



Prevalence of Enterobacteriaceae in Wild Long-Tailed Macaques (*Macaca fascicularis*) in Thailand

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Introduction

An expanding human population has increased the risk of zoonosis (Devaux *et al.* 2019), particularly between humans and wild primates, given our close evolutionary histories and shared ecologies (Fuentes and Hockings 2010). Among the major classes of zoonotic agents, some enteropathogenic bacteria (family Enterobacteriaceae) can cause lethal diarrheal disease and pose a serious threat to all primates (McLennan *et al.* 2018). Enterobacteriaceae may enter primate populations from livestock, soil, human foods, and water (McLennan *et al.* 2018). Epidemiological studies in wild primates, both endangered species (gorillas, *Gorilla gorilla*; chimpanzees, *Pan troglodytes*) and widely distributed species that thrive in (peri)urban environments (rhesus macaques, *Macaca mulatta*; olive baboons, *Papio anubis*) speculate that primates, aside from

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being at risk and showing disease symptoms themselves, also act as reservoirs or carriers of Enterobacteriaceae and pose risks of bacterial spillover into overlapping humans and other wildlife (reviewed in McLennan *et al.* 2018).

Among the most widely distributed, socioecologically flexible primates, long-tailed macaques (*Macaca fascicularis*) are found throughout Southeast Asia, where frequent occurrence of human–macaque interactions have led to calls for macaque population control (Malaivijitnond *et al.* 2011). Such interactions may also increase macaques' exposure to zoonotic Enterobacteriaceae, which nevertheless remain underassessed among wildlife populations in the Asian tropics. Here we report the prevalence of three Enterobacteriaceae [*Salmonella* spp., *Shigella* spp., and Shiga toxin producing *Escherichia coli* (STEC)] in long-tailed macaques living in human-impacted environments in Thailand.

Methods

Between July 22, 2019 and July 26, 2019, we collected and processed 168 samples from 143 macaques from three sites in Thailand—Wat Haad Moon (WHM: 16.3°N, 100.1°E), Wat Ta Sung Tai (WTST: 15.5°N, 99.5°E), and Wat Tam Mi Ka Wat (WTMK: 14.5°N, 100°E)—across which samples were fairly evenly distributed (Electronic Supplementary Material [ESM] Table SI). All three sites are temples or towns connected to fragmented forests that have wild boars (*Sus scrofa*) and feral dogs (Malaivijitnond *pers. obs.*), which may shed Enterobacteriaceae. The monkeys are protected by the Wildlife Act of Thailand (B.E.2562). Macaques at all three sites forage in fragmented forests and rely heavily on human-provisioned foods.

Eighty-three percent of the samples (140/168) were rectal swabs from trapped, anesthetized individuals (Malaivijitnond *et al.* 2008; details in the ESM). For trapped animals, we recorded the identity tag, sex, and body-mass (kg) and estimated their age (yr) from their state of dental eruption (details in Malaivijitnond *et al.* 2008). The remaining samples (28/168, 17%) were fresh feces collected opportunistically from unidentified individuals when they were seen defecating. We collected these samples from the ground without soil contamination. We stored all samples on ice and transported them to the Department of Veterinary Public Health, Chulalongkorn University, on the day of collection.

We followed the procedures of the International Organization for Standardization to test for *Salmonella* spp. (ISO-6579) and *Shigella* spp. (ISO-21567). We used a procedure for *Escherichia coli* and coliforms to test for STEC, in accordance with the U.S. Food and Drug Administration (U.S. FDA) Bacteriological Analytical Manual (BAM) (ESM). We calculated the prevalence of each bacteria for each population as the number of samples in which we confirmed bacterial presence over the total number of samples processed.

To explore the impact of site, and macaque age, sex, and body mass, on bacterial infection, we ran a multivariate logistic general linear model (GLM) on an “effective” sample size of 140 macaques (details in the ESM). The outcome variable was the presence or absence of at least one type of Enterobacteriaceae in a macaque. Given the close evolutionary histories, similar ecologies, and similar epidemiology of the three enterobacterial taxa we processed, we assumed that environmental and host-specific variables will affect the likelihoods of infection with these bacteria in similar ways.

For more information on macaque trapping-and-release procedures, sample processing, the effective sample size, and data analysis, see the [ESM](#).

Ethical Note

All protocols were approved by the Institutional Animal Care and Use Committee of the National Primate Research Centre of Thailand, Chulalongkorn University (NPRCT-CU) (No.1975007) and adhered to the principles for the ethical treatment of primates set by the American Society of Primatologists.

Data Availability The dataset used in this study is also available in the ESM document provided.

Results

The prevalence of each type of Enterobacteriaceae was low in all three populations. Overall, *Salmonella* spp. showed the highest prevalence, followed by STEC and *Shigella* spp. (Table 1). Across sites, *Salmonella* spp. was markedly more prevalent at WTMK than at WHM and WTST, while *Shigella* spp. and STEC showed similar prevalence (Table 1).

The GLM met standard diagnostic criteria (ESM) and was a significantly better fit than a null model (likelihood ratio test: $\chi^2(5) = 14.27, P = 0.01$). The likelihood of infection was significantly negatively associated with body mass ($B = -0.55, P = 0.02$), positively but not significantly associated with age ($B = 0.05, P = 0.09$), and not significantly affected by sex (males vs. females: $B = -0.62, P = 0.24$) or study site (WTMK vs. WHM: $B = 0.47, P = 0.53$; WTST vs. WHM: $B = 0.35, P = 0.64$; WTST vs. WTMK: $B = -0.12, P = 0.80$).

Discussion

Long-tailed macaques at all three sites were exposed to human food and waste, contaminated water, and the feces of other animals with which they overlap spatially (wild boar, feral dogs, other macaques) or interact with socially (other macaques). All

Table 1 Summary of bacterial prevalence among long-tailed macaques at three sites in Thailand, in July 2019

Study site	Sample collection and processing dates	Samples tested	Number of bacterial isolates (% prevalence)		
			<i>Salmonella</i> spp.	<i>Shigella</i> spp.	Shiga toxin producing <i>E. coli</i>
Wat Haad Moon	July 22–23, 2019	39	1 (2)	2 (5)	2 (5)
Wat Ta Sung Tai	July 23–24, 2019	74	5 (7)	2 (3)	6 (8)
Wat Tam Mi Ka	July 25–26, 2019	55	11 (20)	2 (4)	4 (7)
Total		168	17 (10)	6 (4)	12 (7)

these are potential environmental sources of enterobacterial infection (Devaux *et al.* 2019; Malaivijitnond *pers. obs.*).

The low enterobacterial prevalence is consistent with studies of other wild primates (reviewed in McLennan *et al.* 2018). We mostly processed one sample from each macaque, which can decrease pathogen detectability and result in low prevalence. More frequent sampling may also establish location- or pathogen-specific differences in prevalence. However, we detected little evidence for differences in prevalence across pathogen types, except for a relatively high prevalence of *Salmonella* spp. among macaques at WTMK. This finding may relate to the denser macaque population and therefore potentially greater rates of contact/overlap with humans, other macaques, and other animals at WTMK compared to the other locations (Malaivijitnond *pers. obs.*).

The negative relationship between macaque body mass and bacterial infection may have implications for animal health. This pattern is unlikely to be because young, immunologically naive macaques weigh less and are more prone to infection, since we found a positive (rather than a negative) association between age and infection. Instead, lower body mass may be a consequence (rather than a predictor) of bacterial infection, since weight loss is a commonly observed symptom of gastroenteritis and diarrheal disease in humans and other mammals.

Our findings add to existing evidence of Enterobacteriaceae infection in wild primates living in human-impacted environments (McLennan *et al.* 2018). Throughout Southeast Asia, long-tailed macaques live in dense populations that share ecological space with both humans and other wildlife. These primates, in addition to being vulnerable to diarrheal disease themselves, may act as “superspreaders” of pathogens to overlapping humans, livestock, and other wildlife (Devaux *et al.* 2019). From both conservation and public health perspectives, it is therefore imperative to conduct more in-depth epidemiological and socioecological assessments of enterobacterial transmission and pathogenesis at human–nonhuman primate interfaces. Comparison with macaques living in less anthropogenic environments would be valuable to further our understanding of the epidemiology of infection.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10764-021-00209-3>.

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Author contributions KNB, supervised by EA and BJM, designed the study and its aims, analyzed the data, and wrote the manuscript. SM, SJ, and YH all participated in designing the study, supervised the data collection and processing in Thailand, and contributed to writing various sections of the manuscript. TK, SM, JS, MK, and VT participated all helped collect macaque demographic data, processed macaque samples for the screening and confirmation of bacterial pathogens, and contributed to writing the manuscript.

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