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Do humans spread zoonotic enteric bacteria in Antarctica?

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Abstract

Reports of enteric bacteria in Antarctic wildlife have suggested its spread from people to seabirds and seals, but evidence is scarce and fragmentary. We investigated the occurrence of zoonotic enteric bacteria in seabirds across the Antarctic and subantarctic region; for comparison purposes, in addition to seabirds, poultry in a subantarctic island was also sampled. Three findings suggest reverse zoonosis from humans to seabirds: the detection of a zoonotic *Salmonella* serovar (ser. Enteritidis) and *Campylobacter* species (e.g. *C. jejuni*), typical of human infections; the resistance of *C. lari* isolates to ciprofloxacin and enrofloxacin, antibiotics commonly used in human and veterinary medicine; and most importantly, the presence of *C. jejuni* genotypes mostly found in humans and domestic animals but rarely or never found in wild birds so far. We also show further spread of zoonotic agents among Antarctic wildlife is facilitated by substantial connectivity among populations of opportunistic seabirds, notably skuas (*Stercorarius*). Our results highlight the need for even stricter biosecurity measures to limit human impacts in Antarctica.

Keywords: Antarctica, *Campylobacter*, *Salmonella*, seabirds, Southern Ocean, reverse zoonosis.

1. Introduction

The global spread of pathogens is a growing conservation concern because their introduction into novel environments can have dramatic effects on wildlife (Paxton et al., 2016; Van Riper et al., 1986). Pathogens have been dispersed by migratory birds, fish, mammals and other taxa for millions of years, but in recent centuries humans have also contributed to their dispersal (Altizer et al., 2011; Fuller et al., 2012). Antarctica is the only continent where reverse zoonosis transmission has not been documented (Messenger et al., 2014). Despite ongoing concern about human impacts in the region, diseases have not been identified as significant threats (Chown et al., 2012b, 2012a).

To date, the presence of pathogens in Antarctic wildlife has received limited attention (Barbosa and Palacios, 2009; Kerry and Riddle, 2009). It has been assumed that the region's isolation and relatively recent exploration by humans have protected Antarctic wildlife from novel pathogens, although there have been several outbreaks of infectious diseases at Southern Ocean islands (Cooper et al., 2009; Kane et al., 2012; Weimerskirch, 2004). The few surveys of pathogens in Antarctica have been opportunistic, and investigations of occasional mass mortality events to date have not established clear evidence of human-to-animal transmission (Frenot et al., 2005; Gardner et al., 1997; Hernandez et al., 2012; Iveson et al., 2009; Kerry and Riddle, 2009; Vigo et al., 2011).

The mechanisms by which pathogens invaded the Southern Ocean wildlife remain uncertain. Some infectious agents may have invaded the Antarctic and subantarctic region well before the arrival of humans, through migratory birds and their parasites. This is likely to be the case for some pathogens vectored by seabird ticks, such as *Borrelia* spp., as suggested by some authors (McCoy et al 2012; Olsen et al., 1995). However, for other pathogens this may not be the case and humans may be increasing the income of pathogenic agents into that region. Whilst human-mediated transport may be a legacy of exposure in the last few centuries to sealers and whalers or to their domestic animals (Gardner et al., 1997; Griekspoor et al., 2010), several studies indicate that the main risk of pathogen invasion is the

increase in tourism and research activities, which currently account for tens of thousands of visitors each year (Curry et al., 2002; Hughes and Convey, 2010). In this regard, the Protocol on Environmental Protection to the Antarctic Treaty (1991), which came into force in 1996, included a number of measures to prevent the introduction of novel pathogens (Committee for Environmental Protection, 2011). However, it may be of limited value if Antarctic wildlife migrates to areas outside the Antarctic region, where they can be exposed to a wide range of pathogens during their broad scale movements. Many Antarctic seabirds disperse across the Southern Ocean, coming into contact with domestic species in populated areas, and some species that visit the region during the Antarctic summer spend the winter in the northern hemisphere (e.g. Arctic Terns *Sterna paradisaea* and South Polar Skuas *Stercorarius maccormicki*). Such large-scale movements may introduce pathogens to Antarctica, and disperse them within the region. Climate change also may alter the migratory habits of animals, increasing the spread and contact between Antarctic, subantarctic and temperate wildlife (Altizer et al., 2013).

The zoonotic bacteria *Salmonella* spp. and thermotolerant *Campylobacter* spp. are amongst the most important foodborne diarrheal pathogens worldwide (Havelaar et al., 2015). Both agents can spread rapidly in the environment through faecal contamination and can persist in soil or water for long enough to infect wild fauna. We explore the transfer of these zoonotic bacteria from humans and poultry to the subantarctic and Antarctic region by sampling 24 seabird species over a broad geographical range, identifying bacterial species and comparing serovars and genotypes in seabirds with those commonly found in humans and domestic animals, and by testing their resistance to antibiotics commonly used in human and veterinary medicine. We also evaluate whether these pathogens are spreading across wildlife of the Southern Ocean.

2. Materials and methods

2.1. Sampling

From 2008 to 2011 we collected faecal samples from adult seabirds at four Southern Ocean localities: Livingston (Antarctica), Marion, Gough and the Falkland Islands (Figure 1A, Table 1). Additionally, we also sampled backyard poultry at the Falklands, which support a permanent human settlement with a number of farms in close contact with subantarctic and Antarctic wildlife. Birds were caught by hand and faecal samples were collected in duplicate using sterile swabs inserted into the cloaca. Samples were stored refrigerated in Amies transport medium with charcoal (Deltalab, Barcelona, Spain), transported to Spain within two to five weeks after the day of collection and cultured immediately upon arrival to the laboratory.

2.2. Bacterial isolation and identification

We performed *Salmonella* and *Campylobacter* isolation and identification by standard culture methods (Antilles et al., 2015). *Salmonella* serotyping was performed according to the Kauffmann-White scheme (Grimont and Weill, 2007) and carried out at the Laboratori Agroalimentari (Cabrils, Spain) of the Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural. We identified *Campylobacter* isolates to species level by PCR using primers based on the *lpxA* gene (Klena et al., 2004). A multiplex PCR for *C. jejuni* and *C. coli* identification was performed using forward primers lpxA-Cjejuni (5'-ACA ACT TGG TGA CGA TGT TGTA-3') and lpxA-Ccoli (5'-AGA CAA ATA AGA GAG AGA ATC AG-3') and a common reverse primer (lpxARKK2m: 5'-CAA TCA TGD GCD ATA TGA SAA TAH GCC AT-3'). *C. lari* identification was performed with a monoplex PCR using primers lpxA-Clari (5'-TRC CAA ATG TTA AAA TAG GCG A-3') and lpxARKK2m. The type strains of *C. jejuni*, *C. coli* and *C. lari* were used as positive controls in the corresponding species-specific PCRs, and as negative control DNA was replaced by PCR-grade water.

2.3. Antimicrobial susceptibility testing

106 We performed antimicrobial susceptibility testing for both *Salmonella* and *Campylobacter* isolates
107 following the Clinical Laboratory and Standard Institute disc diffusion method (M100-S18) (CLSI, 2016)
108 using Neo-Sensitabs™ (Rosco Diagnostica, Denmark) with CLSI potencies according to the
109 manufacturer's instructions. For *Salmonella* isolates, we used Mueller-Hinton agar (Difco, Madrid, Spain)
110 and plates were incubated at 37°C for 24 h. For *Campylobacter* isolates, we used Mueller-Hinton II agar
111 supplemented with 5% defibrinated sheep blood (BioMérieux, Marcy l'Etoile, France) and plates were
112 incubated at 37°C for 48 h under microaerobic conditions. *E. coli* ATCC 25922 and *C. jejuni* ATCC 33560
113 were used as a quality control for *Salmonella* and *Campylobacter* susceptibility assays, respectively.
114 *Salmonella* isolates were tested against 18 antimicrobials: ampicillin (33 μg), amoxicillin (30 μg),
115 amoxicillin clavulanic (30+15 μg), ceftiofur (30 μg), apramycin (40 μg), streptomycin (100 μg), gentamicin
116 (40 μg), neomycin (120 μg), ciprofloxacin (10 μg), enrofloxacin (10 μg), nalidixic acid (130 μg),
117 norfloxacin (10 μg), colistin (150 μg), chloramphenicol (60 μg), lincomycin + spectinomycin (15 + 200 μg),
118 nitrofurantoin (260 μg), tetracycline (80 μg) and trimethoprim + sulfonamide (5.2 + 240 μg).
119 *Campylobacter* isolates were tested against seven antimicrobials: nalidixic acid (30 μg), ciprofloxacin (5
120 μg), enrofloxacin (10 μg), tetracycline (80 μg), chloramphenicol (60 μg), erythromycin (15 μg) and
121 gentamicin (10 μg).
122
123 *2.4. Salmonella and Campylobacter genotyping*
124 We typed representative bacterial isolates with pulsed-field gel electrophoresis (PFGE) and
125 multilocus sequence typing (MLST). PFGE was carried out according to the standard operating procedure
126 of PulseNet (www.pulsenetinternational.org). We performed restriction enzyme digests for PFGE with
127 XbaI and BlnI enzymes for *Salmonella*, and with SmaI and KpnI enzymes for *Campylobacter* (Roche
128 Applied Science, Indianapolis, IN, USA). *Salmonella* Braenderup H9812 restricted with XbaI was used as
129 molecular size standard for both *Campylobacter* and *Salmonella*. We analysed the resulting PFGE

patterns using Fingerprinting II v3.0 software (Bio-Rad, Hercules, CA, USA). Banding patterns were compared with the UPGMA (Unweighted Pair Group Method with Arithmetic averages) clustering method using the Dice correlation coefficient with a band position tolerance of 1%.

We further characterized *S. enterica* and thermotolerant *Campylobacter* using MLST, which is based on sequencing of seven housekeeping genes (Achtman et al., 2012; Dingle et al., 2001; Miller et al., 2005). Primers used for *Salmonella* were those described in the MLST public database (http://mlst.warwick.ac.uk/mlst) and those used for *Campylobacter* species are indicated in the corresponding MLST database (www.pubmlst.org/campylobacter) and in Miller *et al*. (2005). The sequence types were determined according to the scheme provided on these sites.

To explore potential spill-over from domestic to wild birds, we compared *C. jejuni* and *C. lari* isolates found in the present study with others from ducks and hens from Falkland Is., using PFGE and MLST.

3. Results

3.1. Salmonella and Campylobacter spp. in seabirds

We sampled 666 seabirds from 24 species at Livingston (n= 139), Gough (n= 138), Marion (n= 125) and the Falkland Islands (n= 264) (Figure 1A; Table 1), and isolated three *Salmonella* ser. Enteritidis, 10 *C. jejuni* and 35 *C. lari*. The only other *Salmonella* serovar detected was one Oakey; no other thermotolerant *Campylobacter* species were found.

We isolated *Salmonella* Enteritidis from two kelp gulls (*Larus dominicanus*) and one southern giant petrel (*Macronectes giganteus*) from Livingston Is.; *C. jejuni* from one macaroni penguin (*Eudyptes chrysolophus*), one king penguin (*Aptenodytes patagonicus*), and six brown skuas (*Catharacta antarctica*) at Marion Is. and from single brown skuas at Gough and the Falkland Is (Figure 1B); and *C. lari* from one gentoo penguin (*Pygoscelis papua*), one southern giant petrel and 10 brown skuas at Livingston Is.; from

154 one macaroni penguin, two southern giant petrels and seven brown skuas at Marion Is.; from 10 brown
155 skuas at Gough Is.; and from three brown skuas at the Falkland Is. Marion Is. showed the highest
156 diversity of positive seabird species to *Campylobacter* and was the only locality where co-infections
157 occurred of both *C. jejuni* and *C. lari* (n=3 skuas).
158
159 *3.2. Antimicrobial resistance*
160 We did not detect any antimicrobial resistance in isolates of *Salmonella* or *C. jejuni*. Among *C. lari*
161 isolates, besides nalidixic acid resistance, which is characteristic of this species and was found in all
162 tested isolates, we found ciprofloxacin resistance in isolates from one macaroni penguin and two skuas
163 from Marion Is., and from three skuas from Gough Is. Ciprofloxacin and enrofloxacin resistance was
164 detected in two *C. lari* from skuas at Livingston Is.
165
166 *3.3. Genetic diversity*
167 All three *Salmonella* Enteritidis isolates exhibited identical PFGE patterns and MLST sequence type
168 (ST11). Among *C. jejuni* isolates, PFGE analysis clustered together three isolates: two from brown skuas
169 from the Falklands and Marion Is. and one from a domestic duck from the Falklands. MLST showed
170 these isolates to belong to the widespread ST45. Four other *C. jejuni* ST (ST137, ST227, ST696 and ST883)
171 were isolated from skuas and penguins at Gough and Marion Is. (Figure 2). These ST have been reported
172 in several hosts in developed countries of the northern hemisphere and Australia (Figure 1C).
173 Among *C. lari* isolates**,** PFGE genotyping showed highly similar isolates (> 80% similarity) from
174 several skuas at Livingston, Marion and Gough Is. and from a giant petrel at Marion Is. One cluster was
175 formed by three (GH128-C1, GH131-C1 and MAR5-C1) nearly identical isolates (≥ 95% similarity) found
176 in skuas from Gough and Marion Is. belonging to the same novel ST (Figure 3). In addition, the same
177 genotype was found in two different seabird species, a brown skua and a gentoo penguin from

178 Livingston Is. (isolates AN138-C7 and AN32-C1), which were closely related (81% similarity) to an isolate
179 from a duck (FK72-C1) from the Falklands. One cluster grouped isolates from distant localities, i.e. one
180 isolate from a skua at the Falklands and one from a penguin at Marion Is. (FK54-C1 and MAR18-C1), with
181 an 88% similarity.
182
183 **4. Discussion**
184 Three lines of evidence suggest a reverse zoonosis in Antarctica, whereby zoonotic enteric bacteria
185 have been introduced by humans to Southern Ocean ecosystems: the detection in seabirds of
186 *Salmonella* serovars (e.g. Enteritidis) or *Campylobacter* species (e.g. *C. jejuni*) typically associated with
187 humans (Figure 1B), the antibiotic resistance of some strains, and most importantly, the occurrence of
188 several *Campylobacter* genotypes (ST45, ST137, ST227, ST696 and ST883) previously reported almost
189 exclusively in humans and domestic animals from developed countries. *Salmonella* was only isolated
190 from a few seabirds at Livingston Is. (Antarctic Peninsula), suggesting *Salmonella* is not indigenous to
191 seabirds in the region. *Salmonella* Enteritidis serovar is, together with Typhimurium, the most common
192 serovar causing salmonellosis in humans worldwide (Hendriksen et al., 2011). Our results agree with the
193 scarcity of *Salmonella* isolates previously reported in seabirds and mammals of the Southern Ocean,
194 which mainly belong to serovars commonly found in humans (Figure 1B) (Dougnac et al., 2015; Iveson et
195 al., 2009; Olsen et al., 1996; Palmgren et al., 2000; Retamal et al., 2017; Vigo et al., 2011). The
196 *Salmonella* serovar we found typically occurs in scavenging birds associated with urban areas, such as
197 gulls and raptors, and is relatively uncommon in wildlife from less transformed areas (Ĉížek et al., 1994;
198 Jurado-Tarifa et al., 2016; Ramos et al., 2010). All our *Salmonella* isolates had the same PFGE
199 macrorestriction profile and the same MLST type (ST11), which has also been reported from seabirds
200 and seals in the Antarctic Peninsula (Vigo et al., 2011), and it is the most abundant and widespread ST of

ser. Enteritidis worldwide, further suggesting the clonal spread of this serovar from other continents to Antarctica.

We found thermophilic *Campylobacter* species in all sampled localities, mainly *C. lari*, but also *C. jejuni*, which is a major cause of foodborne diarrhoeal illness in humans worldwide (Havelaar et al., 2015). *C. jejuni* has been isolated only once in penguins from the same colony (3/100; 3/446 of all sampled birds) in the broader Antarctic region, at South Georgia (Broman et al., 2000). In non-remote areas, prevalence of this *Campylobacter* species from scavenging seabirds has been reported at much higher rates (authors, unpublished data) (Kapperud and Rosef, 1983; Keller et al., 2011). We found *C. jejuni* mainly in brown skuas, one of the main opportunistic seabird species of the Southern Ocean. When given the chance, skuas often scavenge on human waste, providing a plausible mechanism for the transfer of *C. jejuni* to this species.

Antimicrobial resistance was generally low, but the presence of at least certain resistance is worrying given that they were found in some of the most remote areas on Earth. A few *C. jejuni* and *C. lari* isolates from poultry at the Falklands (authors, unpublished data) and some *C. lari* isolates from a macaroni penguin and skuas from three islands were resistant to fluoroquinolones (ciprofloxacin, enrofloxacin). These agents belong to the so-called critically important antimicrobials and are therefore seldom used in human or veterinary medicine (WHO AGISAR, 2012). As a result, the development of resistance in backyard poultry or wild seabird populations is very unlikely, strongly suggesting contamination by a resistant strain of anthropogenic origin. Interestingly, the domestic duck which carried a *C. lari* resistant isolate was free ranging most of the day, a practice that may facilitate transmission between the domestic and the wildlife compartments. Resistance also may have developed through spontaneous mutation, acquired by horizontal gene transfer from other microorganisms that constitute natural sources of drug-resistant genes, or may have been imported into the Southern Ocean through bird migration. However, the detection in skuas of several *C. jejuni*

225 genotypes almost exclusively found in humans and livestock supports the likelihood of reverse zoonosis.
226 MLST analysis showed some strains from skuas from Marion Is. to belong to new STs. They could
227 represent host specific strains or strains endemic of the Southern Ocean. However, several other
228 genotypes belonged to STs almost exclusively associated with human disease and asymptomatic
229 infection in livestock (ST45, ST137, ST227, ST696 and ST883) from northern developed countries,
230 strongly supporting their human origin. That is, 70%-85% of the isolates belonging to those STs have
231 been isolated previously from human gastroenteritis cases, and some of them also from chicken or
232 chicken products, but rarely (1-9% of the isolates) or never from wild birds
233 (https://pubmlst.org/campylobacter/). At Gough and Marion Is., introduction likely occurred through
234 personnel based at the South African scientific stations, despite strict biosecurity controls for more than
235 two decades. The introduction of these human-associated strains to these remote islands by migrating
236 birds infected during migrating movements cannot be ruled out, but seems less plausible.

237 The case of the Falkland Is. is particularly relevant, since ST45 was isolated from a skua and a
238 domestic duck. This ST is very common in humans and livestock but has only been reported once in a
239 single bird in the Southern Ocean in a remote site of the Subantarctic region (Griekspoor et al., 2010;
240 Olsen et al., 1996), suggesting movement from the domestic to the wildlife compartment. Inhabited
241 areas close to the Antarctic region with free-ranging livestock, such as Patagonia, the Falklands and
242 Tristan da Cunha, are of particular concern, since in these localities domestic animals come in close
243 contact with Antarctic wildlife, potentially facilitating the spread of infectious diseases. Many Antarctic
244 birds and mammals regularly visit these areas or mix with the local fauna in common wintering grounds
245 (Shirihai, 2007).

246 It is also plausible that zoonotic enteric bacteria and other pathogens can spread and circulate
247 through wildlife across the Southern Ocean. *C. lari*, the most abundant *Campylobacter* species recovered
248 at all four sites, has been reported previously in Southern Ocean penguins, gulls, skuas and seals

(Bonnedahl et al., 2005; García-Peña et al., 2017, 2010; Leotta et al., 2006). The widespread distribution of *C. lari* among host species and localities and its high genetic diversity suggest that it has long been circulating in the region. The genetic similarities among isolates from skuas, penguins and gulls in our study also suggest substantial connectivity across Southern Ocean localities and therefore potential for spreading new pathogens.

Our results provide compelling evidence for reverse zoonosis of pathogens in Antarctica and suggest that zoonotic enteric bacteria can be spread by wildlife across the Southern Ocean. The increasing spread of pathogens, underpinned by globalization and climate change, now affects the most remote areas on Earth. Strict measures to limit human impacts in Antarctica (Chown et al., 2012b, 2012a) should be expanded to zoonotic bacteria and to settled areas in the peri-Antarctic region.

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Conflict of interest statement

The authors declare they have no conflicts of interest.

274 **References**

275 Achtman, M., Wain, J., Weill, F.X., Nair, S., Zhou, Z., Sangal, V., Krauland, M.G., Hale, J.L., Harbottle, H.,
276 Uesbeck, A., Dougan, G., Harrison, L.H., Brisse, S., 2012. Multilocus sequence typing as a
277 replacement for serotyping in *Salmonella enterica*. PLoS Pathog. 8, e1002776.

278 Altizer, S., Bartel, R., Han, B.A., 2011. Animal migration and infectious disease risk. Science 331, 296–
279 302.

280 Altizer, S., Ostfeld, R.S., Johnson, P.T.J., Kutz, S., Harvell, C.D., 2013. Climate Change and Infectious
281 Diseases: From Evidence to a Predictive Framework. Science 341, 514–519.

282 Antilles, N., Sanglas, A., Cerdà-Cuéllar, M., 2015. Free-living Waterfowl as a Source of Zoonotic Bacteria
283 in a Dense Wild Bird Population Area in Northeastern Spain. Transbound. Emerg. Dis. 62, 516–521.

284 Barbosa, A., Palacios, M.J., 2009. Health of Antarctic birds: A review of their parasites, pathogens and
285 diseases. Polar Biol. 32, 1095–1115.

286 Bonnedahl, J., Broman, T., Waldenström, J., Palmgren, H., Niskanen, T., Olsen, B., Bonnedahl, J., Broman,
287 T., Waldenstro, J., 2005. In search of human-associated bacterial pathogens in Antarctic wildlife:
288 report from six penguin colonies regularly visited by tourists. Ambio 34, 430–432.

289 Broman, T., Bergström, S., On, S.L.W., Palmgren, H., McCafferty, D.J., Sellin, M., Olsen, B., 2000. Isolation
290 and Characterization of *Campylobacter jejuni* subsp. *jejuni* from Macaroni penguins (*Eudyptes*
291 *chrysolophus*) in the Subantarctic Region. Appl. Environ. Microbiol. 66, 449–452.

292 Chown, S.L., Huiskes, A.H.L., Gremmen, N.J.M., Lee, J.E., Terauds, A., Crosbie, K., Frenot, Y., Hughes, K.A.,
293 Imura, S., Kiefer, K., Lebouvier, M., Raymond, B., Tsujimoto, M., Ware, C., Van de Vijver, B.,
294 Bergstrom, D.M., 2012a. Continent-wide risk assessment for the establishment of nonindigenous
295 species in Antarctica. Proc. Natl. Acad. Sci. U. S. A. 109, 4938–4943.

296 Chown, S.L., Lee, J.E., Hughes, K.A., Barnes, J., Barrett, P.J., Bergstrom, D.M., Convey, P., Cowan, D.A.,
297 Crosbie, K., Dyer, G., Frenot, Y., Grant, S.M., Herr, D., Kennicutt, M.C., Lamers, M., Murray, A.,

298 Possingham, H.P., Reid, K., Riddle, M.J., Ryan, P.G., Sanson, L., Shaw, J.D., Sparrow, M.D.,
299 Summerhayes, C., Terauds, A., Wall, D.H., 2012b. Challenges to the future conservation of the
300 Antarctic. Science 337, 158–159.
301 Ĉížek, A., Literák, I., Hejlíćek, K., Treml, F., Smola, J., Cizek, A., Literak, I., Hejlicek, K., Treml, F., Smola, J.,
302 1994. *Salmonella* Contamination of the Environment and its Incidence in Wild Birds. J. Vet. Med.
303 Ser. B 41, 320–327.
304 CLSI (Clinical and Laboratory Standards Institute), 2016. Performance Standards for Antimicrobial
305 Susceptibility Testing; Twenty-sixth Informational Supplement (M100-S26). CLSI Publication,
306 Wayne, Pennsylvania, United States.
307 Cooper, J., Crawford, R.J.M., De Villiers, M.S., Dyer, B.M., Hofmeyr, G.J.G., Jonker, A., 2009. Disease
308 outbreaks among penguins at sub-Antarctic Marion Island: A conservation concern. Mar. Ornithol.
309 37, 193–196.
310 Curry, C.H., McCarthy, J.S., Darragh, H.M., Wake, R. a, Todhunter, R., Terris, J., 2002. Could tourist boots
311 act as vectors for disease transmission in Antarctica? J. Travel Med. 9, 190–193.
312 Dingle, K.E., Colles, F.M., Wareing, D.R.A.A., Ure, R., Fox, A.J., Bolton, F.E., Bootsma, H.J., Willems, R.J.L.,
313 Urwin, R., Maiden, M.C.J., 2001. Multilocus Sequence Typing System for *Campylobacter jejuni*. J.
314 Clin. Microbiol. 39, 14–23.
315 Dougnac, C., Pardo, C., Meza, K., Arredondo, C., Blank, O., Abalos, P., Vidal, R., Fernandez, A., Fredes, F.,
316 Retamal, P., 2015. Detection of *Salmonella enterica* in Magellanic penguins (*Spheniscus*
317 *magellanicus*) of Chilean Patagonia: evidences of inter-species transmission. Epidemiol. Infect. 143,
318 1187–1193.
319 Frenot, Y., Chown, S.L., Whinam, J., Selkirk, P.M., Convey, P., Skotnicki, M., Bergstrom, D.M., 2005.
320 Biological invasions in the Antarctic: extent, impacts and implications. Biol. Rev. Camb. Philos. Soc.
321 80, 45–72.

322 Fuller, T., Bensch, S., Müller, I., Novembre, J., Pérez-Tris, J., Ricklefs, R.E., Smith, T.B., Waldenström, J.,
323 2012. The ecology of emerging infectious diseases in migratory birds: An assessment of the role of
324 climate change and priorities for future research. Ecohealth 9, 80–88.
325 García-Peña, F.J., Llorente, M.T., Serrano, T., Ruano, M.J., Belliure, J., Benzal, J., Herrera-León, S., Vidal,
326 V., D'Amico, V., Pérez-Boto, D., Barbosa, A., 2017. Isolation of *Campylobacter* spp. from Three
327 Species of Antarctic Penguins in Different Geographic Locations. Ecohealth 14, 78–87.
328 García-Peña, F.J., Pérez-Boto, D., Jiménez, C., San Miguel, E., Echeita, A., Rengifo-Herrera, C., García-
329 Párraga, D., Ortega-Mora, L.M., Pedraza-Díaz, S., 2010. Isolation and characterization of
330 *Campylobacter* spp. from Antarctic fur seals (*Arctocephalus gazella*) at Deception Island,
331 Antarctica. Appl. Environ. Microbiol. 76, 6013–6016.
332 Gardner, H., Kerry, K.R., Riddle, M., Brouwer, S., Gleeson, L., 1997. Poultry virus infection in Antarctic
333 penguins 387, 245.
334 Griekspoor, P., Engvall, E.O., Olsen, B., Waldenström, J., 2010. Multilocus sequence typing of
335 *Campylobacter jejuni* from broilers. Vet. Microbiol. 140, 180–185.
336 Grimont, P., Weill, F.X., 2007. Antigenic formulae of the Salmonella servovars. WHO Collab. Cent. Ref.
337 Res. *Salmonella* 1–167. Available:
338 http://www.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-
339 Havelaar, A.H., Kirk, M.D., Torgerson, P.R., Gibb, H.J., Hald, T., Lake, R.J., Praet, N., Bellinger, D.C., de
340 Silva, N.R., Gargouri, N., Speybroeck, N., Cawthorne, A., Mathers, C., Stein, C., Angulo, F.J.,
341 Devleesschauwer, B., 2015. World Health Organization Global Estimates and Regional Comparisons
342 of the Burden of Foodborne Disease in 2010. PLOS Med. 12, e1001923.
343 Hendriksen, R.S., Vieira, A.R., Karlsmose, S., Lo Fo Wong, D.M. a, Jensen, A.B., Wegener, H.C., Aarestrup,
344 F.M., 2011. Global monitoring of *Salmonella* serovar distribution from the World Health
345 Organization Global Foodborne Infections Network Country Data Bank: results of quality assured

346 laboratories from 2001 to 2007. Foodborne Pathog. Dis. 8, 887–900.
347 Hernandez, S.M., Keel, K., Sanchez, S., Trees, E., Gerner-Smidt, P., Adams, J.K., Cheng, Y., Ray, A., Martin,
348 G., Presotto, A., Ruder, M.G., Brown, J., Blehert, D.S., Cottrell, W., Maurer, J.J., 2012. Epidemiology
349 of a *Salmonella enterica* subsp. *enterica* serovar Typhimurium strain associated with a songbird
350 outbreak. Appl. Environ. Microbiol. 78, 7290–7298.
351 Hughes, K.A., Convey, P., 2010. The protection of Antarctic terrestrial ecosystems from inter- and intra-
352 continental transfer of non-indigenous species by human activities: A review of current systems
353 and practices. Glob. Environ. Chang. 20, 96–112.
354 Iveson, J.B., Shellam, G.R., Bradshaw, S.D., Smith, D.W., Mackenzie, J.S., Mofflin, R.G., 2009. *Salmonella*
355 infections in Antarctic fauna and island populations of wildlife exposed to human activities in
356 coastal areas of Australia. Epidemiol. Infect. 137, 858–870.
357 Jurado-Tarifa, E., Torralbo, A., Borge, C., Cerdà-Cuéllar, M., Ayats, T., Carbonero, A., García-Bocanegra, I.,
358 2016. Genetic diversity and antimicrobial resistance of *Campylobacter* and *Salmonella* strains
359 isolated from decoys and raptors. Comp. Immunol. Microbiol. Infect. Dis. 48, 14–21.
360 Kane, O.J., Uhart, M.M., Rago, V., Pereda, A.J., Smith, J.R., Van Buren, A., Clark, J.A., Boersma, P.D., 2012.
361 Avian pox in Magellanic Penguins (*Spheniscus magellanicus*). J. Wildl. Dis. 48, 790–794.
362 Kapperud, G., Rosef, O., 1983. Avian wildlife reservoir of *Campylobacter fetus* subsp. *jejuni*, *Yersinia* spp.,
363 and *Salmonella* spp. in Norway. Appl. Environ. Microbiol. 45, 375–380.
364 Keller, J.I., Shriver, W.G., Waldenström, J., Griekspoor, P., Olsen, B., 2011. Prevalence of *Campylobacter*
365 in wild birds of the mid-Atlantic region, USA. J. Wildl. Dis. 47, 750–754.
366 Kerry, K.R., Riddle, M., 2009. Health of Antarctic Wildlife. Springer, Berlin Heidelberg, Germany.
367 Klena, J.D., Parker, C.T., Knibb, K., Claire Ibbitt, J., Devane, P.M.L., Horn, S.T., Miller, W.G., Konkel, M.E.,
368 2004. Differentiation of *Campylobacter coli, Campylobacter jejuni, Campylobacter lari,* and
369 *Campylobacter upsaliensis* by a multiplex PCR developed from the nucleotide sequence of the lipid

370 A gene lpxA. J. Clin. Microbiol. 42, 5549–5557.
371 Leotta, G. a., Vigo, G.B., Giacoboni, G., 2006. Isolation of *Campylobacter lari* from seabirds in Hope Bay,
372 Antarctica. Polish Polar Res. 27, 303–308.
373 McCoy, K., Beis, P., Barbosa, A., Cuervo, J., Fraser, W., González-Solís, J., Jourdain, E., Poisbleau, M.,
374 Quillfeldt, P., Léger, E., 2012. Population genetic structure and colonisation of the western
375 Antarctic Peninsula by the seabird tick *Ixodes uriae*. Mar. Ecol. Prog. Ser. 459, 109-120.
376 Messenger, A.M., Barnes, A.N., Gray, G.C., 2014. Reverse zoonotic disease transmission
377 (Zooanthroponosis): A systematic review of seldom-documented human biological threats to
378 animals. PLoS One 9, e0089055
379 Miller, W.G., On, S.L.W., Wang, G., Fontanoz, S., Lastovica, A.J., Mandrell, R.E., 2005. Extended
380 Multilocus Sequence Typing System for *Campylobacter coli , C. lari, C. upsaliensis,* and C.
381 helveticus. J. Clin. Microbiol. 43, 2315–2329.
382 Olsen, B., Duffy, D.C., Jaenson, T.G.T., Gylfe, A.J., Bonnedahl, J., Bergstrom, S. 1995. Transhemispheric
383 exchange of Lyme Disease spirochetes by seabirds. J. Clin. Microbiol. 33, 3270-3274.
384 Olsen, B., Bergström, S., McCafferty, D., Sellin, M., Wiström, J., 1996. *Salmonella enteritidis* in Antarctica:
385 zoonosis in man or humanosis in penguins? Lancet 348, 1319–1320.
386 Palmgren, H., McCafferty, D., Aspán, A., Broman, T., Sellin, M., Wollin, R., Bergström, S., Olsen, B., 2000.
387 *Salmonella* in sub-Antarctica: low heterogeneity in *Salmonella* serotypes in South Georgian seals
388 and birds. Epidemiol. Infect. 125, 257–262.
389 Paxton, E.H., Camp, R.J., Gorresen, P.M., Crampton, L.H., Jr, D.L.L., Vanderwerf, E.A., 2016. Collapsing
390 avian community on a Hawaiian island. Sci. Adv. 2, e1600029.
391 Ramos, R., Cerdà-Cuéllar, M., Ramírez, F., Jover, L., Ruiz, X., 2010. Influence of refuse sites on the
392 prevalence of *Campylobacter* spp. and *Salmonella* serovars in seagulls. Appl. Environ. Microbiol.
393 76, 3052–3056.

394 Retamal, P., Llanos-Soto, S., Salas, L.M., López, J., Vianna, J., Hernández, J., Medina-Vogel, G., Castañeda,
395 F., Fresno, M., González-Acuña, D., 2017. Isolation of drug-resistant *Salmonella enterica* serovar
396 enteritidis strains in gentoo penguins from Antarctica. Polar Biol. 2531–2536.
397 Shirihai, H., 2007. A Complete Guide to Antarctic Wildlife: the birds and marine mammals of the
398 Antarctic Continent and the Southern Ocean, 2nd ed. Princeton University Press, New Jersey,
399 United States.
400 Van Riper, C., Van Riper, S.G., Goff, L.M., Laird, M., 1986. The Epizootiology and Ecological Significance of
401 Malaria in Hawaiian Land Birds. Ecol. Monogr. 56, 327–344.
402 Vigo, G.B., Leotta, G.A., Caffer, M.I., Salve, A., Binsztein, N., Pichel, M., 2011. Isolation and
403 characterization of *Salmonella enterica* from Antarctic wildlife. Polar Biol. 34, 675–681.
404 Weimerskirch, H., 2004. Diseases threaten Southern Ocean albatrosses. Polar Biol. 27, 374–379.
405 WHO AGISAR (World Health Organization Advisory Group on Integrated Surveillance of Antimicrobial
406 Resistance), 2012. Critically important antimicrobials for human medicine, World Health
407 Organization. Geneva, Switzerland. Available:
408 http://apps.who.int/iris/bitstream/10665/77376/1/9789241504485_eng.pdf
409