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Isolation of *Campylobacter lari* from seabirds in Hope Bay, Antarctica

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Abstract: *Campylobacter* is one of the most common bacterial causes of diarrheal illness in humans. This study describes the isolation of *Campylobacter lari* from seabirds during 4 consecutive summers (2000–2003) in Hope Bay, Antarctic Peninsula. One hundred and twenty-two spontaneously dead Antarctic seabirds were studied. Ten *Campylobacter lari* isolates from 7 skuas (*Stercorarius* spp.), 2 kelp gulls (*Larus dominicanus*), and 1 Adelie penguin (*Pygoscelis adeliae*) were identified by phenotypical characteristics. Human activity in Antarctica was identified as a possible source of infectious agents, and migratory birds could be carriers of infectious diseases. However, nothing is known about zoonotic enteropathogens causing diseases in humans living in the Antarctic region. We demonstrated that seabirds carried *C. lari* in their intestines, and that they were settled around the lakes where humans are supplied with fresh water. Consumption of fresh water from Antarctic lakes contaminated with feces of seabirds could be a risk of human campylobacteriosis. This is the first report of *C. lari* isolated from seabirds in Hope Bay, Antarctica.

Key words: Antarctica, *Campylobacter lari*, skua, kelp gull, Adelie penguin.

Introduction

Campylobacter is a gram-negative microaerophilic bacterium, and is one of the most common bacterial causes of diarrheal illness (CDC 2005). Thermotolerant campylobacters, which grow at 42°C, include *Campylobacter lari*, *C. jejuni*, *C. coli* and *C. upsaliensis*. These bacteria grow best at the body temperature of birds, and seem to be well adapted to those animals, which carry them without becoming ill. *Campylobacter lari* is a phenotypically and genotypically diverse species that comprises the classical nalidixic acid-resistant thermophilic campylobacters (NARTC) and the biochemical *C. lari* variants, including urease-positive campylobacters (UPTC), nalidixic acid-susceptible campylobacters (NASC), and urease-producing nalidixic acid-susceptible campylobacters (Duim *et al.* 2004).

Isolation of *C. lari* from seagulls, chickens, cattle, pigs, dogs, cats, crows, monkeys, fur seals, aquatic birds and the environment has been previously reported (Oyarzabal *et al.* 1997). This organism has been involved in fatal cases of bacteraemia and diarrhea (Nachamkin *et al.* 1984; Tauxe *et al.* 1985), in sporadic cases with gastrointestinal symptoms (Simor and Wilcox 1987; Mégraud *et al.* 1988; Fernández *et al.* 1990), and in a water-borne outbreak (Broczyk *et al.* 1987).

Surface water and mountain streams can become contaminated with *Campylobacter* from infected feces from wild birds. This infection is common in developing countries, and travellers from foreign countries are also at risk of becoming infected (CDC 2005). In the Antarctic and the sub-Antarctic region, *Campylobacter jejuni* subspecies *jejuni* and *C. lari* from animals were isolated (Broman *et al.* 2000; On *et al.* 2001; Bonnedahl *et al.* 2005).

There is a large colony of birds in Hope Bay (Myrcha *et al.* 1987), where there is also a permanent human settlement (Argentine Base Antártica *Esperanza*). In addition, a refuge is used during summers by Uruguayan researchers. Interestingly, water for humans is obtained from lakes where seabirds, principally skuas (*Stercorarius* spp.) and kelp gulls (*Larus dominicanus*), are settled.

The aim of this study was to investigate the presence of *Campylobacter* spp. in dead seabirds found in Hope Bay, Antarctica.

Methods

During the summers of 2000, 2001, 2002 and 2003, 122 dead birds were found in Hope Bay (*Esperanza*). Their carcasses were collected and identified, and necropsied *in situ*. Fifty-eight Adelie penguins (*Pygoscelis adeliae*), 28 brown skuas (*Stercorarius antarctica lonnbergi*), 16 kelp gulls (*Larus dominicanus*), 13 south polar skuas (*Stercorarius maccormicki*), 3 gentoo penguins (*Pygoscelis papua*), 3 storm petrels (*Oceanites oceanicus*), and 1 snowy sheathbill (*Chionis albus*) were studied (see Table 1). The intestine of each of the 122 dead seabirds was conserved at –20°C, and then sent to Laboratorio de Diagnóstico e Investigaciones Bacteriológicas (LADIB), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, where intestinal contents were immediately analyzed. Samples were placed into Brucella broth (Difco Laboratories, Michigan, USA) supplemented with 5% ovine blood, 10 mg of vancomicin (ICN Biomedicals, Ohio, USA) per liter, 5 mg of trimethoprim (ICN) per liter, and 5 mg of cephalothin (ICN) per liter, and incubated at 37°C for 12 h under microaerobic conditions. Later the broth was plated in Skirrow selective medium (Oxoid, Hampshire, England) and incubated at 37°C for 48 h. Colonies grown on Skirrow agar plates were presumptively identified as *Campylobacter* according to colony form, motility, oxidase reaction (Britania, Buenos Aires, Argentina) and fermentation of glucose (ICN). These species were identified by the following tests: catalase (Waco Pure

Table 1

Seabirds dead along 4 consecutive summers (2000–2003) in Hope Bay, and 10 *Campylobacter lari* isolated from these birds.

Summer	Adelie penguin *Pygoscelis adeliae*		Gentoo penguin *Pygoscelis papua*		Kelp gull *Larus dominicanus*		Brown skua *Stercorarius antarctica lonnbergi*		South polar skua *Stercorarius maccormicki*		Snowy sheathbill *Chionis albus*		Storm petrel *Oceanites oceanicus*	
	N	*C. lari* isolation	N	*C. lari* isolation	N	*C. lari* isolation	N	*C. lari* isolation	N	*C. lari* isolation	N	*C. lari* isolation	N	*C. lari* isolation
2000	0	0	0	0	2	1	8	2	0	0	0	0	0	0
2001	51	1	1	0	5	0	16	1	11	1	0	0	0	0
2002	1	0	0	0	8	0	2	1	2	1	0	0	2	0
2003	6	0	2	0	1	1	2	1	0	0	1	0	1	0
Total	58	1	3	0	16	2	28	5	13	2	1	0	3	0

Chemical Industries, Osaka, Japan), nitrate reduction (Britania), H_2S production in triple sugar iron (TSI, Difco) and with lead acetate strips (Waco), hippurate hydrolysis (ICN), indoxil acetate hydrolysis (Sigma, St. Louis, USA), growth at 25°C, 37°C and 42°C, and sensitivity to 30 mg of nalidixic acid (Oxoid) and cephalothin disks (Oxoid). All tests were carried out according to Skirrow and Benjamin (1980).

Results and discussion

All dead seabirds in 2000, and 45 Adelie penguins, 13 brown skuas, 10 south polar skuas, and 3 kelp gulls dead in 2001, were affected by avian cholera. In addition, 2 Adelie penguins dead in 2001 were affected by subcutaneous clostridial infections. These findings were described in separate publications (Leotta *et al.* 2006; Nievas *et al.* 2006). Moreover, traumatic lesions were observed in 4 Adelie penguins dead in 2001 summer, and in all seabirds dead in 2002 and 2003 summers. No other infectious avian disease was diagnosed. Lesions on intestinal mucose was not observed in any of these birds, and endo- and ectoparasites were not detected.

From among 122 dead seabirds we isolated and identified 10 isolates *C. lari* (5 from brown skuas, 2 from south polar skuas, 2 from kelp gulls, and 1 from

Adelie penguins). We found the following percentage of positivity: 17.8% for brown skuas, 15.4% for south polar skuas, 12.5% for kelp gulls, and 1.7% for Adelie penguins. Two kelp gulls and 1 brown skua found in 2000 summer died of avian cholera; the remaining 7 birds died of traumatic causes.

All isolates showed a gram-negative seagull-like cell morphology under light microscopy, motility and positive reactions in catalase and oxidase tests. In addition, all isolates were negative for glucose fermentation and did not grow at 25°C, but did grow at 37°C and 42°C. Isolates reduced nitrate, and were negative to hippurate hydrolysis and indoxil acetate hydrolysis. Moreover, they showed H_2S production in TSI and lead acetate strips, and resistance to nalidixic acid and cephalothin. Therefore, all isolates were identified as classical nalidixic acid-resistant thermophilic *Campylobacter lari*. The avian source of the 10 *C. lari* isolates is shown in Table 1.

For the first time ever in Hope Bay, classical *Campylobacter lari* was isolated from brown skuas, south polar skuas, kelp gulls and Adelie penguins (in order of frequency). *Campylobacter lari* was previously isolated from Adelie penguins, brown skuas and south polar skuas in Antarctic Peninsula (Bonnedahl *et al.* 2005), and from kelp gulls in southern Chile (Fernández *et al.* 1996). However, it is not clear whether such colonization is asymptomatic or subclinical (Fernández *et al.* 1990; Matsuda and Moore 2004). In addition, adapted *C. lari* variants occurring in the sub-Antarctic birds were reported (On *et al.* 2001).

Migratory birds could be carriers of zoonotic enteropathogens to the Antarctic region. Kelp gulls are capable of moving between the southern continents and Antarctica. South polar skuas have been recorded in Greenland and the Aleutian Islands, and brown skuas move around the coast of Antarctica (CEP IV). These birds could be effective carriers of *C. lari* as they come into close contact with humans and many other seabird species; for example, they are opportunistic predators of Adelie penguins.

The possibility of disease introduction into Antarctic wildlife has been recognized since the start of the Antarctic Treaty in 1962, and precautions to prevent the introduction of microorganisms into the region south of 60°S latitude are prescribed by the Protocol on Environmental Protection to the Antarctic Treaty of 1998 (CEP IV). There have been documented events of bacterial diseases causing mortality of Antarctic birds (Leotta *et al.* 2006). Nevertheless, few studies about zoonotic enteropathogens associated with Antarctic birds were reported. *Salmonella* spp. and enteropathogenic *Escherichia coli* (EPEC) from penguins and skuas were isolated (Oelke and Steiniger 1973; Leotta *et al.* 2006). Also *C. lari* from birds in Antarctic Peninsula were isolated (Bonnedahl *et al.* 2005).

We demonstrated that Antarctic seabirds carried *C. lari* in their intestines, and that they were settled around the lakes where humans are supplied with fresh water. Its consumption from Antarctic lakes contaminated with seabird feces could be a risk of human campylobacteriosis. *Campylobacter lari* survived longer in surface waters (Obiri-Danso *et al.* 2001), and was identified as the main protagonist of water-mediated campylobacteriosis (Tauxe *et al.* 1984; Thomas *et al.* 1999). This disease is estimated to affect over 1 million people every year in the USA (CDC 2005), and *C. lari* is thought to be involved (Mishu *et al.* 1992). Human activity in Antarctica was identified as a possible source of infectious agents (CEP IV). However, nothing is known about zoonotic enteropathogens causing diseases in humans living in the Antarctic region.

Further research on the natural microbiological flora of seabirds and in the lakes where the seabirds are settled in the Antarctic region is necessary to determine if the presence of *C. lari* could be a risk of human illness or could be a result of microbial pollution associated with human activity.

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