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Evidence for Kelp Gulls (*Larus dominicanus*) and Franklin's Gulls (*Leucophaeus pipixcan*) as Carriers of *Salmonella* by Real-time Polymerase Chain Reaction

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ABSTRACT: Polymerase chain reaction confirmed that two gull species in Talcahuano, Chile, shed *Salmonella*. Fecal samples from resident *Larus dominicanus* had prevalences of 51.2% for *Salmonella* spp. and 26.3% for *Salmonella* Enteritidis. Prevalences in samples from migratory *Leucophaeus pipixcan* were 75% and 30% respectively. Risks to public health may exist.

Salmonella belong to the family Enterobacteriaceae, which contains pathogens with serotypes that have a high impact on human and animal health (Uhart et al., 2011). Recent environmental microbiology studies have established wild birds, including gulls, as one source of *Salmonella* contamination of water sources. These studies have resulted in the development of preventative techniques to allow handling of wild birds and other *Salmonella* sources (Sinigalliano et al., 2010). Gulls have extended their ranges beyond the coastal zones and have overpopulated urban centers because of the availability of human food waste, mainly at landfills and sewage discharge sites (Belant, 1997). Disease outbreaks in human and animal populations have shown the participation of migratory birds as transmitters of several pathogens (Hubálek, 2004; Albarnaz et al., 2007). We describe the seasonal presence of *Salmonella* spp. in the resident Kelp Gull (*Larus dominicanus*) and migratory Franklin's Gull (*Leucophaeus pipixcan*) over 1 yr, along the coast of Talcahuano, Chile.

Salmonella spp. and *Salmonella* Enteritidis were examined by real-time PCR (RT-PCR) with SYBR Green and TaqMan® probes respectively. Sampling was conducted January–December 2008 in the vicinity of the fish by-products manufacturing plants and wastewater effluents flowing into Talcahuano Bay, Chile (36°43′S, 73°07′W). After observing defecation, we collected 160 feces samples from resident Kelp Gulls (40 per season); 40 stool samples from migratory Franklin's Gulls were taken during the summer of 2008 (Fogarty et al., 2003). Samples were stored at −80 C until their analysis in the Bacterial Pathogenicity Laboratory at the University of Concepción. The DNA from the stool samples was extracted using a commercial kit, UltraClean Fecal DNA Sample (Mo Bio Laboratories, Carlsbad, California, USA); *Salmonella* strain ATCC 13076 was used as positive control. The DNA concentration was measured with the NanoDrop ND-1000 UV-Vis spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, Delaware, USA) and a calibration DNA curve was constructed.

The RT-PCR with SYBR Green, for *Salmonella* spp. was performed using primers ORIC-3s Forward 5′-GTGGATAACCCGGATCCTGTA-3′ Tm: 62.9 C and ORIC-4s Reverse 5′-CCCGTTTTGATCCCAGCTTAT-3′, Tm: 57.9 C (Sigma Genosys Ltd., St. Louis, Missouri USA; *Ori*C, Genebank J01808; Zyskind and Smith, 1980), and the LightCycler 2.0 thermal cycler (Roche Molecular Biochemicals, Penzberg, Germany). The reaction conditions were an initial denatur-

TABLE 1. Detection of *Salmonella* spp. and *Salmonella* Enteritidis by reverse transcriptase polymerase chain reaction in fecal samples of the migratory Franklin's Gull (*Leucophaeus pipixcan*) and resident Kelp Gull (*Larus dominicanus*), including the number of collected samples, number positive, and percentage positive.

	L. pipixcan	*L. dominicanus*				
Characteristic	Summer	Summer	Autumn	Winter	Spring	Total
No. tested						
Salmonella spp.	40	40	40	40	40	200
Salmonella Enteritidis	40	40	40	40	40	200
No. positive						
Salmonella spp.	30	18	35	17	19	119
Salmonella Enteritidis	12	18	22	6	2	60
% positive[a]						
Salmonella spp.	75 (A1)[a,b]	45 (B1)	87 (A1)	42 (B1)	47 (B1)	
Salmonella Enteritidis	30 (B2)	45 (B1)	55 (B2)	15 (A2)	5 (A2)	

[a] Values with different letters are significantly different among seasons ($P<0.05$).

[b] Values with different numbers are significantly different among *Salmonella* taxa ($P<0.05$).

ation cycle of 95 C for 10 min, followed by 45 cycles of amplification at 95 C for 10 min of denaturation, an annealing period at 55 C for 5 min, and an extension at 72 C for 5 min. An additional melting cycle and cooling period were performed. *Salmonella* Enteritidis was tested by RT-PCR with TaqMan probes; primer sequences used were Prot6-E Forward 5-ATATCGTCGTTGCTGCTTCC-3′ Tm: 56.5 C and Prot6-E Reverse 5′-CATTGTTCCACCGTCACTTTG-3′ Tm: 57.6 C, and the TaqMan probe had the sequence Prot6-E FAM-AGGCGCTCATCGGTCCTGCTGT-DQ Tm: 68.2 C (Malorny et al., 2007), provided by Sigma-Genosys Ltd. For TaqMan probe, the reaction conditions were an initial denaturation cycle at 95 C for 15 min, followed by 45 amplification cycles at 95 C for 10 min of denaturation, an annealing period of 45 C for 20 min, an extension of 72 C for 5 min, and a final cooling period at 40 C for 30 min. The primers were tested using a basic local alignment search tool (National Center for Biotechnology Information, 2012). Fisher's exact test was conducted using Epi Info™ 6.04 (Centers for Disease Control and Prevention, Atlanta, Georgia, USA). Differences in the results were considered statistically significant at $P<0.05$.

The amplification analysis of the positive controls of the *Salmonella* strain ATCC 13076 DNA, and its product of 102 bp, showed 83.5±0.5 C for the melting curve. The calculated efficiency for the calibration curve analyzed with LightCycler 2.0 software version 4.0 was 1.977, with an error of 0.0245. The PCR products were verified for size on a 2% agarose gel with 1× Tris-borate-EDTA buffer. Using SYBR Green to amplify *Salmonella* DNA from Franklin's Gull samples, 75% (30/40) of samples were positive for *Salmonella* spp. during the summer (Table 1). In the resident Kelp Gull, 55.6% (89/160) of samples were positive for *Salmonella* spp. Similar frequency was reported for Kelp Gulls and *Salmonella* Typhimurium in Brazil by PCR restriction fragment length polymorphism (Albarnaz et al., 2007). Of the samples analyzed by RT-PCR utilizing TaqMan probes for Franklin's Gull samples, 30% (12/40) were positive for *Salmonella* Enteritidis during the summer. Kelp Gulls had a 15% higher frequency of *Salmonella* Enteritidis in comparison to Franklin's Gulls during the summer ($P<0.05$; Table 1); a lower frequency of fecal samples harboring *Salmonella* Enteritidis was detected for Kelp Gulls during spring in comparison with the

other seasons ($P<0.05$). Palmgren et al. (2006) conducted a study in Sweden using molecular techniques and reported a greater frequency of *Salmonella* in Black-headed Gull (*Chroicocephalus ridibundus*) feces during the summer, associated with the appearance of juveniles nesting in these sites.

In our study, Franklin's Gull feces samples had a 30% higher frequency of *Salmonella* spp. infection in the summer than did Kelp Gull feces samples. When comparing the seasonality of the detection of *Salmonella* spp. in Kelp Gulls, a greater percentage was found in the fall. Similar results were obtained in Scotland, where infection rates for *Salmonella* spp. in Herring Gulls (*Larus argentatus*) were higher during the fall and lower during the winter, which was a consequence of depression in the birds' immune systems during these seasons (Girdwood et al., 1985).

In Chile, 9.44% of poultry product samples were contaminated with *Salmonella* spp.; of these, 7.08% harbored *Salmonella* Enteritidis (Alexandre et al., 2000). The results of our study suggest both gull species have large proportions of birds infected with *Salmonella* spp. and *Salmonella* Enteritidis, which represent a risk for *Salmonella* shedding and a potential public health problem. There may be other serotypes shed that were not investigated in this study. Studies of the other *Salmonella* serotypes and their associated virulence factors are necessary in resident and migratory birds, especially in urban areas and their perimeters.

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