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CAMPYLOBACTERIOSIS, SALMONELLOSIS, AND SHIGELLOSIS IN FREE-RANGING HUMAN-HABITUATED MOUNTAIN GORILLAS OF UGANDA

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ABSTRACT: For conservation purposes and due to growing ecotourism, free-ranging mountain gorillas (*Gorilla gorilla beringei*) have been habituated to humans. Fecal specimens ($n = 62$) collected in January 1999 from mountain gorillas of the Bwindi and Mgahinga National Parks, Uganda, were tested for *Campylobacter* spp., *Salmonella* spp., and *Shigella* spp., and the overall prevalence of infection was 19%, 13%, and 6%, respectively. The prevalence of positive specimens was not related to the year of habituation of a gorilla group to humans. *Campylobacter* spp., *Salmonella,* and *Shigella* spp. infections were not distributed equally among the age classes of gorillas; most of the enteropathogens (80%), and all *Shigella* spp. organisms, *S. sonnei, S. boydii,* and *S. flexneri,* were isolated from subadults and adult gorillas with ages ranging from 6.0 to 11.9 yr. The prevalence of *Campylobacter* spp. and *Salmonella* spp. infections among human-habituated gorillas has doubled during the last 4 yr, and isolation of *Shigella* spp. for the first time from mountain gorillas, may indicate enhanced anthropozoonotic transmission of these enteropathogens.

Key words: Bacterial infection, *Campylobacter* spp., habituated free-ranging mountain gorillas, *Salmonella* spp., *Shigella* spp., survey.

INTRODUCTION

The range of mountain gorillas (*Gorilla gorilla beringei*) is confined within Rwanda, Democratic Republic of the Congo, and Uganda (Butynski and Kalina, 1993). For conservation purposes and due to growing ecotourism, some groups of free-ranging gorillas have been habituated to humans (Butynski et al., 1990; Butynski and Kalina, 1993). *Iodamoeba buetschlii, Giardia lamblia, Chilomastix* sp., *Endolimax nana, Entamoeba coli,* and *Entamoeba histolytica* have been found in the feces of gorillas and people sharing their habitats. It was thought that human presence facilitated anthropozoonotic transmission of these parasites (Ashford et al., 1990; 1996; Hastings et al., 1992; Mudakikwa et al., 1999). *Cryptosporidium* sp. and *Capillaria hepatica* found in gorillas most frequently contacted by people were considered to be a result of anthropozoonotic transmission and human influence on gorilla habitats (Graczyk et al., 1999; Nizeyi et al., 1999). In 1994, feces ($n = 76$) of some groups of free-ranging human-habituated gorillas were tested for enteric pathogens, i.e., *Campylobacter* spp. and *Salmonella* spp. Their prevalence was 8% and 4%, respectively (Kalema, 1995).

Shigellosis and salmonellosis can significantly contribute to morbidity and mortality of captive lowland gorillas (*Gorilla gorilla gorilla*) (Benirschke and Adams, 1980; McClead et al., 1985; Banish et al., 1993; Stetter et al., 1995; Mundy et al., 1998). *Campylobacter* spp. was not reported from captive gorillas; however, this organism has produced serious gastroduodenal disease in other nonhuman primates (Morton et al., 1983; Buck, 1990; Anderson et al., 1993).

TABLE 1. *Campylobacter* spp., *Salmonella* spp., and *Shigella* spp. infections in free-ranging human-habituated mountain gorillas (*Gorilla gorilla beringei*) of the Bwindi Impenetrable Forest and Mgahinga National Parks, Uganda.

National park	Gorilla group	Number of animals	Year of habituation	Number of fecal spp. samples	Number positive		
					Salmonella spp.	*Shigella* spp.	*Campylobacter*
Bwindi	Ibale	27	1995	27	[a]3	[d]1	5
	Mubale	16	1993	16	[a]1	[d,e]2	3
	Nkuringo	17	1999	12	[a]2	0	2
Mgahinga	Nyakagezi	9	1990	7	[b,c]2	[f]1	2

[a] Serogroup B.
[b] Serogroup A.
[c] Serogroup D1.
[d] *Shigella sonnei* (serogroup D).
[e] *Shigella flexneri* (serogroup B).
[f] *Shigella boydii* (serogroup C).

The purpose of the present study was to determine if *Campylobacter* spp., *Salmonella* spp., and *Shigella* spp. are currently present in free-ranging human-habituated populations of mountain gorillas and, if so, to determine their prevalence and the age class distribution of infected animals.

MATERIALS AND METHODS

In January 1999 fecal samples were collected from one and three groups of mountain gorillas of the Mgahinga (30 km^2) (1°17′S 30°10′E) and Bwindi Impenetrable Forest (332 km^2) (1°42′S 31°15′E) National Parks (southwestern Uganda), respectively (Table 1). Gorillas were visualized from a trail, their age and sex were determined, and the visual contact was maintained to observe defecation (Mwebe, 1998). The feces were collected as soon as possible after defecation into a plastic vial containing phosphate-buffered saline (PBS) (pH 7.4) (Miller and Holmes, 1995) and delivered to the laboratory in a cooler. Fecal specimens originated from night and day nests, and from morning and afternoon gorilla trails (Mwebe, 1998). If defecation was not directly observed, the age of the gorilla was determined based on the fecal lobe diameter or based on the presence of silver hairs (Mwebe, 1998).

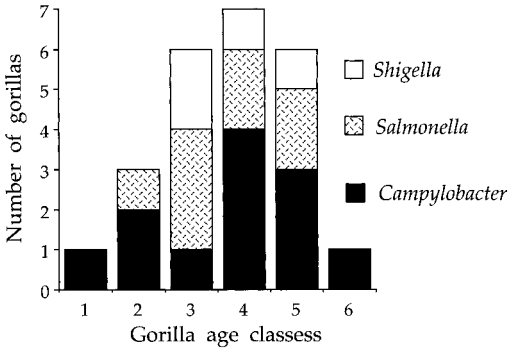

FIGURE 1. Enteric bacteria isolated from feces of free-ranging human-habituated mountain gorillas (*Gorilla gorilla beringei*) of the Bwindi Impenetrable Forest and Mgahinga National Parks, Uganda, (n = 62). Gorilla age classes are (1) infants from 0 to 2.9-yr-old; (2) juveniles at 3.0 to 5.9-yr-old; (3) sub-adults from 6.0 to 7.9-yr-old; (4) sexually mature females adults of >8.0-yr-old; (5) sexually mature males blackbacks from 8.0 to 11.9-yr-old; and (6) sexually mature males silverbacks of >12-yr-old.

In the laboratory, fecal specimens were examined for mucus, blood, or diarrheal appearance, and sorted into 6 age classes consisting of (1) infants from 0 to 2.9-yr-old; (2) juveniles from 3.0 to 5.9-yr-old; (3) sub-adults from 6.0 to 7.9-yr-old; (4) adult sexually mature females of > 8.0-yr-old; (5) sexually mature males blackbacks of > 8.0 to 11.9-yr-old; and (6) sexually mature males silverbacks of > 12-yr-old (Mwebe, 1998) (Fig. 1). Standard techniques for isolation of bacterial enteric pathogens from feces or solid media and broth were used (Gray, 1995; Nachamkin, 1995). Isolation and identification of *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp. were done based on the protocols applied previously to fecal material obtained from lowland gorilla and other nonhuman primates (Morton et al., 1983; Banish et al., 1990; Stetter et al., 1995). All *Shigella*-suspicious slants were tested with *Shigella*-typing antisera (Gibco BRL®, Grand Island, New York, USA), and further serotyped with *Shigella*-grouping antisera (Gibco BRL®, Grand Island, New York, USA) (Gray, 1995;

Stetter et al., 1995) (Table 1., Fig. 1). The serogroup of *Salmonella* spp. isolated from gorilla feces was determined using polyvalent antisera (Gibco BRL®, Grand Island, New York, USA) (McClead et al., 1985; Gray, 1995). Common phenotypic tests, i.e., temperature dependent growth, presence of catalase, H_2S production, and hippurate and indoxyl acetate hydrolysis, were conducted to identify and confirm isolation of *Campylobacter* spp. from gorilla fecal material (Nachamkin, 1995). Statistical analysis was carried out with Statistix 4.1 (Analytical Software, St. Paul, Minnesota, USA). The degree of association between variables was compared using the Partial Correlation test. Fractions of positive fecal specimens were compared using *G*-heterogeneity test, and Kruskal-Wallis analysis of variance (*ANOVA*) was used to determine the significance of differences among variables (Sokal and Rohlf, 1981). Statistical significance was considered at $P \leq 0.05$.

RESULTS

Isolated *Shigella* spp. were determined to be *S. sonnei* (serogroup D), *S. boydii* (serogroup C), and *S. flexneri* (serogroup B) (Table 1, Fig. 1). *Salmonella* spp. isolated from gorilla fecal material represented serogroups B, A, and D1 (Table 1).

The overall prevalence of fecal specimens from which *Campylobacter* spp., *Salmonella* spp., and *Shigella* spp. were isolated was 19%, 13%, and 6%, respectively (Table 1). No enteric illness was observed in any of the gorillas. Significant differences were demonstrated in the number and prevalence among fecal specimens positive for these enteropathogens (Kruskal-Wallis *ANOVA*; $F = 4.35$, $P < 0.047$). Intestinal pathogens were isolated from a total of 35% and 71% of fecal specimens of gorillas from Bwindi and Mgahinga National Parks, respectively (Table 1); these values were significantly different (*G*-heterogeneity test; $F = 25.1$, $P < 0.009$). The overall prevalence of positive specimens from Ibale, Mubale, and Nkuringo gorilla groups (Bwindi National Park) were 33%, 38% and 33%, respectively (Table 1); these values were not significantly different (*G*-heterogeneity test; $F = 25.1$, $P < 0.008$). Neither the prevalence of fecal specimens positive for a specific enteropathogen, nor the overall prevalence (all pathogens together) were related to the year of habituation of a gorilla group to humans or to the age of gorillas (Partial Correlations; $P > 0.045$). No relationship was found between abnormal appearance of the fecal specimens (blood, mucus, or diarrheal consistency) and the presence of enteropathogenic bacteria.

Campylobacter spp., *Salmonella* spp., and *Shigella* spp. infections were not distributed equally among the age classes of gorillas (Fig. 1). Most of these enteropathogens (80%), and all *Shigella* spp., were isolated from fecal specimens of subadults and adult gorillas (age range of 6.0 to 11.9 yr) (Fig. 1). Interestingly, *Campylobacter* spp. was isolated from fecal specimens of gorillas from all age classes (Fig. 1).

DISCUSSION

The present study constitutes the first report of *Shigella* spp. (*S. sonnei, S. boydii,* and *S. flexneri*) isolated from free-ranging gorillas. Morbidity caused by *Shigella* spp., *Salmonella* spp., and *Campylobacter* spp. in captive lowland gorillas and other nonhuman primates is high (Benirschke and Adams, 1980; McClead et al., 1985; Banish et al., 1990; 1993; Stetter et al., 1995; Mundy et al., 1998). Veterinary and medical importance of these enteropathogens is similar due to the their high anthropozoonotic and zoonotic potential (Banish et al., 1990; 1993; Gray-Owens and Schryers, 1993; Stetter et al., 1995).

Habituation of mountain gorillas to humans is a management choice justified for conservation of these endangered animals and economic factors (Butynski et al., 1990; Butynski and Kalina, 1993; Mwebe, 1998). Mountain gorillas represent an important revenue source for African countries and therefore their health and disease status is constantly monitored (Butynski et al., 1990; Butynski and Kalina, 1993). As a result, evidence has accumulated that the habituation process and intensification of human contacts facilitate or enhance an-

thropozoonotic transmission of protozoan and helminthic parasites (Ashford et al., 1990; 1996; Hastings et al., 1992; Graczyk et al., 1999; Nizeyi et al., 1999). For example, most *Cryptosporidium* sp. (73%) were detected in human-habituated gorilla groups, at Ibale and Mubale from Bwindi National Park as compared to non-human-habituated gorillas (Nizeyi et al., 1999).

In 1994, the prevalence of fecal specimens ($n = 76$) of human-habituated gorillas of Bwindi National Park from which *Campylobacter* spp. and *Salmonella* spp. were isolated was 8% and 4%, respectively (Kalema, 1995). The results of the present study yielded prevalences of 18% and 9%, respectively; thus, infections with these enteropathogens has doubled during the last 4 yr. In addition, *Shigella* spp., pathogens that frequently cause mortality in captive lowland gorillas (Banish et al., 1990; 1993; Stetter et al., 1995), were isolated for the first time from mountain gorillas (prevalence 5%). Shigellosis is always an insidious disease in captive gorillas because of the asymptomatic carrier stage, sporadic incidence, multiple etiologies, fulminant manifestation related to animal stress, and antibiotic-resistance (antibiotic-susceptible strains can rapidly acquire resistance) (Banish et al., 1990; Stetter et al., 1995; Mundy et al., 1998). *Shigella flexneri* group B strains caused an outbreak of disease in captive western lowland gorillas that was difficult to manage because the pathogen showed increased antimicrobial resistance over time (Stetter et al., 1995).

The relatively close contact between gorillas and ranger guides, trackers, poachers, tourists, veterinarians, and researchers can enhance anthropozoonotic and zoonotic transmission of *Campylobacter* spp., *Salmonella* spp., and *Shigella* spp. Visitors were considered a plausible source of *S. flexneri* that caused a massive epizootic in a zoological collection of primates (Banish et al., 1990). However, based on the results of the present study, it is difficult to conclude that the observed increased prevalences of these enteropathogens is due to contact with people.

There are multiple etiologies of *Shigella* spp., *Salmonella* spp., and *Campylobacter* spp. infections in non-human primates and multiple routes of anthropozoonotic transmission of these pathogens (Banish et al., 1990; Kalema, 1995; Stetter et al., 1995; Mundy et al., 1998). Surface water has to be considered a factor in the epidemiology of these enteric infections, because gorillas often cross streams and then groom and lick themselves (Nizeyi et al., 1999). Although promiscuous defecation by humans is not allowed in Bwindi and Mgahinga National Parks, this rule may not be followed by people from local communities (Kalema, 1995; Nizeyi et al., 1999). Fecal-oral transmission of pathogens, particularly *Shigella* spp. within a gorilla group could be facilitated by direct contact and coprophagy (Redmond, 1983; Banish et al., 1990).

Mountain gorillas, as strict vegetarians, can fulfill their protein and amino acid requirements by food selection (Casimir, 1975). Diarrhea results when normal food selection is not maintained (Casimir, 1975). In a colony of 10 captive lowland gorillas, protein deficiency disease triggered acute *S. flexneri*-associated gastroenteritis with death in one animal (Mundy et al., 1998). In mountain gorillas, diarrhea may be a result of stress or unbalanced diet (Casimir, 1975), and therefore clinical conclusions on possible enteropathogen infection based on abnormal stool appearance have to be made with caution. In the present study, no relationship was found between abnormal appearance of the fecal specimens and presence of enteropathogenic bacteria.

Ecologic and social factors such as shared water and habitats facilitated transmission of pathogens and parasites between human-and-nonhuman primates (Wolfe et al., 1998). The habituation process of gorillas to humans intensifies some of these factors (Nizeyi et al., 1999). Control of potential anthropozoonotic trans-

mission of enteropathogens associated with this process represents a serious veterinary management challenge.

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