alcohol polyether (Laureth 12; PPG Industries Inc., Gurnee, IL). Proteinase K (Sigma Chemical Co., St. Louis, MO) was added to the resuspension at a concentration of 1 mg/ml, and the samples were incubated overnight at 56°C. The samples were incubated for 10 minutes at 95°C to inactivate the proteinase K, allowed to cool to room temperature, and stored at 4°C until polymerase chain reaction (PCR) amplification. PCR was performed using primers abbreviated as CPBDIAGF/CPBDIAGR to amplify fragments from a variable region coding for the *Cryptosporidium* 18SrRNA.[16] Amplification with these primers produces fragments of 435, 438, and 455 base pairs (bp) of the gene coding for the 18SrRNA of *C. parvum* Genotype 1, *C. parvum* Genotype 2, and *C. felis,* respectively.[17] In addition, two distinct primer pairs were used to amplify polymorphic regions of the gene coding for the *Cryptosporidium* oocyst wall protein (COWP). PCR primer pair CRY9/CRY15 was used to amplify a fragment of 553 bp of the *C. parvum* COWP N-terminal domain. Primers CRY12/CRY14 were used to amplify fragments of 568 and 571 bp of the *C. parvum* Genotype 1 and 2 COWP C-terminal domain, respectively.[18] PCR reactions were performed in 50-μl total volumes, and the conditions for primers CPBDIAGF/CPBDIAGR have been described previously.[17] Amplification with CRY12/CRY14 and CRY9/CRY15 was performed under conditions described previously[16] with PCR mixes containing 50 pmol of each COWP-specific primer and reactions being subjected to 45 cycles. PCR reactions with positive and negative controls were carried out in 50-μl total volumes as described previously.[7,15,16] The positive control was genomic DNA extracted from human stool samples positive for *C. parvum* Genotype 1, and the negative control was a pool of DNA extracted from stools of two baboons (*Papio anubis*) and a single *C. parvum*-negative person. DNA sequencing reactions were done with the Perkin Elmer Big Dye kit and analyzed on the Perkin Elmer ABI 3100 automatic sequencer. Sequences were assembled and aligned by using the SeqMan II, 4.05 (DNASTAR Inc., Madison, WI).

RESULTS

Five of 62 fecal samples (8%) contained *C. parvum* oocysts; this included 4 of 19 park staff members (21%) (all categories except clerks), and 1 of 33 (3%) members of the local community. No oocysts were detected in fecal samples of soldiers or tourists. Three of five people who tested positive for *C. parvum* (all park staff members) and seven who tested negative for the oocysts had experienced at least one diarrheal condition (lasting for at least 5 days) within 1 month before the collection of fecal samples, according to the questionnaire responses.

The age of sampled people varied from 7 to 78 years. Four cases of cryptosporidiosis were found in park staff members (age range = 19–39 years), and a single case was found in a 10-year-old child from a local community.

Fifteen of 19 staff members (79%) reported having frequent contact (i.e., handling, gorilla dung [research purposes or accidental contacts]) within 1 month before the collection of fecal samples. No other participants in the study reported having come in contact with gorilla dung. Of these 15 people, 10 washed their hands and cleaned their shoes of the dung, 3 wiped the dung off their shoes only, and 2 did not do anything. Fourteen of 33 local community people (42%) reported never burying their solid waste while defecating in the bushes within the park boundaries. Three of 19 park staff members (16%) reported not complying with park regulations regarding fecal disposal (i.e., burying their fecal waste within the park territory). Of 62 participants in the study, 49 (79%) reported drinking water directly from streams while being in the park.

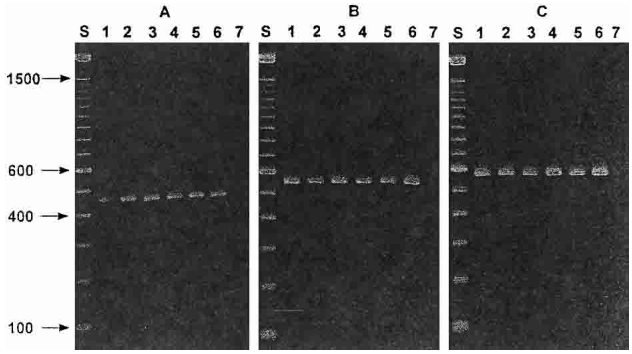

FIGURE 1. Agarose gel analysis of PCR amplification of *Cryptosporidium parvum* DNA from five human stools specimens (lane 1 to 5 in each panel) determined to be oocyst positive by staining with acid-fast and immunofluorescent antibody. Amplified DNA fragments from the Cryptosporidium 18SrRNA coding region (primers CPBDIAGF/CPBDIAGR, panel A) Cryptosporidium oocyst wall protein (COWP) N-terminal domain using primers CRY15/CRY9 (panel B), and COWP C-terminal domain using primers CRY12/CRY14 (panel C) are shown. Lanes 6 and 7 in each panel represent the positive control (genomic DNA extracted from human stool samples positive for *C. parvum* Genotype 1) and the negative control (a pool of DNA extracted from stools of two baboons, Papio anubis, and a single *C. parvum*-negative person). Lane S represents the 100-bp ladder DNA size standard. Numbers on the left side of the gels indicate the DNA fragment sizes (in base pairs).

Positive PCR signal was obtained in DNA templates extracted from all five microscopy positive samples. Fragments of the expected size were amplified from each template using primers CPBDIAGF/CPBDIAGR (Figure 1, panel A, expected size 435 bp), primers CRY15/CRY9 (Figure 1, panel B, expected size 553 bp), and primers CRY12/CRY14 (Figure 1, panel C, expected size 571 bp). Sequencing analysis of the PCR products from the 18SrRNA and from the COWP fragments showed 100% of identity with sequences known for *C. parvum* Genotype 2.

DISCUSSION

Variation among the genotypes of *C. parvum* determines the epidemiology of *C. parvum* infections.[3,4,7] Zoonotic and anthroponotic transmission cycles are distinct because there is no genetic recombination between endogenous parasites originating from Genotype 1 and Genotype 2 oocysts.[4] The epidemiological separation of these two cycles has been explained by (1) the possibility of existence of two parasite species (rather than two genotypes); (2) the existence of geographic barriers or other constraints that limit mixed Genotype 1 and Genotype 2 infections; and (3) the clonal population structure of *C. parvum*.[4]

The current study demonstrates *C. parvum* Genotype 2 infections in members of the local community living in the vicinity of the Bwindi Impenetrable National Park who frequently enter habitats of mountain gorillas and interact with them for a variety of purposes. The same genotype of *C. parvum* has been previously identified in mountain gorillas from socially different groups at various levels (or none) of human habituation.[13] The overall prevalence of human *C. parvum* Genotype 2 infection in the current study was 8%; 11% of gorillas were infected with the same genotype of *C. parvum*.[13] Because *C. parvum* Genotype 2 infections are particularly prevalent in cattle,[5,6] grazing of cattle within the park boundaries can seriously contribute to contamination of gorilla habitats and can initiate anthropozoonotic transmission cycles of this pathogen.

The overall prevalence of *C. parvum* infections of 8% found in the current study falls within the range reported for the immunocompetent population of Uganda.[19] The prevalence of *C. parvum* infections in the park staff (i.e., 21%) was considerably higher than in the local community (3%), although members of the park staff live in the same area. Also, no *C. parvum* infections have been found in clerks and administrative workers (n = 5) who do not enter the park. Together, these data indicate that entering the natural habitat of the park is a predisposing factor for contraction of *C. parvum* infection.

As demonstrated herein, more than one third of the staff members who came in contact with gorilla dung did not undertake appropriate precautions, and not all of the staff members buried their own fecal waste after defecating in the park. In addition, almost half of the local community people did not bury their fecal waste after promiscuous defecation in the park. This means that, on a daily basis, a large percentage of people living near the park boundaries and frequently entering the park do not follow park regulations regarding disposal of their fecal waste. This emphasizes a need for the stronger enforcement of park regulations and mandatory implementation of education into the management of free-ranging gorillas to control transmission of this pathogen against which no effective prophylaxis or therapy exists.[1,2]

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