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Giardia sp. and *Cryptosporidium* sp. Infections in Primates in Fragmented and Undisturbed Forest in Western Uganda

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ABSTRACT: In June 2005, we collected 115 fecal samples from wild primates in western Uganda and examined them for *Cryptosporidium* sp. and *Giardia* sp. with the use of immunofluorescent antibody (IFA) detection. We sampled primates from an undisturbed forest in Kibale National Park and from 3 highly disturbed forest fragments outside the park. Of disturbed forest samples, red colobus (*Pilocolobus tephrosceles*) and red-tailed guenons (*Cercopithecus ascanius*) harbored species of *Cryptosporidium* or *Giardia*, but black-and-white colobus (*Colobus guereza*) did not. All primate samples from undisturbed forest were negative for both parasites. Seven of 35 (20%) red colobus and 1 of 20 red-tailed guenons (5%) from forest fragments were infected with either *Cryptosporidium* sp. or *Giardia* sp. The presence of *Cryptosporidium* and *Giardia* species in primates living in forest fragments, but not in primates in undisturbed forest, suggests that habitat disturbance may play a role in transmission or persistence of these pathogens.

Species of *Cryptosporidium* and *Giardia* have been found in wild apes, and their presence has been argued to indicate enhanced contact with humans and/or domestic livestock (Nizeyi et al., 1999, 2002). Few studies, however, have examined other species of wild nonhuman primates for these pathogens.

We examined *Cryptosporidium* sp. and *Giardia* sp. prevalence and intensity in 3 nonhuman primate species (red colobus, *Pilocolobus tephrosceles*; black-and-white colobus, *Colobus guereza*; and red-tailed guenons, *Cercopithecus ascanius*) living in undisturbed forest in Kibale National Park, Uganda, and from 3 highly disturbed forest fragments outside the park. The fragments range in size from 1.2 to 8.7 ha and occur in areas largely unsuitable for agriculture, i.e., swampy valley bottoms and steep rims of lake craters (Gillespie and Chapman, 2006). These fragments are used by local villagers to varying degrees and are surrounded by tea plantations and small-scale agriculture, including pasture for domestic livestock (Gillespie and Chapman, 2006).

Fecal samples were collected from habituated and semihabituated adult and subadult male and female primates and preserved in 10% neutral buffered formalin (Gillespie, 2006). A Merifluor® *Cryptosporidium*/*Giardia* Direct Immunofluorescent Detection Kit (Meridian Bioscience, Inc, Cincinnati, Ohio) was used to detect both parasites (Johnston et al., 2003). Fecal samples were scored both for presence or absence of the pathogens as well as quantification of oocysts and cysts in feces. Counts were calculated by analyzing 10 μl of a 10-ml solution of 0.1 g of feces from each sample. Oocysts or cysts were then quantified by counting total numbers in 150 microscope fields (×400 magnification) and extrapolating results to the entire sample. This method was validated by spiking negative fecal samples with known numbers of *Cryptosporidium* sp. oocysts.

Results were analyzed statistically with the use of the computer program EpiInfo, Version 3.3.2 (Centers for Disease Control, Atlanta, Georgia). Trends were considered statistically significant at an $\alpha = 0.05$ level.

In total, 115 nonhuman primate samples were collected and analyzed. The overall prevalences of *Cryptosporidium* and *Giardia* spp. in nonhuman primates within forest fragments (n = 80) were 6.3 and 3.8%, respectively. None of the primates within the undisturbed forest (n = 35) was positive for *Giardia* or *Cryptosporidium* spp. infections. No animals were found to be simultaneously infected with both parasites.

Prevalence and mean intensities of infection for both parasites are presented in Table I. Red colobus (*Pilocolobus tephrosceles*) and red-tailed guenons (*Cercopithecus ascanius*) harbored *Cryptosporidium* sp. or *Giardia* sp., but black-and-white colobus (*Colobus guereza*) did not. All nonhuman primate samples from the undisturbed forest were negative for both protozoan parasites. Seven of 35 (20%) red colobus and 1 of 20 red-tailed guenons (5%) sampled from forest fragments were infected with either *Cryptosporidium* sp. or *Giardia* sp. For the primates sampled, *Cryptosporidium* sp. was present only in the red colobus inhabiting the forest fragments (prevalence of 14.3%; Table I). Red colobus inhabiting forest fragments were at a significantly higher risk of infection than were red-tailed guenons and black-and-white colobus within the same fragments (Fisher's exact test; $P = 0.014$). Prevalence of *Giardia* sp. was universally low among species, and no significant differences in *Giardia* sp. prevalence among species were noted (Table I).

Location of sampling was significantly related to the prevalence of infection with both parasites in nonhuman primates. No primates sampled from the undisturbed forest harbored species of *Cryptosporidium* or *Giardia*, whereas prevalence of *Cryptosporidium* and *Giardia* spp. among primate species within the forest fragments ranged from 0 to 14.2% (Table I). This difference was marginally statistically significant (Fisher's exact test; $P = 0.049$).

The results of this study suggest that wild nonhuman primates living in disturbed habitats are at greater risk for infection of *Cryptosporidium* and *Giardia* spp. than are primates living in undisturbed habitats. These results further support previous findings that primates in the Kibale region living in disturbed habitats are at higher risk of infection with directly transmitted gastrointestinal parasites than are primates living in less disturbed habitats (Gillespie et al., 2005; Gillespie and Chapman, 2006).

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TABLE I. Prevalence and intensity of *Cryptosporidium* and *Giardia* spp. in wild primates sampled from 3 forest fragments near Kibale National Park, Uganda.*

Species	No. sampled	*Cryptosporidium*		*Giardia*	
		Prevalence (%)	Mean intensity†	Prevalence (%)	Mean intensity†
Red colobus	35	14.3	13.9 ± 9.1	5.7	3.3 ± 2.6
Black-and-white colobus	25	0	—	0	—
Red-tailed guenon	20	0	—	5.0	3.5 ± 0.0

* Differences in prevalence among the 3 forest fragments were not statistically significant for either parasite or for any host species, so results from all fragments were collapsed for analysis.
† Mean intensities are expressed as 10^4 oocysts (*Cryptosporidium*) or cysts (*Giardia*) per gram, ± standard error of the mean; negative samples are not included in this calculation.

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Human Pseudoterranovosis, an Emerging Infection in Chile

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ABSTRACT: Fifteen cases of human pseudoterranovosis are reported for Chile, representing an emerging parasitic infection in this country caused by larvae of the nematode *Pseudoterranova* sp. Our observations also included an outbreak of pseudoterranovosis in 3 of 4 individuals who shared the same raw fish dish (cebiche). Most of the cases occurred in adult patients. The main source of infection was from consumption raw or fried marine fish, including hakes (*Merluccius australis* or *Merluccius gayi*), pomfret (*Brama australis*), Inca scad (*Trachurus murphyi*), and corvina (*Cilus gilberti*). Seasonal distribution showed most of the cases to occur in fall and spring. Parasite larvae were isolated from the mouths of most of the patients after they reported a pharyngeal tickling sensation, coughing, vomiting, or a foreign body in the mouth or throat.

Human anisakidosis produced by anisakid nematodes is most frequently caused by larvae of *Anisakis* (anisakiosis) and *Pseudoterranova* (pseudoterranovosis) (Takahashi et al., 1998; Audicana et al., 2003). A few cases have been attributed to *Contracaecum osculatum* (Takahashi et al., 1998) and a single case to an immature adult of *Hysterothylacium aduncum* (Yagi et al., 1996).

About 97% of the recorded cases of human anisakidosis have occurred in Japan, with the remaining percentages distributed among 26 countries (Takahashi et al., 1998). In the Western Hemisphere, anisakidosis has been reported in Canada, the United States., Mexico, Brazil, Chile (Takahashi et al., 1998), and Peru (Cabrera et al., 2003). Generally, anisakiosis is more common than human pseudoterranovosis in Japan and Europe (Takahashi et al., 1998; Audicana et al., 2003), although in North America, the greatest prevalence occurs with larvae of *Pseudoterranova* spp. (Amin et al., 2000). In Chile, most of the cases reported also were due to pseudoterranovosis, representing 10 of 13 cases of anisakid worms (Sapunar et al., 1976; Apt et al., 1980; Mercado et al., 1997, 2001; Verhamme and Ramboer, 1988; Canese, 1995; Torres, Canales et al., 2000). Two of the anisakid cases acquired in Chile after consumption of raw (cebiche) or smoked fish were diagnosed in Belgium and Paraguay, respectively; the first patient presented symptoms while traveling from Chile to his country of origin, and the second patient while temporarily occupied professionally in Paraguay (Verhamme and Ramboer, 1988; Canese, 1995).

The definitive hosts of *Anisakis* and *Pseudoterranova* species are marine mammals. McClelland (2002) reviewed the life cycle of *Pseudoterranova decipiens*, the eggs of which are released with the feces of the definitive host (seals). The larvae develop to the third stage (L3), and they are then consumed by benthic and epibenthic copepods. They also may be also transmitted to invertebrates such as mature amphipods, decapods, isopods, nudibranchs, cumaceans, mysids, and polychaetes. Bottom fishes are infected when they consume infected crustaceans; larvae are then localized in the musculature. Seals become infected through consumption of crustaceans or fishes with L3s.

The source of human pseudoterranovosis is the consumption of crustaceans or raw fish (as cebiche, sushi, or sashimi), smoked fish, pickled fish, salad, or other poorly cooked fish dishes containing L3s. The L3s are capable of penetrating the stomach or intestinal wall (Ishikura et al., 1993), and they can migrate from the stomach to the esophagus or pharynx, provoking the tingling throat syndrome, which leads to ex-