Identification of diverse *Salmonella* Serotypes, Virulotypes, and Antimicrobial Resistance Phenotypes in Waterfowl From Chile

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Abstract

Salmonella enterica is a pathogen with a wide host-range that presents great concern in developed and developing countries. To determine and characterize *Salmonella* strains found in Chile's waterfowl, we sampled 758 birds along 2000 km of the Chilean coast. In this sample, 46 isolates from 10 serotypes were detected, several with multidrug resistance phenotypes and different combinations of virulence-associated genes (virulotypes). These results suggest that *Salmonella* infection in waterfowl in Chile could have impacts on public and animal health.

Key Words: *Salmonella enterica*—Waterfowl—Chile—Virulotypes.

Introduction

THE LIST OF FOODBORNE ZOO NOTIC PATHOGENS includes numerous biological agents that have been ranked according to criteria related to public health, animal health, and occurrence in food (Cardoen et al. 2009, Humblet et al. 2012). Among them, *Salmonella enterica* is a pathogen that causes great concern in developed and developing countries, generating periodic outbreaks that result in endemic infection in humans (Cardoen et al. 2009). This bacterium has also gained attention among wildlife-borne pathogens and has been prioritized for active surveillance in wild birds (Tavernier et al. 2011). Although *Salmonella* infection in these animals is asymptomatic in nature, disease outbreaks arise, and direct transmission of the bacterium from wild birds to humans also occurs (Dhama et al. 2008).

The objective of our study was to identify *S. enterica* serotypes infecting waterfowl in Chile and to characterize them through antimicrobial resistance phenotypes and PCR-based virulotyping.

Materials and Methods

From October, 2011, to October, 2012, a sample of 758 birds was collected individually from eight sites and five Chilean Regions (Table 1). This included the following species: *Leucophaeus pipixcan* (29), *Larus dominicanus* (676), *Leucophaeus*

modestus (39), and *Chloephaga melanoptera* (14). Using swabs with Cary–Blair transport medium (COPAN, Murrieta, CA), fresh bird feces were collected from the environment and sent to the Laboratory of Infectious Diseases at the University of Chile in Santiago to be processed. To isolate bacteria, swabs were placed into 5 mL of buffered peptone water (Difco APT broth, Beckton Dickinson, Franklin Lakes, NJ) supplemented with 20 µg/mL novobiocin (Sigma, St. Louis, MO), and incubated for 24 h at 37°C. Then, 100 µL of the suspension were inoculated into modified semisolid Rappaport Vassiliadis basal medium (Oxoid, Sao Paulo, Brazil) supplemented with 20 µg/mL novobiocin and incubated for 24 or 48 h at 41.5°C. Cultures with bacterial growth were plated in Xilose Lysine Deoxicholate agar (Difco XLD broth, Beckton Dickinson, Franklin Lakes, NJ) and incubated for 24 h at 37°C. Suspicious colonies were identified by biochemical tests and *invA* gene detection by PCR (Malorny et al. 2003). *S. enterica* strains were serotyped according to the Kauffman–White scheme (Grimont and Weill 2007). Antimicrobial susceptibility was evaluated by the disk diffusion method following Clinical and Laboratory Standards Institute (CLSI) criteria (CLSI 2010). Antimicrobials tested were (µg/disk) ampicillin (10), amoxicillin–clavulanic acid (20/10), cefotaxime (30), gentamicin (10), trimethoprim–sulfamethoxazol (1.25/23.75), tetracycline (30), cefadroxil (30), (5), cefradine (30), ceftiofur (30), and enrofloxacin (10). *Escherichia coli* ATCC 25922 was used as a

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TABLE 1. *SALMONELLA* SEROTYPES ISOLATED FROM WATERFOWL AND THEIR ANTIMICROBIAL RESISTANCE PHENOTYPES

Sampling location ^a	Hosts (number of samples)	Strain identification	Antimicrobial resistance
Arica			
18°24'S 70°19'W	<i>Leucophaeus pipixcan</i> (29)	<i>S. Enteritidis</i> 95	AMC, AMP
18°26'S 70°18'W	<i>Larus dominicanus</i> (8)	<i>S. Anatum</i> 1	AMP
	<i>Leucophaeus modestus</i> (39)	<i>S. Havana</i> 2	ENR
Coquimbo			
29°57'S 71°20'W	<i>Larus dominicanus</i> (100)	<i>S. Enteritidis</i> 97	AMC, TE
		<i>S. Enteritidis</i> 98	CTX, TE
		<i>S. Enteritidis</i> 99	CE, CN, CTX, TE
		<i>S. Enteritidis</i> 100	TE
		<i>S. Enteritidis</i> 101	TE
		<i>S. Enteritidis</i> 102	TE
		<i>S. Enteritidis</i> 103	AMP, TE
		<i>S. Enteritidis</i> 104	AMP, CN, EFT, ENR, TE
		<i>S. Enteritidis</i> 105	AMP, CN, TE
		<i>S. Enteritidis</i> 106	AMC, AMP, ENR, TE
		<i>S. Enteritidis</i> 107	AMP, CE, TE
Valparaíso			
33°01'S 71°35'W	<i>Larus dominicanus</i> (253)	<i>S. Enteritidis</i> 108	AMP, CN, TE
		<i>S. Enteritidis</i> 109	TE
		<i>S. Enteritidis</i> 110	CE, ENR, STX, TE
		<i>S. Enteritidis</i> 111	AMP, CFR, CN, STX, TE
		<i>S. Enteritidis</i> 112	AMP, CE, TE
		<i>S. Enteritidis</i> 113	TE
		<i>S. Enteritidis</i> 114	Susceptible
		<i>S. Enteritidis</i> 115	TE
		<i>S. Enteritidis</i> 116	Susceptible
		<i>S. Enteritidis</i> 117	TE
		<i>S. Enteritidis</i> 118	TE
		<i>S. Enteritidis</i> 119	TE
		<i>S. Enteritidis</i> 120	TE
		<i>S. Enteritidis</i> 121	CE, TE
		<i>S. Enteritidis</i> 122	EFT, TE
		<i>S. Enteritidis</i> 123	CE, TE
		<i>S. Enteritidis</i> 124	TE
		<i>S. Enteritidis</i> 125	TE
		<i>S. Enteritidis</i> 126	TE
		<i>S. Enteritidis</i> 127	EFT, TE
		<i>S. Heidelberg</i> 1	AMP, ENR
		<i>S. Heidelberg</i> 2	AMP, TE
		<i>S. Heidelberg</i> 3	ENR, TE
		<i>S. Agona</i> 2	Susceptible
33°34'S 71°36'W	<i>Larus dominicanus</i> (125)	<i>S. Infantis</i> 3	AMP
		<i>S. Senftenberg</i> 3	ENR, TE
		<i>S. Dublin</i> 1	Susceptible
33°37'S 71°37'W	<i>Larus dominicanus</i> (165)	<i>S. Senftenberg</i> 2	ENR, TE
		<i>S. enterica</i> Group B ^b	TE
Metropolitana			
33°12'S 70°49'W	<i>Chloephaga melanoptera</i> (14)	<i>S. Heidelberg</i> 4	AMC
Bfo Bfo			
36°42'S 73°6'W	<i>Larus dominicanus</i> (25)	<i>S. Senftenberg</i> 6	Susceptible
		<i>S. Senftenberg</i> 7	Susceptible

^aThere are different sampling sites in regions of Arica and Valparaíso.

^bSerotyped as Group B (I 4,5,12:b:-).

AMC, amoxicillin-clavulanic acid; AMP, ampicillin; CTX, cefotaxime; CFR, cefadroxil; CE, cefradine; EFT, ceftiofur; ENR, enrofloxacin; CN, gentamicin; STX, trimethoprim-sulfamethoxazol; TE, tetracycline.

control strain. Virulence-associated genes *pefA*, *spvC*, *sirA*, *gipA*, *SEN1417*, *trhH*, and *prot6e*, all of which have been variably detected in the *S. Enteritidis* serotype (Pan et al. 2009, Huehn et al. 2010), were used as target sequences for the PCR-based virulotyping. DNA extraction was performed by the High Pure PCR Template preparation Kit[®] (Roche, Mann-

heim, Germany) following the manufacturer's recommendations. The reaction mixture and amplification times were at standard conditions, as previously described (Hughes et al. 2008). After agarose gel electrophoresis, amplified DNA segments were stained with GelRed[®] (Biotium, Hayward, CA) and detected using an ultraviolet (UV) transilluminator.

Categorical data analyses were made through contingency tables with INFOSTAT (2010v) software.

Results and Discussion

Salmonella strains were detected in 46 samples (6% isolation rate), with Enteritidis as the most frequent serotype (70%, Table 1). *S. Dublin*, which is considered a cattle-adapted serovar, was also detected and could represent an evidence of interspecies transmission among terrestrial mammals and waterfowl.

In the study, the largest proportion of samples was represented by Kelp gulls (*L. dominicanus*) (89%), which showed

an infection rate of 6.4% and suggests that this species can be a reservoir of *Salmonella* in aquatic environments along the Chilean coast, in agreement with previous reports from the city of Talcahuano (Lopez-Martin et al. 2011, Rodriguez et al. 2012). Because of nonmigratory habits of Kelp gulls, the potential for periodic dissemination of *Salmonella* between the Americas is represented by Franklin's gulls (*L. pipixcan*), which are latitudinal migratory birds that showed infection with a multidrug-resistant *S. Enteritidis* strain (Table 1).

Almost all isolates found were resistant to at least one antimicrobial, with most of them resistant to tetracycline (74%). This phenotype was found to be serotype associated

FIG. 1. Dendrogram showing genetic similarities (%) between *Salmonella enterica* strains analyzed using PCR. Detection of virulence-associated genes is depicted as grey squares when present. Virulotypes are indicated by letters according to their frequencies (A:20, B:10, C:6, D:3, E to L:1). The tree was constructed using the unweighted pair group method with arithmetic mean (UPGMA) method with TREECON software (1000 replicates). The *Salmonella* reference collection B 16 (SARB16) strain was included as a control.

($p < 0.01$), although more isolates (other than Enteritidis) are required for its characterization. In addition, resistances to tetracycline and amoxicillin-clavulanic acid had contrasting geographical distributions ($p < 0.01$), because the first was mainly detected in strains from Valparaíso, whereas the latter was not detected there. Notably, 21 strains, most of which belonged to the serotype Enteritidis, had multidrug-resistant phenotypes to a range of two to five antimicrobials (Table 1).

The detection of virulence associated genes identified 12 combinations or virulotypes, with a variable distribution among serotypes ($p < 0.01$) (Fig. 1). Variations in pathogenicity functions and genetic repertoire between serotypes explain the differential clustering of *S. Enteritidis* and *S. Heidelberg* strains (Fig. 1). The other serotypes were not clearly discriminated, likely due to both the scarcity of isolates from each one and the *S. Enteritidis*-based criterion for selection of targeted genes. Within *S. Enteritidis*, two virulotypes (A and B) represent the majority of strains and show geographical association ($p < 0.01$) to the Valparaíso and Coquimbo Regions, respectively (Fig. 1), suggesting a heterogeneous distribution of *S. Enteritidis* strains along the Chilean coast.

In Chile, *S. enterica* is an endemic bacterium that produces infections in humans and domestic animals. Among the top 20 most common serotypes isolated from humans in the South American region (Hendriksen et al. 2011), six of them were detected in waterfowl (Enteritidis, Agona, Infantis, Dublin, Anatum, and Heidelberg).

With sampling sites situated along 2000 km of coastline between Arica and Bío Bío, this work constitutes the most extensive survey of *Salmonella* in South American waterfowl and confirms that antimicrobial resistance phenotypes and virulotypes have spatial variations. Further research and monitoring of *Salmonella* infection in wild birds is needed to characterize emerging strains, transmission cycles, and temporal-spatial fluctuations due to global changes, with the aim of reducing potential risks in public health, domestic animals, and wildlife.

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Author Disclosure Statement

No competing financial interests exist.

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