Salmonella Infections in the Common Raccoon (*Procyon lotor*) in Western Pennsylvania[▽]

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Ten *Salmonella enterica* serotypes were isolated from fecal samples collected from anesthetized raccoons ($n = 738$) trapped in six Pennsylvania counties from 2003 to 2005. Comparison of raccoon pulsed-field gel electrophoresis (PFGE) pulse type data with the Pennsylvania Department of Health PFGE database revealed that the patterns of seven *Salmonella* serotypes matched those isolated from humans with salmonellosis.

Common raccoons (*Procyon lotor*) are widely distributed throughout Pennsylvania, occupying agricultural, forested, and suburban areas; they prefer to make their dens in wooded areas near rivers or other water sources (13). Raccoons are nocturnal, omnivorous mammals which have the unique habit of dunking their food in water before consuming it (4). The presence of raccoons in suburban areas is a source of concern because of the increased potential for wildlife-human contact (6). Raccoons can serve as reservoirs for a variety of known zoonotic agents, including rabies virus, *Baylisascaris procyonis*, *Toxoplasma gondii*, leptospires, and salmonellae (2, 5).

Salmonella enterica serotypes have been isolated from numerous species of free-living and captive mammals, with a major emphasis being placed on studies of agricultural animals and avian populations. Increasing attention has turned to wildlife such as skunks, opossum, and deer, as the prevalence of salmonellae in these wildlife populations is unknown due to difficult access, making sampling animals for epidemiologic studies a challenge (12). Many *Salmonella* serotypes can survive in a dormant state in the environment until they encounter conditions suitable for growth; the persistence of salmonellae in the environment is an important characteristic in their epidemiology (14). Therefore, studies which identify potential reservoirs of this zoonotic pathogen are important. This study reports the prevalence of *S. enterica* infections in raccoons in western Pennsylvania from 2003 to 2006.

Raccoon fecal swabs were collected with the assistance of the USDA Wildlife Service. Fecal swabs ($n = 738$) from anesthetized raccoons trapped in three landscapes including rural (Mercer County, $n = 128$), suburban (Erie and Allegheny Counties, $n = 278$), and forested areas (Armstrong, Greene, and Westmoreland Counties, $n = 332$) in western Pennsylvania were collected from 2003 to 2006. Individual rectal swabs were placed into plastic bags and frozen until further analysis. Fecal swabs were rinsed thoroughly and preenriched in 9 ml of buffered peptone water (Difco Laboratories, Detroit, MI) at 37°C

for 24 h. One milliliter of preenriched broth was transferred to 9 ml of tetrathionate broth (Difco) and incubated at 37°C for 48 h. In addition, 0.1 ml of the preenriched broth was transferred to 9.9 ml of Rappaport-Vassiliadis broth (Difco) and incubated at 42°C for 24 h. The selective enrichment broths were vortexed and streaked onto Hektoen Enteric agar (Oxoid, Unipath Ltd., United Kingdom) and xylose lysine deoxycholate agar (Oxoid) plates. The selective agar plates were incubated at 37°C for 24 h. Presumptive *Salmonella* colonies were subcultured onto MacConkey agar (Difco) and incubated at 37°C for 24 h. This was followed by inoculation of presumptive positive *Salmonella* colonies onto triple sugar iron agar slants (Difco), reaction with Salmonella Polyvalent O antisera (Difco), and species identification with API 20E (BioMérieux, St. Louis, MO).

Salmonella isolates were serotyped at the National Veterinary Services Laboratory, Ames, IA. Confirmed *Salmonella* isolates were analyzed by pulsed-field gel electrophoresis (PFGE) with XbaI following the PulseNet protocol (<http://www.cdc.gov/pulsenet/index.htm>) at Pennsylvania State University and the Molecular Microbiology section of the Bureau of Laboratories at the Pennsylvania Department of Health, Lionville. The PFGE patterns of *Salmonella* serotypes from raccoons were compared with *Salmonella* serotypes of human origin in the Pennsylvania Department of Health PFGE database.

Salmonellae were isolated from 7.4% (55 of 738) of the raccoon fecal samples examined, including 7.8% (10 of 128), 8.7% (29 of 332), and 5.7% (16 of 278) of those from rural, forested, and suburban areas, respectively (Table 1). Most studies of salmonellae in wildlife focus on animals in rehabilitation centers or zoos, with fewer studies examining free-ranging animals (8, 12). The prevalence in this study is lower than the data reported by Bigler et al. (2), who isolated salmonellae from 16% of the free-ranging raccoons sampled. However, the prevalence in our study is higher than that reported in studies of salmonellae in free-ranging wildlife (10, 11). In our study, the *Salmonella* isolates belonged to 10 serotypes and *S. enterica* serovar Newport accounted for 12 (21.8%) of the 55 *Salmonella* isolates found in this study.

PFGE analysis classified the 55 *Salmonella* isolates into 13

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TABLE 1. *Salmonella* serovars isolated from raccoons

<i>Salmonella enterica</i> serovar	No. of isolates			Total
	Rural (n = 128) ^a	Forest (n = 332) ^a	Suburban (n = 278) ^a	
Bardo			1	1
Berta		3		3
Hartford	9	1		10
Infantis		8		8
Newport		6	6	12
Oranienburg		2	1	3
Paratyphi B var. L-tartrate+		1	8	9
Thompson	1			1
Typhimurium		6		6
Typhimurium var. Copenhagen		2		2
Total	10 (7.8) ^b	29 (8.7) ^b	16 (5.7) ^b	55 (7.4) ^c

^a Number of raccoons sampled by habitat.^b Percentage with respect to raccoons sampled by habitat in parentheses.^c Percentage of total isolates in parentheses.

distinct pulse types (Table 2). The raccoon PFGE pulse types were compared with *Salmonella* pulse types of human origin in the Pennsylvania Department of Health PFGE database with respect to location and time of isolation. The criterion for a match in location was that the two pulse types be from the same county, and the criterion for a match in time was that the two pulse types occur within 3 months of each other. There were a total of 70 human *Salmonella* isolates belonging to seven serovars with PFGE pulse types that matched pulse types isolated from raccoons (Table 2). A total of 28 human *Salmo-*

nella isolates matched pulse types with respect to time only, including 19 *S. enterica* serovar Typhimurium isolates. There were four instances in which human *Salmonella* pulse types matched pulse types from raccoons with respect to both location and time: one *S. enterica* serovar Infantis isolate in Westmorland County, one *S. enterica* serovar Newport isolate in Allegheny County, one *S. enterica* serovar Paratyphi B var. L-tartrate+ isolate in Allegheny County, and one *S. enterica* serovar Typhimurium isolate in Greene County.

Raccoons likely acquire salmonellae from their immediate surroundings. Raccoons may acquire an infection and become intermediate hosts through the consumption of infected wildlife, poultry, fish, or shellfish or contaminated soil, water, or plant material. The *Salmonella* serotypes isolated in this study include several known pathogenic serotypes which not only cause gastroenteritis in humans but can lead to more severe illnesses or death (3). The link between wildlife and human cases of salmonellosis has been documented (1, 7). A recent study by Meinersmann et al. (9) examined the presence of salmonellae in river water and reported finding 7 of the 10 *Salmonella* serotypes isolated in our study. The unique behavior of dunking their food in water before consuming it suggests that raccoons could play significant roles in dispersing salmonellae from contaminated water sources.

Based on the findings of this study, it can be inferred that raccoons are asymptomatic carriers of salmonellae. This could have public health implications, particularly in locations that are shared by humans and wildlife. Studies which use molecular methods such as DNA fingerprinting of salmonellae by PFGE, which can help identify the source of contamination in the wild, are extremely important. The same serotype found in

TABLE 2. PFGE pulse types of *Salmonella* serovars isolated from raccoons and comparison to human pulse types in the Pennsylvania Department of Health PFGE database

<i>Salmonella enterica</i> serovar	Habitat type	PFGE type	No. of isolates	Comparison of raccoon and human <i>Salmonella</i> PFGE pulse types			
				No. of human isolates that match pulse type	No. of matches for time only ^a	No. of matches for location only ^b	No. of matches for time and location
Bardo	Suburban	1	1	0	0	0	0
Berta	Forested	2	3	0	0	0	0
Hartford	Forested	3	1	0	0	0	0
Hartford	Agricultural	3	8			0	0
Hartford	Agricultural	4	1	0	0	0	0
Infantis	Forested	5	8	2	0	0	1
Newport	Suburban	6	2	3	2	0	0
Newport	Forested	6	1			0	0
Newport	Suburban	7	1	7	0	0	0
Newport	Forested	7	2			0	0
Newport	Suburban	8	3	5	1	1	1
Newport	Forested	8	3			0	0
Oranienburg	Suburban	9	1	3	2	1	0
Oranienburg	Forested	9	2			0	0
Paratyphi B var. L-tartrate+	Suburban	10	8	3	0	0	1
Paratyphi B var. L-tartrate+	Forested	10	1			0	0
Thompson	Agricultural	11	1	10	4	0	0
Typhimurium	Forested	12	6	29	19	0	1
Typhimurium var. Copenhagen	Forested	13	2	8	0	0	0
Total			55	70	28	2	5

^a A match in time was considered to be within 3 months (90 days).^b A match in location was considered to be the same county.

multiple animals from the same location suggests a point source of contamination. Identification of a point source may allow the development of a plan to manage and minimize ongoing environmental contamination. Routine monitoring of *Salmonella* serotypes in wildlife and their habitats can lead to a better understanding of the epidemiology and patterns of transmission between wildlife and humans.

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