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Detection, Identification, and Antimicrobial Susceptibility of *Campylobacter* spp. and *Salmonella* spp. from Free-ranging Nonhuman Primates in Sri Lanka

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ABSTRACT: Infections with *Campylobacter* spp. and *Salmonella* spp. are the most frequently reported causes of human bacterial enteritis. Warm-blooded animals, including livestock, pets, and wildlife, can be carriers of the bacteria and may contaminate the environment and food products. The present study investigated the occurrence of *Campylobacter* spp. and *Salmonella* spp. in fecal pat samples from free-ranging toque macaques (*Macaca sinica*) and tufted gray langurs (*Semnopithecus priam*) collected in March–May 2015 in Sri Lanka. In 58 samples from toque macaques, *Campylobacter jejuni* was isolated in 10 (17%), *Campylobacter coli* in four (7%), and *Salmonella enterica* subsp. *enterica* serovar Virchow in two (3%). None of the bacteria were isolated in the 40 samples from tufted gray langurs. Pulse-field gel electrophoresis and multilocus sequence typing identified six profiles and four clonal complexes of *C. jejuni*. The isolated *Campylobacter* spp. showed varying susceptibility to antimicrobial substances. All *Campylobacter* spp. isolates were susceptible to chloramphenicol, erythromycin, florfenicol, gentamicin, and streptomycin. Four of the *C. jejuni* were resistant to at least one of the following: ampicillin, ciprofloxacin, nalidixic acid, and tetracycline, and one of the isolates was multidrug resistant. All four *C. coli* were resistant to ampicillin, whereas the two *Salmonella* Virchow strains were susceptible to all antibiotics tested. The presence of *Campylobacter* spp. and *Salmonella* spp. in toque macaques may have an impact on the conservation of endangered primates and public health in Sri Lanka.

Key words: Antimicrobial resistance, *Campylobacter* spp., conservation, nonhuman primates, PFGE, *Salmonella* spp.

Zoonotic diseases are a major concern in today's globalized world. Sri Lanka is a biological hotspot (Myers et al. 2000) with moderate to high risk of transmission of zoonotic disease from wildlife to humans (Jones et al. 2008). All primate species in Sri Lanka have decreasing population trends due to anthropogenic disturbance and increasing human-primate conflicts. However, at temples and other holy places, primates can roam relatively undisturbed where they may consume human waste and food offerings, potentially exposing themselves to foodborne pathogens (Nahallage and Huffman 2013).

Campylobacter spp. and *Salmonella* spp. can colonize or infect many species, including primates that may become asymptomatic carriers or develop clinical disease (Nizeyi et al. 2001; Ngotho et al. 2006). The bacteria can be spread by direct contact between animals and humans or indirectly via the environment. Most infected humans recover without specific treatment. Antimicrobials are warranted for patients with severe, prolonged enteritis, or life-threatening conditions.

The presence of these bacteria and antibiotic resistance has not been reported in Sri Lankan nonhuman primates. The aim of our study was to investigate the occurrence of *Campylobacter* spp. and *Salmonella* spp. in toque macaques (*Macaca sinica*) and tufted gray langurs (*Semnopithecus priam*) in Sri Lanka, to assess the antibiotic susceptibility of detected isolates, and to genetically subtype the isolates.

During March–May 2015, fresh fecal pat samples were collected from endemic, endangered toque macaques and near-threatened tufted gray langurs in five locations in Sri Lanka (Fig. 1 and Table 1). The primates were

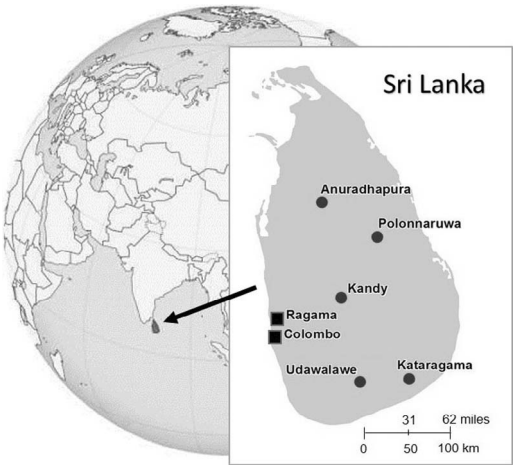


FIGURE 1. Five sampling locations (circles) in Sri Lanka where fecal pat samples were collected in March–May 2015 for isolation of *Salmonella* spp. and *Campylobacter* spp. in free-ranging tufted gray langurs (*Semnopithecus priam*) and toque macaques (*Macaca sinica*). Laboratory analyses were conducted at the University of Kelaniya in Ragama, near the capital Colombo (squares).

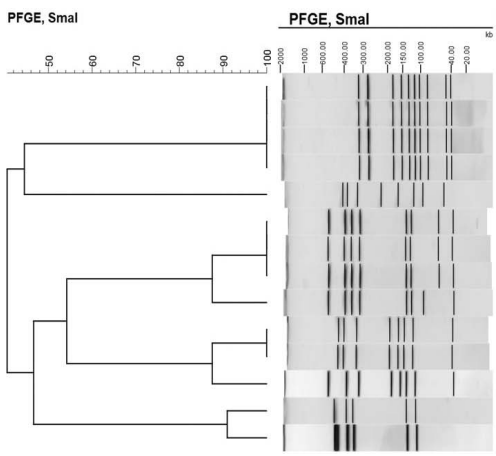
resident at temples or archeological sites frequented by local and international visitors. To minimize risk of double sampling, no specimens within 2 m of each other were sampled, unless they were directly observed defecations from different individuals. The samples were collected in duplicates using Copan FecalSwab™ (Copan Diagnostics Inc., Murrieta, California, USA) and immediately stored in a cooler box with ice packs (5±3 C), followed by refrigeration for up to 2 days, until analyses at the University of Kelaniya (Ragama, Sri Lanka; Fig. 1).

Culturing of *Campylobacter* spp. and *Salmonella* spp. was performed according to the International Organization for Standardization (ISO 10272, 2017) and Nordic Committee on Food Analysis (NMKL no. 71, 1999), respectively. Suspected isolates were stored in serum broth with 15% glycerol at –20 C for later transport on dry ice to the National Veterinary Institute (SVA, Uppsala, Sweden). Species identification of *Campylobacter* spp. was performed with matrix-associated laser desorption/ionization-time of flight mass spectrometry. Suspected *Salmonella* isolates were identified by serotyping with antisera to determine species and serovar according to the Kauffman-White classification system (Popoff and Minor 1997). Susceptibility to selected antimicrobial substances was assessed with VetMIC™ panel analysis systems (SVA), determining the antimicrobial minimum inhibitory concentration. Multidrug resistance was defined as resistance to three or more antibiotic classes. For example, resistance to ciprofloxacin, enrofloxacin, and nalidixic acid was considered resistance to one antibiotic class (fluoroquinolones). Genetic subtyping was performed by pulsed field gel electrophoresis (PFGE). Computer-assisted

TABLE 1. Distribution and number (*n*) of fecal pat samples collected in March–May 2015 from tufted gray langurs (*Semnopithecus priam*) and toque macaques (*Macaca sinica*) at five locations in Sri Lanka. *Salmonella enterica* subsp. *enterica* serovar Virchow, *Campylobacter jejuni*, and *Campylobacter coli* were found in fecal pat samples from toque macaques but not from tufted gray langurs.

			Toque macaque			
				Positive samples		
Location	Total no. of samples	Tufted gray langur, no. of samples	No. of samples	*Salmonella* Virchow	*C. jejuni*	*C. coli*
Anuradhapura	30	17	13	0	4	0
Kandy	19	NA[a]	19	2	4	4
Polonnaruwa	21	9	12	0	2	0
Kataragama	14	14	NA	NA	NA	NA
Udawalawe	14	NA	14	0	0	0
Total	98	40	58	2	10	4

[a] NA = not applicable since the species was not resident at the location.



Campylobacter species		Location	Sequence type	Clonal complex	PFGE
Campylobacter	coli	Kandy	-	ST-828 complex	7
Campylobacter	coli	Kandy	-	ST-828 complex	7
Campylobacter	coli	Kandy	-	ST-828 complex	7
Campylobacter	coli	Kandy	-	ST-828 complex	7
Campylobacter	jejuni	Anuradhapura	2100	ST-52 complex	1
Campylobacter	jejuni	Anuradhapura	403	ST-403 complex	4
Campylobacter	jejuni	Anuradhapura	403	ST-403 complex	4
Campylobacter	jejuni	Anuradhapura	403	ST-403 complex	4
Campylobacter	jejuni	Kandy	403	ST-403 complex	5
Campylobacter	jejuni	Polonnaruwa	451	ST-21 complex	2
Campylobacter	jejuni	Polonnaruwa	451	ST-21 complex	2
Campylobacter	jejuni	Kandy	-	ST-21 complex	3
Campylobacter	jejuni	Kandy	362	ST 362-complex	6
Campylobacter	jejuni	Kandy	362	ST 362-complex	6

FIGURE 2. Dendrogram of *Campylobacter* spp. isolates originating from fecal pat samples collected in March–May 2015 from toque macaques (*Macaca sinica*) in Sri Lanka. PFGE=pulsed field gel electrophoresis.

identification (BioNumerics version 7.5, Applied Maths, Sint-Martens-Latem, Belgium) was used to construct a dendrogram of *Campylobacter* isolates (Fig. 2). A dendrogram was constructed based on PFGE band pattern similarities according to the standardized Campynet protocol (On et al. 2000). Sequence types (STs) and clonal complexes are based on multilocus sequence typing (MLST) using the standardized PubMLST *Campylobacter* method (Dingle et al. 2001).

Out of 58 toque macaque samples, *Salmonella enterica* subsp. *enterica* serovar Virchow (*Salmonella* Virchow) was isolated in two (3%), *C. jejuni* in 10 (17%), and *C. coli* in four (7%). The *C. jejuni* isolates were classified into four clonal complexes: ST-21, ST-52, ST-362, and ST-404, and divided in six unique PFGE profiles (Fig. 2). All four *C. coli* isolates and both *Salmonella* Virchow isolates originated from the same location (Table 1), and the isolates were indistinguishable from each other within each species, based on PFGE band patterns (Fig. 2). Two *C. jejuni* isolates (ST-21, PFGE profile 2) were resistant to ciprofloxacin, tetracycline, and nalidixic acid. The third (ST-52) was resistant to ampicillin, ciprofloxacin, tetracycline, and nalidixic acid and was therefore considered multidrug resistant. The four *C. coli* isolates (ST 828) were resistant only to ampicillin, and the two *Salmonella* Virchow isolates were susceptible to all antibiotics tested (Table 2). Neither *Campylobacter* spp. nor *Salmonella* spp. were detected in the 40 samples from tufted gray langurs (Table 1).

Nonhuman primates can be asymptomatic carriers of these bacteria, but fatal illness may develop (Nizeyi et al. 2001; Ngotho et al. 2006). This is a reason for concern regarding the conservation of free-ranging primate populations. In 58 fecal samples from toque macaques, *C. jejuni*, *C. coli*, and *Salmonella* Virchow were detected in 17, 3, and 3% of the samples, respectively, whereas none of the pathogens were detected in 40 samples from tufted gray langurs. Varying prevalence of *C. jejuni* has been documented in Peru (21%) and China (2%) in wild monkeys and in Uganda (19%) in mountain gorillas *(Gorilla gorilla beringei*; Tresierra-Ayala and Fernandez 1997; Nizeyi et al. 2001; Zeng et al. 2016).

Possible transmission routes of the bacteria to the primates in our study could have been human food waste, contaminated water, soil, feces, or direct contact with other species or conspecifics. Poultry meat is an important vehicle for both *Salmonella* spp. and *Campylobacter* spp. in Sri Lanka (Coorey and Perera 2007; Jayatilleke et al. 2015), where an incidence of *Campylobacter* greater than 70% in broiler flocks has been reported (Kottawatta et al. 2007). In Brazil, four subtypes of *C. jejuni* that were found among humans were

TABLE 2. Distribution of susceptibility of isolates of *Salmonella enterica* subsp. *enterica* serovar Virchow from fecal pat samples from toque macaques (*Macaca sinica*) in Sri Lanka collected in March–May 2015. Isolates of *Salmonella* Virchow, *Campylobacter jejuni*, and *Campylobacter coli* were tested for susceptibility of selected antimicrobial substances. Values for susceptibility were obtained from the European Committee on Antimicrobial Susceptibility Testing (2015).

	Salmonella Virchow		*C. jejuni*		*C. coli*	
Antimicrobial	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible
Ampicillin	0	2	2	8	4	0
Amoxicillin/Clavulanic acid	0	2	NA[a]	NA	NA	NA
Cefotaxime	0	2	NA	NA	NA	NA
Chloramphenicol	NA	NA	0	10	0	4
Ciprofloxacin	NA	NA	3	7	0	4
Colistin	0	2	NA	NA	NA	NA
Enrofloxacin	0	2	NA	NA	NA	NA
Erythromycin	NA	NA	0	10	0	4
Florfenicol	NA	NA	0	10	0	4
Gentamicin	0	2	0	10	0	4
Nalidixic acid	NA	NA	3	7	0	4
Neomycin	0	2	NA	NA	NA	NA
Nitrofurantoin	0	2	NA	NA	NA	NA
Streptomycin	0	2	0	10	0	4
Tetracycline	0	2	3	7	0	4
Trimethoprim/Sulfamethoxazole	0	2	NA	NA	NA	NA

[a] NA = not applicable since the bacteria was not tested for the antimicrobial substance.

also found in captive marmosets (*Callitrix* spp.) that were given chicken (*Gallus domesticus*) as part of their diet (Scarcelli et al. 2005). Contaminated water is another possible route, because *Campylobacter* and *Salmonella* have been isolated from both surface and bottled water (Mannapperuma et al. 2013; Kuhn et al. 2017). *Salmonella* form a large group of bacteria with varying pathogenicity in different animal species depending on serovar (Ohl and Miller 2001). *Salmonella* Virchow has been isolated from both humans and other animals (Salisbury et al. 2011). For determining transmission routes and identifying sources of infection, bacterial characterization below the species level, so-called subtyping, such as PFGE and MLST, is useful (Fig. 2). The sequence types identified in this study have been isolated from humans with gastrointestinal disease and chickens in different parts of the world (Dingle et al. 2001; Scarcelli et al. 2005; Islam et al. 2009).

The study could have been strengthened by sampling of more individuals and additional sampling sites, which possibly could have detected the bacteria in both species of nonhuman primates. However, langurs are frugivorous foregut fermenters, with high levels of bacteriolytic lysozymes in the stomach (Stewart et al. 1987), whereas macaques are hindgut fermenters. The differences between the two species' digestive systems may influence bacterial colonization. Toque macaques, being less selective in their foraging, may be subjected to a higher risk of food borne transmission.

The emergence of resistant bacteria has been strongly linked with the use of antibiotics in animal production (Aarestrup and Engberg 2001). Despite national guidelines on the use of antimicrobials in Sri Lanka (Sri Lanka College of Microbiologists 2014), the use of antibiotics as growth promoters in animal production and aquaculture is still practiced, leading to increasing problems with antibiotic resistance (Patabendige et al. 2011). To the best of our knowledge the primates in this study had never been treated with antibiotics.

Despite this, 30% (3/10) *C. jejuni* isolates showed resistance to both quinolones (ciprofloxacin and nalidixic acid) and tetracycline. In comparison, in human *Campylobacter* isolates in Sri Lanka, 38% were resistant to ciprofloxacin and 69% to nalidixic acid (Cooray and Perera 2007). Animals living in proximity to human settlements tend to harbor a higher number of resistant bacteria (Allen et al. 2010). In conclusion, the presence of zoonotic, multidrug-resistant bacteria in toque macaques may affect conservation of endangered primates and public health in Sri Lanka.

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