Patterns of gastrointestinal bacterial exchange between chimpanzees and humans involved in research and tourism in western Uganda

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ABSTRACT

Ecological overlap may increase the risks of microbial exchange between humans and wild non-human primates. *Escherichia coli* bacteria were collected from chimpanzees and humans in Kibale National Park, western Uganda, in May and June 2004, in order to examine whether interaction between humans and apes in the wild might affect gastrointestinal bacterial communities in the two species. Chimpanzees harbored bacteria genetically more similar to those of humans employed in chimpanzee-directed research and tourism than to those of humans from a local village. Most humans (81.6%) and 4.4% of chimpanzees harbored at least one isolate resistant to locally available antibiotics. In isolates from both humans and chimpanzees, resistance was higher to five of these antibiotics than to Ceftiofur, an antibiotic not available in the region. These data indicate that humans and apes interacting in the wild can share genetically and phenotypically similar gastrointestinal bacteria, presumably originating from common environmental sources. Strategies to limit transmission of pathogens between humans and primates, whether that transmission is direct or indirect, would benefit both human health and primate conservation.

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1. Introduction

Infectious agents transmitted between humans and non-human primates pose a risk to both human health and primate conservation. Human behavior modifies this risk. For example, hunting likely accounted for the introduction into humans of the progenitor of the HIV-1 virus from chimpanzees in Cameroon in approximately 1931 (Gao et al., 1999; Keele et al., 2006).

Modern-day African hunters harbor retroviral pathogens originating from a diversity of primate species (Wolfe et al., 2004, 2005). Hunting and butchering of primates is also a known risk for Ebola virus transmission (Rouquet et al., 2005).

In the great apes, several instances of pathogen transmission to and from humans have been documented in the context of research and tourism, such that these activities have been implicated as anthropogenic factors increasing transmis-

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sion risk (Gagneux et al., 2001). Between 1966 and 1997, polio-like virus, pneumonia, respiratory disease, and scabies, probably originating from local humans, caused epidemic mortality in chimpanzees in Gombe National Park, Tanzania (Wallis, 2000). Gastrointestinal parasites of likely human origin occur in gorilla and chimpanzee populations that are the subjects of research and tourism elsewhere (Nizeyi et al., 1999; Graczyk et al., 2002; Lilly et al., 2002). In 1994, Ebola virus from a chimpanzee in Cote d'Ivoire infected a chimpanzee researcher (Formenty et al., 1999). Such pathogens are now appreciated as drivers of population declines in wild ape populations (Leendertz et al., 2006), as well as threats to human health.

Despite growing evidence that non-human primates and humans can exchange pathogens in the wild, information is lacking on the range of conditions under which such exchange can occur. Primates today generally live in small protected areas or in habitat mosaics of forest fragments and agricultural land (Marsh, 2003). Under such conditions, humans and wild non-human primates interact in ways that potentially increase the risks of microbial exchange between the species. Such exchange may be direct or indirect (through environmental sources or intermediary host species), and epidemics of clinical disease might not always ensue. Nevertheless, knowing the general conditions that promote the exchange of microbes between humans and non-human primates could help predict and prevent epidemics of clinical disease, thus aiding conservation planning.

The goal of this study was to investigate whether habitat overlap between apes and humans involved in research and tourism might influence the genetic and phenotypic structure of gastrointestinal bacterial communities in the two species. Gastrointestinal microbes are known to pose health threats to wild non-human primates, and their occurrence in primates is associated with anthropogenic habitat disturbance (Nizeyi et al., 1999; Lilly et al., 2002; Gillespie and Chapman, 2006). The study focused on western Uganda, where chimpanzee-directed research and tourism have experienced a recent renaissance and where tourism is being promoted as a strategy for the long-term protection of the region's apes. The common gastrointestinal bacterium *Escherichia coli* (*E. coli*) was chosen as a study system because it is ubiquitous, can be transmitted via a variety of direct and indirect routes, is known to be zoonotic, and, although usually benign, can cause clinical disease in its pathogenic form (Trabulsi et al., 2002; Wasteson, 2001). Examining genetic relationships among bacteria from humans and chimpanzees and measuring their resistance to locally available antibiotics provided information on whether humans employed in chimpanzee research and tourism tended to share similar bacteria with the chimpanzees whose habitats they frequent.

2. Methods

2.1. Study site and populations

The study took place in and around Kibale National Park, Uganda, a mid-altitude forested park of 795 km^2 located in western Uganda near the foothills of the Ruwenzori Mountains (0°13′–0°41′N, 30°19′–30°32′; Chapman et al., 2000).

Approximately 10 communities of chimpanzees inhabit Kibale, three of which are the focus of intensive research efforts or tourism ventures. This study focused on two of these communities: Kanyawara, a research community that has been studied continuously for approximately 20 years (Wrangham et al., 1992), and Kanyanchu, a community that has been the focus of a Uganda Wildlife Authority sponsored tourism venture since 1991.

At the time of the study, 13 people (12 men, 1 woman) were employed as full-time field assistants or ranger-guides at the research and tourism sites, respectively. These people were all Ugandans between approximately 20 and 50 years of age who live outside the park but typically spent between 3 and 8 h each working day in forests frequented by chimpanzees, often observing the animals at close range. For comparison, a population of people from a local village approximately five km from the research station and 25 km from the tourism station was included in the study. People from the village would have had no regular interaction with chimpanzees.

2.2. Collection of bacterial isolates

In May and June of 2004, volunteers from each of the two human populations (high and low chimpanzee contact) were given self-contained, sterile bacterial transport systems containing Cary-Blair agar (BD CultureSwab, Becton, Dickinson and Company, Franklin Lakes, NJ) and were instructed in the proper methods for self-administering a rectal swab. Inoculated swabs were collected anonymously within 24 h of distribution. Chimpanzees were tracked in the forest until individuals were observed to defecate. Fecal material was then collected into sterile tubes. To avoid environmental contamination, care was taken to collect only portions of the material that had not contacted the ground.

Swabs and fecal samples were streaked for isolation onto individual MacConkey agar plates and incubated at 37 °C for 24 h. Up to six putative *E. coli* colonies from each sample were transferred into tubes containing 0.1 ml tryptic soy agar and were stored at room temperature for up to four weeks. Once in the United States, bacteria were re-isolated, subjected to standard biochemical tests for positive identification (MacFaddin, 1980), and stored in 20% glycerol at −80 °C.

2.3. Bacterial genotyping

E. coli isolates were genotyped using Rep-PCR, which targets repetitive sequences dispersed throughout bacterial chromosomes (Versalovic et al., 1991) and has high power for discriminating among closely related *E. coli* isolates (Goldberg et al., 2006; Johnson and O'Bryan, 2000; Woods et al., 1993). DNA extraction, PCR, and electrophoresis protocols are described in detail elsewhere (Goldberg et al., 2006).

2.4. Antibiotic susceptibility testing

By surveying people in the study populations and visiting local health care facilities in May 2004, it was determined that nine antibiotics (and closely-related antibiotics from the same class) were available for purchase in the region, were used commonly by local people, and would be effective

against gram-negative bacteria such as *E. coli*. The susceptibility of all *E. coli* isolates to each of these antibiotics was measured. For comparison, susceptibility was also measured to Ceftiofur, a broad-spectrum, third-generation cephalosporin antibiotic used in veterinary medicine that is not available in the region. Susceptibility testing was performed using the "disc diffusion" method on Mueller Hinton agar, according to Clinical and Laboratory Standards Institute (CLSI) protocols, incorporating recommended quality controls and resistance cutoffs (Clinical and Laboratory Standards Institute, 2005).

2.5. Analysis

Rep-PCR genotypes were stored in a database in the computer program BioNumerics, version 4.0 (Applied Maths, Austin, TX). Optimal analytical parameters for inferring relationships among isolates from Rep-PCR genotypes were chosen based on published guidelines (Goldberg et al., 2006). Population genetic analyses were performed with the computer programs Arlequin, version 3.0 (Excoffier et al., 2005) and MEGA3 (Kumar et al., 2004). Phylogenetic analyses were performed with the computer programs Phylip, version 3.57c (Felsenstein, 1995), PAUP*, version 4.0b10 (Swofford, 2000), and MacClade, version 4 (Maddison and Maddison, 2000). Statistical analyses were performed with the computer programs SAS, version 9 (SAS Institute, Cary, NC) and Systat, version 10.2 (Systat Software, Richmond, CA).

3. Results

Two hundred and fifty *E. coli* isolates were collected from 25 humans and 23 chimpanzees; this included 100% of people employed in chimpanzee research and tourism (Table 1). Data from the human research and tourism populations were combined because of small sample sizes. Eighty-nine unique Rep-PCR genotypes were found among these 250 *E. coli* isolates. Phylogenetic relationships among genotypes were inferred using the neighbor-joining algorithm (Saitou and Nei, 1987) with optimal analytical parameters (Goldberg et al., 2006). Isolates tended to cluster by both species and location (Fig. 1); however, isolates from humans and chimpanzees did not form exclusive clades.

To quantify the apportionment of bacterial genetic diversity within and among individuals, populations, and species, analyses of molecular variance (AMOVA) were performed (Excoffier et al., 1992; Table 2). In both humans and chimpanzees, most bacterial genetic diversity (74.3% and 61.8%, respectively) was contained within individuals. However, differences among individuals accounted for a lower proportion of bacterial genetic diversity in humans (24.9%) than in chimpanzees (37.2%). Differences among species accounted for a still lower proportion of bacterial genetic diversity (12.7%), and differences among locations accounted for only a very small proportion of bacterial genetic diversity (less than 1% in both cases).

Pairwise F_{ST} values, which can be interpreted as short-term genetic distances between populations (Reynolds et al., 1983; Slatkin, 1995), were calculated between bacterial populations using Arlequin. F_{ST} between bacteria collected from humans engaged in chimpanzee-directed research/tourism and bacteria collected from chimpanzees was 0.119. This was significantly lower than F_{ST} between humans from the local village and the same chimpanzees (0.170; $t = 2.75$, $P = 0.003$).

Phylogenetic analyses were used to investigate directional biases in bacterial transmission within and between species (methods are described in Goldberg, 2003). Briefly, observed proportions of different classes of inferred directional transmission events were compared to null distributions derived from 1000 trees of equal size and host species composition randomly generated using the computer program MacClade (Maddison and Maddison, 2000). Interspecific transmission events were inferred significantly less frequently than would be expected by chance (8.2% of all inferred transmission events were interspecific, which was significantly lower than the null expectation of 17.7%; $P = 0.002$). There was no statistically significant bias in the direction of interspecific transmission (human-to-chimpanzee versus chimpanzee-to-human; Fisher's exact test; $P = 0.36$). The frequencies of inferred intra-specific transmission events (human-to-human and chimpanzee-to-chimpanzee) were not significantly different from null expectations (Fisher's exact tests; $P = 0.152$ and 0.715, respectively).

Fifty-four percent of isolates from humans and 3.8% of isolates from chimpanzees were clinically resistant to at least one antibiotic (Fig. 2). At least one isolate clinically resistant to at least one antibiotic was recovered from 81.6% of humans and 4.4% of chimpanzees. No isolates were resistant to Ceftiofur, which is not available in the region (Fig. 2). Resistance to Ciprofloxacin, Gentamicin, and Neomycin was not appreciably different from that to Ceftiofur in isolates collected from either humans or chimpanzees.

Table 1 – Description of E. coli isolates collected from humans and chimpanzees		
	Humans	Chimpanzees
Individuals sampled	25	23
Locations sampled[a]	3	2
Isolates collected and analyzed	130	120
Isolates per individual[b]	4.80 ± 0.34	5.65 ± 0.19
Unique genotypes per individual[b]	2.56 ± 0.29	2.22 ± 0.22

a Locations included a research site and a tourism site from which both humans and chimpanzees were sampled ($n = 6$ humans (6 males) and 14 chimpanzees (6 males, 5 females, 2 unknown), and 7 humans (6 males, one female) and 9 chimpanzees (2 males, 2 females, 5 unknown) from the research and tourism sites, respectively), and a nearby village from which only humans ($n = 12$; 8 males, 4 females) were sampled.
b Mean ± standard error; unique genotypes were defined by their characteristic Rep-PCR banding patterns.
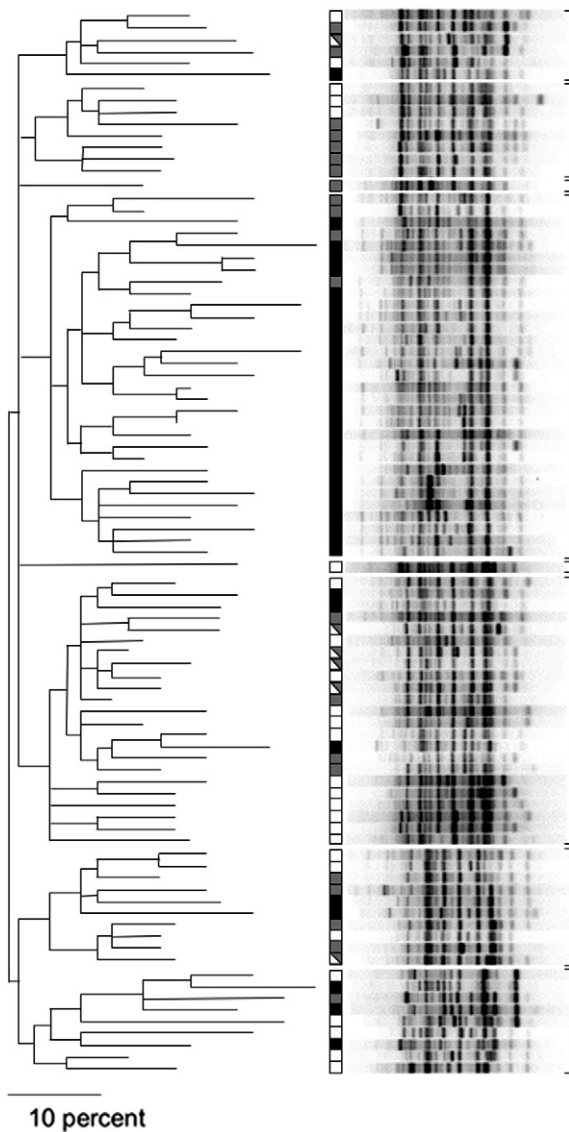
Fig. 1 – Phylogram of 89 unique genotypes found among 250 *E. coli* isolates collected from humans and chimpanzees in the region of Kibale National Park, Uganda. Genetic distances were calculated using optimized parameters according to the methods of Goldberg et al. (2006). The tree was constructed using the neighbor-joining algorithm (Saitou and Nei, 1987), and the analysis was performed with the computer program BioNumerics, version 4.0 (Applied Maths, Inc). Boxes indicate species and locations from which genotypes were recovered: white = humans from a local village with no regular interaction with chimpanzees; gray = humans employed in chimpanzee research or tourism; black = chimpanzees that are the focus of research and tourism. Divided boxes indicate genotypes found in individuals from more than one population. Brackets indicate major clusters.

Of genetically distinct isolates within individuals, 30.1% were resistant to multiple antibiotics. Fifteen different multiple antibiotic resistance patterns were observed, but only three of these occurred at a frequency greater than 1%.

Multiple resistance to SOX-TET-TMP was observed in 3.3% of genetically distinct isolates, multiple resistance to AMP-CHL-SOX-STR-TET-TMP was observed in 8.1% of genetically distinct isolates, and multiple resistance to AMP-SOX-STR-TET-TMP was observed in 9.8% of genetically distinct isolates. Only the last, most frequently observed pattern was found in chimpanzees; two genetically distinct isolates displaying this pattern were recovered from a single chimpanzee at the research site (Yogi, an adult male). This same pattern was observed in 8.3% of genetically distinct isolates from humans at the village site and 20.6% of genetically distinct isolates from humans employed in chimpanzee research or tourism.

4.   Discussion

The results of this study show that chimpanzees in Kibale National Park, Uganda, tend to harbor bacteria that are more similar genetically to bacteria of humans engaged in research and tourism than to bacteria of people from a nearby village with limited interaction with chimpanzees. Phylogenetic and population genetic analyses both indicate that bacterial gene flow among individuals within chimpanzees and humans is high, and that bacterial gene flow between the species is comparatively lower. Nevertheless, bacterial gene flow between the species is not zero, and this gene flow appears to be higher between populations of the two species that interact in the wild.

Levels of antibiotic resistance were surprisingly high in humans in this study, considering that rural Ugandans generally have small incomes and limited access to health care. However, antibiotics are available "over the counter" in Uganda and may be used indiscriminately. Antibiotics have never been administered to Kibale chimpanzees. The presence of clinically resistant isolates in chimpanzees provides evidence for the transmission of resistant bacteria or resistance-conferring genetic elements from humans to chimpanzees. The lack of appreciable resistance to Ciprofloxacin, Neomycin, Gentamicin, and Ceftiofur in either humans or chimpanzees suggests that local antibiotic use is responsible for the patterns observed. Of the nine locally available antibiotics included in the study, Neomycin and Gentamicin were the only ones not available in oral formulations (they were available in topical and ocular formulations only). Enteric bacteria such as *E. coli* would therefore not be expected to experience significant selection for resistance to these antibiotics. Ciprofloxacin, although available, is expensive and used infrequently by local people, and Ceftiofur is not available in the region.

Inferences about transmission from the results of this study should be drawn with caution. For example, similar bacterial genes may or may not be responsible for the shared patterns of antibiotic resistance seen in humans and chimpanzees in Kibale. Although preliminary data (unpublished) show a high (approximately 50%) prevalence of class 1 integrons (see Mazel, 2006) among resistant isolates in Kibale, full characterization of these and other genes would be required to draw stronger inferences about transmission. Furthermore, transmission need not be direct. Chimpanzees and the humans who observe them probably acquired genetically and

Table 2 – Hierarchical analyses of molecular variance (AMOVA) for E. coli isolates collected from humans and chimpanzees in the region of Kibale National Park, Uganda[a]

Variance component		Observed partition		ϕ statistic	P[b]
		Variance	% Total		
Humans (n = 120 isolates from 25 individuals at three locations)					
Among locations	σ_a^2	0.058	0.81	$\phi_{CT} = 0.008$	<0.001
Among individuals within locations	σ_b^2	1.795	24.89	$\phi_{SC} = 0.251$	<0.001
Within individuals	σ_c^2	5.359	74.30	$\phi_{ST} = 0.257$	0.192
Chimpanzees (n = 130 isolates from 23 individuals at two locations)					
Between locations	σ_a^2	0.080	0.95	$\phi_{CT} = 0.010$	<0.001
Among individuals within locations	σ_b^2	3.132	37.21	$\phi_{SC} = 0.376$	<0.001
Within individuals	σ_c^2	5.206	61.84	$\phi_{ST} = 0.382$	0.238
Humans and chimpanzees (n = 250 isolates from 48 individuals at three locations)					
Between species	σ_a^2	1.174	13.07	$\phi_{CT} = 0.131$	<0.001
Among individuals within species	σ_b^2	2.531	28.17	$\phi_{SC} = 0.324$	<0.001
Within individuals	σ_c^2	5.278	58.76	$\phi_{ST} = 0.412$	<0.001

a Isolates were analyzed from humans at three locations (a research site, a tourism site, and a nearby village) and from chimpanzees at two locations (the same research and tourism sites). Data consisted of bacterial genotypes represented as series of binary variables indicating the presence/absence of bands at each of 85 band positions, identified and scored using the "bandmatch" procedure of the computer program BioNumerics, version 4.0 (Applied Maths, Inc.), employing optimal analytical parameters (Goldberg et al., 2006). AMOVA was performed with the computer program Arlequin, version 3.0 (Excoffier et al., 2005).
b 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 using Arlequin.

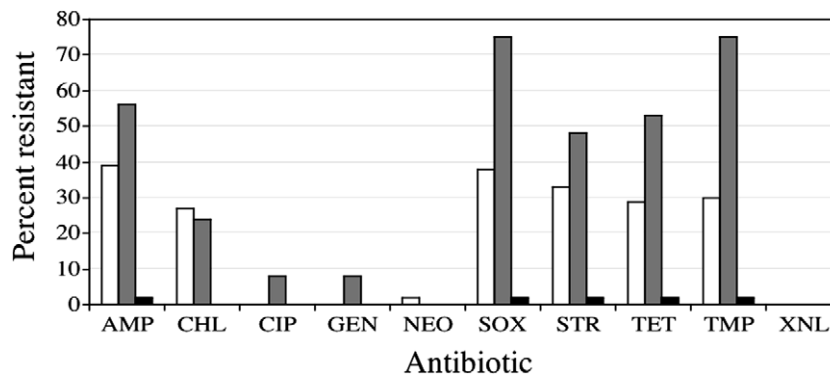**Fig. 2 – Antibiotic resistance in E. coli isolates collected from humans and chimpanzees in the region of Kibale National Park, Uganda. White bars represent isolates (n = 36) from humans in a local village with no regular interaction with chimpanzees, gray bars represent isolates (n = 34) from humans employed in chimpanzee research or tourism, and black bars represent isolates (n = 53) from chimpanzees that are the focus of research and tourism. Heights of bars indicate percent of isolates resistant to each of nine antibiotics commonly available in the region (ampicillin, AMP; cloramphenicol, CHL; ciprofloxacin, CIP; gentamicin, GEN; neomycin, NEO; sulfisoxazole, SOX; streptomycin, STR; tetracycline, TET; and trimethoprim, TMP), plus a tenth antibiotic (Ceftiofur, XNL) not available in the region. Duplicate isolates with indistinguishable Rep-PCR genotypes and antibiotic resistance patterns recovered from the same individual were removed prior to the analysis to avoid pseudoreplication; however, results from the full data set were essentially the same.**

phenotypically similar bacteria through contact with common environmental sources. The data presented here do not permit estimation of how recently such indirect bacterial exchange might have occurred, or whether it is still occurring. Although they spend much of their time in forests, chimpanzees often venture into other habitats such as woodland, grassland, and human settlements to travel and forage (Kortlandt, 1983). Members of the chimpanzee communities studied are known periodically to enter villages near the edge of the forest to raid crops (Naughton-Treves et al., 1998). Contact

with environmental reservoirs of human bacteria could have occurred during such incursions.

Overall, the results of this study suggest that humans and chimpanzees that interact in the wild can share enteric bacteria that are genetically and phenotypically more similar than would be expected by chance. Regardless of whether this represents direct transmission or, more likely, indirect transmission through common environmental sources, this conclusion urges increased caution in the operation of ape-focused research and tourism endeavors. "Simple" strategies

such as discouraging defecation in the forest, encouraging hand washing before entering or leaving the forest, placing strict limits on the distance at which chimpanzees can be observed, and regularly monitoring the health status of employees of research and tourism ventures (Mountain Gorilla Veterinary Project 2002 Health Group, 2004) should prove useful. Because of the growing benefits of ape-directed research and tourism to the local economies and wildlife of developing nations, the disease-associated risks of these activities need to be minimized.

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