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SALMONELLA IN RACCOONS (*PROCYON LOTOR*) IN SOUTHERN ONTARIO, CANADA

Claire Jardine,[1,5] Richard J. Reid-Smith,[2,3] Nicol Janecko,[3] Mike Allan,[4] and Scott A. McEwen[2]

[1] Department of Pathobiology, University of Guelph, Guelph, Ontario N1G 2W1, Canada
[2] Department of Population Medicine, University of Guelph, Guelph, Ontario N1G 2W1, Canada
[3] Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ontario N1G 3W4, Canada
[4] Ontario Ministry of Natural Resources, Rabies Research and Development Unit, Trent University, Peterborough, Ontario K9J 7B8, Canada
[5] Corresponding author (email: cjardi01@uoguelph.ca)

ABSTRACT: Numerous serotypes of *Salmonella* have been detected in a variety of wild animals, including raccoons (*Procyon lotor*). Raccoons are common, mid-size omnivores that live in close association with people in urban and rural areas in Ontario. Although raccoons are known to shed *Salmonella*, little is known about their potential long-term role in maintaining *Salmonella* infections. We sampled feces from raccoons in three areas of Ontario: one primarily urban site around Niagara, one primarily rural site north of Guelph, and the grounds of the Toronto Zoo, in 2007 to identify which serotypes of *Salmonella* were commonly shed by raccoons in southern Ontario. In addition, we conducted a longitudinal study at the Toronto Zoo site to determine if raccoons remain persistently infected with *Salmonella*. *Salmonella* was found in 45% of samples. The prevalence of *Salmonella* in raccoon feces ranged from 27% at the rural site to 65% at the urban site. We detected 16 serotypes of *Salmonella* in 83 positive samples. The most common serotype detected in raccoons from the rural and zoo sites was *Salmonella enterica* serotype Typhimurium, whereas *Salmonella* Newport was detected most commonly in the urban site. Only one raccoon of 11 that were captured in four or more consecutive trapping sessions shed the same *Salmonella* serotype for two consecutive months, suggesting that raccoons regularly acquire new *Salmonella* serotypes from the environment.

Key words: Maintenance host, *Procyon lotor*, raccoons, *Salmonella*, serotypes.

INTRODUCTION

Salmonellosis is one of the most important food-borne bacterial zoonoses in the world (Forshell and Wierup, 2006), with an estimated 1.4 million human cases and 582 deaths annually in the United States (Mead et al., 1999). Nontyphoidal *Salmonella* serotypes are transmitted to humans primarily through consumption of animal-derived food products as a result of fecal contamination during processing (Forshell and Wierup, 2006), although contaminated fresh produce is increasingly recognized as an important food vehicle (Beuchat 2002). Other sources of infection include direct contact with reptiles and contaminated pet products (Friedman et al., 1998; Campbell et al., 2001; Pitout et al., 2003).

A wide variety of *Salmonella* serotypes have been detected in wild animals throughout the world (Everard et al., 1979; Wilson et al., 2003, Millan et al., 2004) and *Salmonella* contamination by wild animals has been linked to human outbreaks (Handeland et al., 2002) and disease in production animals (Humphrey and Bygrave, 1988). Wild mice and opossums (species names not specified) have been implicated as potential sources of *Salmonella* contamination for greenhouse tomatoes (Orozco et al., 2008) and exposure to wildlife feces has been implicated as a risk factor related to outbreaks of salmonellosis associated with other fresh produce (Doyle and Erikson, 2008). In addition, *Salmonella* has been detected in wildlife living close to farms, suggesting that there is a risk of transmission of *Salmonella* between production animals and wildlife (Skov et al., 2008).

Raccoons (*Procyon lotor*) are common, mid-size, omnivorous mammals that occupy a variety of habitats including rural and urban areas. The population density of raccoons in urban areas of Ontario has been estimated to be as high as 37–94

raccoons/km^2 (Broadfoot et al., 2001), leading to concerns about increased disease transmission risk between animals and to humans. Previous studies have reported that raccoons shed a variety of *Salmonella* serotypes with observed prevalences of *Salmonella* in raccoon feces ranging from 7 to 52% (Bigler et al., 1974; White et al., 1975; Morse et al., 1983; Compton et al., 2008). The role of raccoons in the maintenance of *Salmonella* in the environment is not clear as most previous studies have only sampled populations at one point in time. Understanding the role of wild animals in the epidemiology of *Salmonella* is essential for the development of appropriate control and prevention strategies to reduce the risk of *Salmonella* contamination of the environment. Our purpose was to identify the serotypes of *Salmonella* that are commonly found in wild raccoons in southern Ontario and to determine the duration of shedding in naturally infected raccoons.

MATERIALS AND METHODS

Procedures for trapping and handling raccoons were approved by the Animal Care Committee at the University of Guelph following the guidelines of the Canadian Committee on Animal Care. Raccoons were live-trapped using size 106 and 108 Tomahawk traps (Tomahawk Live Trap Co. Tomahawk, Wisconsin, USA) set in three areas: on the grounds of the Toronto Zoo (43°49′N, 79°11′W); in an urban setting in Niagara, Ontario (43°3′N, 79°2′W); and in a rural setting north of Guelph, Ontario (43°57′N, 80°24′W).

At the zoo site, a total of 40 traps was set in two areas for 3 nights/mo from June to October 2007. Traps were set at sites where raccoons were known to be present and where there was limited public access. Traps were baited with cat food, set in the evening, and checked the following morning. Captured raccoons were brought to a centralized holding area for processing, unless they had already been caught that month, in which case they were released immediately at the point of capture.

Raccoons were anesthetized using an intramuscular injection of 0.05 mg/kg medetomidine hydrochloride (Domitor 1 mg/ml; Pfizer Animal Health, Pfizer Canada Inc., Kirkland, Quebec, Canada) and 5 mg/kg ketamine hydrochloride (Ketaset 100 mg/ml; Wyeth Animal Health, Guelph, Ontario, Canada) before removal from traps. A numbered metal ear tag (1005-3, National Band and Tag Co. Newport, Kentucky, USA) was placed in one ear and a passive integrated transponder tag (AVID Canada, Calgary, Alberta, Canada) was injected subcutaneously between the shoulder blades for subsequent identification. Sex, age class (adult or juvenile, on the basis of animal size and teeth wear/staining), and mass were recorded for each animal. Fecal samples were collected per rectum. After sampling animals were given an anesthetic reversal agent, 0.25 mg/kg atipamazole (Antisedan 5 mg/ml; Pfizer Animal Health) and placed back in the traps to recover from the anesthetic before release at the point of capture. Individual animals were sampled only once per monthly trapping session; however, multiple samples were collected from the same individual if they were caught in subsequent months.

As part of separate, ongoing management procedures at the Toronto Zoo the majority of raccoons were sterilized (vasectomy or tubal ligation), vaccinated for distemper and rabies, and treated for parasites upon first capture. Nine animals were given 1 ml/animal of penicillin, (Penicillin G, Benzathine and Procaine Sterile Aqueous Suspension, Vetoquinol Canada Inc, Lavaltrie, Quebec, Canada) as a precaution against infection after surgery in June. Animals that underwent surgery were released at the point of capture the following morning.

Samples from the other two sites were obtained opportunistically from raccoons trapped as part of the Ontario Ministry of Natural Resources Rabies Research and Control Program using the same methods that were used for the raccoons at the zoo site. Samples from 20 urban animals were obtained from animals trapped 29–31 August 2007 and samples from 30 rural animals were obtained from animals trapped 2–4 October 2007.

Fecal sample volume ranged from 0.1 g to approximately 5 g. All fecal samples were placed in sterile vials, kept in a cooler in the field, and refrigerated at the submission laboratory. Upon arrival at the *Salmonella* testing laboratory (within 3 days of collection), samples were placed in a buffered peptone water enrichment broth (Becton, Dickinson Co., Sparks, Maryland, USA) at a 1:10 ratio, homogenized, and incubated at 37 C for 24 hr. The enrichment broth was injected into modified semisolid Rappaport Vassiliadis agar

(Oxoid Ltd., Besingstoke, Hampshire, UK) and incubated at 42 C for ≤72 hr. All subsequent incubation conditions were in an aerobic humidity-controlled incubator at 37 C with incubation periods of 24 hr. Presumptive positive plates were further plated onto MacConkey and XLT4 agars (Becton, Dickinson), incubated, and two colonies were purified on tryptic soy agar (Becton, Dickinson). The suspected colonies were tested using triple sugar iron slant and urea slant agars (Becton, Dickinson) as biochemical reaction indicators. Final confirmation testing was conducted using Salmonella O poly A-I & Vi antiserum (Becton, Dickinson).

One *Salmonella* spp. isolate from each positive sample was submitted for serotyping and phagetyping at the Laboratory for Foodborne Zoonoses (LFZ) *Salmonella* Reference Laboratory, Guelph, Ontario, Canada. A modified Kauffmann–White scheme for designation of *Salmonella* serotypes was used and phagetypes were identified for specific serotypes.

Antimicrobial susceptibility was conducted at the LFZ Antimicrobial Resistance Reference Laboratory using an automated microbroth dilution method (Sensititre®, Trek Diagnostics, Westlake, Ohio, USA). Minimum inhibition concentrations (MICs) for each isolate were identified using the National Antimicrobial Resistance Monitoring System (NARMS) microtiter antimicrobial plate configuration CMV1AGNF using the following antimicrobials: amikacin, amoxicillin–clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfizoxazole, tetracycline, and trimethoprim–sulfamethoxazole. MIC break points were those used by the Canadian Integrated Program for Antimicrobial Resistance Surveillance and NARMS, which are derived from Clinical Laboratory Standards Institute break points.

The effect of age, sex, month, site, and their interactions on *Salmonella* status were examined using generalized linear mixed models (SAS 9.2, SAS Institute Inc. Cary, North Carolina, USA). Repeated measures of individual animals over time were taken into account by including a random effect for individual animals in the model. Nonsignificant terms ($P \geq 0.10$) were removed from the model using backward elimination. *P* values were adjusted for multiple comparisons using Tukey's honestly significant difference test. Differences were considered significant at $P < 0.05$.

RESULTS

We collected 132 fecal samples from 59 raccoons trapped on the grounds of the Toronto Zoo from June to October 2007 and detected 14 serotypes of *Salmonella* in 62 of 132 fecal samples. Five serotypes of *Salmonella* were detected in 13 of 20 urban animals and seven serotypes were detected in eight of 30 rural animals (Table 1).

Salmonella enterica serotype Typhimurium was the most commonly detected serotype from the zoo and rural raccoons (27% and 25% of isolates, respectively), whereas *Salmonella* Newport was the most commonly detected serotype from the urban animals (54% of isolates; Table 1). Resistance to antimicrobials was detected in *Salmonella* from five raccoons trapped at the zoo in July (Table 2). One animal that was colonized with ampicillin- and tetracycline-resistant *Salmonella* Hadar had been given penicillin after vasectomy the previous month. No other animals with resistant serotypes of *Salmonella* had been given antibiotics in June.

Age and sex were not significantly associated with *Salmonella* status and were not included in our final model ($P > 0.10$). Site and month were significantly associated with *Salmonella* status ($P = 0.01$, $P = 0.002$, respectively). Taking into account month, samples from animals at the urban site were significantly more likely to be positive for *Salmonella* compared with samples from animals at either the rural or zoo site (odds ratio [OR], 14.71; 95% confidence interval [CI] 2.56–83.33; $P = 0.003$ and OR 5.36; 95% CI 1.45–19.82; $P = 0.01$, respectively). The odds of a sample from the zoo site being found positive for *Salmonella* were not statistically different from those of the rural site (OR 2.76; 95% CI 0.85–9.01; $P = 0.09$).

The prevalence of *Salmonella* in raccoons trapped at the Toronto Zoo was lowest in June (22%; 95% CI 6–48), highest in July (77%; 95% CI 60–90), and increased from 26% (95% CI 11–46)

TABLE 1. Number of samples positive for each *Salmonella* serotype from raccoons (*Procyon lotor*) trapped in Canada: on Toronto Zoo grounds, in the Niagara area (urban), and north of Guelph (rural) in 2007. Toronto Zoo site includes results from animals sampled on multiple occasions.

	Number of positive samples		
Salmonella serotype	Toronto Zoo (n=132)	Niagara (urban) (n=20)	North of Guelph (rural) (n=30)
Anatum	7	-	-
Berta	4	-	1
Hadar	3	-	-
Hartford	-	2	1
Heidelberg	2	-	-
I 4,5,12:b:-	7	2	1
I 6,7,14:-:1,5	1	-	-
I Rough-O:k:1,5	-	-	1
I Rough-O:y:e,n,x	1	1	-
Muenchen	4	-	-
Newport	6	7	1
Oranienburg	1	1	-
Schwarzengrund	1	-	-
Thompson	6	-	-
Typhimurium	18	-	2
Typhimurium var. Copenhagen	1	-	1
Total	62	13	8

in August to 43% (95% CI 25–63) in September and up to 50% (95% CI 29–70) in October. *Salmonella* Typhimurium was the most commonly detected serotype in July, accounting for 41% of July isolates (Table 2). Ten of these 11 isolates were the same phagetype (10), whereas one was phagetype 193. Taking into account site,

TABLE 2. Number of samples positive for each *Salmonella* serotype by month from raccoons (*Procyon lotor*) trapped on the grounds of the Toronto Zoo, Toronto, Canada, June–October 2007.

	Session				
Salmonella serotype	June (n=18)	July (n=35)	August (n=27)	September (n=28)	October (n=24)
Anatum	-	-	-	3	4
Berta	-	1	-	2	1
Hadar	1	2[a]	-	-	-
Heidelberg	-	2[b]	-	-	-
I 4,5,12:b:-	2	2	-	1	2
I 6,7,14:-:1,5	-	1	-	-	-
I Rough-O:y:e,n,x	-	-	-	1	-
Muenchen	-	2	-	1	1
Newport	1	1	3	-	1
Oranienburg	-	1	-	-	-
Schwarzengrund	-	1[c]	-	-	-
Thompson	-	2	2	1	1
Typhimurium	-	11	2	3	2
Typhimurium var. Copenhagen	-	1	-	-	-
Total	4	27	7	12	12

[a] Both isolates resistant to tetracycline, and one isolate also resistant to ampicillin.

[b] Both isolates resistant to ampicillin, amoxicillin–clavulanic acid, cefoxitin, and ceftiofur.

[c] Isolate resistant to ampicillin and streptomycin.

TABLE 3. Serotypes of *Salmonella* detected in raccoons (*Procyon lotor*) sampled in four or more consecutive months on the grounds of the Toronto Zoo, Toronto, Canada in 2007.

ID	Age and sex	Session[a]				
		June	July	August	September	October
504	Adult male	0	Oranienburg	0	Anatum	0
508	Adult female	0	Typhimurium var. Copenhagen	0	Muenchen	0
510	Adult male	0	0	0	I 4,5,12:b:-	0
515	Adult male	0	0	0	0	0
516	Adult female	Hadar	Heidelberg	0	I Rough-O:y:e,n,x	NS
507	Adult male	NS	I 4,5,12:b:-	0	Anatum	0
514	Adult female	NS	0	0	0	0
526	Juvenile female	NS	Newport	Typhimurium	0	0
527	Juvenile male	NS	Berta	Newport	Anatum	Newport
532	Juvenile male	NS	Typhimurium	Typhimurium	0	Typhimurium
543	Juvenile male	NS	Typhimurium	Thompson	0	0

[a] NS = no sample collected; 0 = sample negative.

samples collected from animals in July were significantly more likely to be positive for *Salmonella* compared with samples collected in June, August, or September (OR 11.62; 95% CI 2.87–47.62; $P=0.004$ and OR 9.83; 95% CI 2.97–32.54; $P=0.002$ and OR 4.49; 95% CI 1.47–13.75; $P=0.044$, respectively). The odds of a sample being positive for *Salmonella* in July were not significantly different compared with October (OR 3.40; 95% CI 1.07–10.82; $P=0.160$) and no other significant differences between months were detected. We were unable to examine possible interactions between month and location because samples were collected only in 1 mo at two sites.

Of 33 raccoons caught on multiple occasions, 14 were *Salmonella* positive on more than one occasion. Two of these 14 animals were colonized with the same serotype on all occasions, and one animal was positive for the same serotype on two of four occasions. The remaining animals shed different serotypes each sampling period. *Salmonella* was not detected in two of 11 animals sampled for four or more consecutive months. One of these 11 animals was positive with the *Salmonella* Typhimurium (phagetype 10) in all sessions where *Salmonella* was detected (Table 3).

DISCUSSION

The prevalence of *Salmonella* in raccoons at the urban site (65%) was higher than what has been reported in the literature (Bigler et al., 1974; White et al., 1975; Morse et al., 1983; Compton et al., 2008) and was significantly higher than what we found at the rural and zoo sites in this study. Investigators in two previous studies did not detect a difference in the prevalence of *Salmonella* in raccoons living in urban/suburban and rural areas (Morse et al., 1983; Compton et al., 2008); however, it has been hypothesized that animals living in urban areas may be more likely to be exposed to *Salmonella* in garbage and pet animal feces (Morse et al., 1983). In addition to differences in prevalence we also found differences in the serotypes found in the urban and rural sites. *Salmonella* Typhimurium was the most commonly detected serotype in both the zoo and rural animals, but was not detected in the urban animals, where *Salmonella* Newport dominated. In contrast, Compton et al. (2008) found *Salmonella* Newport commonly in animals from a forest and a suburban site in western Pennsylvania, but not from a rural site. On the basis of our current results and the results of previous studies, it is evident

that there are no consistent associations between environmental type (urban, rural) and *Salmonella* prevalence and serotypes. Replicated field studies of *Salmonella* in raccoons living in urban and rural areas are required to determine if our results are generally applicable to urban and rural areas in southern Ontario or if they are specific to the sampling time and sites we used.

All the named serotypes we detected have been associated with clinical disease in humans (Centers for Disease Control [CDC], 2006). *Salmonella* Typhimurium was the dominant serotype found in raccoons in this study and was the most frequently reported serotype isolated from human sources reported to the CDC in 2006 and second most frequently reported in Canada in 2006 (CDC, 2006; Public Health Agency of Canada, 2007). Similar to previous reports, *Salmonella*-positive raccoons in this study appeared to be clinically normal, suggesting that they were asymptomatic carriers of *Salmonella* (Compton et al., 2008). Previous studies have suggested that as asymptomatic carriers of *Salmonella*, raccoons might play a significant role in dispersing the agent into new areas (Compton et al., 2008). This includes areas that may result in exposure of humans, as wild animals have been linked to *Salmonella* contamination of fresh produce (Orozco et al., 2008) and outbreaks of *Salmonella* in humans have been associated with contamination of private gardens by wild animals (Handeland et al., 2002).

In this study, five *Salmonella* isolates from raccoons at the zoo site exhibited resistance to ≥ 1 antimicrobials. Four of these five isolates were resistant to drugs that are considered to be of high or very high importance to human health by the Veterinary Drug Directorate of Health Canada (Health Canada, 2009). The high prevalence of *Salmonella* in raccoons and the detection of several multidrug-resistant isolates in animals living in proximity to humans in southern Ontario is a potential public health concern. We presume that the only deliberate antimicrobial treatment of these raccoons was the prophylactic penicillin treatments of the spayed and neutered zoo-caught animals; therefore it is probable that they acquired the resistant salmonellae from their environment or other animals.

The results of the longitudinal study conducted at the Toronto Zoo suggest that raccoons may not remain colonized with the same serotype of *Salmonella* for long periods. Only one animal of 11 that were caught and sampled for four or more consecutive periods shed the same serotype for two consecutive months. Most raccoons shed *Salmonella* intermittently and were colonized with different serotypes of *Salmonella* each time they were sample positive. Raccoons may be similar to dogs in that they shed *Salmonella* in feces only intermittently, often acquiring new serotypes of *Salmonella* over relatively short periods (Mackel et al., 1952). Colonization in both species may reflect transient dietary exposures. So, although raccoons might play an important role in dispersing *Salmonella* into new areas (Compton et al., 2008), they may not maintain long-term colonization of these bacteria without re-exposure from the environment. Alternatively, since we submitted only one isolate from each positive sample for serotyping, it is possible that raccoons remain colonized with one serotype while acquiring new ones. As many as four different serotypes of *Salmonella* have been found in dogs at one time (Galton et al., 1952), so it is possible that we are missing some cases of longer-term shedding. Further studies looking at multiple isolates from individuals will help distinguish between these two hypotheses.

The prevalence of *Salmonella* in the animals trapped on zoo grounds was significantly higher in July (77%) compared with June (22%), August (26%), and September (43%). In July, 41% of the *Salmonella*-positive animals shed serotype *Salmonella* Typhimurium (primarily phage-

type 10), but no animals were shedding *Salmonella* Typhimurium in June, suggesting that there may have been a point source of contamination in July. The source of exposure is impossible to determine, but *Salmonella* Typhimurium is typically associated with a wide range of animal species in both diseased and clinically normal circumstances. *Salmonella* has been isolated from captive zoo animals (Gopee et al., 2000) and it is possible that animal feed or feeding areas that were accessible to raccoons were contaminated with *Salmonella*. Wildlife may act as indicators of *Salmonella* contamination in the local environment. For example, Handeland et al. (2008) found that foxes (*Vulpes vulpes*) in Norway were most commonly infected with a *Salmonella* Typhimurium 4,12:i:1,2, which was associated with disease outbreaks in small passerines, and suggested that *Salmonella* infections in foxes were primarily acquired through ingestion of infected birds. Further studies investigating potential sources of *Salmonella* for wildlife in southern Ontario and determining the role of wildlife in the dissemination of *Salmonella* into the environment are warranted.

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