Contributed Paper

Gastrointestinal Bacterial Transmission among Humans, Mountain Gorillas, and Livestock in Bwindi Impenetrable National Park, Uganda

INNOCENT B. RWEGO,*§ GILBERT ISABIRYE-BASUTA,* THOMAS R. GILLESPIE,†‡ AND TONY L. GOLDBERG*†‡

*Makerere University, Department of Zoology, P.O. Box 7062, Kampala, Uganda
†Department of Pathobiology, College of Veterinary Medicine, University of Illinois, 2001 South Lincoln Avenue, Urbana, IL 61802, U.S.A.
‡Department of Anthropology, University of Illinois, Urbana, IL 61801, U.S.A.

Abstract: *Habitat overlap can increase the risks of anthroponotic and zoonotic pathogen transmission between humans, livestock, and wild apes. We collected* Escherichia coli *bacteria from humans, livestock, and mountain gorillas (*Gorilla gorilla beringei*) in Bwindi Impenetrable National Park, Uganda, from May to August 2005 to examine whether habitat overlap influences rates and patterns of pathogen transmission between humans and apes and whether livestock might facilitate transmission. We genotyped 496* E. coli *isolates with repetitive extragenic palindromic polymerase chain reaction fingerprinting and measured susceptibility to 11 antibiotics with the disc-diffusion method. We conducted population genetic analyses to examine genetic differences among populations of bacteria from different hosts and locations. Gorilla populations that overlapped in their use of habitat at high rates with people and livestock harbored* E. coli *that were genetically similar to* E. coli *from those people and livestock, whereas* E. coli *from gorillas that did not overlap in their use of habitats with people and livestock were more distantly related to human or livestock bacteria. Thirty-five percent of isolates from humans, 27% of isolates from livestock, and 17% of isolates from gorillas were clinically resistant to at least one antibiotic used by local people, and the proportion of individual gorillas harboring resistant isolates declined across populations in proportion to decreasing degrees of habitat overlap with humans. These patterns of genetic similarity and antibiotic resistance among* E. coli *from populations of apes, humans, and livestock indicate that habitat overlap between species affects the dynamics of gastrointestinal bacterial transmission, perhaps through domestic animal intermediates and the physical environment. Limiting such transmission would benefit human and domestic animal health and ape conservation.*

Keywords: disease ecology, ecosystem health, *Escherichia coli*, primates, zoonoses

Transmisión de Bacterias Gastrointestinales entre Humanos, Gorilas de Montaña y Ganado en el Parque Nacional Bwindi Impenetrable, Uganda

Resumen: *El traslape de hábitats puede incrementar los riesgos de transmisión de patógenos antroponótica y zoonótica entre humanos, ganado y simios silvestres. Recolectamos bacterias* Escherichia coli *de humanos, ganado y gorilas de montaña (*Gorilla gorilla beringei*) en el Parque Nacional Bwindi Impenetrable, Uganda, de mayo a agosto 2005 para examinar sí el traslape de hábitat influye en las tasas y patrones de transmisión de patógenos entre humanos y simios y sí el ganado facilita esa transmisión. Determinamos el genotipo de 496 aislados de* E. coli *con marcaje de reacción en cadena de polimerasa palindrómica extragénica (rep-PCR) y medimos la susceptibilidad a 11 antibióticos con el método de difusión de disco. Realizamos análisis de genética poblacional para examinar las diferencias genéticas entre poblaciones de bacterias de huéspedes y*

§*email rwegovet@yahoo.co.uk*
Paper submitted October 15, 2007; revised manuscript accepted March 10, 2008.

Conservation Biology, Volume 22, No. 6, 1600–1607

DOI: 10.1111/j.1523-1739.2008.01018.x

localidades diferentes. Las poblaciones de gorilas con alto grado de traslape en el uso de hábitat con humanos y ganado presentaron E. coli *genéticamente similar a* E. coli *de humanos y ganado, mientras que* E. coli *de gorilas sin traslape en el uso hábitat con humanos y ganado tuvo relación lejana con las bacterias de humanos y ganado. Treinta y cinco porciento de los aislados de humanos, 27% de los aislados de ganado y 17% de los aislados de gorilas fueron clínicamente resistentes a por lo menos un antibiótico utilizado por habitantes locales, y la proporción de gorilas individuales con presencia de aislados resistentes declinó en las poblaciones proporcionalmente con la disminución en el grado de traslape con humanos. Estos de patrones de similitud genética y resistencia a antibióticos entre* E. coli *de poblaciones de simios, humanos y ganado indican que el traslape de hábitat entre especies afecta la dinámica de transmisión de bacterias gastrointestinales, probablemente a través de animales domésticos intermediarios y el ambiente físico. La limitación de esa transmisión beneficiaría a la salud de humanos y animales domésticos y a la conservación de simios.*

Palabras Clave: ecología de enfermedades, *Escherichia coli*, primates, salud del ecosistema, zoonosis

Introduction

The nature and frequency of human contact with wild primates is changing as a result of hunting, human encroachment on wildlife habitats, research, ecotourism, and other activities that bring people and primates into close proximity or direct contact (Adams et al. 2001). Such interaction may increase the risks of anthroponotic and zoonotic pathogen transmission, which can reduce human health and the health and viability of wild primate populations (Wallis & Lee 1999). Apes may be particularly susceptible to exchanging pathogens with people. Chimpanzees (*Pan troglodytes*), for example, share 98.77% nucleotide and 99% amino acid identity with humans (Fujiyama et al. 2002). They are also susceptible because they range widely into human habitats (Naughton-Treves et al. 1998; Goldsmith 2000) and are hunted and typically surrounded by high human-population densities (McCallum & Dobson 1995; Gubelman et al. 1995). In addition, many groups of free-ranging mountain gorillas (*Gorilla gorilla beringei*) and chimpanzees have been habituated to humans for purposes of research and ecotourism (Butynski et al. 1990; Butynski & Kalina 1993), which brings them into close proximity to people on a regular basis (Butynski & Kalina 1998; Sleeman et al. 2000; Graczyk et al. 2001).

Certain pathogens have already demonstrated their ability to threaten wild apes. In Gombe National Park, Tanzania, poliomyelitis-like epidemics of possible human origin caused widespread mortality in chimpanzees (Goodall 1983, 1986). In addition, between 1966 and 1997, pneumonia, respiratory disease, and scabies—probably originating from local human populations—caused epidemic mortality in these chimpanzees (Wallis 2000). Ebola virus has been the main infectious killer of gorillas in central Africa, having caused nearly 80% reductions in gorilla populations in border regions of Gabon and the Democratic Republic of Congo (Huijbregts et al. 2003; Walsh et al. 2003; Leroy et al. 2004). Gastrointestinal pathogens of presumed human origin occur in gorilla and chimpanzee populations that are subjects of research and tourism (Nizeyi et al. 1999; Graczyk et al. 2002; Lilly et al. 2002). Pathogens transmitted to apes in such contexts are now appreciated as drivers of ape population declines (Leendertz et al. 2006).

Much of our understanding of the risks of disease transmission between people and apes comes from anecdotal descriptions and retrospective investigations of epidemics (Goodall 1986; Kortlandt 1996; Wallis & Lee 1999), which have typically been sudden and severe (e.g., Ebola). With the exception of scabies and Ebola (Kalema-Zikusoka et al. 2002; Leroy et al. 2004), it has not been proven that lethal microbes such as respiratory pathogens are transmitted between humans and apes, although good anecdotal evidence for such transmission exists, and publications citing this fact usually draw conclusions from the presence of humans at the time of outbreaks. Our current appreciation of infectious disease risks to apes is therefore biased toward directly transmitted and highly virulent pathogens. Less is known about the risks posed by pathogens with chronic or mild clinical manifestations. Pathogens transmitted through the environment and "indirectly" between people and apes may fit into this category.

Our goal was to investigate whether habitat overlap influences rates and patterns of transmission of environmentally persistent and indirectly transmitted microbes between humans and wild apes. We focused on mountain gorillas, an endangered species experiencing frequent contact with people and their livestock (goats, sheep, and cattle). Using a common gastrointestinal bacterium (*Escherichia coli*) as a model system, we investigated the nature of bacterial transmission across ape populations as a function of habitat overlap with people and livestock. The information gathered may be helpful in formulating conservation recommendations to reduce pathogen transmission in areas where apes, humans, and livestock interact at high rates, such as in small protected reserves surrounded by human settlements and ecotourism and research sites.

Methods

Bwindi Impenetrable National Park, a 331-km^2 mid-altitude in southwestern Uganda (0°53′–1°08′N, 29°35′–29°50′E, 1160–2607 m in elevation), contains approximately 320 mountain gorillas (approximately 45% of the world's population). The area was initially designated a central forest reserve in 1932 (Howard 1991) and became a national park in 1991 (Butynski & Kalina 1993). The park is characterized by lowland and montane forests in a continuum and contains numerous steep-sided hills and narrow valleys (McNeilage et al. 2001). The area surrounding the park has a high human population density (average 220 people/km^2 according to a 1991 population census) and is growing at a rate of about 2.7% per year (Gubelman et al. 1995). People living at the park boundary are primarily subsistence farmers from the Bakiga tribe, and they practice mixed-crop agriculture and rear livestock (mainly goats, sheep, and cattle).

We focused on 3 groups of mountain gorillas: Nkuringo, a group of 19 individuals that has been the focus of a tourism venture since 2004 and spends more than 67% of its time outside the park boundary (Goldsmith 2000; Rwego 2004); Kyaguriro, a group of 16 individuals that has been studied continuously for approximately 15 years by researchers but that is not visited by tourists; and a wild, unhabituated gorilla group that has no regular contact with humans and is not the subject of research. The population size of the wild gorilla group is unknown, but it is estimated at approximately 6 individuals on the basis of nest counts we made when we sampled the population. Because of its interior location within the park and the fact that no people live inside the park boundary, the wild gorilla group would not be expected to come into contact with people at the park boundary.

We also focused on people who interact with the mountain gorillas at high frequency as research workers or tour guides or because gorillas raid crops on their land. People employed in gorilla research and tourism consisted of 9 field assistants and 22 guides or porters. All employees were men who ranged in age from 19 to 66 years. They lived outside the park but typically spent from 1 to 8 h each working day in habitats frequented by gorillas. The village of Nkuringo at the park boundary (8 households consisting of 34 individuals, 14 males and 20 females, age range 0.75–70 years) experienced frequent crop raiding by gorillas. We systematically sampled livestock (cattle, goats, sheep) owned by the people from Nkuringo ($n = 48$) to investigate the possible role of domestic animals in human–gorilla bacterial exchange.

We collected fecal samples from human volunteers, their livestock, and mountain gorillas from May to August 2005. We gave human volunteers self-contained, sterile bacterial transport systems containing Cary–Blair agar (BD CultureSwab, Becton Dickinson , Franklin Lakes, New Jersey) and instructed them on the proper method for administering a rectal swab. We collected the swabs within 24 h of distribution. We used gloves to collect fresh fecal samples from livestock and placed the fecal samples in sterile tubes for transport to our field laboratory. We used sterile tongue depressors to collect gorilla fecal samples noninvasively from gorilla night nests not more than 6 h old. We avoided contamination of domestic animal and gorilla samples by collecting only those portions of the fecal material that had not contacted the ground. Prior to the full study, we conducted pilot studies in which we collected samples of soil, water, and vegetation to assess the potential for contamination with environmentally ubiquitous *E. coli* in western Ugandan forests.

We streaked samples and swabs onto MacConkey agar plates for isolation of *E. coli* and incubated plates at 37 °C for 24 h. We transferred up to 4 putative *E. coli* colonies (based on colony morphology) from each sample on which we observed growth in tubes containing 0.1-mL tryptic soy agar, and we stored the tubes at room temperature for up to 4 weeks prior to transport to the United States. Once isolates were in the United States, we reisolated bacteria on MacConkey agar and confirmed the bacteria were *E. coli* with standard biochemical tests (MacFaddin 1980). We then stored the samples in 20% glycerol at −80 °C prior to further analysis.

We used the DNeasy Mini kit (QIAGEN, Valencia, California), eluted in 100 μL volume, to extract DNA from bacterial isolates grown overnight in broth culture. To generate DNA fingerprints for bacterial isolates, we used repetitive extragenic palindromic polymerase chain reaction (rep-PCR). This technique targets consensus sequences of the repetitive extragenic palindromic element, which is dispersed throughout bacterial chromosomes and is useful for inferring relationships among bacterial isolates (Versalovic et al. 1991; Woods et al. 1993). We performed rep-PCRs with previously described amplification protocols (Goldberg et al. 2006) and electrophoresed PCR products in 20 × 30 cm gels of 200 mL 2.0% GenePure LE agarose (ISC BioExpress, Kaysville, Utah) in 1× TAE (Tris-acetate-EDTA) buffer (model A2 gel electrophoresis system [Owl Separation Systems, Portsmouth, New Hampshire]) at 4 °C for 15 h at 62 volts. We loaded 10 μL of DNA length marker (GeneRuler 100-bp DNA ladder plus; Fermentas, Hanover, Maryland) after every sixth sample. We stained gels for 20 min in 1× TAE containing 0.5 mg/L ethidium bromide, destained for 60 min in 1× TAE, and photographed gels immediately under UV light with a Gel Doc XR system (Bio-Rad, Hercules, California). We stored fingerprint images in a database in the computer program Bionumerics (version 4.0; Applied Maths, Austin, Texas), which we also used for band scoring and for calculating genetic distances among bacterial isolates. We used

Table 1. Description of *E. coli* isolates collected from livestock, mountain gorillas, and humans.

Description	*Livestock*	*Mountain gorillas*	*Humans*
No. of individuals sampled	48	66	65
No. of locations sampled	1	3	3
No. of isolates collected and analyzed	139	204	153
No. of genetically distinct isolates	71	129	114
Mean no. of isolates recovered per individual (SE)	2.90 (0.13)	3.09 (0.13)	2.35 (0.12)
Mean no. of genetically distinct isolates per individual (SE)*	1.75 (0.09)	1.95 (0.11)	1.48 (0.10)

**Characteristic rep-PCR banding patterns were used to define unique genotypes.*

parameters specific to the BioNumerics program that were objectively chosen to maximize the correspondence of rep-PCR data to the set standards of multilocus sequence typing. These parameters and the details of their application to infer relationships among *E. coli* isolates are described in detail elsewhere (Goldberg et al. 2006).

We measured susceptibility of all isolates to 11 antibiotics (Ampicillin, Cephalothin, Chloramphenical, Ciprofloxacin, Doxycycline, Gentamycin, Nalidixic acid, Neomycin, Streptomycin, Trimethoprim-sulfaxazole, and Tetracycline) readily available to people in and around Bwindi. We used the disc-diffusion method on Mueller Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) protocols and recommended quality controls and resistance cutoffs (CLSI 2006). For comparison, we also measured susceptibility to Ceftiofur, a broad-spectrum, third-generation cephalosporin antibiotic used in veterinary medicine and not available in the study area.

We used analytical methods available in the computer programs Arlequin (version 3.0; Excoffier et al. 1992), MEGA3 (Kumar et al. 2004), and BioNumerics to measure genetic differences among bacterial populations. We used analysis of molecular variance (AMOVA) to apportion genetic variation among different populations of *E. coli* and thus to examine bacterial population genetic subdivisioning. To quantify genetic distance among bacterial populations, we used the Arlequin program to calculate F_{ST}, which can be interpreted as short-term genetic distances between populations (Reynolds et al. 1983; Slatkin 1995). To examine associations between bacterial genetic distance and distance in other dimensions (host species, location, household), we performed tests of matrix correlation. To test the matrix, we used Mantel's test (Mantel 1967) and a matrix correlation row-wise test (de Vries 1993) with matrix cells containing either F_{ST} values to represent genetic distance or binary values to represent identity or nonidentity of host species, location, or household (MatMan, version 1.1, Noldus Information Technology, Wageninzen, The Netherlands). We used 10,000 permutations of the original matrices to assess the statistical significance of matrix correlations.

Results

Four hundred ninety-six *E. coli* isolates were collected from 48 domestic animals samples, 65 human samples, and 66 mountain gorilla samples. Samples included 100% of humans employed in tourism and research centered around the Nkuringo and Kyaguriro gorilla groups, respectively, people in 8 households from Nkuringo village (representing those who were willing to participate, or approximately 13% of households in the village), and a random sample of livestock owned by these households (Table 1). Porters, trackers, rangers, field assistants, and guides working with Nkuringo and Kyaguriro gorilla groups were combined in the analyses because of the low number of people in each group. In our pilot studies we did not isolate any *E. coli* from the physical environment (soil, water, vegetation).

Genetic differences among bacteria within individual people or animals accounted for the majority of bacterial genetic diversity within mountain gorillas (70.6%) and humans (69.5%) (Table 2). Differences among individuals also accounted for an approximately equal proportion of bacterial genetic diversity in humans (29.6%) and in mountain gorillas (26.1%). Differences among species accounted for only a very small proportion of overall bacterial genetic diversity (5.3%), and differences among locations accounted for a still lower proportion of bacterial genetic diversity of 3.3% and 0.9% for bacteria in mountain gorillas and humans, respectively (all values were statistically significant at $p < 0.001$).

Three hundred fourteen unique rep-PCR genotypes were found among the 496 *E. coli* isolates genotyped (Table 1). Humans and livestock harbored bacteria that were very closely related to each other ($F_{ST} = 0.02$; Table 3). Bacteria from all 3 groups of gorillas were more closely related to bacteria from people employed in gorilla research and tourism than to bacteria from people in local villages. Across gorilla groups, genetic similarity between gorilla bacteria and human bacteria was highest for the tourism group (highest human contact), lower for the research group (intermediate human contact), and lowest for the wild group (lowest or no human contact). No clear trends were observed for genetic relationships between bacteria from gorillas and those from livestock.

Table 2. Hierarchical analyses of molecular variance (AMOVA) for *E. coli* isolates collected from mountain gorillas, humans, and livestock in Bwindi Impenetrable National Park.

Variance component[a]	*Observed partition*		Φ[b]	p[c]
	variance	*% total*		
Mountain gorillas				
between locations	0.278	03.31	CT 0.033	<0.001
among individuals within locations	2.190	26.08	SC 0.270	<0.001
within individuals	5.930	70.61	ST 0.294	<0.001
Humans				
between locations	0.066	0.91	CT 0.009	<0.001
among individuals within locations	2.173	29.62	SC 0.299	<0.001
within individuals	5.096	69.47	ST 0.305	0.032
Gorillas, humans, and livestock				
between species	0.436	05.33	CT 0.534	<0.001
among individuals within species	2.275	27.86	SC 0.294	<0.001
within individuals	5.454	66.80	ST 0.332	<0.001

[a]*Data consist of presence or absence of bands at each of 103 band positions identified and scored with empirically optimized analytical parameters (Goldberg et al. 2006).*
[b]*Phi tested under CT, random permutations of whole populations across locations; SC, random permutations of individuals across populations but within the same location; ST, random permutations of individuals across populations without regard to either their original populations or location (Excoffier et al. 1992).*
[c]*Probability of having a more extreme variance component and Φ statistic than the observed value by chance alone; probabilities were calculated from 16,000 random permutations of the data in the Arlequin computer program.*

Bacteria from both groups of humans and from livestock showed the same pattern of decreasing genetic similarity to *E. coli* from the 3 gorilla groups (Fig. 1). Specifically, *E. coli* from the tourism gorilla group was consistently most similar genetically to *E. coli* from humans. *E. coli* from the research gorilla group was of intermediate genetic similarity to *E. coli* in humans, and *E. coli* from the wild gorilla group was the least similar to *E. coli* from humans. This trend was identical for livestock.

Gorillas from the same group tended to share genetically similar bacteria (Mantel test of matrix correlation, $r = 0.110, p < 0.001$). Nevertheless, people working with the same gorilla group did not tend to share genetically similar bacteria with each other than would be expected by chance ($r = 0.058$, $p = 0.195$). Livestock from the same household also did not tend to share genetically similar bacteria ($r = 0.054$, $p = 0.097$). Humans in the local village living in the same household, however, did tend to share genetically similar bacteria ($r = 0.269$, $p < 0.001$). Finally, row-wise matrix correlation analysis demonstrated that people working with a particular gorilla group tended to harbor bacteria that were more similar genetically to the bacteria of the gorillas they worked with than would be expected by chance (Zr statistic $= -673.2, p < 0.001$).

Thirty-five percent of bacterial isolates from humans, 27% of isolates from livestock, and 17% of isolates from

Table 3. Interpopulation F_{ST} values (SE) for *E. coli* bacteria from humans, livestock, and mountain gorillas in Bwindi Impenetrable National Park, Uganda.*

Population (n)	*Population*					
	1	*2*	*3*	*4*	*5*	*6*
Gorilla workers ($n = 64$)						
Humans from village ($n = 89$)	0.0122					
	(0.074)					
Livestock ($n = 139$)	0.0205	0.0273				
	(0.125)	(0.166)				
Tourism gorillas ($n = 132$)	0.0395	0.0546	0.0645			
	(0.240)	(0.332)	(0.393)			
Research gorillas ($n = 96$)	0.0761	0.0905	0.0250	0.1116		
	(0.463)	(0.551)	(0.152)	(0.679)		
Wild gorillas ($n = 24$)	0.0899	0.1002	0.0773	0.0663	0.0954	
	(0.547)	(0.610)	(0.470)	(0.403)	(0.580)	

**Standard errors were estimated from bootstrap analysis with 10,000 permutations. Sample sizes represent numbers of bacterial isolates per population. The F_{ST} values are statistically significant at $p < 0.05$.*

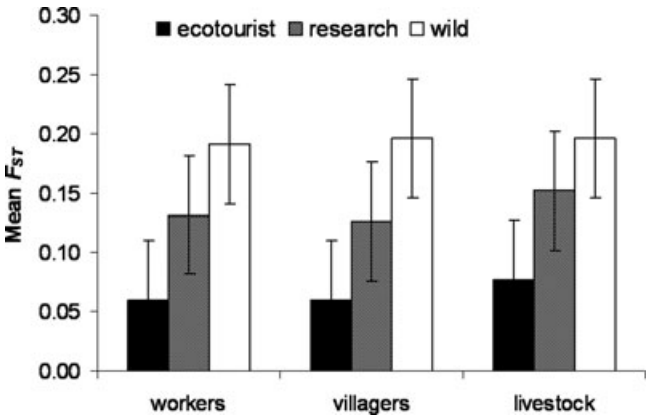

Figure 1. Genetic distance (F_{ST}) between bacteria from mountain gorillas and bacteria from humans and livestock in and around Bwindi Impenetrable National Park, Uganda. Error bars are standard errors. The F_{ST} values were calculated between each person involved in research of mountain gorillas or tourism and the gorillas in different groups (ecotourist, research, and wild).

gorillas were clinically resistant to at least one antibiotic (Fig. 2). There was no resistance to Ciprofloxacin and only one isolate collected from wild gorillas was resistant to Ceftiofur (Fig. 2). Multiple resistances to Chloramphenicol, Streptomycin, Trimethoprim-sulfaxazole,

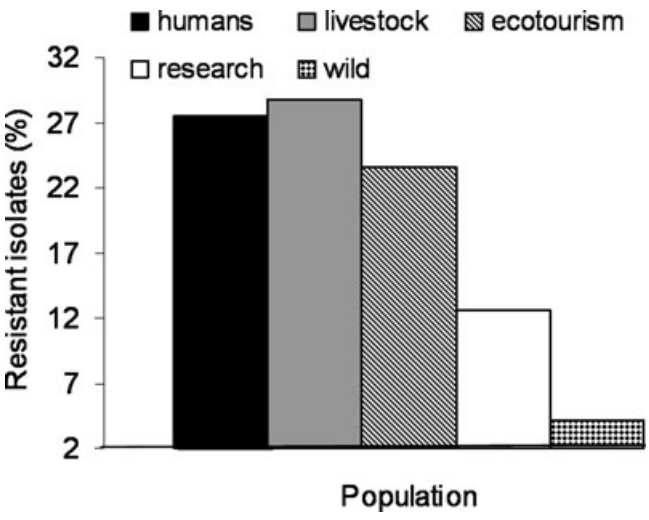

Figure 2. Antibiotic resistance in E. coli *isolates collected from humans, livestock, and different populations of mountain gorillas (ecotourism, research, and wild) in and near Bwindi Impenetrable National Park. Antibiotic resistance testing was done on isolates from humans (*n = *153), livestock (*n = *139), gorillas exposed to tourism (ecotourism,* n = *109) and research (research,* n = *71), and wild gorillas (*n = *24).*

and Tetracycline was observed in 4.2% of genetically distinct isolates, and multiple resistance to Ampicillin, Trimethoprim-sulfathaxazole, and Tetracycline was observed in 7.2% of all genetically distinct isolates. This same pattern was observed in 20.3% of isolates from humans involved in gorilla work and 11.2% of genetically distinct isolates from humans from the village.

Discussion

Our results provide evidence that habitat overlap among humans, livestock, and mountain gorillas can influence patterns of gastrointestinal bacterial exchange among species. Overall, gorilla populations that overlap in their use of habitat with people and livestock tend to harbor *E. coli* bacteria that are genetically similar to *E. coli* from those people or livestock. *E. coli* from the Nkuringo (tourism) gorilla group in particular were consistently most genetically similar to *E. coli* from local people and livestock. Mountain gorillas in the Nkuringo group spend a large percentage of their time outside the park boundary venturing into areas used by humans (Goldsmith 2000) and thus come into direct or indirect contact with villagers and their livestock. Conversely, gorillas in the Kyaguriro group interact with the field assistants working with the group but not with local villagers, and gorillas from the wild group would rarely contact people or their habitats. Our documentation of significant effects of household and gorilla group on relative degrees of human–gorilla bacterial genetic similarity underscore that frequent contact and shared habitats, even on very fine scales, can influence bacterial transmission rates within and among populations of humans, apes, and livestock.

Antibiotic resistance was high in humans in this study. In rural Uganda antibiotics are easily obtained over the counter and may be used indiscriminately. Antibiotics are rarely used for livestock in the Bwindi area, and administration of antibiotics to gorillas has been exceptionally rare. The presence of clinically resistant bacteria in gorillas (especially isolates resistant to multiple antibiotics) implies that antibiotic-resistant bacteria or resistance-conferring genetic elements are diffusing from humans into the gorilla population. Such transmission appears to occur even between humans and gorilla groups that do not overlap with humans, although at a low rate, as evidenced by the presence of an isolate resistant to multiple antibiotics in the wild gorilla group. Our finding of lack of appreciable resistance to Ciprofloxacin, Neomycin, Gentamycin, and Ceftiofur in humans, livestock, and gorillas suggests that local antibiotic use by humans is responsible for the trends observed (Goldberg et al. 2007). Neomycin and Gentamycin are only available in topical and ocular formulations and therefore enteric bacteria like *E. coli*

would not be expected to experience significant selection for resistance to these antibiotics. Ciprofloxacin is expensive and is therefore rarely used by local people, and Ceftiofur is not available in the region. Nearly the same patterns of antibiotic resistance were found in *E. coli* from humans and chimpanzees in a study carried out by Goldberg et al. (2007) in Kibale National Park, Uganda (approximately 200 km north of Bwindi and separated by a densely populated agricultural landscape).

Our results should be interpreted cautiously with respect to transmission. Genetic similarity between bacterial populations does not necessarily imply transmission in the conventional sense (i.e., direct exchange of microbes through direct or immediate contact). Transmission in the Bwindi system may occur indirectly and over extended time periods, perhaps through contaminated environmental sources such as soil and water. Our inability to isolate *E. coli* from environmental sources during the pilot phase of our study may have resulted from limited sampling or seasonal effects; we sampled during the dry season.

Goldberg et al. (2007) examined a different ape species (chimpanzees) in and near Kibale National Park, Uganda, and showed that bacterial gene flow was higher between chimpanzees and humans employed in chimpanzee research and tourism than between chimpanzees and people from local villages who rarely, if ever, share habitats with chimpanzees. This previous study also documented surprisingly high levels of antibiotic resistance in local people and the diffusion of antibiotic resistance to apes. Our findings extend these results to a different ape species and to a different tropical forest location. Like chimpanzees, gorillas that are the subjects of research and tourism appear to be at increased risk of exchanging gastrointestinal microbes with people. In addition, we studied a population of gorillas with little or no contact with humans. Goldberg et al. (2007) did not include a population of chimpanzees with low or no human contact (such a population does not exist in Kibale National Park). That *E. coli* from the wild gorilla group were least similar to those of humans and had the lowest prevalence of antibiotic resistance demonstrates that apes with little or no contact with humans may be at a much-reduced risk of exchanging gastrointestinal microbes with people.

Overall, the patterns of genetic similarity and antibiotic resistance we found reflect the degrees to which apes, humans, and livestock interact. Habituation of mountain gorillas to humans for the purposes of research and tourism (Butynski & Kalina 1993) also appears to be associated with increased risks of gastrointestinal bacterial transmission between the species. Research and tourism on apes in Uganda have been decidedly positive for conservation, in that they have attracted considerable public attention and foreign currency. Moreover, concerns about pathogen transmission already underlie many of the regulations in place governing interactions between people and apes (e.g. minimum observational distances, maximum observation times). Our results suggest, however, that apes even in well-managed situations may be at increased risk of pathogen exchange with humans and livestock. If, as we speculate, common sources of environmental contamination underlie the trends we have documented, then preventing direct or even close contact between people and mountain gorillas may not be sufficient for preventing microbial exchange. This conclusion may apply to gastrointestinal pathogens and to pathogens transmitted by other modes, such as through the respiratory system, that represent serious and potentially epidemic disease threats to wild apes. Strategies such as discouraging people from defecating in the forest, encouraging hand washing before and after entering the forest (Goldberg et al. 2007), mandating the wearing of aerosol-limiting face masks for people entering ape habitats, and encouraging employee health programs (Mountain Gorilla Veterinary Project Health Group 2004) would be reasonable strategies to limit bacterial exchange between people and apes that would safeguard ape health and aid conservation efforts.

Acknowledgments

We gratefully acknowledge the Uganda Wildlife Authority and the Uganda National Council for Science and Technology for granting us permission to conduct this research, and we thank the Institute of Tropical Forest Conservation and especially A. McNeilage for providing facilities and logistic support. We also thank F. Nizeyimana, D. Maniragaba, S. Mbonigaba, D. Mabesha, V. Kobusheshe, and J. Byarugaba for providing invaluable assistance in the field and E. Estoff, K. Inendino, J. Salzer, E. Wheeler, K. Knuffman, and S. Hampson for providing assistance in the laboratory. We based this manuscript on work supported by a Wildlife Conservation Society Research Fellowship (I.B.R.) and by the Morris Animal Foundation (award D04ZO-67 and D07ZO-024).

Literature Cited

Adams, H. R., J. Sleeman, I. Rwego, and J. C. New. 2001. Self-reported medical history survey of humans as a measure of health risks to chimpanzees (*Pan troglodytes schweinfurthii*) of Kibale National Park. Oryx **35:**308–312.

Butynski, T. M., and J. Kalina. 1993. Three new mountain gorilla National Parks for Uganda. Oryx **27:**212–224.

Butynski, T. M., and J. Kalina. 1998. Gorilla tourism: a critical look. Pages 280–300 in E. J. M.-G. A. R. Mace, editor. Conservation of biological resources. Blackwell Science, Oxford, United Kingdom.

Butynski, T. M., E. Werikheis, and J. Kalina. 1990. Status, distribution and conservation of the mountain gorilla in the Gorilla Game Reserve, Uganda. Primate Conservation **11:**31–40.

CLSI (Clinical and Laboratory Standards Institute). 2006. Performance standards for antimicrobial disc susceptibility tests; approved standard. 9th Edition. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania.

de Vries, H. 1993. The rowwise correlation between two proximity matrices and the partial rowwise correlation. Psychometrika **58:**53–69.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distance among DNA haplotypes: applications to human mitochondrial DNA restriction data. Genetics **131:**479–491.

Fujiyama, A., et al. 2002. Construction and analysis of a human-chimpanzee comparative clone map. Science **295:**131–134.

Goldberg, T. L., T. R. Gillespie, I. B. Rwego, E. Wheeler, E. L. Estoff, and C. A. Chapman. 2007. Patterns of gastrointestinal bacterial exchange between chimpanzees and humans involved in research and tourism in western Uganda. Biological Conservation **135:**511–517.

Goldberg, T. L., T. R. Gillespie, and R. S. Singer. 2006. Optimization of analytical parameters for inferring relationships among *Escherichia coli* isolates from repetitive-element PCR by maximizing correspondence with multilocus sequence typing data. Applied and Environmental Microbiology **72:**6049–6052.

Goldsmith, M. L. 2000. Effects of ecotourism on behavioral ecology of Bwindi gorillas, Uganda: preliminary results. American Journal of Physical Anthropology **30:**161.

Goodall, J. 1983. Population dynamics during a fifteen year period in one community of free-living chimpanzee in the Gombe Stream national Park, Tanzania. Zeitschrift fur Tierpsychologie **61:**1–60.

Goodall, J. 1986. The Chimpanzee of Gombe: patterns of behavior. Harvard University Press, Cambridge, Massachusetts.

Graczyk, T. K., J. Bosco-Nizeyi, B. Ssebide, R. C. Thompson, C. Read, and M. R. Cranfield. 2002. Anthropozoonotic *Giardia duodenalis* genotype (assemblage) A infections in habitats of free-ranging human-habituated gorillas, Uganda. Journal of Parasitology **88:**905–909.

Graczyk, T. K., 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.

Mountain Gorilla Veterinary Project Health Group. 2004. Risk of disease transmission between conservation personnel and mountain gorillas: results from an employee health program in Rwanda. EcoHealth **1:**351–361.

Gubelman, E., J. Schoorl, and I. Ochoka. 1995. Bwindi Impenetrable National Park Management Plan 1995–1999. Unpublished report, Uganda National Parks, Kampala, Uganda.

Howard, P. C. 1991. Nature conservation in Uganda's tropical forest reserves. IUCN, Gland.

Huijbregts, B., P. De Wachter, S. Ndong Obiang, and M. Akou Ella. 2003. Ebola and the decline of gorilla (*Gorilla gorilla*) and chimpanzee (*Pan troglodytes*) populations in Minkebe Forest, northeastern Gabon. Oryx **37:**437–443.

Kalema-Zikusoka, G., R. A. Kock, and E. J. Macfie. 2002. Scabies in free-ranging mountain gorillas (*Gorilla beringei beringei*) in Bwindi Impenetrable National Park, Uganda. Veterinary Record **150:**12–15.

Kortlandt, A. 1996. An epidemic of limb paresis (polio?) among the chimpanzee population at Beni (Zaire) in 1964, possibly transmitted by humans. Pan Africa News **3:**9–10.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics **5:**150–163.

Leendertz, F. H., G. Pauli, K. Maetz-Rensing, W. Boardman, C. Nunn, H. Ellerbrok, S. A. Jensen, S. Junglen, and C. Boesch. 2006. Pathogens as drivers of population declines: the importance of systematic monitoring in great apes and other threatened mammals. Biological Conservation **131:**325–337.

Leroy, E. M., et al. 2004. Multiple Ebola virus transmission events and rapid decline of central African wildlife. Science **303:**387–390.

Lilly, A. A., P. T. Mehlmann, and D. Doran. 2002. Intestinal parasites in gorillas, chimpanzees, and humans at Mondika Research site, Dzanga-Ndoki National Park, Central African Republic. International Journal of Primatology **23:**555–573.

MacFaddin, J. F. 1980. Enterobacteriaceace and other intestinal bacteria. Pages 439–464 in J. F. MacFaddin, editor. Biochemical tests for identification of medical bacteria. Williams & Wilkins, Baltimore, Maryland.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research **27:**209–220.

McCallum, H., and A. Dobson. 1995. Detecting disease and parasite threats to endangered species and ecosystems. Trends in Ecology & Evolution **6:**190–194.

McNeilage, A., A. J. Plumptre, A. Brock-Doyle, and A. Vedder. 2001. Bwindi Impenetrable National Park, Uganda: gorilla census 1997. Oryx **35:**39–47.

Naughton-Treves, L., A. Treves, C. Chapman, and R. Wrangham. 1998. Temporal patterns of crop-raiding by primates: linking food availability in croplands and adjacent forest. Journal of Applied Ecology **35:**596–606.

Nizeyi, J. B., R. Mwebe, A. Nanteza, M. R. Cranfield, G. R. 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.

Reynolds, J., B. S. Weir, and C. C. Cockerham. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. Genetics **105:**767–779.

Rwego, I. B. 2004. Prevalence of clinical signs in mountain gorillas, Bwindi Impenetrable National Park. Wildlife and Animal Resources Management, Makerere University, Kampala.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics **139:**457–462.

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 Parc National des Volcans, Rwanda. Journal of Zoo Wildlife Medicine **31:**322–328.

Versalovic, J., T. Koeuth, and J. R. Lupski. 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Research **19:**6823–6831.

Wallis, J. 2000. Prevention of disease transmission in primate conservation. Annals of the New York Academy of Sciences **916:**691–693.

Wallis, J., and D. R. Lee. 1999. Primate conservation: the prevention of disease transmission. International Journal of Primatology **20:**803–826.

Walsh, P. D., et al. 2003. Catastrophic ape decline in western equatorial Africa. Nature **422:**611–614.

Woods, C. R., J. Versalovic, T. Koeuth, and J. R. Lupski. 1993. Whole cell repetitive element sequence based polymerase chain reaction allows rapid assessment of clonal relationships of bacterial isolation. Journal of Clinical Microbiology **31:**1927–1931.