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## **SALMONELLA ENTERICA ISOLATED FROM WILDLIFE AT TWO OHIO REHABILITATION CENTERS**

**Steffani Jijón, Amy Wetzel, B.S., Ph.D., and Jeffrey LeJeune, D.V.M., Ph.D., Dipl. A.C.V.M.**

**Abstract:** Between May and September 2004, fecal samples from various wildlife species admitted to two rehabilitation centers in Ohio were cultured for *Salmonella enterica* and *Escherichia coli* O157:H7. Eight of 71 (11%) samples, including specimens from three opossums (*Didelphis virginiana*), two gray squirrels (*Sciurus carolinensis*), a woodchuck (*Marmota monax*), a Harris hawk (*Parabuteo unicinctus*), and a screech owl (*Otus asio*) tested positive for *Salmonella* serovars Braenderup, Senftenberg, Oranienburg, and Kentucky. The *Salmonella* Oranienburg isolates were indistinguishable by pulsed-field gel electrophoresis. Most isolates were susceptible to commonly used antibiotics; however, the *Salmonella* Kentucky isolate was resistant to multiple beta-lactam antibiotics (amoxicillin/clavulanic acid and ampicillin), cefoxitin, and ceftiofur, a third-generation cephalosporin. *Escherichia coli* O157:H7 was not isolated from any sample. Transmission of *Salmonella* from wildlife may occur between animals at rehabilitation centers.

**Key words:** nosocomial, rehabilitation, *Salmonella*, wildlife.

### **INTRODUCTION**

Free-ranging animals have been implicated as reservoirs and vectors for many important historic, emerging, and reemerging human viral, parasitic, and bacterial diseases such as rabies, avian influenza, cryptosporidiosis, Lyme disease, and salmonellosis.<sup>2,13</sup> Transmission of *Salmonella* is of particular concern because of the worldwide distribution of this bacterium and the large variety of different host species, including insects, fish, amphibians, reptiles, birds, and mammals that can harbor this organism, often without clinical signs of illness.<sup>21</sup> Transmission of *Salmonella* from wildlife to humans may occur following direct contact with animals.<sup>13</sup> However, indirect exposure, through human contact with environmental niches contaminated with zoonotic pathogens (including water sources) or contamination of the food supply as a result of interactions between wildlife and food-producing animals or crops, may also serve as less obvious routes of zoonotic transmission. Several factors, including the frequency, types, and degree of interaction between humans and wild animals and their environment; the environmental survival of particular pathogens; the hygienic practices taken to prevent transmission; and the prevalence of pathogens in the wildlife population may impact the frequency of wildlife-associated salmonellosis in humans. The objective of this study was to determine the prevalence of *Salmonella* and *Escherichia coli* O157 in the feces of different species of wildlife admitted

to two rehabilitation centers in Ohio. Fecal material was collected for culture and sensitivity, and salmonella isolates were characterized by pulse-field gel electrophoresis.

### **MATERIALS AND METHODS**

Between May and September 2004, freshly voided fecal samples were collected on sterile cotton-tipped swabs from the floors of cages of recently admitted (within 3 days) animals at two nonprofit wildlife rehabilitation centers in Ohio that treated native wildlife. Staffed by a team of eight volunteers and an individual with over 20 yr experience in animal rehabilitation, one facility admitted between 600 and 700 animals of various species each year. Animals were housed either in plastic animal crates or stainless-steel bank cages that were scrubbed with detergent and water then sprayed with an alkyl dimethyl benzyl ammonium chloride disinfectant (Lysol®, Reckitt Benckiser, Inc., Parsippany, New Jersey 07054) prior to placement of animals. Waste was removed and fresh bedding materials provided daily or more often if needed. Equipment such as bottles, feeding tubes, and syringes, were washed with hot soapy water between pens of animals. Staffed by one full-time individual, several part-time workers, and numerous volunteers, the second facility rehabilitated only birds. Severely debilitated birds were housed in stainless steel cages or crates of appropriate size for each patient. Cages were cleaned with soap and water prior to animal placement then disinfected with a 10% solution of household bleach or a quaternary ammonium disinfectant 0.4% vol/vol (Rocal® D-plus, Pfizer Inc., New York, New York 10017). Larger outdoor cages constructed with pressure-treated lumber and vinyl-clad wire were provided

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for rehabilitation of birds. Hard surfaces were hosed down and soil areas were raked two–three times weekly.

Animal species were identified based upon characteristic markings according to North American wildlife field manuals.<sup>5,12</sup> Members of the surrounding small urban communities brought young orphans and debilitated animals (many with traumatic injuries) to the dedicated rehabilitation facilities for care. When multiple animals of the same species, such as littermates, were admitted at the same time, only one pooled sample was collected per cage. One facility housed a resident Harris hawk (*Parabuteo unicinctus*), which was also sampled.

Samples of approximately 0.5 g each were enriched overnight at 42°C in buffered peptone water (BPW). Enrichments were tested immediately for *E. coli* O157:H7 using automated immunomagnetic separation according to the recommendation of the manufacturer (Dynal Biotech ASA, Oslo NO-0379, Norway). One milliliter of each enrichment culture was frozen in 30% buffered glycerol and stored at –70°C. To detect *Salmonella*, the frozen enrichment cultures were added to tetrathionate broth, sequentially transferred at 48-hr intervals after incubation at 42°C to Rappaport Vassiliadis broth, and then plated onto XLT-4 agar. Black colonies that grew on XLT-4 agar plates after overnight incubation at 37°C were screened for characteristic biochemical reactions of *Salmonella* on triple-sugar iron agar, Christensen's urea broth, Simmon's citrate agar, and agglutination with antisalmonella antiserum.<sup>14</sup> Overnight broth cultures of *Salmonella*-presumptive colonies were frozen in 30% buffered glycerol and stored at –70°C, until they were re-plated and sent for serotyping at the National Veterinary Services Laboratory (United States Department of Agriculture, Ames, Iowa 50010, USA) and for antibiotic susceptibility testing at the National Antibiotic Resistance Unit (United States Department of Agriculture, Athens, Georgia 30605, USA). Antibiotic susceptibility was determined by the broth microdilution method using NCCLS interpretive criteria to the following antibiotics: sulfathiazine, tetracycline, ampicillin, cefoxitin, naladixic acid, streptomycin, chloramphenicol, amoxicillin/clavulanic acid, trimethoprim-sulfamethoxazole, amikacin, ceftriaxone, ciprofloxacin, gentamycin, kanamycin, and ceftiofur.<sup>3,15</sup> Pulsed-field gel electrophoresis (PFGE) of all isolates was performed according to PulseNet (CDC) Protocol.<sup>7</sup>

## RESULTS

Nineteen different animal species (11 avian and 8 mammalian) were tested for *E. coli* O157:H7 and

*Salmonella enterica* (Table 1). *Escherichia coli* O157:H7 was not isolated from any fecal samples. *Salmonella* was isolated from 8 of 71 (11%) fecal samples. *Salmonella* was isolated from 5 of the 19 (26%) animal species admitted on different occasions over the course of the 3-month study (Table 1). Notably, we identified the presence of four serovars of *Salmonella*. Seven of the eight (88%) isolates recovered were susceptible to the entire panel of antibiotics tested. However, isolate 134, *Salmonella* Kentucky obtained from an Eastern gray squirrel (*Sciurus carolinensis*), was resistant to ampicillin, the combination of amoxicillin and clavulanic acid, cefoxitin, and ceftiofur. Isolates of different serovars produced unique PFGE banding patterns (Fig. 1), but the five *Salmonella* Oranienburg isolates were indistinguishable from one another. All the *Salmonella* Oranienburg isolates originated from orphaned mammals that, upon subsequent investigation, were identified as having been fed infant formula with a common feeding tube. This feeding tube was washed with hot soapy water between animals in different cages, but no specific treatment to disinfect or sanitize the tube had been performed.

## DISCUSSION

All the *Salmonella* serovars identified in this study have previously been reported as causes of human disease and have been isolated from food-producing animals. Presently, the number of human *Salmonella* infections linked directly, or indirectly, to wildlife sources is undefined. Although the frequency of human–wildlife interactions is not known, the number of animals cared for by rehabilitators is increasing.<sup>4</sup> One estimate reported an average of 360,000 human contact–days with wildlife each year by rehabilitators in licensed rehabilitation centers in Colorado, a state with about 4.3 million inhabitants.<sup>6</sup> Importantly, this same report identified 95% of this contact occurred in home-based facilities, locations where food preparation and consumption may be more likely and where children or immune-compromised individuals might be present. These estimates do not account for individuals who might occasionally try to rehabilitate wildlife without permits or other types of human–wildlife interactions. Given the frequency of *Salmonella* carriage and the amount of contact required to foster juvenile or debilitated wildlife, transmission from animals to humans is possible.

In this study, it was not possible to definitively determine whether animals entered the rehabilitation facilities carrying *Salmonella* or if they became colonized after admission. However, the indistin-

**Table 1.** Isolation of *Salmonella* in 71 fecal samples collected from wildlife species at two wildlife rehabilitation centers in Ohio, May–September 2004.

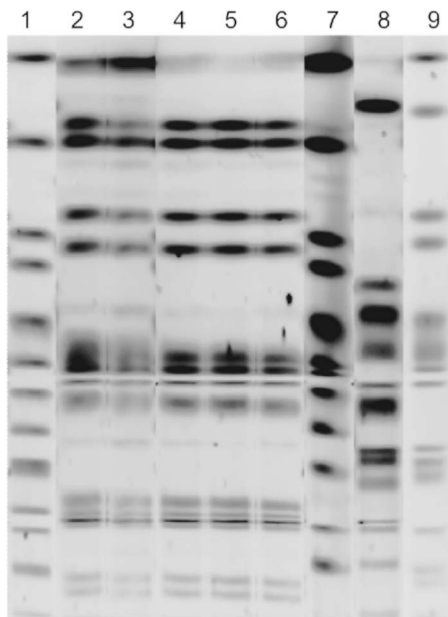
Animal	<i>n</i>	Date	<i>Salmonella</i> serovar	Antibiotic resistance phenotype
Turkey vulture ( <i>Cathartes aura</i> )	1			
Eastern cottontail ( <i>Sylvilagus floridanus</i> )	4			
Opossum ( <i>Didelphis virginiana</i> )	7	Aug 13	Oranienburg	Pansusceptible <sup>a</sup>
		Aug 15	Oranienburg	Pansusceptible
		Aug 28	Oranienburg	Pansusceptible
Common raccoon ( <i>Procyon lotor</i> )	4			
Canada goose ( <i>Branta canadensis</i> )	4			
Mallard duck ( <i>Anas platyrhynchos</i> )	2			
Red-tailed hawk ( <i>Buteo jamaicensis</i> )	14			
Harris's hawk <sup>b</sup> ( <i>Parabuteo unicinctus</i> )	1	Aug 28	Senftenberg	Pansusceptible
Cooper's hawk ( <i>Accipiter cooperii</i> )	3			
Eastern screech-owl ( <i>Otus asio</i> )	8	May 29	Braenderup	Pansusceptible
Barred owl ( <i>Strix varia</i> )	3			
Great horned owl ( <i>Bubo virginianus</i> )	1			
American kestrel ( <i>Falco sparverius</i> )	6			
Mourning dove ( <i>Zenaidura macroura</i> )	3			
Jumping meadow mouse ( <i>Zapus hudsonius luteus</i> )	1			
White-tailed deer ( <i>Odocoileus virginianus</i> )	1			
Woodchuck ( <i>Marmota monax</i> )	3	Sep 9	Oranienburg	Pansusceptible
Eastern gray squirrel ( <i>Sciurus carolinensis</i> )	4	Aug 23	Oranienburg	Pansusceptible
		Jul 9	Kentucky	Resistant <sup>c,d,e,f</sup>
Eastern fox squirrel ( <i>Sciurus niger</i> )	1			
Total samples	71			

<sup>a</sup> Pansusceptible = serovar susceptible to all antibiotics tested.<sup>b</sup> Permanent center resident.<sup>c</sup> Amo = serovar resistant to amoxicillin/clavulanic acid.<sup>d</sup> Amp = serovar resistant to amoxicillin.<sup>e</sup> Fox = serovar resistant to ceftiofur.<sup>f</sup> Tio = serovar resistant to ceftiofur.

guishable PFGE result among *Salmonella* Oranienburg isolates suggests a common (nosocomial) source of exposure. The remaining isolates (*Salmonella* Braenderup, *Salmonella* Kentucky, and *Salmonella* Senftenberg) may have been acquired while in the facility or been present in the animals upon admission. Wildlife may acquire *Salmonella* from feed or from other environmental sources, such as livestock operations, feed manufacturers, and human waste treatment plants.<sup>10,16</sup> The prevalence of *Salmonella* in the wildlife in a particular geographic region is probably a reflection of the frequency that these potential sources of contamination are present in their home range.<sup>8</sup> Estimates of prevalence of pathogens may also be influenced by the sensitivity of the microbiological assay used. In this study, previously frozen enrichment specimens were cultured for *S. enterica*. Despite the fact that the frozen samples contained glycerol, the freezing of specimens may have reduced the recovery of *S. enterica* from the specimen, thereby lim-

iting the sensitivity of detection. Nevertheless, our prevalence estimates are slightly higher than those previously reported in wildlife in California (4%) and in Spain (4.19%), and are similar to that reported in a rehabilitation center in Italy.<sup>1,17,18</sup>

The animals in this study were recovered from urban environments, such as private residences, city streets, and public parks. *Salmonella* Senftenberg and *Salmonella* Brandenburg have been previously reported from gulls and a kestrel.<sup>16,17</sup> Reports of *Salmonella* Kentucky and *Salmonella* Oranienburg isolated from wildlife are not available in the peer-review literature. However, given the occurrence of these two serovars in livestock, humans, and the environment, these serovars, as well as the two aforementioned serovars, may have originated from any number of diverse sources.<sup>11,20,22</sup> It was interesting to have found even a single *Salmonella* isolate that was resistant to ceftiofur, a third-generation cephalosporin antibiotic, in a wild animal that had no known history of antibiotic treatment. This find-



**Figure 1.** Pulse-field gel electrophoresis of *Salmonella enterica* isolates obtained from 8 of 71 fecal samples from wildlife recently admitted to two wildlife rehabilitation centers in Ohio, May–September 2004. Lane 1, Reference *Salmonella* Braenderup strain (H9812); lanes 2–6, *Salmonella* Oranienburg isolates 132, 133, 136, 141, and 142, respectively; lane 7, isolate 135, *Salmonella* Braenderup; lane 8, isolate 134, *Salmonella* Kentucky; lane 9, isolate 138, *Salmonella* Senftenberg.

ing underscores the importance of controlling the transmission of *S. enterica* between animals and from patient to caretakers through adequate hygienic practices. Antibiotic treatment is not a prerequisite for the occurrence of drug-resistant organisms to be present.<sup>9</sup> Regardless of the origin of the drug-resistant organism, the presence and potential nosocomial transmission of *S. enterica*, including one strain that exhibited resistance to multiple antibiotics, in these rehabilitation centers is important for several reasons: 1) *Salmonella* may be pathogenic to wildlife, 2) antibiotic resistance may make treatment of animal disease difficult, and 3) human contact with infected animals may result in zoonotic disease.<sup>1</sup> For example, the *Salmonella*-positive hawk was occasionally taken to public events and used as an educational exhibit. Two outbreaks of salmonellosis have been tracked to youth educational programs where children dissected pellets obtained from an owl held at a rehabilitation center.<sup>19</sup> In addition, a goal of most rehabilitation centers is release of as many animals back to native habitat as possible (typically more than 50%),

which may increase the likelihood of *Salmonella* strains in free-ranging populations.<sup>6</sup>

## CONCLUSION

Efforts should be made to increase the public and rehabilitator awareness of the zoonotic hazards associated with wildlife contact. Specifically, wildlife contact should be considered in cases of human salmonellosis. Furthermore, we emphasize the need for increased education among individuals who have contact with wildlife. Individuals working with wildlife should know and practice appropriate measures of environmental and personal hygiene to prevent nosocomial infections among animal patients, as well as to reduce the potential of zoonotic infections among animal handlers, rehabilitators, veterinarians, and other people who might have contact with these animals.

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