Isolation and Characterization of *Campylobacter* spp. from Antarctic Fur Seals (*Arctocephalus gazella*) at Deception Island, Antarctica[▽]

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The presence of *Campylobacter* spp. was investigated in 41 Antarctic fur seals (*Arctocephalus gazella*) and 9 Weddell seals (*Leptonychotes weddellii*) at Deception Island, Antarctica. Infections were encountered in six Antarctic fur seals. The isolates, the first reported from marine mammals in the Antarctic region, were identified as *Campylobacter insulaenigrae* and *Campylobacter lari*.

The Antarctic and sub-Antarctic regions are often regarded as pristine landscapes, unaffected by human activity. A limited number of surveys have been carried out to investigate the possible occurrence of zoonotic enteropathogens and if certain bacteria could be used as tools for evaluating biological pollution in this area (4, 11). In the case of *Campylobacter* species, there have been only three reports in the literature, but in all of them *Campylobacter* was isolated from marine seabirds but not from marine mammals. *Campylobacter jejuni* was isolated in Antarctic and sub-Antarctic areas from Macaroni penguins (*Eudyptes chrysolophus*) (4), and *Campylobacter lari* was isolated from Brown skuas, South Polar skuas, and Adelie penguins (2, 11).

Reports of *Campylobacter* species isolated from marine mammals are rare. *Campylobacter insulaenigrae* was isolated from three harbor seals (*Phoca vitulina*) and a harbor porpoise (*Phocoena phocoena*) in Scotland (7). The isolation of *C. jejuni*, *C. lari*, and an unknown *Campylobacter* species from juvenile northern elephant seals (*Mirounga angustirostris*) in California was also reported (22). Finally, 71 isolates of *C. insulaenigrae* and 1 isolate similar to but distinct from both *Campylobacter upsaliensis* and *Campylobacter helveticus* were isolated from northern elephant seals in California (23). In the South Georgia Archipelago, fecal swabs were taken from 206 Antarctic fur seal pups, but no isolates could be obtained (4). In this study, we successfully isolated *C. lari* from 7.3% of Antarctic fur seals (*Arctocephalus gazella*) sampled and *C. insulaenigrae* from a further 7.3%. On the other hand, *Campylobacter* was not detected in the nine Weddell seals (*Leptonychotes weddellii*) sampled. To our knowledge, this is the first report on the isolation of *C. lari* and *C. insulaenigrae* from marine mammals in the Antarctic region.

Fieldwork was conducted at Deception Island (latitude of 62°58′S and longitude of 60°40′W), in the South Shetland Islands. During January to February 2007, Antarctic fur seals

(*Arctocephalus gazella*) and Weddell seals (*Leptonychotes weddellii*) were captured and fecal samples were collected by insertion of sterile cotton wool swabs into the rectum of the marine mammals. A total of 41 Antarctic fur seals and 9 Weddell seals were sampled. The distribution by ages was of 7 adults (over 4 years of age with breeding activity), 19 subadults (2 to 4 years of age), and 15 juvenile Antarctic fur seals (less than 2 years of age), and 8 adult Weddell seals and 1 juvenile. All animals presented a good body condition and showed no symptoms at the time of sampling.

Three swabs were taken from each animal and were placed in FBP medium (8) with 0.5% active charcoal (Sigma Ltd.), Amies transport medium with charcoal, and Cary Blair transport medium, respectively. All samples were kept at +4 to 8°C until culture in the lab. The number of days between sampling and cultivation varied from 96 to 124 days, with a median value of 105 days.

Each swab was placed in 10 ml of *Campylobacter* enrichment broth (Lab M) with 5% laked horse blood and CAT supplement (cefoperazone [8 μg/ml], teicoplanin [4 μg/ml], and amphotericin B [10 μg/ml]) at 37°C. The broth was incubated at 37°C for 48 h and 5 days in 3.5-liter anaerobic containers using CampyGen sachets (Oxoid), before an aliquot of 100 μl was plated onto CAT agar and the plates were incubated at 37°C for 72 h in a microaerobic atmosphere. In addition, a 47-mm-diameter cellulose membrane with 0.60-μm pores was placed on the surface of an anaerobe agar base (Oxoid) with 5% laked horse blood. Eight to 10 drops of enrichment broth (200 μl) were placed onto the surface of the membrane. The membrane was left for 20 to 30 min on the agar surface at room temperature until all of the fluid had passed through (20). The plates were incubated as described above, but for 5 days to isolate the less common, slower growing species.

Isolates were examined by dark-field microscopy to determine morphology and motility and tested to determine whether oxidase was produced. For each sample, five isolates from each of the solid media that had typical morphology and motility and for which the oxidase test was positive were frozen at −80°C in FBP medium (8) until they were tested by phenotypic and genotypic methods.

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Original *Campylobacter* identification was done by Gram staining, catalase activity, hippurate hydrolysis, ability to hydrolyze indoxyl acetate, urease activity, H_2S production on triple-sugar iron slants, growth at 25°C and 42°C in a microaerophilc environment, growth at 37°C in an aerobic atmosphere, and agglutination with Microscreen latex (Microgen, Camberley, United Kingdom).

No differences between the strains were observed in any of the phenotypic tests used. All isolates showed a Gram-negative, slender, curved, seagull wing-like morphology under light microscopy and positive reactions in the catalase test. They were negative for hippurate and indoxyl acetate hydrolysis and urease and did not show H_2S production. In addition, they grew at 42°C but did not grow at 25°C or 37°C in an aerobic atmosphere. Finally, all of them were positive in the agglutination test.

Because phenotypic results commonly lead to misidentification of *Campylobacter* species, it is recommended that a molecular method be included in the identification scheme for *Campylobacter* (5, 15). Identification of the isolates was performed using 16S rRNA gene PCR and sequence analysis (15, 21). Forward and reverse conserved 16S rRNA eubacterial primers 8F (5′-AGAGTTTGATCCTGGCTCA G-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) were used to amplify the 16S rRNA according to the protocol described by Jang et al. (9). Forward and reverse sequencing reactions were performed by the Laboratorio Central de Veterinaria's DNA sequencing facility (LCV Algete, Madrid, Spain). Three strains were identified as *C. lari* and the other three as *C. insulaenigrae* based on both forward and reverse sequence analysis.

Molecular characterization of strains was carried out using a combination of pulsed-field gel electrophoresis (PFGE) using KpnI enzyme and multilocus sequence typing (MLST). Preparation of intact *Campylobacter* DNA for PFGE was performed following the Pulsenet protocol (17, 24). PFGE for the restriction enzyme KpnI (Takara, Conda, Spain) was performed following the protocol described by Ribot et al. (17). DNA fragments were resolved on 0.9% Seakem Gold agarose gels (Iberlabo, Spain) with a Bio-Rad CHEF DRIII system (Bio-Rad, Spain) at 14°C and 6 V/cm. Electrophoresis was carried out for 22 h with pulse times ramping from 4 s to 20 s. The fingerprinting experiments were analyzed using the Info-Quest FP software (Bio-Rad, Spain), and the dendrogram was constructed using the unweighted-pair group method using average linkages (UPGMA).

MLST of *C. lari* strains was performed as described by Miller et al. (13). In the case of *C. insulaenigrae* strains, MLST was performed following the protocol described by Stoddard et al. (23). All amplicons were sequenced by the Sequencing Service of the Instituto de Salud Carlos III (Madrid, Spain). Sequence data were collated, and alleles were assigned using the *Campylobacter* PubMLST database (http://pubmlst.org/campylobacter/). Novel alleles and sequence types were submitted for allele and sequence type (ST) designations when appropriate.

Regarding the age distribution of animals, *C. lari* was isolated from 1 of 7 adult (14.3%), 1 of 19 subadult (5.3%), and 1 of 15 juvenile (6.6%) Antarctic fur seals. *C. insulaenigrae* was isolated from 1 of 7 adults (14.3%) and 2 of 19 of subadults (10.5%) but not from juvenile animals (Table 1). All strains

TABLE 1. Source of *Campylobacter* isolates

Animal	Age category and sex	Date (mo/day/yr) of:		*Campylobacter* sp. and isolate no.
		Sampling	Culture	
L 06/56	Adult male	2/15/07	5/30/07	*C. insulaenigrae* FR-07
L 06/78	Subadult male	2/15/07	5/30/07	*C. insulaenigrae* FR-15
L 06/102	Subadult male	2/22/07	5/30/07	*C. lari* FR-28
L 06/134	Juvenile male	2/21/07	5/30/07	*C. lari* FR-36
L 06/146	Subadult male	2/22/07	5/30/07	*C. insulaenigrae* FR-38
L 06/48	Adult male	2/22/07	5/30/07	*C. lari* FR-48

were obtained from the swabs kept in FBP transport medium.

Campylobacter is very sensitive to excessive amounts of oxygen and has little capacity to survive in the environment. It is therefore possible that the prevalence of *Campylobacter* species in Antarctic fur seals is greater than that obtained in our survey and that we have isolated more-resistant strains with a larger ability to survive a prolonged transport. Nevertheless, we think that the freezing medium described by Gorman and Adley (8) modified by the addition of 0.5% of activated charcoal is a very good transport medium since the bacteria remained viable for 3 months at refrigeration temperature, whereas they did not survive in the transport media routinely used for the preservation of fecal samples such as Amies and Cary Blair media.

PFGE is a useful tool for conducting epidemiological studies of *Campylobacter* species. We used digestion with KpnI because it has been reported to have greater power of discrimination than digestion with SmaI (16). All isolates showed very different patterns (Fig. 1), indicating different sources of infection and circulation of different clones on Deception Island. These data were confirmed by the results of MLST, in which each strain belonged to a different ST, none of which had been previously reported. We submitted to the MLST database 12 new sequences of alleles tested for *C. insulaenigrae* and 10 new sequences of *C. lari* obtained (Table 2).

The introduction of *C. lari* in the Antarctic fur seal colonies may have occurred through seabirds. *C. lari* has been isolated from Adelie penguins (*Pygoscelis adeliae*), kelp gull (*Larus dominicanus*), Brown skuas (*Stercorarius antarctica lonnbergi*), and South Polar skuas (*Stercorarius maccormicki*) in Hope Bay (11) and in the Antarctic Peninsula (2). Gulls can travel between South America and Antarctica and are potential carriers of enteric pathogens (1). Thus, *C. lari* has been isolated from kelp gulls in southern Chile (6). Also, South Polar skuas have been reported in Greenland and the Aleutian Islands and Brown skuas move around the Antarctic coast. Therefore, it is possible that these birds acquire infectious organisms when they move to areas with high levels of human activity. These birds have been reported on Deception Island (10), and it is common to find skuas and giant petrels on beaches where Antarctic fur seal colonies rest. The carrier birds could eliminate *Campylobacter* and pollute these areas. Alternatively, these birds could be occasional prey for Antarctic fur seals.

C. insulaenigrae is a new *Campylobacter* species whose host range might be restricted to marine mammals (23). It could be hypothesized that *C. insulaenigrae* evolved from *C. lari* based on the presence of both species in sea lions and their sharing
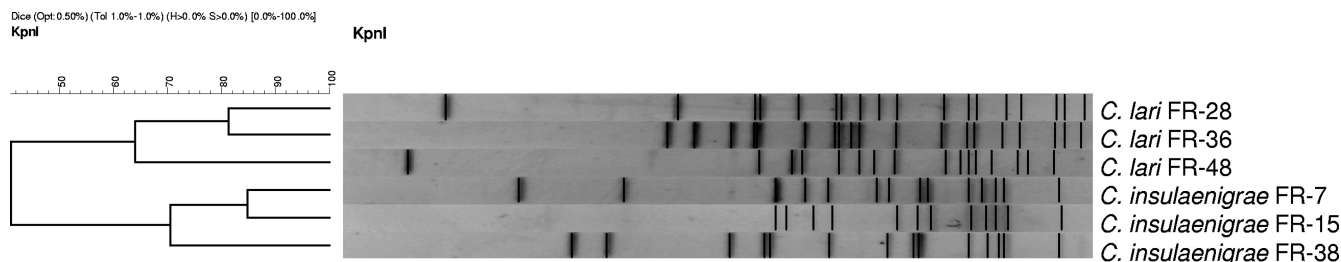
FIG. 1. UPGMA dendrogram of PFGE profiles.

other characteristics such as the absence of the citrate synthase gene (23). In addition, considering that *C. insulaenigrae* has not been isolated from seabirds or shellfish and the migration ranges of sea lions are generally not very large, Antarctic fur seals could have been initially infected with *C. lari*, and subsequently this species has evolved, adapting to mammals. Alternatively both species could share an ancestor and have adapted to different hosts.

The Antarctic fur seals captured showed no weight loss, diarrhea, or other symptoms at the time of sampling. However, due to the nature of our study, it is not possible to know whether the animal had been ill before the time of collection and was subsequently a carrier. Taking into account previous reports (7, 23) and our results, pinnipeds could possibly act as reservoir of *C. insulaenigrae*.

The presence of *Campylobacter* in Antarctic fur seals could also be important due to the zoonotic potential of both species (5, 12, 18, 19). Therefore, researchers should continue to exercise caution when working with these animals. In addition, *C. lari* has been involved in waterborne outbreaks (3) and some reports have identified this species as the most frequently isolated from surface water (25). Most of the Antarctic stations' catchwater from lakes generated by meltwater and the water treatment cannot be accomplished by chemical products to prevent marine pollution. In general, water is not treated or is treated only by filtration and UV light. Antarctic fur seals can nevertheless pollute the water of these lakes and/or infect other species such as penguins and other birds, which in turn could also act as a source of infection for humans. Furthermore, Obiri-Danso et al. (14) have reported that *C. lari* survives for longer in surface waters than *C. jejuni* and *Campy-*

lobacter coli, so it would have a greater chance of surviving the water treatment. Finally, in case of infection, the therapy may be complicated because in many of the stations there are only basic medical services.

In summary, we describe here the first isolation and characterization of two species of *Campylobacter*, *C. lari* and *C. insulaenigrae*, from Antarctic fur seals. Further studies are needed to determine the prevalence of *Campylobacter* spp. in Antarctic pinnipeds, the possible sources of infection and if the presence of *Campylobacter* in marine mammals could be a risk for human illness or could be a result of microbial pollution associated with human activity.

TABLE 2. Alelle numbers and sequence types of *Campylobacter* isolates

Species and isolate no.	ST	Allele no.[a]						
		aspA or *adk*	*atpA*	*glnA*	*glyA*	*pgi*	*pgm*	*tkt*
C. insulaenigrae								
FR-7	41	2 (*aspA*)	16*	12*	2	15*	15*	11*
FR-15	42	4 (*aspA*)	10	11*	12*	14*	15*	12*
FR-38	43	7 (*aspA*)	17*	11*	13*	14*	3	13*
C. lari								
FR-28	17	52* (*adk*)	57*	2	50*	56*	51*	31*
FR-36	16	52* (*adk*)	57*	2	2	56*	52*	31*
FR-48	18	53* (*adk*)	58*	1	2	57*	52*	32*

[a] Asterisks indicate new alleles.

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