ENTERIC BACTERIA IN OLROG'S GULL (*LARUS ATLANTICUS*) AND KELP GULL (*LARUS DOMINICANUS*) FROM THE BAHÍA BLANCA ESTUARY, ARGENTINA

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ABSTRACT.— The Bahía Blanca Estuary in Argentina is under the effect of chronic microbiological pollution with untreated raw sewage and the pathogens associated with it. In this estuary, there are breeding colonies of Olrog's Gull (*Larus atlanticus*) and Kelp Gull (*Larus dominicanus*), where parents feed chicks and themselves on prey and food items foraged at or near sewage outfalls and refuse tips where they can be exposed to pathogens of human and animal origin. The objective of this study was to assess the role of Olrog's Gull and Kelp Gull as carriers of zoonotic pathogens of faecal origin. Bacterial recovery from faecal samples of both gull species was positive for *Salmonella enterica* var. Typhimurium, *Salmonella enterica* var. Gallinarum, *Escherichia coli*, *Shigella dysenteriae*, *Enterobacter cloacae* and *Klebsiella pneumoniae*. The prevalence and diversity of pathogens varied between gull species, and Olrog's Gull tested positive for a wider range of pathogens than Kelp Gull. *Escherichia coli* was the most prevalent bacteria in chicks and adults of both gull species, followed by *Salmonella* spp. in Olrog's Gull chicks, and *Enterobacter cloacae* and *Shigella dysenteriae* in Olrog's Gull adults. The potential use of these gull species as sentinels of microbiological pollution in the Bahía Blanca Estuary is proposed.

KEY WORDS: *Bahía Blanca Estuary, Kelp Gull,* Larus atlanticus, Larus dominicanus, *microbiological pollution, Olrog's Gull, sentinel species, zoonotic pathogens.*

RESUMEN. BACTERIAS ENTÉRICAS EN LA GAVIOTA CANGREJERA (*LARUS ATLANTICUS*) Y LA GAVIOTA COCINERA (*LARUS DOMINICANUS*) EN EL ESTUARIO DE BAHÍA BLANCA, ARGENTINA.— El estuario de Bahía Blanca en Argentina se encuentra bajo el efecto de la contaminación microbiológica a partir del vertido de desagües cloacales sin tratamiento previo y los patógenos asociados. En este estuario existen colonias reproductivas de Gaviota Cangrejera (*Larus atlanticus*) y Gaviota Cocinera (*Larus dominicanus*) y los adultos alimentan a sus crías con presas obtenidas en sitios contaminados con desagües cloacales y en basurales a cielo abierto donde pueden estar expuestas a patógenos de origen humano y animal. El objetivo de este estudio fue evaluar el rol de la Gaviota Cangrejera y de la Gaviota Cocinera como portadores de patógenos zoonóticos de origen fecal. A partir de las muestras de material fecal se recuperaron *Salmonella enterica* var. Typhimurium, *Salmonella enterica* var. Gallinarum, *Escherichia coli*, *Shigella dysenteriae*, *Enterobacter cloacae* y *Klebsiella pneumoniae*. La prevalencia y diversidad de patógenos varió entre las dos especies de gaviotas, y la Gaviota Cangrejera fue positiva para una mayor variedad de patógenos que la Gaviota Cocinera. *Escherichia coli* fue la bacteria más prevalente en pichones y adultos de ambas especies, seguida por *Salmonella* spp. en pichones de Gaviota Cangrejera, y *Enterobacter cloacae* y *Shigella dysenteriae* en adultos de Gaviota Cangrejera. Se propone el uso de estas especies de gaviotas como centinelas de contaminación microbiológica en el estuario de Bahía Blanca.

PALABRAS CLAVE: *contaminación microbiológica, especies centinela, estuario de Bahía Blanca, Gaviota Cangrejera, Gaviota Cocinera,* Larus atlanticus, Larus dominicanus, *patógenos zoonóticos.*

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Point and nonpoint-sources of pollution are major factors compromising coastal marine habitats worldwide, and the first includes sewage treatment plant discharges. These sources can contribute pollution in various forms, including human pathogens (viruses, bacteria, protozoans, helminths, and funguses), which represent potentially serious health hazards in drinking water and bathing areas. There is large epidemiological evidence that enteric and respiratory diseases can be associated with bathing or swimming at marine coastal beaches contaminated with pathogenic micro-organisms (i.e., exposure to pollution from domestic wastewater sources; Kay et al. 1994, Fleisher et al. 1998, Pruss 1998).

The Bahía Blanca Estuary is a mesotidal coastal plain of 2300 km^2 which has long been impacted by the injection of raw sewage (approximately 1500 m^3/h), and it has been suggested that sewage pollution in the area represents a health risk for the people using the area for recreational activities (Streitenberger and Baldini 2010). These authors found *Escherichia coli* at very high concentrations (up to 14000 CFU/100 ml) in 81% of samples collected in the estuary, and reported levels above those allowed for bathing waters (US Environmental Protection Agency 2003). Also, they detected a 23.5-fold increase in the concentration of *Escherichia coli* between 1993 and 2008-2009, and in a previous research (Brezina and Baldini 2008) demonstrated the presence of a spatial trend in the concentration of somatic coliphage *Escherichia coli* ATCC 13706, with highest concentrations in sites near raw sewage input.

Gulls can act as carriers of zoonotic pathogens such as *Salmonella* spp., *Campylobacter* spp. and *Listeria* spp. from contaminated sources to humans and domestic animals (Reilly et al. 1981, Coulson et al. 1983, Quessy and Messier 1992, Tizard 2004), and they can be used as indicators of environmental microbial contamination (Girdwood et al. 1985). Notably, larids can carry *Salmonella* spp. over long distances through migratory movements during the non-breeding season or during foraging trips away from their breeding colonies (Coulson et al. 1983, Cramp 1983). Other human pathogens found in gull faeces include, but are not limited to, *Shigella* spp. (Karagüzel et al. 1993) and pathogenic forms of *Escherichia coli* (Makino et al. 2000, Hubálek 2004). Among the latter, strains of Shiga toxin-producing *Escherichia coli*, including enterohemorrhagic strain *Escherichia coli* O157, enteropathogenic *Escherichia coli* and other pathogenic serogroups have been isolated from gulls (Wallace et al. 1997, Makino et al. 2000, Kobayashi et al. 2002, Foster et al. 2006), which act as carriers when they feed at or near sewage effluents (Ferns and Mudge 2000) or at refuse tips (Coulson et al. 1983).

The Kelp Gull (*Larus dominicanus*) is a feeding generalist species that uses artificial food sources resulting from human activities such as refuse tips, sewage outfalls, slaughter houses and fisheries bycatch (Coulson and Coulson 1993). Also, the species' feeding ecology has been associated with its role as a carrier of zoonotic pathogens in different countries, such as *Salmonella enterica* var. Typhimurium in Brazil (Albarnaz et al. 2007), *Salmonella enterica* var. Enteritidis, *Salmonella enterica* var. Senfteberg, *Salmonella enterica* var. Anatum, and *Salmonella enterica* var. Infantis in Chile (López-Martín et al. 2011), and *Escherichia coli*, *Salmonella typhimurium*, and *Shigella* spp. in Argentina (Frere et al. 2000). The occurrence of large concentrations of the Herring Gull (*Larus argentatus*) close to urban centres has been proposed as a risk for human health in the Northern Hemisphere (Butterfield et al. 1983). Despite the current growth and expansion of Kelp Gull populations in coastal Argentina during at least the last two decades (Yorio et al. 2005), there is only one published study on zoonotic bacteria in gulls from Argentina (Frere et al. 2000). Large numbers of Olrog's Gull (*Larus atlanticus*) and Kelp Gull use the Bahía Blanca Estuary as wintering and breeding areas. Over 80% of the total breeding population of Olrog's Gull is concentrated as the largest known colony (approximately 3600 pairs) in the estuary, and is surrounded by a large colony of Kelp Gull (Delhey et al. 2001b) with approximately 5000 pairs (Petracci, unpublished data). These two colonies are the largest for their species in the area and are located approximately 3.5 km from outfalls draining raw sewage from the city of Bahía Blanca (human population: 400 000). The ample evidence of intense sewage pollution in the estuary, coupled with the associated health risks posed to people using this wetland for recreational purposes and the existence of large populations of gulls in the

Table 1. Prevalence (95% confidence interval in brackets) for enteric bacteria isolated in chicks and adults from Olrog's Gull (*Larus atlanticus*) and Kelp Gull (*Larus dominicanus*) breeding colonies at Isla del Puerto, Bahía Blanca Estuary, Argentina. Number of positives is shown in parentheses.

	Olrog's Gull		Kelp Gull	
	Chicks	Adults	Chicks	Adults
Mixed flora	58.5 [45.1–70.8] (31)	67.5 [51.9–80.0] (27)	59.1 [44.4–72.3] (26)	50.0 [15.0–85.0] (2)
Salmonella enterica var. Typhimurium	7.5 [2.5–18.5] (4)	2.5 [~0–14.0] (1)	-	-
Salmonella enterica var. Gallinarum	1.9 [~0–10.9] (1)	-	-	-
Escherichia coli	32.1 [21.0–45.5] (17)	20.0 [10.2–35.0] (8)	38.6 [25.7–53.4] (17)	50.0 [15.0–85.0] (2)
Shigella dysenteriae	-	2.5 [~0–14.0] (1)	-	-
Enterobacter cloacae	-	7.5 [1.9–20.6] (3)	-	-
Klebsiella pneumoniae	-	-	2.3 [~0–12.9] (1)	-

area should encourage research regarding the potential role of Olrog's Gull and Kelp Gull as sentinels of microbiological pollution. The objectives of this study were (1) to investigate the presence of bacteria with zoonotic potential in the faeces of Olrog's Gull and Kelp Gull from the Bahía Blanca Estuary, and (2) to assess the potential role of these species as sentinels of sewage pollution in the estuary.

METHODS

Field work was conducted during the breeding season of 2003 on the Isla del Puerto breeding colony in the Bahía Blanca Estuary (38°48'S, 62°15'W), Buenos Aires Province, Argentina. Adult individuals of Olrog's Gull ($n = 40$) and Kelp Gull ($n = 4$) were captured during late incubation period using funnel traps placed above active nests containing eggs. Live chicks (approximately 45 days old) of Olrog's Gull ($n = 53$) and Kelp Gull ($n = 44$) were captured using boat landing nets. Culture specimens for bacteriology were collected by cloacal swabbing. When the bird was safely contained, a sterile swab was inserted into the cloacal orifice. The swab was softly rotated against the cloacal lining six times and it was placed into tubes with sterile Cary Blair transport media (Copan Diagnostics 132C.US) and kept cool (8–10 °C) for a maximum of 24 h until processed.

Bacteriological specimens were cultured in cystine lactose electrolyte deficient agar plates for Enterobacteriaciae, *Salmonella-Shigella* agar, and blood agar. Finally, specimens were placed in selenite enrichment broth for 24 h after which colonies were picked and transferred to *Salmonella-Shigella* agar. Based on morphological and biochemical characteristics and growth patterns, the colonies compatible with Enterobacteriaceae were further typified using API 20 E strips (Biomérieux) for Gram negative. Identification of the isolated *Salmonella* spp. and *Shigella* spp. strains was achieved by serotyping (serological typing) at the Instituto Nacional de Enfermedades Infecciosas Dr. Carlos G. Malbrán (Administración Nacional de Laboratorios e Institutos de Salud, Argentina).

RESULTS

Bacterial recovery from faecal samples of Olrog's Gull and Kelp Gull was positive for *Salmonella enterica* var. Typhimurium, *Salmonella enterica* var. Gallinarum, *Escherichia coli*, *Shigella dysenteriae*, *Enterobacter cloacae* and

Table 2. *Salmonella* spp. carriage in gulls of the genus *Larus*. Data are presented from the lowest prevalence to the highest.

Species	Prevalence	Country	Source
Larus argentatus	2.9	Scotland	Coulson et al. (1983)
Larus canus	2.9	Czech Republic	Cízek et al. (1994)
Larus argentatus	3.4	United Kingdom	Butterfield et al. (1983)
Larus argentatus	3.7	Scotland	Coulson et al. (1983)
Larus dominicanus	4.0	Argentina	Frere et al. (2000)
Larus ridibundus	6.3	Whales	Ferns and Mudge (2000)
Larus atlanticus	6.5	Argentina	This study
Larus argentatus, *L. fuscus*, *L. rudibundus*	7.8	Scotland	Girdwood et al. (1985)
Larus delawarensis	8.7	Canada	Quessy and Messier (1992)
Larus argentatus	9.6	Scotland	Monaghan et al. (1985)
Larus fuscus	12.0	Scotland	Coulson et al. (1983)
Larus dominicanus, *L. novaehollandiae*	18.2	New Zealand	Robinson and Daniel (1968)
Larus sp.	55.0	Scotland	Fenlon (1983)

Klebsiella pneumoniae (Table 1). Carriage rate and diversity of pathogens found varied between gull species. Most cultures from both species and age groups grew mixed flora. *Escherichia coli* was the most prevalent bacteria in chicks and adults of both gull species, and prevalence was not different between chicks and adults of either Olrog's Gull or Kelp Gull (Fisher's Exact Test; $P > 0.05$). *Salmonella* spp. were only detected among Olrog's Gull adults and chicks. Prevalence of *Salmonella* spp. was not different between chicks and adults of Olrog's Gull (Fisher's Exact Test; $P > 0.05$). *Shigella dysenteriae* and *Enterobacter cloacae* were detected in one and three adults of Olrog's Gull, respectively, whereas *Klebsiella pneumoniae* was found in one chick of Kelp Gull.

DISCUSSION

This study shows that Olrog's Gull and Kelp Gull feeding in areas polluted with raw domestic sewage and garbage carry different pathogenic bacteria, both human-specific and zoonotic such as *Salmonella enterica* var. Typhimurium, *Salmonella enterica* var. Gallinarum, *Escherichia coli*, *Shigella dysenteriae*, *Enterobacter cloacae* and *Klebsiella pneumoniae*. These findings suggest the potential use of Olrog's Gull and Kelp Gull as sentinels of microbiological pollution in the Bahía Blanca Estuary, and their possible role as spreaders of zoonotic pathogens to the human and domestic animal populations.

The gull species studied here differ in their feeding ecology: during their breeding season in the Bahía Blanca Estuary, Olrog's Gull adults feed chicks and themselves almost exclusively on bottom feeding, *Neohelice granulata* (Delhey et al. 2001a) and *Cyrtograpsus angulatus* crabs (La Sala, pers. obs.), which they prey in mudflats impacted by raw sewage. Contrarily, Kelp Gull is a generalist and opportunistic forager that feeds on a wide range of prey such as fish, fish discards, cattle carrion, garbage from open-sky refuse tips, and rodents (Petracci et al. 2004). Considering that the diet of Kelp Gull is more diverse than that of Olrog's Gull, we had anticipated the detection of a larger variety of pathogens in the first species. However, Olrog's Gull (chicks and adults combined) tested positive for a wider range of microorganisms than Kelp Gull. This finding could be explained by the feeding ecology of Olrog's Gull parents, who prey mostly on *Cyrtograpsus angulatus* and *Neohelice granulata* in areas impacted by raw sewage where bottom-dwelling organisms, such as crabs, are exposed to a wide group of human pathogens. This hypothesis would be supported by recent *in vivo* and *in vitro* results (Miyakawa et al., unpublished data) showing that *Neohelice granulata* can act as transient carrier of highly pathogenic human bacteria in sewage polluted areas of the Bahía Blanca Estuary.

Salmonella enterica is commonly acquired from contaminated food and has been related

with human and animal illness worldwide. The serovar Typhimurium is a leading cause of gastroenteric disease, and with the exception of the Oceania and North American regions, it ranks as the second most prevalent serovar in all regions of the world (Hendriksen et al. 2011). The role of wild birds as reservoirs of *Salmonella enterica* var. Typhimurium, as source of salmonellosis outbreaks in humans, and their importance in maintaining salmonellae in the environment, have long been suggested (Kapperud et al. 1998). According to Butterfield et al. (1983) and Ferns and Mudge (2000), the rate of salmonellae carriage detected in Olrog's Gull from this study (6.5%) should not be considered as high. However, 50% of the studies included in a literature review reported lower *Salmonella* spp. prevalence than found in this study (Table 2).

Shigella spp. is a human-specific pathogen with its greatest burden in resource-poor countries where the disease causes 167 million cases of diarrhoea and over a million deaths annually (Kotloff et al. 1999). As much as 99% of cases occur in developing countries, and in the latter, 69% occur in children under five years of age. Although prevalence of *Shigella dysenteriae* was low in birds from Bahía Blanca Estuary, this is the second report of *Shigella* spp. in gulls from Argentina and the first account of *Shigella dysenteriae* in Olrog's Gull.

Our general findings, and in particular the report of bacteria that can be highly pathogenic for humans such as *Salmonella enterica* var. Typhimurium and *Shigella dysenteriae*, underline the need for further research into the role of gulls as sentinels of sewage-related pathogen pollution in the Bahía Blanca Estuary. Additionally, new research should be encouraged to gain a better appreciation of circulating pathogen strains both in the environment and in gull populations from this area.

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