Salmonella in Raptors and Aquatic Wild Birds in Chile

Article *in* Journal of wildlife diseases · April 2020

DOI: 10.7589/2019-08-198

SHORT COMMUNICATIONS

DOI: 10.7589/2019-08-198

Journal of Wildlife Diseases, 56(3), 2020, pp. 000–000

Salmonella in Raptors and Aquatic Wild Birds in Chile

Rodolfo Tardone,[1] Dácil Rivera,[1] Fernando Dueñas,[1] Nicole Sallaberry-Pincheira,[1] Christopher Hamilton-West,[2] Aiko D. Adell,[1,3] and Andrea I. Moreno-Switt[1,3,4] [1]Escuela de Medicina Veterinaria, Facultad de Ciencias de la Vida, Universidad Andres Bello, República 440, Santiago 8320000, Chile; [2]Departamento de Medicina Preventiva Animal, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santa Rosa 11735, La Pintana, Santiago 8820000, Chile; [3]Millennium Initiative for Collaborative Research on Bacterial Resistance (MICROB-R), Las Condes 12461, Las Condes, Santiago 7550000, Chile; [4]Corresponding author (email: andrea.moreno@unab.cl)

ABSTRACT: *Salmonella enterica* is one of the main causes of gastrointestinal disease worldwide. Wild birds are capable of harboring a variety of *Salmonella* serovars, which could have an important role in the epidemiology of salmonellosis in humans and production animals. We tested 519 fecal samples from raptors and aquatic birds from different regions of central (three rehabilitation centers for wildlife and the coastal area) and southern areas of Chile for *Salmonella*. All samples were obtained in 2015 and 2017, covering all four seasons. *Salmonella* was isolated from 12 of the 519 samples (2%) analyzed, from two carnivorous birds, four birds with generalist habits, and six waterfowl. Among the isolates obtained, one showed resistance to gentamicin, and one showed a multidrug-resistance phenotype, with resistance to ampicillin, ceftriaxone, ciprofloxacin, chloramphenicol, streptomycin, gentamicin, kanamycin, trimethoprim-sulfamethoxazole, and tetracycline. These results demonstrated the importance of characterizing *Salmonella* in wild birds because previous studies have shown genetic and phenotypic evidence suggesting interspecies transmission of *Salmonella enterica* that is resistant to antimicrobials between humans and wild and domestic birds.

Key words: Antimicrobial resistance, rehabilitation centers, *Salmonella enterica*, wild birds.

Salmonella enterica is one of the most relevant foodborne pathogens worldwide, with estimates of 93.8 million human cases resulting in 155,000 deaths/yr (Majowicz et al. 2010). Although production animals are considered the main reservoir and source of infection for humans, *Salmonella* is also present in various species of wild animals (Plym Forshell and Wierup 2006; Hoelzer et al. 2011). Wild birds have roles in the dissemination of *Salmonella*, including multidrug-resistant (MDR) strains (Mohsin et al. 2017). Wild birds are capable of harboring a large variety of *Salmonella* serovars, such as Typhimurium, Enteritidis, and Anatum (Tizard 2004; Fresno et al. 2013), including serovars that are important in human and domestic livestock salmonellosis (Navarro-Gonzalez et al. 2016). In Chile, *Salmonella* was reported in aquatic wild birds, with an isolation frequency in water birds, such as Kelp Gulls (*Larus dominicanus*) and Franklin's Gulls (*Leucophaeus pipixcan*) of 6–25% (López-Martín et al. 2011; Fresno et al. 2013). In those studies, *Salmonella* Enteritidis was the main serovar detected. To expand our knowledge of the role of wild birds as carriers of *Salmonella*, we aimed to determine the presence and risk factors of *S. enterica* in wild birds arriving at wildlife rehabilitation centers and in free-living wild birds in Chile.

This study was conducted in central and southern Chile. Samples for *Salmonella* isolation were obtained from birds in rehabilitation centers, wild birds, and in feces from wild birds. Three rehabilitation centers were included in the study: the Zoological Safari Park (RZ), the Metropolitan Zoo of Santiago (ZM), and the Wildlife Rehabilitation Center of Universidad Andres Bello/Buin Zoo (UFAS); all three are located in central Chile. Samples of wild birds were obtained from three locations in southern Chile, and fecal samples were obtained in the coastal city of Concon, in central Chile. In total, 519 samples were processed for *Salmonella* isolation; 313 samples were collected from wild birds that entered the rehabilitation centers from March 2015 to August 2016. These samples were obtained within the first 24 h of the arrival of the birds at the rehabilitation centers, before any pharmacological treatment. For samples from birds in the wild, 45 cloacal swabs were obtained in southern Chile during the months

of August 2015 and April 2016 (data on the collection of these samples is available in Verdugo et al. 2019). Finally, 161 samples collected directly from fresh bird feces were obtained; these represented all fresh feces available at the sampling visit. These samples were obtained in the coastal city of Concon, which was selected because of its high diversity and confluence of wild water birds. These samples were collected from June 2017 to November 2017.

All fecal samples were collected in Cary Blair transport medium (Copan Italia Spa, Brescia, Italia) under sterile conditions and were transported within 24 h of collection at 4 C for processing at Universidad Andres Bello. For *Salmonella* isolation, 10 g of each sample was enriched with 90 mL of peptone water (Becton-Dickinson, Franklin Lakes, New Jersey, USA) and incubated at 37 C overnight. Subsequently, 100 µL of each sample was transferred to Rappaport Vassiliadis (Becton-Dickinson) supplemented with 20 µg/mL novobiocin (Sigma-Aldrich, St. Louis, Missouri, USA), and 1 mL of the sample was transferred on Tetrathionate (Becton-Dickinson) and incubated at 42 C. After incubation, 100 µL was transferred into agar XLT-4 (Becton-Dickinson), supplemented with sodium tetradecyl sulfate, and incubated at 37 C for 24 h. Presumptive colonies were confirmed as previously described by *inv*A PCR (Kim et al. 2007) and stored at –80 C with glycerol.

The Kirby-Bauer technique was used to evaluate antimicrobial resistance for the following 14 antimicrobials (OXOID™, Hampshire, UK): gentamicin (10 µg), streptomycin (10 µg), amoxicillin-clavulanic acid (20/10 µg), cefoxitin (30 µg), ceftiofur (30 µg), ceftriaxone (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), azithromycin (15 µg), ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), tetracycline (30 µg), and kanamycin (30 µg). *Escherichia coli* ATCC 25922 was used as a control. Results were interpreted according to the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute 2017). In the *Salmonella* isolates, we used a molecular scheme (PCR-based) previously described to identify *Salmonella* serogroups B, C1, C2, D, and E1 (Ranieri et al. 2013).

A statistical analysis was conducted to determine a possible association of seasons and site with the presence of *Salmonella*. The variables considered for the model were site, season, and detection of *Salmonella*. For the site variable, only sites with positive and negative results were included (i.e., Concon, UFAS, and ZM), and sites with all negative results were excluded (i.e., samples from birds from the wild and the RZ). The variables, *season* (spring, summer, fall, and winter), *dry season* (spring and summer), and *wet season* (fall and winter) were also used, with the variable *detection* of *Salmonella* as the outcome. A model with the variables of interest was then evaluated with generalized linear model multivariable analysis to determine the association among these variables jointly and the outcome of interest. $P \leq 0.05$ was considered significant. The statistical software R Studio (version 1.1.456, RStudio, Boston, Massachusetts, USA) was used to perform all the analyses.

Among the 519 analyzed samples, 12 (2.3%) tested positive for *Salmonella* (Table 1). In samples from the rehabilitation centers, *Salmonella* was identified in 1.7% (2/121) of the birds of the ZM and in 2.2% (4/182) of the birds of the UFAS. In samples from birds in the wild, *Salmonella* was not detected (0/45), and in wild bird feces, 3.7% (6/161) of the Concon samples tested positive. Among the samples from the rehabilitation center, we found positive samples from carnivorous and generalist birds. Because of factors such as stress and the rehabilitation process suffered by wild animals that arrive at rehabilitation centers, there might be an increase in *Salmonella* shedding, so the sampling of these individuals might better reflect the prevalence of *Salmonella* in wildlife, in comparison with the samplings performed only on asymptomatic animals (Smith et al. 2002).

Twelve isolates were characterized by their serogroup and antimicrobial-resistant profiles (Table 2). Most of the isolates were pansusceptible (10/12, 83%); one isolate from a

TABLE 1. Origin, source, number of samples, and positivity of samples obtained in this study in wild birds in Chile in 2015 and 2017.

Sample origin	Food categories of birds	No. of samples	% Positive samples
Metropolitan zoo	Carnivorous	65	1.6
	Generalist	38	0
	Granivorous	12	0
	Insectivorous	3	0
	Folivorous	1	0
	Vermivorous	2	0
UFAS[a]	Carnivorous	78	0
	Generalist	61	2.2
	Granivorous	26	0
	Insectivorous	3	0
	Folivorous	3	0
	Vermivorous	4	0
	Piscivorous	1	0
Rancagua Zoo	Carnivorous	7	0
	Generalist	6	0
	Granivorous	2	0
	Insectivorous	1	0
Central Chile[b]	Insectivorous		
	Piscivorous	161	3.7
	Generalist		
Southern Chile	Piscivorous	41	0
	Generalist	3	0
	Carnivorous	1	0
Total		519	2.3

[a] UFAS = Wildlife Rehabilitation Center of Universidad Andres Bello/Buin Zoo

[b] The 161 samples of birds dropping were from the feces of birds belonging to the three food categories.

[c] Based on diet categories of each species of bird.

carnivorous bird (Harris's hawk [*Parabuteo unicinctus*] obtained from the ZM) showed resistance to gentamicin, and one isolate from a generalist bird (Kelp Gull [*Larus dominicanus*] obtained from the UFAS) showed resistance to nine antimicrobials (ampicillin, ceftriaxone, ciprofloxacin, chloramphenicol, streptomycin, gentamicin, kanamycin, trimethoprim-sulfamethoxazole, and tetracycline). A previous study identified resistance to gentamicin, tetracycline, and ampicillin in *Salmonella* isolates obtained from waterfowl in Chile (Fresno et al. 2013). These results show the presence in Chile of MDR *Salmonella* in wild birds, which might possess a potential role as disseminators of these microorganisms and could be a reflection of the anthropogenic effect on the habitat of these birds (Mohsin et al. 2017). Within the identified serogroups, three isolates from UFAS were classified as serogroup B, and two isolates (one from ZM and one from Concon) were classified as serogroup C1; two isolates from Concon were classified as serogroup D. The other three isolates could not be classified in any of the five serogroups that we tested for. Although the *Salmonella* serotypes most commonly isolated in wild birds of different regions of the world and Chile are Typhimurium and Enteritidis, wild birds can also harbor a great diversity of serovars not included in the serogroups tested with the scheme used here (Tizard 2004).

The multivariable model was run for the *season* and *site* variables (Table 2); this analysis showed that only the variable *season* had at least one level that was significant ($P \leq 0.05$). The multivariable model indicated

TABLE 2. Serogroup and antimicrobial resistance of isolated *Salmonella* from two birds at rehabilitation centers that tested positive and from samples from fresh feces from birds at Concón, Chile.

Isolate	Origin	Bird species/type[a]	Serogroup	Antimicrobial resistance profile[b,c]
RT-002	Metropolitan Zoo	*Parabuteo unicinctus*/raptor	ND[e]	GEN
RT-008	Metropolitan Zoo	*Vultur gryphus*/raptor	C1	Pansusceptible
RT-022	UFAS[d]	*Nycticorax nycticorax*/aquatic	B	Pansusceptible
RT-023	UFAS	*Bubulcus ibis*/aquatic	B	Pansusceptible
RT-024	UFAS	*Bubulcus ibis*/aquatic	B	Pansusceptible
RT-027	UFAS	*Larus dominicanus*/aquatic	ND	AMP, CRO, CIP, GEN, CHL, STR, KAN, SXT, TET
RT-028	Concon	Aquatic birds	B	Pansusceptible
RT-029	Concon	Aquatic birds	C1	Pansusceptible
RT-030	Concon	Aquatic birds	B	Pansusceptible
RT-031	Concon	Aquatic birds	ND	Pansusceptible
RT-032	Concon	Aquatic birds	D	Pansusceptible
RT-033	Concon	Aquatic birds	D	Pansusceptible

[a] Data on bird species are reported for samples obtained at rehabilitation centers and for samples obtained from fresh feces; only the type of bird was recorded.

[b] Pansusceptible = susceptible to all antibiotics tested.

[c] Abbreviations: GEN = gentamicin; STR = streptomycin; CRO = ceftriaxone; SXT = trimethoprim-sulfamethoxazole; AMP = ampicillin; CHL = chloramphenicol; CIP = ciprofloxacin; TET = tetracycline; KAN = kanamycin.

[d] UFAS = Wildlife Rehabilitation Center of Universidad Andres Bello/Buin Zoo.

[e] ND = not determined; these isolates could not be classified in any of the serogroups in the scheme (B, C1, C2, D, and E1).

that detecting *Salmonella* in birds in spring was 0.19× lower than in winter ($P \leq 0.0471$; 95% confidence interval [CI], 0.03–0.83%). The seasonal presence of *Salmonella* has been previously evaluated in waterbirds in Chile (López-Martín et al. 2011); those authors also found a greater number of isolates in the winter and fall. However, the authors attributed those results to the different feeding habits of the birds (López-Martín et al. 2011). A higher frequency of *Salmonella* in winter was associated with wild-bird mortality events, mainly in passerine birds using garden feeders (Tizard 2004).

In our study, the variable *site* was not associated with *Salmonella* (Table 3). In other studies, fecal and anthropogenic contamination has been linked with the presence of *Salmonella* in wild birds, thus reflecting the role of the environment in *Salmonella* carriage (Tizard 2004). Interestingly, the MDR isolate found here was isolated from a *Larus dominicanus* from the UFAS, which presented a resistance phenotype against nine antimicrobials; MDR *Salmonella* isolates have been previously reported in wildlife (Molina-López et al. 2015).

Previous studies have used genetic and phenotypic evidence of isolates obtained from aquatic birds, poultry, and humans in Chile to suggest interspecies transmission of *Salmonella* Enteritidis (Fresno et al. 2013; Retamal et al. 2015; Toro et al. 2016). Importantly, anthropogenic contamination of the environment may facilitate infection in wild birds, which could act as an important reservoir of *Salmonella* Enteritidis, in which humans would be an accidental host (Toro et al. 2016). Additionally, these same factors that facilitate interspecies transmission in *Salmonella* Enteritidis can facilitate the transmission of antimicrobial resistance present in the *Salmonella* that inhabits other hosts, which are under selective pressure from contaminated environments, such as farmed animals and sewage, many of which are frequented by wild bird, such as the Kelp Gull (Retamal et al. 2015; Masarikova et al. 2016). The findings reported here contribute to our knowledge of the role of wildlife animals in the epidemiol-

Table 3. Multivariable generalized linear model showing risk-factor associations of *site* and *season* for *Salmonella detection* in samples from birds in Chile in 2015 and 2017.

Variable	Categories or levels[a]	Odds ratio estimates[b]	95% Confidence intervals	*P* value
Site	Concon[c]			
	UFAS[d]	1.11	0.02–0.13	0.8878
	Metropolitan Zoo	1.06	0.25–4.30	0.9561
Season	Winter			
	Fall	0.56	0.03–5.10	0.6437
	Spring	0.19	0.03–0.83	0.0471[e]
	Summer	0.13	0.006–1.03	0.0848

[a] Only sites with positive and negative results for *Salmonella* presence were included.

[b] Akaike information criterion = 115.21; R^2=0.0170551.

[c] Used as the reference category for statistical comparisons.

[d] UFAS = Wildlife Rehabilitation Center of Universidad Andres Bello/Buin Zoo.

[e] Risk factors with statistically significant results.

ogy of *Salmonella* with characteristics of public health interest, such as the presence of MDR.

The authors thank Isabel Campos and Carolina Sánchez from the Wildlife Rehabilitation Center (UFAS), Diego Peñaloza from the Rancagua Zoo, Carolina Ibarra and Consuelo Foerster from the Metropolitan Zoo of Santiago, and Claudio Verdugo from the Universidad Austral for their assistance in the sampling activities. Funding was from FONDECYT 11140108 to A.I.M.-S., UNAB Initiation, and the Millennium Science Initiative of the Ministry of Economy, Development and Tourism, Government of Chile.

LITERATURE CITED

Fresno M, Barrera V, Gornall V, Lillo P, Paredes N, Abalos P, Fernández A, Retamal P. 2013. Identification of diverse *Salmonella* serotypes, virulotypes, and antimicrobial resistance phenotypes in waterfowl from Chile. *Vector-Borne Zoonot Dis* 13:884–887.

Hoelzer K, Switt AIM, Wiedmann M. 2011. Animal contact as a source of human non-typhoidal salmonellosis. *Vet Res* 42:34.

Kim JS, Lee GG, Park JS, Jung YH, Kwak HS, Kim SB, Nam YS, Kwon ST. 2007. A novel multiplex PCR assay for rapid and simultaneous detection of five pathogenic bacteria: *Escherichia coli* O157:H7, *Salmonella, Staphylococcus aureus, Listeria monocytogenes, and Vibrio parahaemolyticus*. *J Food Prot* 70: 1656–1662.

López-Martín J, Junod T, Riquelme F, Contreras C, González-Acuña D. 2011. Detection of *Salmonella* and *Mycobacterium* species in seagulls captured in Talcahuano, Chile. *Rev Med Chile* 139:1496–1502.

Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra RM. 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin Infect Dis* 50:882–889.

Masarikova M, Manga I, Cizek A, Dolejska M, Oravcova V, Myskova P, Karpiskova R, Literak I. 2016. *Salmonella enterica* resistant to antimicrobials in wastewater effluents and black-headed gulls in the Czech Republic, 2012. *Sci Total Environ* 542:102–107.

Mohsin M, Raza S, Schaufler K, Roschanski N, Sarwar F, Semmler T, Schierack P, Guenther S. 2017. High prevalence of CTX-M-15-Type ESBL-producing *E. coli* from migratory avian species in Pakistan. *Front Microbiol* 8:2476.

Molina-López RA, Vidal A, Obón E, Martín M, Darwich L. 2015. Multidrug-resistant *Salmonella enterica* serovar Typhimurium monophasic variant 4,12:i:— Isolated from asymptomatic wildlife in a Catalonian wildlife rehabilitation center, Spain. *J Wildl Dis* 51: 759–763.

Navarro-Gonzalez N, Ugarte-Ruiz M, Domínguez L, Ruiz-Fons F. 2016. A European perspective on the transmission of foodborne pathogens at the wildlife–livestock–human interface. In: *Food Safety Risks from Wildlife*, Jay-Russell M., Doyle M, editors. Springer, Cham, Switzerland, pp. 59–88.

Plym Forshell L, Wierup M. 2006. *Salmonella* contamination: A significant challenge to the global marketing of animal food products. *Rev Sci Tech Off Int Epizoot* 25: 541–554

Ranieri ML, Shi C, Moreno Switt AI, den Bakker HC, Wiedmann M. 2013. *Salmonella* serovar prediction: Comparison of typing methods with a new procedure based on sequence characterization. *J Clin Microbiol*. 52:1786–1797

Retamal P, Fresno M, Dougnac C, Gutierrez S, Gornall V, Vidal R, Vernal R, Pujol M, Barreto M, Gonález-Acuña D, et al. 2015. Genetic and phenotypic

evidence of the *Salmonella enterica* serotype Enteritidis human-animal interface in Chile. *Front Microbiol* 6:464.

Smith WA, Mazet JAK, Hirsh DC. 2002. *Salmonella* in California wildlife species: prevalence in rehabilitation center and characterization of isolates. *J Zoo Wildl Med* 33:228–235.

Tizard I. 2004. Salmonellosis in wild birds. *Semin Avian Exot Pet Med* 13:50–66.

Toro M, Retamal P, Ayers S, Barreto M, Allard M, Brown EW, Gonzalez-Escalona N. 2016. Whole-genome sequencing analysis of *Salmonella enterica* serovar Enteritidis isolates in Chile provides insights into possible transmission between gulls, poultry, and humans. *Appl Environ Microbiol* 82:6223–6232.

Verdugo C, Pinto A, Ariyama N, Moroni M, Hernandez C. 2019. Molecular identification of avian viruses in neotropic cormorants (*Phalacrocorax brasilianus*) in Chile. *J Wildl Dis* 55:105–112.

Submitted for publication 7 August 2019.
Accepted 17 January 2020.