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, being the most frequent secondary diagnosis in people with AIDS and significantly contributing to their mortality. Human Cryptosporidium parvum-associated disease is the result of zoonotic or anthropogenic transmission of the parasite’s infectious stages, the oocysts. The parasite is transmitted via a fecal-oral route and very frequently via contaminated water and food. Molecular studies showed that the oocysts causing zoonotic cryptosporidiosis and those originating from anthropogenic sources are genetically and immunologically distinct, although they are morphologically and morphometrically indistinguishable. 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.

Cryptosporidium species infections have been identified in free-ranging mountain gorillas (Gorilla gorilla beringei) of the Bwindi Impenetrable National Park, Uganda. 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, including Cryptosporidium. Molecular analysis of Cryptosporidium species isolates originating from free-ranging gorillas of Uganda yielded positive identification of C. parvum Genotype 2. 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 anthropogenic transmission cycles. 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 km², is situated in southwestern Uganda and supports the existence of approximately 300 mountain gorillas that free range within the park boundaries. 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 sawmill- ing. The main illegal human activities within the park boundaries include cattle grazing and unlicensed harvesting of the forest.

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), non-park 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 and acid-fast stain (AFS) test kit (Meridian Diagnostic, Cincinnati, OH). Slides were examined for Cryptosporidium oocysts by light microscopy as described previously. The DNA from C. parvum oocysts from specimens determined to be positive by AFS and IFA was extracted as described previously with minor modifications. Samples were
described previously. 7, 15, 16 The positive control was genomic
negative controls were carried out in 50-
being subjected to 45 cycles. PCR reactions with positive and
reactions containing 50 pmol of each COWP-specific primer and reactions
with these primers produces fragments of 435, 438, and 455
base pairs (bp) of the gene coding for the 18SrRNA of
Cryptosporidium (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). Lane 5 represents the 100-
bp ladder DNA size standard. Numbers on the left side of the gels
indicate the DNA fragment sizes (in base pairs).

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.

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. 7
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

Figure 1. Agarose gel analysis of PCR amplification of Cryptosporidium parvum DNA from five human stool 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
proteins (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 5 represents the 100-
bp ladder DNA size standard. Numbers on the left side of the gels
indicate the DNA fragment sizes (in base pairs).
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 habitation. 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*. Because *C. parvum* Genotype 2 infections are particularly prevalent in cattle, 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. 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.

Acknowledgments: The authors thank the Uganda Wildlife Authority, Kampala-Uganda, for permission to access gorilla fecal samples and Makerere University, Kampala-Uganda, for facilitating this study.

Financial support: The study was supported by Morris Animal Foundation Grant 98MG-11 (Englewood, CO) and Maryland Zoological Society Grant H680-951-2118 (Baltimore, MD).

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