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Original Contribution

Isolation of *Campylobacter* spp. from Three Species of Antarctic Penguins in Different Geographic Locations

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Abstract: The presence of *Campylobacter* species was studied in three Antarctic penguin species, Adélie (*Pygoscelis adeliae*), chinstrap (*Pygoscelis antarctica*) and gentoo (*Pygoscelis papua*). A total of 390 penguins were captured in 12 different rookeries along the Antarctic Peninsula with differences in the amount of human visitation: six colonies were highly visited [Stranger Point, King George Island (*P. papua* and *P. adeliae*); Hannah Point, Livingston Island (*P. papua* and *P. antarctica*); Deception Island (*P. antarctica*); and Paradise Bay, Antarctic Peninsula (*P. papua*)], and six colonies were rarely visited [Devil's Point, Byers Peninsula, Livingston Island (*P. papua*); Cierva Cove, Antarctic Peninsula (*P. papua*); Rongé Island (*P. papua* and *P. antarctica*); Yalour Island (*P. adeliae*); and Avian Island (*P. adeliae*)]. A total of 23 strains were isolated from penguins from nine different rookeries. *Campylobacter lari* subsp. *lari* was isolated from eight samples (seven from *P. papua* and one from *P. adeliae*); *C. lari* subsp. *concheus* from 13 (ten from *P. adeliae* and three from *P. antarctica*) and *C. volucris* from two samples (both from *P. papua*). We did not find any significant differences in the prevalence of *Campylobacter* spp. between the populations in highly and rarely visited areas. This is the first report of *C. lari* subsp. *concheus* and *C. volucris* isolation from penguins in the Antarctic region.

Keywords: *Campylobacter*, pygoscelids penguins, Antarctica

Introduction and Purpose

Movement of pathogens into new geographic locations by human carriers is one of the main factors triggering the emergence of infectious diseases in wildlife. This phenomenon has been termed "pathogen pollution" by some scientists (Daszak et al. 2000) and may be defined as a disease resulting from human biological invasion and introductions (Vitousek et al. 1997).

The Antarctic region is often regarded as a pristine landscape, unaffected by human activity. However, human

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activities, such as research stations and tourism, have been increasing in recent years and could be facilitating the spread of diseases (Curry et al. 2002). Research activities are usually concentrated in relatively small areas or specific locations, while tourists visit a large number of colonies in a short period of time (Barbosa and Palacios 2009). Global change is another of the main factors affecting ecosystem health, due to the relationship between climate change and global human travel, and favoring the increase in distribution ranges, and also the abundance and/or virulence of parasites and pathogens (Daszak et al. 2000; Harvell et al. 2002). In Antarctica, especially the west coast of the Antarctic Peninsula, temperatures have increased 0.53 degree per decade from 1956 to 2006 (Turner et al. 2004; Steig et al. 2009).

In this region, a limited number of surveys have been done to explore the possible occurrence of zoonotic enteropathogens and find out whether certain bacteria could be used as biological indicators for evaluating biological pollution. So, several studies have been done on the presence and/or prevalence of *Campylobacter* spp. (Broman et al. 2000; Leotta et al. 2006; Griekspoor et al. 2009; González Acuña et al. 2013), *Salmonella* spp. (Oelke and Steiniger 1973; Olsen et al. 1996; Palmgren et al. 2000; Bonnedahl et al. 2005), and extended-spectrum β-lactamase bacteria (Hernández et al. 2012).

Campylobacter species are gram-negative spiral-rod bacteria commonly associated with human gastroenteritis, specifically in the case of *C. jejuni* and *C. coli* (World Health Organization 2012). They colonize a wide range of hosts, including domestic and wild animals, and are generally considered commensals of livestock, domestic pets and birds. *Campylobacter* species have been isolated from a wide variety of wild birds, although variation among taxa is considerable (Broman et al. 2002; Waldenström et al. 2002; Waldenström et al. 2007; Keller et al. 2011)

Reports on isolation of *Campylobacter* species in Antarctic and subantarctic marine mammals and seabirds are scarce in the literature (Barbosa et al. 2015). In marine mammals, there is only one report about isolation of *Campylobacter insulaenigrae* and *Campylobacter lari* from Antarctic fur seals (*Arctocephalus gazella*) (García-Peña et al 2010). Although there are more reports on seabirds, those on isolation of *Campylobacter* species from birds in the Antarctic and subantarctic are also rare. *C. lari* has been isolated from the Adélie (*Pygoscelis adeliae*) and gentoo (*Pygoscelis papua*) penguins, Antarctic sheathbill (*Chionis albus*), Brown skuas (*Catharacta lonnbergi*), South Polar skuas (*Catharacta maccormicki*), kelp gull (*Larus dominicanus*) and Antarctic shag (*Phalacrocorax bransfieldensis*) (Bonnedahl et al. 2005; Leotta et al. 2006; Barbosa and Palacios 2009; Barbosa et al. 2013; González Acuña et al. 2013). *Campylobacter jejuni* and *Campylobacter coli* have not been isolated from birds in the Antarctic region, and isolation of *C. jejuni* has only been reported for Macaroni penguins (*Eudyptes chrysolophus*) in the subantarctic region (Bird Island, South Georgia) (Broman et al. 2000; Griekspoor et al. 2009).

The aims of this study were to determine the presence of *Campylobacter* species in pygoscelid penguins, to compare the prevalence of *Campylobacter* in highly and rarely visited locations of the South Shetland Islands and the Antarctic Peninsula, and finally, to characterize these isolates by different methods in order to establish the relationship between them and the possible origin of infection.

Material and Methods

General Methods and Sample Collection

In January 2008, 2009 and 2010, 12 breeding penguin colonies of chinstrap, gentoo and Adélie penguins along the west coast of the Antarctic Peninsula were visited over a geographic range from 62°10′S to 67°46′S (Table 1). Adult penguins (390 individuals) were chosen at random and captured on the beach to minimize disturbance in the breeding colonies (Barbosa et al. 2007). Cloacal swabs were collected from each individual and preserved in Amies transport medium with charcoal (2008 and 2009 samples) or in FBP medium (Gorman and Adley 2004) with 0.5% active charcoal (Sigma Ltd.) (2010 samples). Samples were frozen at −20°C for five months until analysis.

Laboratory Analyses

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 5 days in 3.5-l anaerobic containers using CampyGen sachets (Oxoid). An aliquot of 100 µl was plated on CAT agar plates at 48 h and 5 days of incubation, 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

Table 1. Sampling points and distribution of the samples by location, year, and penguin species.

Location	Latitude	Year	Number of animals sampled		
			P. papua	*P. antarctica*	*P. adeliae*
Stranger Point[a] (King George Island)	62°2′S, 58°21′W	2008, 2009	40 (2008)	–	23 (2009)
Byers Peninsula[b] (Livingston Island)	62°38′S, 61°5′W	2009	24	–	–
Hannah Point[a] (Livingston Island)	62°40′S, 60°36′29″W	2008, 2009	40 (2009)	15 (2008)	–
Deception Island[a]	62°58′37″S, 60°39′W	2008	–	52	
Rongé Island[b]	64°43′S, 62°41′W	2008	38	39	–
Paradise Bay[a] (Antarctic Peninsula)	64°49′S, 62°52′W	2008	15	–	–
Cierva Cove[b] (Antarctic Peninsula)	64°9′S, 60°53′W	2008	39	–	–
Yalour Island[b]	65°14′S, 64°10′W	2008	–	–	40
Avian Island[b]	67°46′S, 68°54′W	2010	–	–	25
TOTAL/penguin species			196	106	88

Each species at each location means a colony.
[a]Highly visited locations.
[b]Rarely visited locations.

anaerobe agar base (Oxoid) with 5% laked horse blood plate. Eight to ten drops of enrichment broth (200 µl) were placed on the surface of the membrane at 48 h and 5 days of incubation. The membrane was left for 20–30 min on the agar surface at room temperature until all of the fluid had passed through. 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 having typical morphology and motility and oxidase positive were frozen at −80°C in FBP medium until testing by phenotypic and genotypic methods.

For preliminary *Campylobacter* identification, Gram staining, catalase activity, hippurate hydrolysis, ability to hydrolyze indoxyl acetate, growth at 25 and 42°C in a microaerobic environment and growth at 37°C in an aerobic atmosphere were tested.

Since phenotypic results commonly lead to misidentification of *Campylobacter* species, a molecular method was also included (On 1996). Identification of the isolates was performed in successive steps. First, the multiplex PCR assay described by Wang et al. (2002) was used. In a second step, the positive strains were identified by *gro*EL gene PCR and sequence analysis according to the protocol described by Kärelampi et al. (2004). Forward and reverse sequencing reactions were performed by the Laboratorio Central de Veterinaria's DNA sequencing facility (LCV Algete, Madrid, Spain).

Molecular characterization of strains was carried out using a combination of pulsed-field gel electrophoresis (PFGE) with *Sma*I and *Kpn*I enzymes and multilocus sequence typing (MLST). PFGE for the *Sma*I restriction enzyme (ThermoFisher, UK) was performed following the protocol described by Ribot et al. (2001). PFGE for the KpnI restriction enzyme (ThermoFisher, UK) was performed following the protocol described by On et al. (1998). The fingerprinting experiments in this study were analyzed using an average estimation from experiments in a global comparison with BioNumerics v 6.6 software (Applied Maths, Sint-Martens-Latem, Belgium).

For MLST, primers and PCR conditions available on the MLST website (http://pubmlst.org/campylobacter/info/clari_primers.shtml) were used. DNA for every *C. lari* strain was purified using the NZY Tissue gDNA Isolation kit (NZYTech, Portugal) and amplified with all primer sets

Table 2. Prevalence of the *Campylobacter* species/subspecies isolated by location and penguin species.

Location	*Campylobacter* prevalence		
	P. papua	*P. antarctica*	*P. adeliae*
Stranger Point[a]	2.5% (1/40) *C. lari* subsp. *lari*	–	4.3% (1/23) *C. lari* subsp. *lari*
Byers Peninsula[b]	4.2% (1/24) *C. volucris*	–	–
Hannah Point[a]	2.5% (1/40) *C. volucris*	0% (0/15)	–
Deception Island[a]	–	1.9% (1/52) *C. lari* subsp. *concheus*	–
Rongé Island[b]	0% (0/38)	5.1% (2/39) *C. lari* subsp. *concheus*	–
Paradise Bay[a]	20% (3/15) *C. lari* subsp. *lari*	–	–
Cierva Cove[b]	7.7% (3/39) *C. lari* subsp. *lari*	–	–
Yalour Island[a]	–	–	0% (0/40)
Avian Island[b]	–	–	40% (10/25) *C. lari* subsp. *concheus*
Prevalence	4.6% (9/196)	2.8% (3/106)	12.5% (11/88)

Numbers in brackets are number of positives/total number of samples.
[a]Highly visited locations.
[b]Rarely visited locations.

for the seven housekeeping genes: *adk, atp*A,*gln*A, *gly*A, *pgi, pgm* and *tkt*. PCR products obtained were purified using ExoSap-IT (Amersham, USB). All purified amplicons were sequenced by the Instituto de Salud Carlos III Sequencing Service (Madrid, Spain). Sequences found were compared with the MLST database, available at http://pubmlst.org/campylobacter/ for allele assignment. Novel alleles and sequence types (STs) were submitted to the MLST web for allele and sequence type (ST) assignment.

Results

None of the birds had obvious clinical signs associated with campylobacteriosis at time of capture. A total of 23 *Campylobacter* strains were isolated, 11 from Adélie penguins, nine from gentoo penguins and three from chinstrap penguins.

All isolates showed *Campylobacter* morphology, motility and phenotypic characteristics. In addition, all isolates were negative to hippurate and indoxyl acetate hydrolysis and grew at 42°C in a microaerobic environment.

All strains were identified as *Campylobacter* spp. by PCR as described by Wang et al. (2002) by the *Campylobacter* 23S rRNA primers which was present in all *Campylobacter* strains tested. The amplicon for *C. lari glyA* primers was detected in eight strains. These strains were confirmed as *C. lari* subsp. *lari*, and the other 15 strains were identified as *C. lari* subsp. *concheus* (13 strains) and *C. volucris* (two strains) using *groEL* gene PCR and sequence analysis.

General *Campylobacter* spp. prevalence was 5.9% (23/390). *C. lari* subsp. *lari* was isolated from seven samples taken from *P. papua* and one from *P. adeliae* [2.1% (8/390)], *C. lari* subsp. *concheus* from ten samples from *P. adeliae* and three from *P. antarctica* [3.3% (13/390)] and *C. volucris* from two samples from *P. papua* [0.5% (2/390)]. Other pathogenic species of *Campylobacter*, such as *C. jejuni* and *C. coli*, were not detected in any sample taken from the three penguin species.

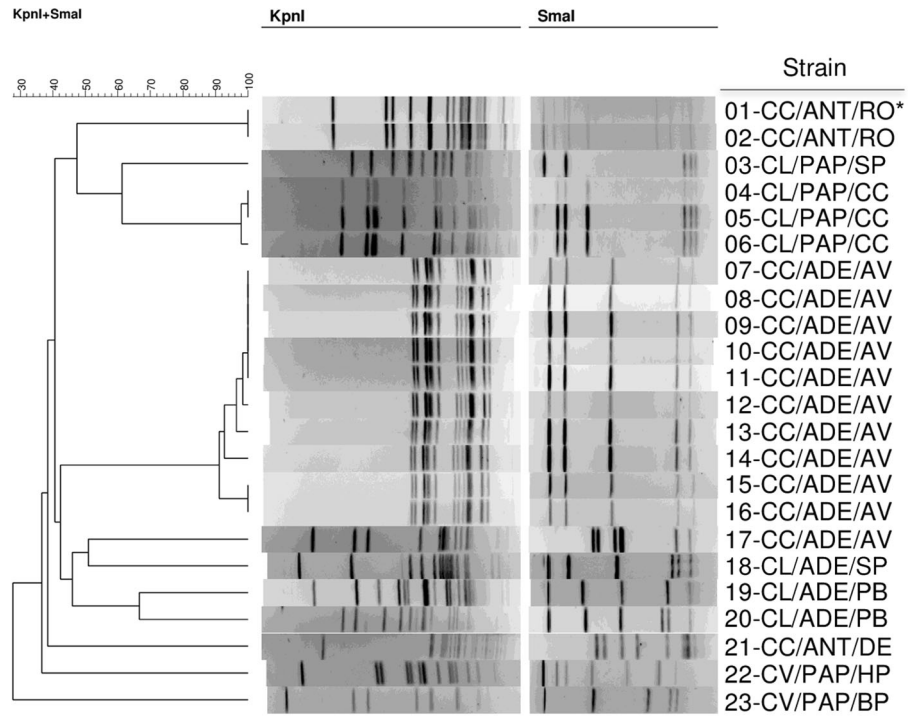

Figure 1. UPGMA dendrogram of PFGE profiles.

The presence of *Campylobacter* was detected in 9 out of the 12 rookeries analyzed (75%), five of them located in highly visited areas (83.3%) and four in rarely visited locations (66.6%). *C. lari* subsp. *lari* was found in four rookeries (33.3%), *C. lari* subsp. *concheus* in three (25%) and *C. volucris* in two rookeries (16.7%). However, no mixed infections were observed in individuals.

Prevalence by penguin species and geographic location is summarized in Table 2. Total *Campylobacter* prevalence in highly visited colonies was 3.1% (7/225) and in rarely visited locations 9.7% (16/165).

When Amies with charcoal was used as the transport medium for the cloacal swabs (in 2008 and 2009), 3.6% (13/365) of samples were positive, and when FBP medium was used (2010) 40% (10/25) were positive.

Strain characterization by PFGE was very similar for both *Sam*I and *Kpn*I restriction endonucleases. A total of 11 different pulsetypes were found, six for *C. lari* subsp. *lari*, three for *C. lari* subsp. *concheus* and two pulsetypes for *Campylobacter volucris* (Fig. 1). Strains from the same penguin colony belonged to the same pulsetype, except in the Paradise Bay and Stranger Point rookeries where three and two different pulsetypes were observed, respectively. Similar pulsetypes were not found in the same penguin species in different rookeries.

Approximately 45% of the alleles found by MLST were novel, 25% were already assigned and the remaining 30% could not be assigned because the sequences found were non-optimal. A total of seven putative different and novel sequence types were found: five for *C. lari* subsp. *lari* and two for *C. lari* subsp. *concheus* (Table 3). The only new ST (ST-19) found was assigned to two strains of *C. lari* subsp. *concheus*. With these MLST results a complete agreement between PFGE pulsetypes and MLST STs may be observed since the same *Campylobacter* spp. strains are grouped in the different STs and pulsetypes.

Table 3. Allele numbers and sequence types of *Campylobacter* isolates.

Strains[a]	*adk*	*atp*A	*gln*A	*gly*A	*pgi*	*pgm*	*tkt*	ST
01-CC/ANT/RO	52	**60**[b]	**53**	10	**63**	**53**	**34**	19
02-CC/ANT/RO	52	**60**	**53**	10	**63**	**53**	**34**	19
03-CL/PAP/SP	NA[c]	**64**	**51**	NA	**61**	NA	NA	–
04-CL/PAP/CC	2	57	2	NA	**58**	NA	**33**	–
05-CL/PAP/CC	2	57	2	NA	**58**	NA	**33**	–
06-CL/PAP/CC	2	57	2	NA	**58**	NA	**33**	–
07-CC/ADE/AV	**55**	**59**	5	4	**60**	NA	**35**	–
08-CC/ADE/AV	**55**	**59**	5	4	**60**	NA	**35**	–
09-CC/ADE/AV	**55**	**59**	5	4	**60**	NA	**35**	–
10-CC/ADE/AV	**55**	**59**	5	4	**60**	NA	**35**	–
11-CC/ADE/AV	**55**	**59**	5	4	**60**	NA	**35**	–
12-CC/ADE/AV	**55**	**59**	5	4	**60**	NA	**35**	–
13-CC/ADE/AV	**55**	**59**	5	4	**60**	NA	**35**	–
14-CC/ADE/AV	**55**	**59**	5	4	**60**	NA	**35**	–
15-CC/ADE/AV	**55**	**59**	5	4	**60**	NA	**35**	–
16-CC/ADE/AV	**55**	**59**	5	4	**60**	NA	**35**	–
17-CL/ADE/PB	NA	NA	NA	NA	NA	NA	NA	–
18-CL/ADE/SP	8	57	1	NA	**59**	32	**33**	–
19-CL/ADE/PB	**56**	**62**	**52**	NA	**62**	NA	NA	–
20-CL/ADE/PB	NA	**61**	NA	NA	**62**	NA	NA	–
21-CC/ANT/DE	NA	NA	NA	NA	NA	NA	NA	–

CC, *C. lari* susp *concheus*; CL, *Campylobacter lari* subsp. *lari*; ANT, *P. antarctica*; PAP, *P. papua*; ADE, *P. adeliae*; RO, Roger Island; SP, Stranger Point; CC, Cierva Cove; AV, Avian Island; PB, Paradise Bay; DE, Deception Island.

[a]Strain number-*Campylobacter* species/Penguin species/Location.

[b]New alleles in bold.

[c]Not optimal sequence obtained.

Discussion

Campylobacter species colonize a wide range of hosts, including human, domestic animals and wild birds and mammals. In Antarctic and subantarctic animals, *Campylobacter* species prevalence studies are scarce and exceptional (Broman et al. 2000; Bonnedahl et al. 2005; Leotta et al. 2006; Barbosa et al. 2013). This study analyzed the presence of *Campylobacter* spp. in 12 different rookeries of gentoo (six colonies), chinstrap (three colonies) and Adélie (three colonies) penguins.

We found *Campylobacter* spp. in at least one infected animal in 75% of the rookeries sampled, suggesting that *Campylobacter* spp. could be widely disseminated and circulating in penguin colonies in the Antarctic Peninsula region. *Campylobacter* occurrence in 100% of the Antarctic penguin colonies has previously been reported, but these studies were limited to only one (Broman et al. 2000; Leotta et al. 2006), two (Barbosa et al. 2013) or three locations (González Acuña et al. 2013).

The overall *Campylobacter* prevalence in penguins (5.9%) was similar to that reported by González Acuña et al. (2013) in gentoo penguins (6.5%), but higher than reported by other authors. Leotta et al. (2006) reported 1.7% positives in Adélie penguins, while *Campylobacter* spp. was not found in gentoo penguins, and Broman et al. (2000) reported a prevalence of 3% in Macaroni penguins. Even so, the prevalence in our study is probably underestimated, because *Campylobacter* is very sensitive to excessive amounts of oxygen and has little capacity to survive in the environment, so we probably only isolated the most resistant strains able to survive long transport.

There were great differences in prevalence in each penguin colony in different areas, ranging from 0% (Adélie penguin in Yalour Island) to 40% (Adélie penguin in Avian Island). One possible explanation for the difference might

be that the Avian Island isolate is more adapted to penguins and may be more able to colonize them than the other *Campylobacter* isolates of the study. These strain-specific properties in colonization abilities have been noted previously for *Campylobacter jejuni* in chickens (Stern et al. 1998) and adapted *C. lari* strains in subantarctic birds (On et al. 2001). However, the change in transport media between 2008 and 2009 sampling (Amies with charcoal) and 2010 sampling (FBP) could also explain such differences. Thus, while the highest prevalence was 20% in samples preserved in Amies with charcoal (2008 and 2009 samples), it was 40% in FBP with charcoal (2010 samples). In fact, García-Peña et al. (2010) tested differences in the use of both media in *Campylobacter* strains from Antarctic marine mammals and they found differences among them. We did not perform such tests, but these results suggest the influence of the change in media transport. In any case, change in the transport media would affect only the population sampled in 2010, Avian Island, which means that the remaining 11 populations were not affected by such change and then results are reliable.

The *Campylobacter* species isolated in our study were *C. lari* (21 strains) and *C. volucris* (two strains). Two *C. lari* subspecies were described and accepted in 2009, *C. lari* subsp. *lari* and *C. lari* subsp. *concheus* (Debruyne et al. 2009). Strains of subspecies *lari* have been isolated from cases of human diarrhea and bacteremia, from horse intestines, from the feces of wild birds, dogs, chickens, Antarctic fur seals and from shellfish (Debruyne et al. 2009; García-Peña et al. 2010). Strains of *C. lari* subsp. *concheus* have been isolated from human, seagull and shellfish feces (Debruyne et al. 2009). Both subspecies have also been isolated from environmental samples, mainly surface waters (Obiri-Danso et al. 2001; Van Dyke et al. 2010). In our study, *C. lari* subsp. *lari* was isolated from one Adélie and seven gentoo penguins, but not from chinstrap penguins. C. *lari* subsp. *concheus* was isolated from three chinstrap and ten Adélie penguins but not from gentoo penguins. To our knowledge, these are the first reports of *Campylobacter* spp. in chinstrap penguins and of *Campylobacter lari* subsp. *concheus* in any penguin species.

Campylobacter volucris was described in 2010 as a new species of *Campylobacter*. Three strains were found in black-headed gulls (*Larus ridibundus*) during a sampling campaign in Malmö, southern Sweden, in March 1999 (Debruyne et al. 2010). In our study, *C. volucris* was found in two samples from gentoo penguins on Livingstone Island, and as far as we know this would be the second report of this *Campylobacter* species and the first in the Southern hemisphere and in penguin species.

The results of macrorestriction and multilocus sequence typing methods were similar, and a total of nine *C. lari* and two *C. volucris* clones were observed, thus corroborating the high genetic diversity within the *C. lari* group reported in previous studies (On et al. 2001; Duim et al. 2004).

In the penguin colonies from Rongé Island, Cierva Cove and Avian Island, we detected *Campylobacter* spp. in more than one animal and all strains from each one of the rookeries belonged to the same PFGE and MLST clusters. These results are a strong indication that the strains from each of the rookeries represent a single clone and may be genetically identical. One explanation of the isolation of only one clone might be because these strains are highly host-adapted and would outcompete and eventually exclude less well-adapted *Campylobacter* strains. Another possibility could be that these strains were selected because of their greater ability to survive prolonged transport. For instance, *C. lari* survives longer in water than either *C. jejuni* or *C. coli,* particularly in the dark and at low temperatures (Obiri-Danso et al. 2001).

The similarity of the isolates from each rookery could also be the result of a common source of infection for in each penguin colony and subsequent dissemination within the rookery. In a penguin colony, large numbers of individuals congregate, breeding in a relatively small, dense area and produce a large amount of feces in the colony. Once the first individual is infected, the *Campylobacter* strain would spread through the colony quickly, because of the natural penguin behavior of pecking at each other and picking up objects like stones which might be contaminated.

Only in Paradise Bay rookery, the three 2008 isolates of *C. lari* subsp. *lari* were not similar. In this case, the variation in genotypes may be due to multiple sources of contamination or to *Campylobacter* ability to incorporate exogenous DNA or to undergo genomic rearrangement by multiple recombination (Rivoal et al. 2005). The two Stranger Point isolates were different, but they were obtained in different years (2008 and 2009) and from colonies of different penguin species.

The two *C. volucris* strains were isolated from gentoo penguins on Livingstone Island, but one of them was isolated from the Hannah Point rookery sampled in 2008, and the other from Peninsula Byers colony sampled in 2009. The two isolates showed different profiles in pulsed-field

gel electrophoresis with both *Sma*I and *Kpn*I restriction enzymes, indicating that the source of infection was probably different.

Our results strongly suggest that human activity in Antarctica has little or no impact on the microbiological pollution of penguin rookeries by *Campylobacter* spp. Firstly, we did not detect significant differences in either overall prevalence of *Campylobacter* spp. or percentage of positive colonies between locations with a relatively high number of visitors and locations rarely visited by humans. Also, in agreement with previous reports (Bonnedahl et al. 2005; Leotta et al. 2006; Barbosa et al. 2013; González Acuña et al. 2013), the two most common human-associated *Campylobacter* (*C. jejuni* and *C. coli*) were not detected in any of the colonies. Finally, the results of MLST did not find any association between the sequence types and human host, since a high percentage of allele sequences were new and generated sequence types not described previously. However, human impact needs further research, since little is known about the epidemiology of *C. lari* (Duim et al. 2004), the number of sequence types in the MLST database is relatively small (http://pubmlst.org), and it is difficult to relate specific sequence types to hosts or geographic areas.

One likely route to introduction of *Campylobacter* in the penguin colonies may have been through other seabird species. *C. lari* has been isolated from the kelp gull (*L. dominicanus*), Brown skuas (*C. lonnbergi*) and South Polar skuas (*C. maccormicki*) in the Antarctic Peninsula (Bonnedahl et al. 2005; Leotta et al. 2006). Gulls travel between South America and Antarctica and are potential carriers of enteric pathogens (Abulreesh et al. 2007), and *C. lari* has been isolated from kelp gulls in southern Chile (Fernández et al. 1996). South Polar skuas have also been reported in Greenland and the Aleutian Islands, and Brown skuas move around the Antarctic coast (Kendall et al. 2003). Therefore, these birds could acquire infectious organisms when they move to areas with high levels of human activity, transmitting them to penguins in Antarctica.

Another possible source of *Campylobacter* infection could be through penguin prey contaminated in areas of human activity. Once in coastal waters, *Campylobacter* spp. would be naturally active and could persist in unculturable but viable states (Abulreesh et al. 2006) and contaminate Antarctic krill (*Euphausia* spp.), the main food of penguins. Contaminated krill could reach the Antarctic Peninsula since circulation of *E. triacantha* and *E. superba* between Antarctica and Chilean fjords has been reported (Clarke et al. 2005).

In wild birds, *Campylobacter* spp. has been isolated from a wide variety of taxa (Broman et al. 2002; Keller et al. 2011; Waldenström et al. 2002; Waldenström et al. 2007) and in several studies, high prevalence of *C. jejuni*, *C. coli* and *C. lari* was found in apparently healthy birds (Pacha et al. 1988; Waldenström et al. 2002). For this reason, they are also considered part of the normal microbiota, although disease manifestation has not been extensively evaluated. Only one recent study showed a slight reduction in body mass in European robins (*Erithacus rubecula*) challenged with a *C. jejuni* strain isolated from another songbird species (Waldenström et al. 2010). Similarly, *Campylobacter* spp. has previously been identified in many penguin species, but there have been no studies linking the presence of this bacterium to disease, so their pathogenicity or how long infection is maintained in subantarctic penguins is unknown (Broman et al. 2000; Leotta et al. 2006; Griekspoor et al. 2009; González Acuña et al. 2013). Further research is necessary to determine whether infection and intestinal persistence of *Campylobacter* spp. are actually of significant concern due to their potential impact on wild penguin health and survival.

The presence of *Campylobacter* spp. in penguins could be important due to their possible role as a potential source of infection for wild Antarctic mammals. Although *C. lari* has been isolated from Antarctic fur seals that did not show any weight loss, diarrhea or other symptoms at the time of capture (García-Peña et al. 2010), some strains could potentially cause outbreaks of overt disease. Most of the New Zealand sea lion (*Phocarctos hookeri*) adults examined in the mass mortality event on the Auckland Islands in 1998 died as a result of bacterial septicemia likely caused by an organism of the *Campylobacter* genus (Duignan 1998).

Finally, *C. lari* has been described as causing clinical infection in humans (Debruyne et al. 2009), and penguins could be important reservoirs of these bacteria. Therefore, researchers should continue to exercise caution when working with these animals. In addition, penguins can pollute the water of lakes generated by meltwater, which is used in Antarctic stations. Since water is usually not treated or is treated only by filtration and UV light, it could also act as a source of *Campylobacter* infection for humans.

Conclusions

From this study, we can conclude that the circulation of *Campylobacter* spp. in populations of pygoscelid penguins

in the Antarctic Peninsula is very frequent, but there are no differences between colonies highly and rarely visited by humans. The intracolony prevalence may also be underestimated because it depends on the transport media used for conservation of the samples.

The only species detected were *C. lari* and *C. volucris,* and when the strains could be characterized by MLST, all of them were new sequence types. In light of the above and genotyping results, we suggest that human activity in Antarctica has little or no impact on the microbiological pollution of penguin rookeries regarding *Campylobacter* spp.

Further research is required to determine the true prevalence of *Campylobacter* infection in penguins in Antarctica, but researchers and visitors should take appropriate hygiene precautions when handling these birds or when coming into contact with fecal matter in the rookeries.

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