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SALMONELLA ISOLATED FROM CENTRAL NEW YORK WILDLIFE ADMITTED TO A VETERINARY MEDICAL TEACHING HOSPITAL

Kevin J. Cummings,[1,3] Julie D. Siler,[1] Noha Abou-Madi,[2] Laura B. Goodman,[1] Patrick K. Mitchell,[1] Lauren Palena,[1] and Sara E. Childs-Sanford[2]

[1] Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, 240 Farrier Road, Ithaca, New York 14853, USA
[2] Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, 131 Swanson Drive, Ithaca, New York 14853, USA
[3] Corresponding author (email: kjc39@cornell.edu)

ABSTRACT: The role of wildlife as a source of zoonotic *Salmonella* transmission is poorly understood, as are the clinical implications of this pathogen among wildlife species. Wildlife hospitals represent an important location to conduct *Salmonella* surveillance, given the wide variety of species admitted for medical and surgical care. Our objectives were to estimate the prevalence of fecal *Salmonella* shedding among wildlife admitted to a veterinary medical teaching hospital, to identify risk factors for infection, and to fully characterize the isolates. Voided fecal samples (birds and mammals) and cloacal swab samples (reptiles and amphibians) were collected between May 2018 and March 2020. Standard bacteriologic culture methods were used to detect *Salmonella*, and isolates were characterized via serotyping, antimicrobial susceptibility testing, and whole-genome sequencing. Samples were collected from 348 wildlife patients representing 74 wildlife species, and the apparent prevalence of fecal *Salmonella* shedding was 1.4% (5/348; 95% confidence interval, 0.5–3.3%). Four serotypes were identified, and isolates were phenotypically susceptible to all antimicrobial agents tested. Two isolates were closely related to human clinical isolates, demonstrating the overlap between wildlife and human pathogens. Fecal *Salmonella* shedding among hospitalized wildlife appears to be uncommon, and the risk of either nosocomial or zoonotic *Salmonella* transmission is presumably low. Nevertheless, the occurrence of *Salmonella* in wildlife, particularly among common species found in a wide array of habitats, poses a potential threat to public health and may result in transmission to more-vulnerable wildlife populations.

Key words: Epidemiology, public health, *Salmonella* spp., surveillance, wildlife, zoonoses.

INTRODUCTION

Salmonella enterica causes an estimated 1.2 million illnesses, 22,000 hospitalizations, and 425 deaths annually in the US (Scallan et al. 2011; Hale et al. 2012). Progress in reducing the incidence of human salmonellosis has not been achieved in recent years (Tack et al. 2020). Although mainly associated with self-limiting acute enteritis, *Salmonella* can also cause invasive infections that may be fatal (Crump et al. 2015). Transmission is usually through foodborne exposure (Scallan et al. 2011). However, *Salmonella* is also transmitted via direct contact with feces from infected animals (Hoelzer et al. 2011). Research on the role of wildlife in *Salmonella* ecology and transmission has been relatively limited, particularly for studies utilizing molecular methods to better ascertain the potential threat to public health (Greig et al. 2015). Zoonotic transmission is a risk for people with occupational contact with wildlife, including veterinary personnel, rehabilitators, and wildlife biologists. Other forms of wildlife exposure, such as hunting, present similar opportunities for pathogen transmission. Additionally, fecal *Salmonella* shedding results in environmental contamination that may pose a broader risk of zoonotic transmission. Human encroachment upon wildlife habitat, driven by human population growth and associated land use changes, presumably increases this risk. Pathogens shed by wildlife may also enter the food production chain through transmission to livestock or fecal contamination of crop fields and irrigation water.

Salmonella is also an important cause of gastrointestinal disease in domestic animals. Although many infections remain subclinical,

Salmonella can cause diarrhea and fever in cattle, horses, pigs, dogs, and others (Marks et al. 2011; Burgess and Morley 2014; Holschbach and Peek 2018). Bacteremia commonly occurs among neonatal animals with salmonellosis and may result in secondary infections. Among wildlife, salmonellosis is an emerging disease in passerines (Tyson-Pello and Olsen 2020), and outbreaks are recognized as an important cause of mortality (Hall and Saito 2008; Hernandez et al. 2012). Generally, however, the clinical implications of *Salmonella* among wildlife are poorly understood.

Wildlife hospitals represent an important location to conduct surveillance for shedding of *Salmonella* or other zoonotic pathogens among wildlife. A wide variety of wildlife species are admitted for medical and surgical care, creating an excellent opportunity for comprehensive sampling initiatives. Risk of pathogen shedding in wildlife patients is probably enhanced by concurrent disease and stress associated with transport, handling, and an unfamiliar environment. Thus, there is potential for both nosocomial and zoonotic transmission of *Salmonella*.

We investigated epidemiologic and clinical features of *Salmonella* infection among wildlife admitted to a veterinary medical teaching hospital in Ithaca, New York. Our objectives were to estimate the prevalence of fecal *Salmonella* shedding among wildlife, to identify clinical and other risk factors for infection, and to fully characterize the isolates and assess their relatedness using whole-genome sequencing.

MATERIALS AND METHODS

Study design

Wildlife patients admitted to Cornell University's Janet L. Swanson Wildlife Hospital in Ithaca, New York were sampled between May 2018 and March 2020. Voided fecal samples were collected from birds and mammals; cloacal swab samples were collected from reptiles and amphibians. All admitted patients were eligible for sample collection. Samples were collected upon admission or shortly thereafter (within 24 h), placed in a vial with Cary-Blair medium, and held at room temperature until processing. Samples were processed in the research laboratory twice weekly. Vials were labeled with data corresponding to each sampled animal including case number, species, and sampling date. Additional relevant data were obtained from the electronic medical records database including admission date, original location, historical data, clinical presentation, diagnosis, and sex and age group when possible. The sampling protocol was approved by the Cornell University Institutional Animal Care and Use Committee.

Microbiologic procedure for *Salmonella* detection

Standard bacteriologic culture methods were used to isolate *Salmonella* from samples. Larger fecal samples (e.g., from waterfowl, meso-mammals) were enriched in 10 mL of tetrathionate broth (Thermo Fisher Scientific, Waltham, Massachusetts, USA) containing 0.2 mL of iodine solution; smaller fecal samples (e.g., passerines, rodents) and all cloacal swab samples were enriched in 5 mL of tetrathionate broth containing 0.1 mL of iodine solution. The sample-broth mixture was incubated at 42 C for 18–24 h and then streaked onto brilliant green and xylose-lysine-deoxycholate selective media (Hardy Diagnostics, Santa Maria, California, USA). Both plates were incubated at 37 C for 18–24 h. Presumptive *Salmonella* colonies were inoculated into Kligler iron agar slants and incubated at 37 C for 18–24 h. Those xylose-lysine-deoxycholate plates without suspected colonies were reincubated at 37 C for an additional 18–24 h. For Kligler iron agar slants with colonies exhibiting the biochemical properties of *Salmonella*, a colony from one of the original selective agar plates was subcultured onto tryptic soy agar with 5% sheep blood (Northeast Laboratory Services, Winslow, Maine, USA) and incubated overnight at 37 C. An isolated colony from the tryptic soy agar plate was then inoculated into Luria-Bertani broth (Thermo Fisher Scientific) and frozen in 15% glycerol for subsequent characterization. Confirmed *Salmonella* isolates were sent to the National Veterinary Services Laboratories (Animal and Plant Health Inspection Service, US Department of Agriculture, Ames, Iowa, USA) for serotyping using standard protocols.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of confirmed *Salmonella* isolates was determined using the broth microdilution method. Minimal inhibitory concentrations (MIC) were established for each isolate against the National Antimicrobial Resistance Monitoring System (NARMS) Gram-neg-

ative panel of 14 antimicrobial agents (Sensititre, TREK Diagnostic Systems, Cleveland, Ohio, USA): amoxicillin/clavulanic acid, ampicillin, azithromycin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. For each agent, MIC values were interpreted using NARMS breakpoints (CDC 2019). Quality control was performed weekly using *Escherichia coli* ATCC 25922, *Staphylococcus aureus* 29213, *Enterococcus faecalis* 29212, and *Pseudomonas aeruginosa* 27853. The MIC ranges for quality control recommended by the Clinical and Laboratory Standards Institute were used, and results were accepted if the MIC values were within expected ranges for these bacterial strains.

Whole-genome sequencing

From pure colonies, DNA was extracted using an automated magnetic bead-based process (MagMAX CORE; Thermo Fisher Scientific) and quantified with fluorometry (Qubit 2.0; Thermo Fisher Scientific). Genomic libraries were prepared and barcoded using the Nextera XT DNA Library Preparation Kit (Illumina, Inc., San Diego, California, USA), then sequenced on the Illumina MiSeq platform using the MiSeq Reagent Kit version 3 (Illumina, Inc.) with 2×250 base pair chemistry.

Sequence analysis

Sequencing reads for each isolate were assembled using SKESA version 2.3.0 (Souvorov et al. 2018). Resulting assemblies were screened for antimicrobial resistance (AMR) genes using the National Center for Biotechnology Information (NCBI) AMRFinderPlus v. 3.0.12 (Feldgarden et al. 2019). Multilocus sequence typing (MLST) profiles were identified using mlst v. 2.16.1 (https://github.com/tseemann/mlst; Jolley and Maiden 2010); single nucleotide polymorphism (SNP) distances between isolates sharing an MLST profile were calculated using the CFSAN SNP pipeline (Davis et al. 2015). Sequence data were submitted to the NCBI Pathogen Detection database (NCBI 2021) for comparison with other genomes. Isolates in this database are classified as either "clinical" or "environmental/other;" we assumed clinical isolates to be from human cases if a nonhuman host was not specified.

Statistical analysis

Data were imported into a commercial statistical software program (version 9.4, SAS Institute Inc., Cary, North Carolina, USA) for variable coding and analysis. Date of sample collection was used to create a variable for season (winter, December–February; spring, March–May; summer, June–August; fall, September–November). Descriptive analysis of relevant variables was performed. An estimate of the prevalence of fecal *Salmonella* shedding and its 95% confidence interval were calculated.

RESULTS

Individual fecal samples or cloacal swab samples were collected from 348 wildlife patients. A total of 74 species were represented, with the most common being Rock Pigeon (*Columba livia*; *n*=35, 10.1%), Red-tailed Hawk (*Buteo jamaicensis*; *n*=29, 8.3%), Eastern Screech-Owl (*Megascops asio*; *n*=15, 4.3%), and Mourning Dove (*Zenaida macroura*; *n*=15, 4.3%). Avian patients were most common (*n*=260, 74.7%) followed by mammals (*n*=70, 20.1%), reptiles (*n*=17, 4.9%), and amphibians (*n*=1, 0.3%). Seasonal distribution of sampling was as follows: summer (*n*=120, 34.5%), fall (*n*=83, 23.9%), spring (*n*=74, 21.3%), and winter (*n*=71, 20.4%).

The prevalence of fecal *Salmonella* shedding was 1.4% (5/348; 95% confidence interval, 0.5–3.3%). Positive patients included two Rock Pigeons (both juveniles of unknown sex), a red fox (*Vulpes vulpes*; adult female), a Virginia opossum (*Didelphis virginiana*; adult male), and a wood turtle (*Glyptemys insculpta*; adult female). Serotypes identified were *Salmonella* Cerro (Rock Pigeon and Virginia opossum), *Salmonella* Infantis (red fox), *Salmonella* Kentucky (Rock Pigeon), and *Salmonella* Thompson (wood turtle). The serotype Cerro isolates shared the same MLST profile (ST367) but were separated by more than 100 SNPs. The five isolates were phenotypically susceptible to all antimicrobial agents tested, and no AMR genes were detected (Table 1).

The Kentucky and opossum Cerro isolates did not cluster with any other isolates in the NCBI Pathogen Detection database. The Cerro isolate from the Rock Pigeon was in a SNP cluster with an isolate from a dairy cow in New York; these isolates were separated by 29 SNPs. The Thompson isolate was part of a very large SNP cluster (more than 3,000 isolates) and within 5 SNPs of 10 human

TABLE 1. *Salmonella* isolates from central New York wildlife admitted to a veterinary medical teaching hospital, 2018–20.[a]

NCBI isolate ID	Species	Date of sampling	Serotype	MLST	AMR genes
SAMN12174600	Wood turtle (*Glyptemys insculpta*)	31 May 2018	Thompson	26	None
SAMN12174604	Red fox (*Vulpes vulpes*)	26 March 2019	Infantis	32	None
SAMN16089648	Rock Pigeon (*Columba livia*)	22 January 2020	Kentucky	152	None
SAMN16089649	Rock Pigeon (*Columba livia*)	14 February 2020	Cerro	367	None
SAMN16089650	Virginia opossum (*Didelphis virginiana*)	4 March 2020	Cerro	367	None

[a] MLST = multilocus sequence typing; AMR = antimicrobial resistance genes.

clinical isolates and five food isolates. The Infantis isolate was part of another large SNP cluster (more than 1,000 isolates) and within 5 SNPs of two human clinical isolates.

DISCUSSION

Salmonella shedding among wildlife in central New York has not previously been comprehensively investigated. The animals sampled might not be representative of the source population of wildlife in this region, given the potential for bias associated with sick and injured wildlife presenting to a hospital facility. However, previous work indicated that pathogen surveillance at wildlife rehabilitation centers is useful for monitoring overall prevalence in common species (Camacho et al. 2016).

Salmonella was isolated from avian, mammalian, and reptilian hosts, but the apparent prevalence of fecal shedding overall was less than 2%. This is presumably an underestimate of the true prevalence, based on the sensitivity of fecal *Salmonella* culture (House et al. 1993; Smith et al. 1994). An investigation of *Salmonella* shedding among hospitalized wildlife patients in California yielded a similar prevalence estimate (2.4%, 8/338; Siembieda et al. 2011). Collectively, these studies suggest that fecal *Salmonella* shedding among hospitalized wildlife in the US is uncommon, with an apparently low risk of either nosocomial or zoonotic *Salmonella* transmission. Nevertheless, the occurrence of *Salmonella* in wildlife, particularly among common species found in a wide array of habitats, poses a potential threat to public health and may result in transmission to more-vulnerable wildlife populations. Studies of *Salmonella* shedding among wildlife at rehabilitation centers in California (Smith et al. 2002) and Ohio (Jijón et al. 2007) revealed prevalence estimates of 4% and 11%, respectively, although a focus on marine birds and mammals (California study) and a relatively small sample (Ohio study) limit comparison with our current study. Another Ohio study (Farias et al. 2015) reported a considerably higher estimated prevalence of fecal *Salmonella* shedding among nondomestic animals (24.9%), but the study sample consisted of captive wildlife plus a variety of exotic animals.

We had hoped to identify risk factors for *Salmonella*-positive status in wildlife, including clinical signs and diagnoses; the relatively small number of positive patients precluded such an analysis. Four of the five positive patients (opossum, wood turtle, and both Rock Pigeons) presented with fractures and other evidence of trauma, probably vehicular trauma. The severity of injuries resulted in the death of two of these patients (the opossum was euthanized and the wood turtle died during hospitalization). The red fox was obtunded, hypothermic, and dehydrated on presentation, with bilateral nasal discharge and a wound at the base of the right ear. Euthanasia was performed due to the poor prognosis; necropsy revealed cerebral and meningeal hemorrhage, suspected to be caused by trauma.

Two serotypes detected in this study, *Salmonella* Infantis and *Salmonella* Thompson, are among the top 10 serotypes isolated from human patients with laboratory-con-

firmed salmonellosis in the US (CDC 2018). Both have a broad host range. The *Salmonella* Infantis and *Salmonella* Thompson isolates were closely related to human clinical isolates, suggesting the possibilities of either zoonotic transmission or exposure to a common source of infection. However, integration of supportive epidemiologic data would be necessary to make reliable inferences. *Salmonella* Kentucky is a common serotype among chickens and dairy cattle (Cummings et al. 2013; Velasquez et al. 2018) but an infrequent cause of clinical disease among humans in the US. *Salmonella* Cerro is strongly associated with dairy cattle and rarely causes salmonellosis in humans. It has emerged as the leading serotype among bovine *Salmonella* isolates from clinical samples submitted to veterinary diagnostic laboratories in the northeastern US (Tewari et al. 2012; Cummings et al. 2013), and recent data indicate more-widespread geographic dissemination (Hong et al. 2016; Valenzuela et al. 2017). Rock Pigeons and opossums are habitat generalists; it is possible that the patients shedding *Salmonella* Cerro and *Salmonella* Kentucky had epidemiologic links to dairy farms.

Phenotypic resistance to antimicrobial agents that are included on the NARMS panel was not detected, and no known AMR genes were identified. This is consistent with our previous work documenting minimal or no resistance among *Salmonella* isolated from various wildlife species (Cummings et al. 2016; Grigar et al. 2016, 2017). Antimicrobial selection pressure faced by enteric bacteria of wildlife is presumably negligible in most environments versus animals raised in agricultural settings. However, further research to generate a larger number of isolates would be needed to adequately evaluate the prevalence of AMR among *Salmonella* isolated from wildlife in this region.

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