EcoHealth 15, 143–147, 2018
https://doi.org/10.1007/s10393-017-1303-4

ECOHEALTH

Short Communication

Zoonotic Enterobacterial Pathogens Detected in Wild Chimpanzees

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Abstract: Infectious diseases including those acquired through direct or indirect contact with people and livestock threaten the survival of wild great apes. Few studies have reported enterobacterial pathogens in chimpanzees. We used multiplex PCR to screen faeces of chimpanzees sharing a landscape with villagers and livestock in Bulindi, Uganda for *Salmonella* spp., enterohemorrhagic *Escherichia coli* (*E. coli*) and *Shigella* spp./enteroinvasive *E. coli*. All three potentially zoonotic pathogens were detected. Individual prevalence ranged between 7 and 20%, with most infections observed in mature male chimpanzees. These preliminary findings suggest detailed investigation of enterobacterial infections in people, primates and livestock in this ecosystem is warranted.

Keywords: *Salmonella*, *Shigella*, *Escherichia coli*, *Pan troglodytes*, Health monitoring, Pathogen screening

Enterobacterial pathogens including *Shigella* spp., *Salmonella* spp. and enterovirulent *Escherichia coli* (*E. coli*) are leading causes of diarrhoeal diseases in humans worldwide (Beutin 2006; Majowicz et al. 2010; Kotloff et al. 2013). Surveys of enteric bacteria infecting wild nonhuman primates have been few relative to helminth, protozoan and viral parasites (Nunn and Altizer 2006). However, increasing contact between people, domestic animals and wild primates enhances conditions for transmission of zoonotic parasites including bacteria (Chapman et al. 2005). Exchanges of benign forms of *E. coli* have been documented between humans, livestock and primates including great apes sharing high levels of ecological overlap (Goldberg et al. 2007, 2008; Rwego et al. 2008). The few reports of enterobacterial infections with strong pathogenic potential in wild primates suggest these likely arise out of direct or indirect contact with humans or livestock (Nizeyi et al. 2001; Kaur et al. 2011; Bublitz et al. 2015; Beisner et al. 2016).

At Bulindi (1°29′N, 31°28′E), Uganda, chimpanzees (*Pan troglodytes schweinfurthii*) inhabit small fragments of degraded forest amid agricultural fields, villages, roads, schools and trading centres (McLennan and Asiimwe 2016). The chimpanzees enter villages habitually to forage on agricultural crops and have daily encounters with people and domestic animals, including cattle, pigs, goats, chickens, dogs and cats (McLennan 2013). Villagers use forest

Published online: November 30, 2017

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fragments for timber, fuelwood and cattle grazing, and collect water from forest streams. Other primates in Bulindi include black and white colobus monkeys (*Colobus guereza*), tantalus monkeys (*Chlorocebus tantalus*), and olive baboons (*Papio anubis*). Surveys of gastrointestinal helminths and protozoa infecting the chimpanzees are reported in Ota et al. (2015), Hasegawa et al. (2016, 2017) and McLennan et al. (2017).

The aim of this preliminary study was to screen faeces of chimpanzees inhabiting the 'high-risk' environment at Bulindi for three bacterial pathogens that can cause severe disease in humans and have known zoonotic potential: *Salmonella* spp., enterohemorrhagic *E. coli* (EHEC), and *Shigella* spp./enteroinvasive *E. coli* (EIEC). Forty faecal samples were collected noninvasively between late-March and early-June 2015, coinciding with the April–May wet season (see McLennan et al. 2017 for climate details). The chimpanzees are habituated to researchers and were observed at distances of 15 m or less, including when chimpanzees were in trees directly overhead. Samples were collected only from fully identified individuals, and as quickly as possible post-defecation to minimize potential environmental contamination. All samples were collected within forest fragments, and never in agricultural fields or village areas. Between 1 and 5 samples were obtained from 15 of the 21 individuals in the Bulindi chimpanzee 'community', including all adult and subadult males ($N = 4$), all adult and subadult females ($N = 8$), 3 of 4 juveniles, but none of 5 infants. Approximately 1–2 g faeces were placed in 15-ml tubes prefilled with 10 ml RNA*later*® (Ambion). Faecal consistency was noted for soft or diarrhoeic stools. No blood was noted in any faecal sample. Samples were stored in a refrigerator (≤ 5°C), transported in a cold condition, and preserved at − 80°C until DNA extraction at Mahidol University, Thailand.

DNA was extracted from 200 mg faeces using the commercially available PSP Spin Stool DNA Kit (Stratec Inc., Germany) according to the manufacturer's instructions. The final elution volume was 100 µl. The screening of *Salmonella* spp., EHEC, and *Shigella* spp./EIEC targeting the invasion protein (*invA*) gene, verocytotoxin (VT) genes (*VT1*, *VT2*, *VT2vha*, *VT2vhb*, *VT2vp1*), and invasion plasmid antigen H (*ipaH*) gene, respectively, was performed using a ready-to-use multiplex PCR kit (Toyobo, FIK-101) according to the manufacturer's recommendations. Lengths of the PCR products for target genes were 610–655, 430–480, and 260–290 bp, respectively. The amount of template DNA in one reaction was 2 µl, and the total reaction volume was 20 µl. Each PCR series included an extraction control, a positive control for all tested organisms, and a negative control. In addition, each reaction included an internal amplification control (length of PCR products 685–735 bp) which indicates the quality of the sample, and the detection of PCR inhibitors and false negatives. The multiplex PCR profiles consisted of heat activation at 20°C for 10 min and 95°C for 2 min; ten cycles of 94°C for 2 s, 57°C for 30 s and 68°C for 30 s; fifteen cycles of 94°C for 10 s and 55°C for 30 s and 68°C for 30 s; twenty cycles of 94°C for 10 s and 53°C for 30 s and 68°C for 30 s. Five microlitres of PCR products were separated by 1.5% agarose gel electrophoresis and visualized after staining for 10 min in a 1 µg/ml ethidium bromide solution. A standard 100-bp DNA size marker (Fermentus, USA) was included in every gel.

All three bacterial pathogens were detected in ≥ 1 faecal sample (Table 1). Overall, 8 samples (20%) tested positive for ≥ 1 target bacteria with dual infections detected in 2 samples. Individual prevalence (% of chimpanzees positive) was 7, 20 and 20% for *Salmonella* spp., EHEC, and *Shigella* spp./EIEC, respectively, and in each case was higher than sample prevalence (% of total faecal samples positive).

Four chimpanzees tested positive for at least 1 target bacteria. Three of 4 adult or subadult males tested positive, with plural infections detected in 2 males. Conversely, only 1 of 8 adult and subadult females tested positive. None of 3 juveniles tested positive (Table 1).

Six faeces were partially diarrhoeic, including 3 that were positive (EHEC once and *Shigella*/EIEC twice). Four partially diarrhoeic faeces comprised undigested agricultural fruit pulp (jackfruit and mango), as is often observed in loose stools at Bulindi (unpublished data) and may not signify gastric upset. However, 2 partially diarrhoeic faeces from 2 different males lacked undigested pulp and tested positive for *Shigella*/EIEC (2 of 4 positive samples for this bacterium). One was coinfected with *Cryptosporidium* sp. (unpublished data). The other contained > 10 undigested whole leaves of *Desmodium velutinum* and *Aneilema nyasense*. Ingestion of bristly leaves of these plants is associated with expulsion of nodular worms (*Oesophagostomum* spp.) in chimpanzee faeces at Bulindi (McLennan and Huffman 2012). However, this behaviour might also be stimulated by abdominal discomfort caused by other pathogens (McLennan et al. 2017).

We are unaware of previous reports of *Salmonella* spp., EHEC, and *Shigella* spp./EIEC infections in wild chim-

Table 1. Prevalence of *Salmonella* spp., Enterohemorrhagic *E. coli* (EHEC) and *Shigella* spp./Enteroinvasive *E. coli* (EIEC) in 15 Chimpanzees of the Bulindi Community, Uganda.

Chimpanzee			No. (%) samples positive[2]			
	Age class[1]	No. samples collected	*Salmonella*	EHEC	*Shigella*/EIEC	No. infections detected
Males						
SL	Adult	3	–	1 (33.3%)	2 (66.6%)	2
MR	Adult	2	–	–	1 (50%)	1
TM	Adult	5	–	–	–	0
MO	Subadult	5	1 (20%)	1 (20%)	1 (20%)	3
AR	Juvenile	2	–	–	–	0
GD	Juvenile	1	–	–	–	0
Females						
JY	Adult	3	–	–	–	0
MN	Adult	3	–	3 (100%)	–	1
TD	Adult	1	–	–	–	0
OL	Adult	1	–	–	–	0
MD	Adult	5	–	–	–	0
LL	Adult	2	–	–	–	0
JM	Subadult	4	–	–	–	0
JN	Subadult	2	–	–	–	0
TB	Juvenile	1	–	–	–	0
Sample prevalence ($N = 40$)			1 (2.5%)	5 (12.5%)	4 (10%)	
Individual prevalence ($N = 15$)			1 (6.6%)	3 (20%)	3 (20%)	

[1]Juvenile (4–7 years), Subadult (8–12 years); Adult (> 12 years).
[2]A dash indicates an individual's samples all tested negative for the pathogen.

Figure 1. Adult male chimpanzees eating mangoes in a vegetable garden at Bulindi. Photo by Jacqueline Rohen.

panzees. Based on their sequence of the 16S rRNA-gene, *Shigella* and *E. coli* are highly related. They can be differentiated on physiological and biochemical characteristics, but not by screening for the presence of the *ipaH* gene alone (van den Beld and Reubsaet 2012). Thus, we could not separate them here. Post-defecation environmental contamination is an unlikely explanation for our findings, given faeces were collected quickly after defecation. How-

ever, a disadvantage of PCR screening is that it does not discriminate dead from live bacteria. It remains possible that the infections detected result from ingestion of foods containing bacterial DNA, particularly vertebrate prey (De Nys et al. 2015). But since the Bulindi chimpanzees exhibit negligible levels of carnivory (Cibot et al. 2017), this is highly unlikely. Moreover, some consistency was evident in samples collected from infected chimpanzees, i.e. each of 3 samples collected over a period of 6 weeks from female MN (the only infected female in our study) tested positive for EHEC; 2 of 3 samples from male SL tested positive for *Shigella* spp./EIEC (Table 1).

Prevalence of enterobacterial pathogens in the chimpanzees was overall similar to prevalence reported in other wild primates sharing habitats closely with people and domestic animals (Nizeyi et al. 2001; Bublitz et al. 2015; Beisner et al. 2016). However, relatively few samples were collected per individual chimpanzee in this short study. Given the intermittent nature of faecal pathogen shedding, individual prevalence would potentially increase with additional sampling.

There was some indication that enterobacterial pathogens were more prevalent in adult and subadult male chimpanzees compared to females and juveniles. Mature males are most likely to enter village areas including livestock holdings in search of agricultural crops (Krief et al. 2010; McLennan 2010; Hockings et al. 2012) (Fig. 1). Such behaviour might increase their exposure to potential environmental reservoirs of zoonotic bacteria from people and livestock.

Like all great apes, wild chimpanzee populations are declining rapidly. Infectious diseases including those acquired through direct or indirect contact with people and domestic animals pose substantial threats to their survival (Gilardi et al. 2015). Deaths of captive apes have previously been attributed to shigellosis and salmonellosis (Rewell 1949; Ocholi et al. 1987; Enurah et al. 1988). However, the pathogenesis of the target bacteria in wild apes warrants further investigation. In this study, three of 8 positive samples (37.5%) were partially diarrhoeic, including one faeces that contained evidence of self-medication (whole leaf swallowing). No other behavioural indications of ill health were noted in infected individuals. This preliminary study underscores the need to screen local people and livestock for bacterial pathogens, alongside further sampling of chimpanzees and other primates in the Bulindi ecosystem. Bacterial culture with molecular genotypic methods will be required to determine isolated strains, evaluate host specificity, and establish potential transmission pathways.

Acknowledgements

This research was approved by the Uganda National Council for Science and Technology, the President's Office, and the Uganda Wildlife Authority. All applicable institutional and/or national guidelines for the care and use of animals were followed. Sample collection was conducted with assistance from Tom Sabiiti. Matthew McLennan was supported by an Early Career Fellowship from the Leverhulme Trust, UK. Laboratory analysis was supported partly by JSPS Core-to-Core Program (B. Asia-Africa Science Platforms). The manuscript was improved by comments from two anonymous reviewers.

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