Official master's degree in Zoonoses and One Health (2020 – 2021)

Epidemiology of zoonotic enteric bacteria in seabirds from Southern Ocean

Authorship:
Irene Ribes Mengual

Supervisor: Marta Cerdà-Cuéllar

Tutor: Laila Darwich Soliva

Centre de Recerca en Sanitat Animal – Institut de Recerca i Tecnologia Agroalimentàries

IRENE RIBES MENGUAL - DNI 74012225L

Firmado digitalmente por IRENE RIBES MENGUAL - DNI 74012225L
Fecha: 2021.07.08 23:58:05 +02'00'

Marta Cerdà Cuellar - DNI 46132625F (SIG)

2021.07.09 07:55:52 +02'00'

LAILA DARWICH SOLIVA

Firmado digitalmente por LAILA DARWICH SOLIVA
Fecha: 2021.07.09 08:49:48 +02'00'

firma digital	firma digital	firma digital
o manuscrita escaneada	o manuscrita escaneada	o manuscrita escaneada
Irene Ribes Mengual	Marta Cerdà-Cuéllar	Laila Darwich Soliva

TABLE OF CONTENTS

1. Abstract

Campylobacter and *Salmonella* are among the most commonly reported zoonoses in the EU and USA and have a strong impact on public health. Poultry are considered the main reservoir and source of infection for humans, but wild birds also play an important role in the epidemiology of these pathogens as they are usually asymptomatic carriers able to maintain infection and disperse them over long distances through migratory movements. Antarctic and sub-Antarctic regions were long thought to be protected from pathogens introduction, but recent studies have reported the presence of human and animal pathogens. To gain insight into the epidemiology of *Campylobacter* and *Salmonella* in seabirds inhabiting the Southern Ocean, 60 samples from different species of seabirds were sampled at Crozet, Amsterdam and Kerguelen islands during December 2020. We analysed them by direct PCR detection, obtaining an overall prevalence of 16.67% for *Campylobacter* and 6.67% for *Salmonella*. Furthermore, 54 *Campylobacter* isolates and 29 *Salmonella* isolates recovered from previous samplings were also included in the study to determine the *Campylobacter* species and *Salmonella* serovars, and to assess their genetic diversity by means of RFLP, PFGE and ERIC-PCR. *Campylobacter* species mainly included were *C. lari* and *C. jejuni*. Molecular typing techniques showed a high genetic diversity among the *Campylobacter* isolates, but also some clusters suggest a certain connectivity among Southern Ocean localities and a lack of host specificity. *Salmonella* serovars identified were Typhimurium, Enteritidis, Newport and Chincol, which showed a low genetic diversity. The presence of some *Campylobacter* genotypes and *Salmonella* serovars suggest that events of reverse zoonoses have occurred in the region. Our results highlight the role of seabirds, especially skuas, as disseminators of pathogens through their foraging and migration movements, as well as the need of further studies to assess the impact of human activity on the introduction of new pathogens in these remote areas.

2. Introduction

2.1 Zoonoses and reverse zoonoses

A zoonosis is defined by World Health Organization (WHO) as an infectious disease that has jump from a non-human animal to humans. It can be caused by any kind of pathogen (bacterial, viral or parasitic, or may involve unconventional agents), and can spread to humans through direct contact or through food, water or the environment. It has been estimated that the 75% of all emerging infectious diseases (EIDs) have a zoonotic origin and so it has become a huge public health concern (L. H. Taylor et al., 2001). This new pathogen-host scenario that has been observed in recent years became evident in the current health crisis due to COVID-19 pandemic, but many other major pathogens such as Ebola, Nipah virus or Influenza have also caused serious consequences on the health of the population.

The mechanism by which pathogen jumping between species occurs is complex and influenced by microbial, environmental, socioeconomic and political factors (Gebreyes et al., 2014). Over the last century, new variants have appeared such as the increase in world population which has led to an increase in the use of land for agriculture and livestock farming with the consequent loss of biodiversity and habitat for wildlife. Furthermore, while globalization has brought many advantages, it has also facilitated the spread of pathogens over greater distances in less time, making their detection a challenge. All of this coupled with climate change has increased contact between humans, farm animals and wildlife, thus increasing the likelihood of a zoonotic spill over from wildlife reservoirs into humans (Daszak et al., 2000; Mackenzie & Jeggo, 2019).

Similarly, reverse zoonosis can also occur when the pathogen is transmitted from humans to non-human animals, this can pose a risk to wild animal populations, especially those that are endangered. There are some evidences of *Giardia duodenalis* transmission from humans to wild population of African painted dog (Ash et al., 2010) and of *Campylobacter* strains and *Salmonella* serovars commonly isolated in humans or livestock found in seabirds (Cerdà-Cuéllar et al., 2019). Although there is a high burden of pathogens considered “multiple species pathogens”, the research in this field is still very limited and not well documented (Messenger et al., 2014).

The new multifactorial scenario we are currently witnessing highlights the importance of addressing public health and animal health problems in a multidisciplinary approach where different disciplines work together for a comprehensive solution. This is the reason why the One Health concept has gained importance in recent years and has been demanded by major international organisations such as WHO, World Organization for Animal Health (OIE) and Food and Agriculture of the United Nations (FAO) (Gebreyes et al., 2014).

2.2 Campylobacter

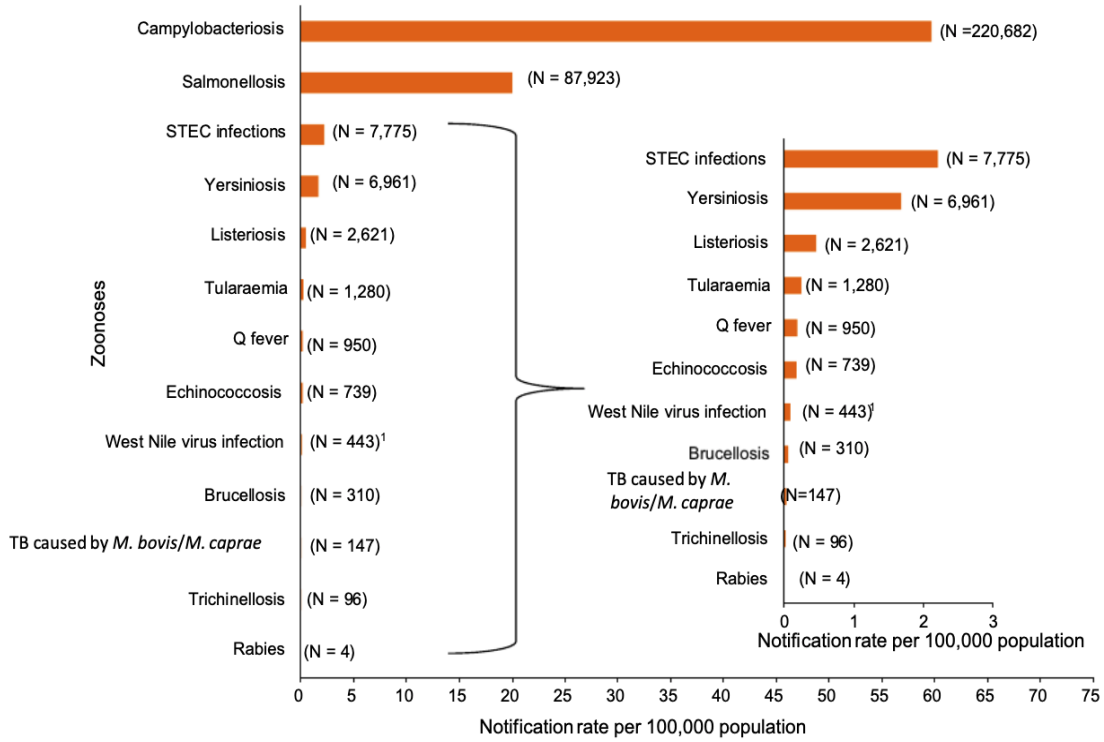
The genus *Campylobacter* belongs to the family *Campylobacteriacae* and consists of a large and diverse group of more than 28 species and 9 subspecies. They are a small (0.2-0.9 µm wide and 0.2-5.0 µm long) gram-negative, spirally curved that do not form spores. Its growth characteristics include its ability to grow in microaerophilia (atmosphere containing nearly 10% of CO_2 and 5% of O_2) with an optimum pH of 6.5 – 7.5 and water activity above 0.987 (Chlebicz & Śliżewska, 2018). Also, it is considered a nutritionally fastidious bacteria, which means that require a complex nutritional environment, thus, selective media and pre-enrichment are necessary for their isolation (Man, 2011; Silva et al., 2011).

Growth characteristics of *Campylobacter* vary according to species with a wide range of growth temperatures from 30 to 46ºC. The species considered thermotolerant (e.g., *C. jejuni, C. coli, C. lari* and *C. upsaliensis*) have their optimum growth temperature between 40 – 42ºC (Man, 2011). Furthermore, it has been observed they be able to survive outside the host (in water, food or faeces) if conditions are suitable (humidity, $\%O_2$, nutrient concentration, temperature…) (Epps et al., 2013; Whiley et al., 2013).

2.2.1 Campylobacteriosis and Public Health

Campylobacteriosis is the most common foodborne illness reported in Europe (Figure 1) and United States since 2005 and one of the four leading causes of diarrhoeal disease worldwide. There has been an increasing trend of confirmed cases in the European Union (EU) over a decade, but during the last few years this tendency has remained flat. Two thirds of all reported cases in the EU member states have food of animal origin as

a source of infection, with fresh meat from broilers and turkeys accounting for the highest percentage of reports, followed by milk and milk products (European Food Safety Authority [EFSA] & European Centre for Disease Prevention and Control [ECDC], 2018; Tack et al., 2019; World Health Organization [WHO], 2020).



Note: The total number of confirmed cases is indicated between parentheses at the end of each bar.
[1] Exception: West Nile virus infection for which the total number of cases was used.

Figure 1 Reported numbers and notification rates of confirmed human zoonoses in the EU, 2019 (EFSA & ECDC, 2021)

The species with the highest prevalence is *C. jejuni* subsp. *jejuni* with 84.4% of the total cases reported in Europe, followed by *C. coli* (9.2%), *C. lari*, *C. fetus* and *C. upsaliensis* with 0.1% each one. Moreover, it is not only a major public health concern, but also entails large economic losses, with an estimated cost around 2.4 billion euros per year (EFSA, 2014).

The target organ of *Campylobacter* is the colon, causing in infected people watery or bloody diarrhoea, fever and/or weight loss for an average of 6 days (Kaakoush et al., 2015; Silva et al., 2011). It has also been associated as a causative agent of the syndrome of *Guilllain-Barré*, a type of autoimmune-mediated paralysis (Ramos et al., 2021) and, also, it can cause extragastrointestinal infections such as septicaemia, meningitis and

urinary tract infections (Man, 2011). It is estimated that around 10% of infected persons require hospitalisation, with higher values when those affected are at-risk population, but rarely is associated with mortality (24 deaths per 10,000 confirmed cases) (Ruiz-Palacios, 2007).

2.3 *Salmonella*

The genus *Salmonella* belongs to the family of *Enterobacteriaceae*, which are Gram-negative, flagellated facultatively anaerobic bacilli. Two species, *S. enterica* and *S. bongori*, and six subspecies have currently been identified by molecular techniques due to differences in 16S rRNA sequence (Table 1). *Salmonella* is characterized by the presence of the antigens O (somatic), H (flagellar) and Vi, which combine to form more than 2500 serotypes according Kauffmann-White classification (Giannella, 1996; Pui et al., 2011). Pasteur Institute is the one in charge of updating the recognized antigenic formulae of *Salmonella* serotypes reported every year in a document called Kauffmann-White scheme. In order to have a standard nomenclature and to avoid confusion, each new strain of a new serovar is named after the geographical place of origin (e.g., serovar Dublin) (Grimont & Weill, 2007).

Growth conditions of *Salmonella* vary according to the different serotype and environmental conditions, the temperature range goes from 5 to 47 ºC with an optimum temperature of 35 to 37ºC. Likewise, the pH range is also wide (4 to 9) with an optimum in 6.5 – 7.5, but available moisture (aw) inhibits growth at values below 0.94 in neutral pH media and higher aw is needed if pH declines (Harris et al., 2003; Pui et al., 2011).

Table 1 *Salmonella* species, subspecies, serotypes, and their usual habitats, adapted from Brenner et al., 2000.

***Salmonella* species and subspecies**	**No. of serotypes within subspecies**	**Usual habitat**
S. enterica subsp. *enterica* (I)	1,454	Warm-blooded animals
S. enterica subsp. *salamae* (II)	489	Cold-blooded animals and the environment
S. enterica subsp. *arizonae* (IIIa)	94	Cold-blooded animals and the environment
S. enterica subsp. *diarizonae* (IIIb)	323	Cold-blooded animals and the environment
S. enterica subsp. *houtenae* (IV)	70	Cold-blooded animals and the environment
S. enterica subsp. *indica* (VI)	12	Cold-blooded animals and the environment
S. bongori (V)	20	Cold-blooded animals and the environment

2.3.1 Salmonellosis and Public Health

After campylobacteriosis, salmonellosis is the most commonly reported gastrointestinal infection in humans in the EU with a rate of 20 cases per 100.000 population. It is an important food-borne pathogen mainly transmitted by egg and egg products followed by meat and bakery products. The majority of the outbreaks are caused by *Salmonella* serovar Enteritidis, which is mainly related with laying hens, followed by serovars Typhimurium and Infantis (EFSA & ECDC, 2021). The start of vaccination in 2007 in animal production resulted in a downward trend up to 2016 in the EU, following a flat trend since then (Chlebicz & Śliżewska, 2018; EFSA & ECDC, 2021).

Pathogenic bacteria belonging to the *Salmonella* genus can cause three types of infection: typhoid fever and nontyphoid invasive or noninvasive. Serotypes causing typhoid fever are related with food or water contaminated with human waste and they cause a severe illness that can be fatal. Nontyphoid serotypes are closely linked to

animal origin infection as they are commonly isolated from both humans and animals and they cause the infection called salmonellosis (Chlebicz & Śliżewska, 2018).

Salmonellosis is usually limited to gastrointestinal symptoms, but 5-10% of cases may develop bacteremia leading to focal infections in different organs (meningitis, osteomyelitis, endocarditis…) (Su & Chiu, 2007). Severity of infection depends on several factors such as the pathogenicity of the serotype. Serovars Cholearaesuis or Dublin have been identified as highly invasive serotypes, (Pui et al., 2011), and also antimicrobial resistant strains play an important role in the patient's recovery (Su et al., 2004).

2.4 Reservoirs

Thermotolerant *Campylobacter* and non-typhoidal serovars of *Salmonella* are part of the normal microbiota of healthy warm-blooded animals both livestock and wildlife. As indicated above, food production animals are considered the main source of human infections for both pathogens, with poultry playing a major role as asymptomatic carriers with high *Campylobacter* prevalence in broiler carcases (41%) (Dunkley et al., 2009; Epps et al., 2013; EFSA & ECDC, 2021; Moore et al., 2005). Other farm animals such as pig, cattle and sheep and domestic animals as cats and dogs have also been shown to be a risk factor for human infection (Baker et al., 1999; Stevens et al., 2009; Whiley et al., 2013).

Environmental contamination also plays an important role in the spread of both pathogens, their presence in drinking and recreational water reservoirs are well documented (Daczkowska-Kozon & Brzostek-Nowakowska, 2001; Polo et al., 1998; Szczepanska et al., 2017). Wild-living birds, especially those that share habitat with humans, can function as animal vectors and participate in the epidemiology of these bacteria by coming in contact with food production animals or refuse sites and covering long distances during annual movements (Hald et al., 2016). Pathogenic strains of both *Campylobacter* and *Salmonella* that have been linked to sporadic cases of infection or outbreaks in humans have also been isolated from different wild birds, such as raptors, gulls, passerines, waterfowl, crows or pigeons (Iglesias-Torrens et al., 2018; Kaczorek-

Łukowska et al., 2020; Keller et al., 2011; Quessy & Messier, 1992; Ramos et al., 2010; Refsum et al., 2002).

It is not only in the most populated areas where human-wildlife pathogen interaction has been observed. Some studies have identified *Campylobacter* strains and *Salmonella* serovars commonly associated with humans in seabirds and seals from remote areas such as the sub-Antarctic islands (Cerdà-Cuéllar et al., 2019; Palmgren et al., 2000). These areas have experienced an increase of human activity due to tourism and research activities during the last century (Frenot et al., 2005) with an impact on the microbiota carried by the animals but whose long-term effects are not known (Coetzee & Chown, 2016).

2.4.1 Antarctic and Sub-Antarctic seabirds

The Southern Ocean it is the habitat of a large number of seabirds, many of them forming colonies on the sub-Antarctic islands (e.g., Crozet Is. and Kerguelen Is.) and dispersing throughout the ocean. Some of these species also fly far of their breeding latitudes in summer or spend the winter in the northern hemisphere (Munro & Burg, 2017; Woods et al., 2009). During migratory movements these seabirds may come into contact with farm animals, other wildlife and human settlements. Furthermore, these movements are sensitive to shift due to climate change, potentially leading to the entry of new pathogens into the remote breeding areas (Altizer et al., 2013).

The species most commonly sampled in our study was the Subantarctic skua, or also known as Brown skua (*Stercorarius antarticus)*, which is the largest and top predator seabird that breed in the Sub-Antarctic and Antarctic area (Borghello et al., 2019). Their migratory movements range from Sub-Antarctic to mixed Sub-Antarctic and Sub-Tropical waters, spending most of the time sitting on the water and return to land only to nest (Carneiro et al., 2016). Their diet has seasonal variations depending on different physiological demands but typically penguin eggs, fish and molluscs are found in the pellet (Borghello et al., 2019).

2.5 Molecular epidemiology

Typing techniques have become an essential tool to discriminate between different bacterial isolates, characterize and classify them at the species or subspecies level (Sabat et al., 2013). We can divide these methods between phenotypic techniques based on the detection of the characteristics expressed by the bacteria and genotypic techniques based on DNA and RNA analysis. Both provide different information and are therefore complementary, but genotyping allows to differentiate many subtypes within a species as well as define phylogenetic relationships, increasing sensitivity in strain differentiation (Eberle & Kiess, 2012).

Focusing on genetic typing techniques, there are several methods depending on the information we want to obtain, and many are being used for the characterisation of *Campylobacter* and *Salmonella* species. The most common ones are based on Polymerase Chain Reaction (PCR) which amplifies repetitive target segments of DNA (e.g. enterobacterial repetitive intergenic consensus (ERIC)-PCR), flagellin gene typing including Restriction Fragment Length Polymorphism (RFLP)-*flaA*, pulse-field gel electrophoresis (PFGE) based on the study of genomic bacterial DNA restriction patterns, multilocus sequence typing (MLST) and whole-genome sequencing (WGS) (Lin et al., 2014; Saxena et al., 2002; Taboada et al., 2013).

RFLP-*fla*A is based in the digestion of PCR-amplified *fla*A gene DNA with specific restriction endonucleases to produce a profile that can be used in genetic analysis. It is one of the simplest and most cost-effective genotyping and has also demonstrated a high capacity for discrimination among *Campylobacter* isolates. However, for epidemiological and phylogenetic studies, we need complementary methods (Nebola & Steinhauserova, 2006; Taboada et al., 2013).

For typing Enterobacteriaceae, such as *Salmonella* isolates, Versalovic et al. (1991) described the presence of repetitive sequences of DNA in some eubacteria and showed that they can produce clear bands in agarose gel after PCR amplification with ERIC-sequence primers. Later it was demonstrated its capability as a tool for typing within a short period of time as well as its ability to describe clusters, but not to determine different serotypes (Secundo de Souza et al., 2015; Swanenburg et al., 1998).

PFGE is based in the study of the entire chromosomal DNA and subsequent electrophoretically separation of restriction fragments based on size, providing for each isolate a different banding pattern (Lin et al., 2014). Several studies have shown that this technique confers a high sensitivity in differentiating between closely related strains of *Campylobacter*, *Salmonella* and other bacteria as well as more stable patterns (Lin et al., 2014; Nebola & Steinhauserova, 2006). For this reason, together with MLST, is the "gold standard" typing method for tracking foodborne pathogens and has been widely applied in epidemiological investigation (Fakhr et al., 2005). The main problem found in PFGE is the difficulty in comparing results with other laboratories. To solve it, new typing techniques as MLST or WGS appeared as alternative when comparing and evolutionary history is needed (Llarena et al., 2017).

MLST is a DNA sequence method based on the characterisation of seven housekeeping genes of the isolated strain. Because there are many alleles at each of the seven *loci*, the discriminatory capacity is really high and same allelic profile can be assigned as members of the same clone (Lin et al., 2014). But its major advantage is the ease of comparison of results with those of other laboratories (Eberle & Kiess, 2012).

Finally, new genetic technologies such as WGS have transformed the field of molecular epidemiology. This technique analyses the entire genome of the bacteria providing much more detailed information and thus, being able to generate high-resolution full genome data to differentially characterize bacterial strains that only differ in one base (Sabat et al., 2013). Among the several applications of this technique it is worth mentioning in the field of *Campylobacter* and *Salmonella* its integration into outbreak surveillance to differentiate between clusters and sporadic cases (Llarena et al., 2017), as well as the ability to predict antimicrobial-resistant phenotypes in isolates of *C. jejuni* and *C. coli* (Painset et al., 2020; Taylor et al., 2015).

3. Hypothesis and objectives

Over the last century, the remote regions of the Southern Ocean and Antarctica have experienced a raise of human presence, which increases the risk of the entry of new pathogens that can alter the health of wildlife populations.

The aim of this study is to determine the occurrence of the zoonotic enteric bacteria *Campylobacter* and *Salmonella* in seabirds from Southern Ocean sampled during 2020, to characterize isolates from previous years in terms of genetic diversity and to assess a potential reverse zoonosis in the region.

4. Materials and methods

4.1 Study area

The samples analysed in this study had previously been recovered from 2017 to 2020 from cloacal swabs and fresh faecal swabs from different species of seabirds and a Southern elephant seal that inhabit several islands in the Southern Ocean (Figure 2).

Figure 2. Map of the species sampled at each location.

4.2 Samples and isolates

Samples from 2020 had been collected from three different islands: Crozet (n=9), Kerguelen (n=20) and Amsterdam (n=31) and from 6 different seabird species and one elephant seal. All of them were culture-negative for the pathogens tested, thus in the present work we further analysed these samples for direct PCR detection of pathogens.

Campylobacter isolates selected for genotyping were recovered from cloacal samples of 5 different species of seabirds inhabiting King George Is. (n=1), Gough Is. (n=11), Crozet Is. (n=17), Kerguelen Is. (n=7), Amsterdam Is. (n=4) and Falkland Is. (n=14), with up to 5 isolates from each bird (Table 2).

Table 2. Isolates selected for *Campylobacter* genotyping

Location	Sampling date	N[a] (n)[b]	*C. lari*	*C. jejuni*	*C. coli*
King George Is.					
South polar skua	2017	1(1)	1	0	0
Gough base					
Subantarctic skua	2017	11(2)	3	8	0
Crozet Is.					
Lesser sheathbill	2017	4(1)	0	4	0
Southern giant Petrel	2019	3(1)	0	3	0
Subantarctic skua	2019	10(2)	0	10	0
Kerguelen					
Subantarctic skua	2018	2(1)	1	1	0
	2019	5(1)	5	0	0
Amsterdam Is.					
Subantarctic skua	2018	4(1)	0	4	0
Falkland Is.					
Subantarctic skua	2018	12(3)	9	0	2
	2019	2(2)	2	0	0
Total		54	22	30	2

[a] number of isolates recovered; [b] number of positive individuals

Salmonella isolates characterized in the present study were recovered from cloacal swab samples of skuas with up to 4 isolates from each bird. They were collected in Amsterdam Is. (n=21), Adelaide Is. (n=4) and Gough Is. (n=4) from 2017 to 2020.

Isolates from both pathogens had been stored in Brain heart infusion (BHI) broth with 20% of glycerol at – 75 ºC.

4.2 Genomic DNA extraction

4.2.1 Genomic DNA extraction from samples

Two different kits were tested for direct PCR detection from samples: Wizard Genomic DNA Purification Kit (TMO50, Promega corporations, Madison, WI, USA) and NucleoSpin (Rev.03, Macherey-Nagel, Mountain View, CA, USA). From an overall 60 samples, 31 were tested with both kits and the remaining 29 only with Wizard-Promega as better results were observed. DNA extraction was performed directly from the enriched samples in Bolton broth (Oxoid).

Wizard Genomic DNA purification kit was used following the protocol for isolating genomic DNA from gram-negative bacteria. Thus, 1ml of the sample was centrifuged at 13.000 rpm for 2 min and the supernatant was discarded. Then 600 µl of Nuclei Lysis Solution was added to resuspend the pellet and the mixture was incubated at 80ºC for 5 min. Next, 3 µl of RNase solution was added and it was incubated at 37ºC for 20 min. Afterwards 200 µl of Protein Precipitation Solution was added and the mixture was vortexed at high-speed, and incubated for 5 min in ice, followed by centrifugation at 13.000 rpm for 3 min. Next, the supernatant containing the DNA was transferred to a tube containing 600 µl of isopropanol, centrifuged with the same conditions and the supernatant was poured off to drain the tube, and 600 µl of 70% ethanol was added. Finally, we centrifuged to remove the supernatant. The pellet containing the DNA was rehydrated with 100 µl of DNA rehydration solution and was incubated at 4ºC overnight. The obtained DNA was stored at -20ºC until used.

The NucleoSpin kit was used following the protocol for genomic DNA purification from whole blood. First of all, we mixed and vortexed 200 µl of the sample with 25 µl of Proteinase K and 200 µl of B3 buffer solution and the resulting mixture was incubated at 70ºC for 15 min to allow cellular lysis. Next, the binding DNA step was performed by adding 210 µl of ethanol 96% and placing each preparation in a NucleoSpin Blood Column to centrifuge at 13,600 rpm for 3 min. Afterwards, 2 washes of the silica membrane present in the NucleoSpin Blood column were carried out by adding 500 µl of Buffer BW and 600 µl of Buffer B5 and centrifuging with the same conditions after

adding each solution. Finally, the silica membrane was dried by centrifuging again with the same conditions. The DNA was finally eluted with 100 µl of Buffer BE (70ºC) dispensed directly onto the silica membrane and centrifuging again and was stored at -20ºC until used.

4.2.2 Nucleic acid extraction from isolates

For the genetic characterization of *Campylobacter* and *Salmonella* isolates, DNA extraction was performed using the InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA, USA), following the recommendations of the manufacturer. Briefly, 250 µl of a bacterial suspension in PBS was mixed with 100 µl of matrix. The mixture was incubated 30 min at 56ºC and vortexed at high speed for 10 sec before placing it in a 100ºC heat block. The final mixture was centrifuged at 12.000 rpm for 2 min and the supernatant was collected and transferred to a new Eppendorf tube and stored at -20ºC until used.

4.3 *Campylobacter* spp. and *Salmonella* spp. identification

PCR detection of *Campylobacter* genus was conducted with specific primers C412F and CAMP R2 (Table 3). The reaction mix contained 12.5 µl of a PCR Master mix (M7502, Promega Corporation, Madison, WI, USA), 2.5 µl of a BSA (1 µg/ul), 1 µl (10 pmol/µl) from each primer and nuclease-free water up to total mix per sample of 22.5 µl, and 2.5 µl of DNA. The PCR cycling conditions used were: one cycle at 94ºC for 5 min, followed by 30 cycles at 94ºC for 1 min, 58ºC for 1 min and 72ºC for 1 min and final extension step at 72ºC for 7 min.

Campylobacter identification at species level was performed by a multiplex PCR for *C. jejuni/C. lari* and *C. jejuni/C. coli* using primers targeting the lipid A gene *lpxA* (Table 3). The reaction mixture was the same as for *Campylobacter* spp. and the PCR cycling conditions for both PCRs were: one cycle at 94ºC for 5 min, followed by 30 cycles at 94ºC for 1 min, 50ºC for 1 min and 72ºC for 1 min, with a final extension step at 72ºC for 7 min.

Salmonella spp. identification was performed by PCR with specific primers targeting the *invA* gene and including an internal control using primers targeting the 16S rDNA (Table

3). The reaction mixture was the same as above but with the corresponding primers. The PCR cycling conditions were: one cycle at 95ºC for 5 min, followed by 35 cycles at 95ºC for 30", 55ºC for 45" and 72ºC for 45" and a final extension step at 72ºC for 4 min.

As a positive control we used the type strains *C. jejuni* DSM 4688^T, *C. coli* DSM 4689^T, *C. lari* DSM 11375^T and *Salmonella* Typhimurium LT2, and as a negative control DNA was replaced by PCR-grade water.

PCR products were detected by gel electrophoresis using 1.8% agarose gel containing 0.2 µg/ml of ethidium bromide (BrEt) and running the gel at 120 V for 20 – 40 min. The DNA molecular weight marker used was 2-log DNA ladder (0.1 – 10.0 kb) (New England Biolabs, Ipswich, MA, USA).

4.4 *Campylobacter* genotyping

The genetic diversity of the *C. jejuni* isolates (n=30) was first studied by *fla*A-RFLP according to standardized method of Harrington et al.(2003) with the primers described in the Table 3 and the enzyme D*del*. The first step was to perform a PCR with the following reaction mixture: 25 µl Master Mix (Promega), 1.25 µl of each primer (10pmol/µl) and water up to a total volume of 47.5 µl per sample. The cycling conditions were: one cycle at 94ºC for 1 min, followed by 35 cycles at 94ºC for 15", 45ºC for 45" and 72ºC for 105" and final cycle at 72ºC for 5 min. The electrophoresis conditions to confirm the presence of amplified DNA were the same as indicated above.

This was followed by enzymatic digestion of the *fla*A gene, where 5 µl of the *flaA* amplicon was mixed with 1 µl of the restriction enzyme *Ddel* (HypF3I; FastDigest®, Thermo Fisher Scientific), 2 µl Buffer Green Day and 12 µ of nuclease-free water during 10 min at 37ºC and 5 min at 65ºC. Digest products were separated by electrophoresis on a 2.5% agarose gel in 1x TAE buffer at 90V for 180 min and staining with BrEt. DNA band patterns were then visualized under UV light. Isolates from the same animal showing identical *flaA*-RFLP profile were considered as the same clone and only one was included in the PFGE analyses.

The PFGE analysis was performed according to the standard operating procedure of PulseNet (www.pulsenetinternational.org) with *Sma*I restriction enzyme. *Salmonella Braenderup* H9812 restricted with *Xba*I was used as molecular size standard. Electrophoresis was performed in a CHEF-DR III System (Bio-Rad, Hercules, CA, USA).

4.5 *Salmonella* genotyping

We performed ERIC-PCR from a subset of *Salmonella* isolates (n=45) with the primers described in the Table 3. The reaction mix contained: 5 µl of 5x Green GoTaq Flexi Buffer (Promega, Madison, WI, USA), 3 µl of 25 mM $MgCl_2$ solution, 1.15 µl of each deoxynucleotide triphosphate (5 mM), 1.5 µl of each primer (20 µM), 0.15 units of *Taq* DNA polymerase and DNA quality water (sufficient to make final volume up to 25 µl per sample). PCR cycling conditions were: one cycle at 95ºC for 2 min, followed by 30 cycles at 94ºC for 3 sec, 50ºC for 1 min and 72ºC for 2.5 min and one more cycle of 72ºC for 20 min.

PCR products were separated by electrophoresis on a 2% Pure Agarose gel in TAE buffer 1X at 60 V for 180 min, then dyed with BrEt (0.05 µl/ml) and visualized under UV light. One isolate from each clone (isolates showing identical DNA band pattern) from each animal was chosen for subsequent serotyping (see below).

According to the serovars, isolates were further genotyped by PFGE following the standard operating procedure of PulseNet (www.pulsenetinternational.org). The restriction enzyme used was *Xba*I and the molecular size standard was the same as above. Electrophoresis conditions consisted of an initial switch time of 6.8s and a final switch time of 35.4s and gradient of 6 V/cm for 18h on a CHEF-DR III System (Bio-Rad).

Table 3. Primers sets used for different techniques

Technique	Gene	Primer	Sequence 5' – 3'	Annealing Tº (ºC)	Amplicon size (bp)	References
***Campylobacter* spp. PCR**	*16S* RNA	C412	GGTTACACTTTTCGGAGC	58	857	Linton et al., 1996
		CAMP R2	GGCTTCATGCTCTCGAGTT			
***Salmonella* spp. PCR**	*invA*	139	GTGAAATTATCGCCACGTTCGGGCAA	55	284	Rahn et al., 1992
		141	TCATCGCACCGTCAAAGGAACC			
	16S rDNA	Salmo-16S-F	CCGTCWATTCMTTTGAGTTT		560 - 580	Lane, 1991
		Salmo-16S-R	CTACGGGAGGCAGCAGT			
Campylobacter	*lpxA*	lpxA-*C. jejuni*	ACAACTTGGTGACGATGTTGTA	50	331	Klena et al., 2004
species-specific PCR		lpxA-RKK2m	CAATCATGDGCDATATGSAATAHGCCAT		521	
	lpxA	lpxA-*C. coli*	AGACAAATAAGAGAGAATCAG	50	391	Klena et al., 2004
		lpxA-RKK2m	CAATCATGDGCDATATGASAATAHGCCAT		521	
	lpxA	lpxA-*C. lari*	TRCCAAATGTTAAAATAGGCGA	50	233	Klena et al., 2004
		lpxA-RKK2m	CAATCATGDGCDATATGASAATAHGCCAT		521	
RFLP-flaA	*flaA*	A1	GGATTTCGTATTAACACAAATGGTGC	45	1700	Nachamkin et al., 1993, 1996
		A2	CTGTAGTAATCTTAAAACATTTTG			
ERIC-PCR	*hsdR*	ERIC-1R	CAGCCATGAACAACTGGTGGCG	50		Versalovic et al., 1991
		ERIC-2	TGCTTTGCGCAGGGAAGATTCC			

4.6 Analysis and comparison of band patterns

The RFLP and PFGE band patterns were analyzed using Fingerprinting II v3.0 software (Bio-Rad, Hercules, CA, USA). Similarity matrices were calculated using the Dice coefficient with tolerance and optimization values of 1.0%. Dendrograms were constructed based on an unweighted-pair group method with arithmetic mean (UPGMA) cluster analysis. A cut-off of 90% was used for the determination of the different profiles. PFGE data from previous studies from additional sampling sites from the Southern Ocean and Antarctic region were included in the analysis.

4.5 *Salmonella* serotyping

Salmonella serotyping was performed according to the Kauffmann-White scheme (Grimont & Weill, 2007) and carried out at the Laboratori Agroalimentari (Cabrils, Spain) of the Departament d'Acció Climàtica, Alimentació i Agenda Rural.

5. Results

No *Campylobacter* nor *Salmonella* were isolated from samples obtained during 2020, therefore we assayed the direct PCR detection of both pathogens from swab samples by using two DNA extraction kits. Initially, with a subset of 31 samples we used both kits and we obtained the same positive samples, but the Promega kit provided better results as stronger signals were obtained from the PCR. We also measured the amount and purity of DNA samples with BioDrop µLite (Fisher Scientific SL, Spain) from both extractions, observing a higher concentration of DNA in the samples extracted with Promega kit and a similar purity in both. Thus, with the remaining 29 samples we used only the Promega kit.

By direct PCR detection from swabs, we obtained an overall prevalence of *Campylobacter* of 16.67% and of 6.67% for *Salmonella.* Kerguelen Is. showed the highest *Campylobacter* prevalence (25%) whilst *Salmonella* was only detected in Amsterdam Is. with a prevalence of 12.9% (Table 4). Positive samples were mainly isolated from Subantarctic skua and Kelp gull. Also, multiplex PCR was carried out to determine the *Campylobacter* species and the results were negative.

Table 4. Sampling sites, hosts, sample size (November-December 2020 sampling) and pathogen occurrence

Location / Source	N	*Campylobacter sp.* (%)[a]	*Salmonella sp.* (%)[a]
Crozet Is.			
Subantarctic skua	9	2 (22,22)	0 (0%)
Kerguelen			
Subantarctic skua	1	1 (100)	0 (0%)
Kelp gull	8	4 (50)	0 (0%)
Kerguelen shag	7	0	0 (0%)
Southern elephant seal	3	0	0 (0%)
Southern giant petrel	1	0	0 (0%)
Total	20	5 (25)	0 (0%)
Amsterdam Is.			
Subantarctic skua	31	3 (9,68%)	4 (12,9%)
Total	60	10 (16,67%)	4 (6,67%)

[a] Promega DNA extraction

From previous samplings, where *Campylobacter* and *Salmonella* were isolated, we studied the genetic diversity of the isolates. For this we first determined the *Campylobacter* species from those isolates not previously identified at the species level (n= 13), and *Salmonella* serovars were determined at the Laboratori Agroalimentari. Once isolates were identified at specie level, in total we genotyped 54 isolates: 30 *C. jejuni,* 22 *C. lari* and 2 *C.coli*.

We used *flaA*-RFLP in the first instance to determine the genotypic diversity among 25 out of 30 *C. jejuni* isolates (the remaining 5 isolates had been previously typed). In most of the cases, isolates from the same individual showed identical RFLP profile, and therefore only one isolate per profile and bird was further analyzed by PFGE. Also, those isolates not typeable by RFLP (no band pattern obtained) were included in the PFGE analyses.

An overall of 18 *C. jejuni* isolates, 2 *C. coli* isolates and 12 *C. lari* isolates were selected for the PFGE analysis. All of them showed a high genetic diversity with a total of 8

different *KpnI*-PFGE pulsotypes (band patterns with ≥ 90% similarity) in the *C. jejuni* isolates, 10 in the *C. lari* isolates and 1 in the *C. coli* isolates. Despite the overall high genetic diversity, we found one *C. jejuni* pulsotype that included isolates from different years from the same locality, Crozet Is., and from different bird species, Subantarctic skua and Lesser sheathbill (Figure 3).

We also compared all these isolates with those from the Southern Ocean, Antarctic region and South Africa previously analyzed by our group and therefore present in our group PFGE database. We found that one *C. jejuni* isolate (MAR93-C1) from a King penguin (*Aptenodytes patagonicus*) sampled in Marion Is. was closely related (similarity of 86%) to the above-mentioned isolates from Crozet Is. (Figure 3). We also observed an 88% of similarity between one of our *C. lari* isolates (FK462-C1) and two isolates previously sampled on islands located thousands of Km apart (Marion Is. and Amsterdam Is.). Moreover, we found the same pulsotype among *C. lari* isolates from different bird species {Brown skua, Gentoo penguin (*Pygoscelis papua*), Kelp gull and Duck} and years (from 2009 to 2019) from Southern Ocean localities (Gough Is., Marion Is., Livingston and Falkland) and South Africa (Strandfontein, Cape Town). We found the same pulsotype (11 in total) in different localities of which three of them were from different bird species (Figure 4, highlighted in blue and yellow).

Salmonella isolates were genotyped with ERIC-PCR to determine genetic diversity among isolates from the same individual. In all but one individual, isolates from the same bird showed identical ERIC-PCR profile, but we also observed the same profile in three different individuals. One isolate per profile and individual was serotyped and was also further analysed by PFGE.

Overall, we found 4 different *Salmonella* serovars: Newport (44.8%), Enteritidis (31%), Typhimurium (13.8%) and Chincol (10.4%) (Table 5). Each *Salmonella*-positive bird carried a single *Salmonella* serovar, except those which were carriers of ser. Chincol, which also carried ser. Newport. Serovar Enteritidis was present in all the localities where a positive bird was found, while Newport and Typhimurium were present only in two locations (Gough Is. and Amsterdam) and Chincol was only isolated in Amsterdam Is.

Table 5: Sampling sites, hosts, year and *Salmonella* serovars occurrence

Location/Source	Year	N	Serovars			
			Enteritidis	Newport	Typhimurium	Chincol
Gough Is.						
Subantarctic skua	2017	4	2	1	1	0
Adelaide Is.						
South polar skua	2017	4	4	0	0	0
Amsterdam Is.						
Great skua	2017	9	0	6	0	3
Subantarctic skua	2018	1	0	1	0	0
	2019	9	3	5	1	0
	2020	2	0	0	2	0
Total		29	9	13	4	3

The *Salmonella* PFGE gel could not be analysed by Fingerprinting II v3.0 software due to technical problems. But in the images obtained we observed low genetic diversity within serovars. Serovar Enteritidis showed the same pulsotype among isolates from different individuals inhabiting two different localities (Adelaide Is. and Livingstone), and belonging also to different bird species. All but one isolate of ser. Typhimurium, showed the same pulsotype and all isolates of ser. Chincol showed the same pulsotype. Serovar Newport also showed a low clonality as the same profile was found in different individuals living on Amsterdam Is. and in one individual from a Gough Is. In addition, many of the isolates showing the same pulsotype were sampled in different years (data not shown).

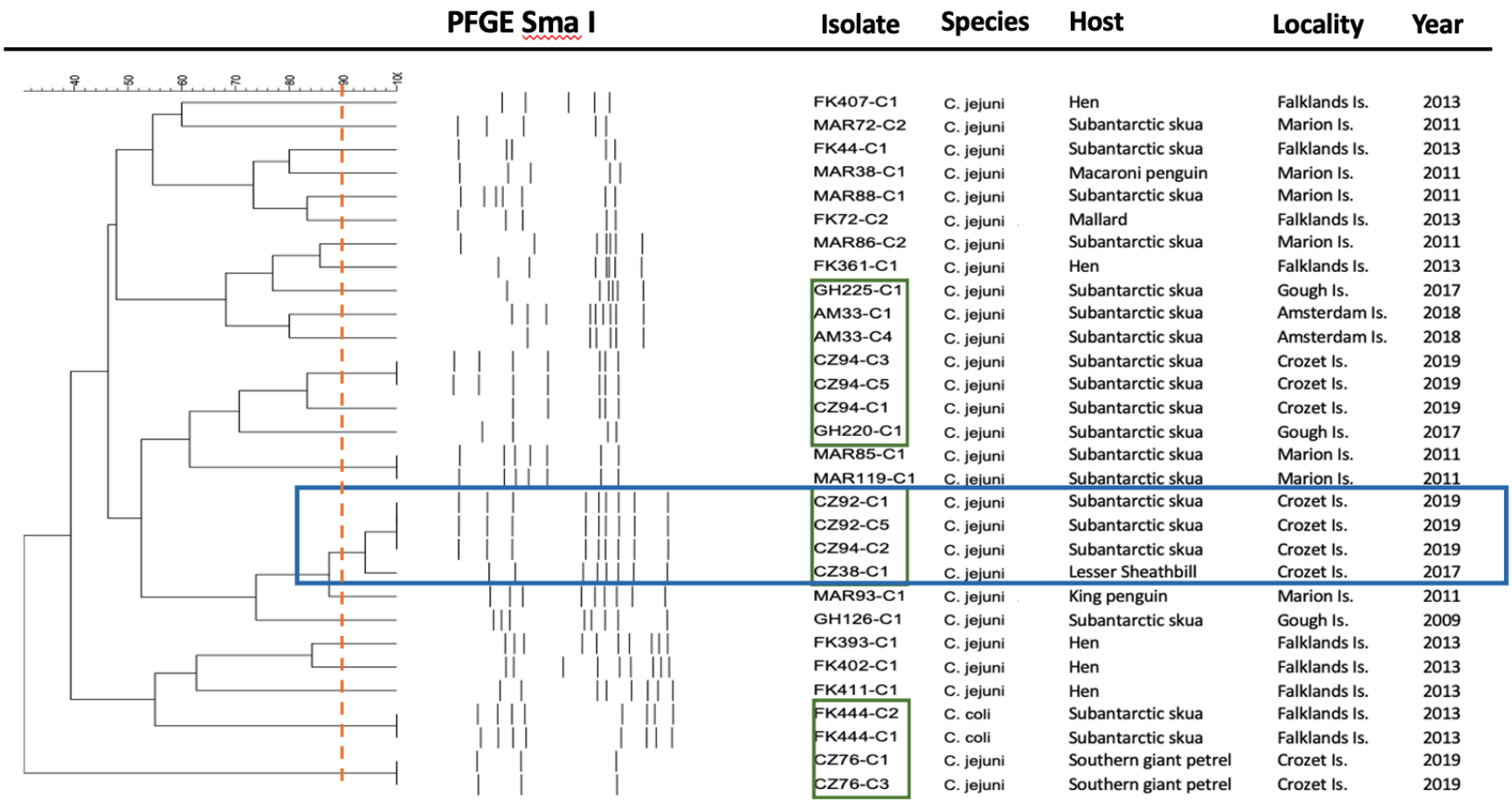


Figure 3. PFGE dendrogram of *C. jejuni* and C. coli isolates from sampling conducted between 2009 and 2019 at different Antarctic and Sub-Antarctic localities. Pulsotypes were assigned with a similarity level ≥ 90%. Blue squares highlight isolates from different seabird species showing ≥ 90% similarity and green squares highlight isolates genotyped in this study.

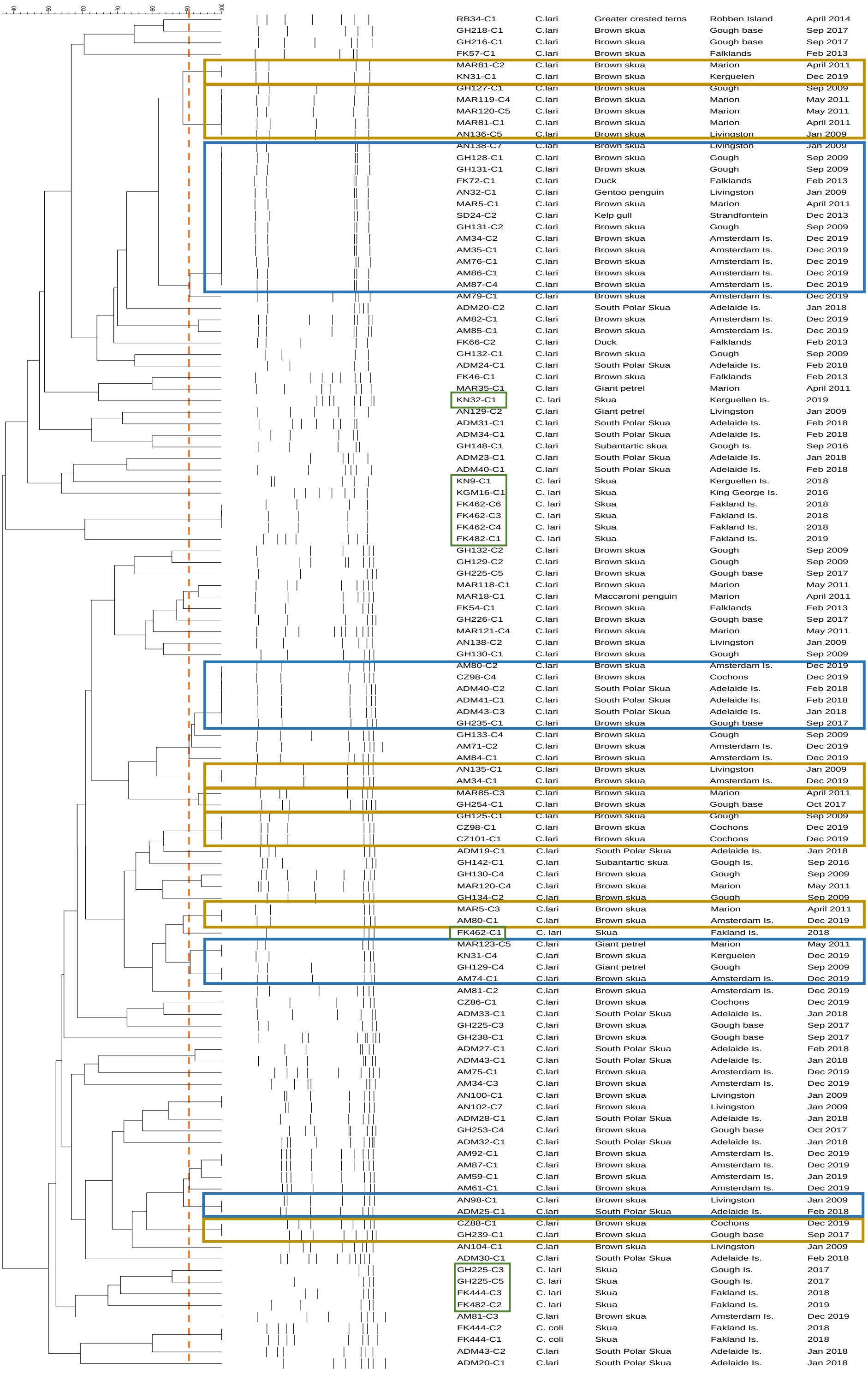


Figure 4. PFGE dendrogram of *C. lari* and *C. coli* isolates from sampling conducted between 2009 and 2019 at different Antarctic and sub-Antarctic localities. Pulsotypes were assigned with a similarity level ≥ 90%. Yellow squares highlight isolates from different localities showing ≥ 90% similarity and blue squares highlight isolates from different seabird species and localities showing ≥ 90%. Green squares highlight isolates analyzed in this study.

6. Discussion

Campylobacter spp. and *Salmonella* spp. are present in a wide range of reservoirs, but birds are ideal carriers as their body temperature is optimum for their growth and they usually act as asymptomatic carriers of these pathogens. A variety of studies have assessed their presence in poultry farms (Hald et al., 2016; Nair & Kollanoor Johny, 2019; Newell & Fearnley, 2003) as well as in wild birds inhabiting populated areas (Antilles et al., 2015; Keller et al., 2011). But in order to fully understand the epidemiology of these pathogens and the role of wildlife in their maintenance and spread in the environment, and the potential occurrence of reverse zoonosis, fauna inhabiting remote and unpopulated areas have also been studied (Cerdà-Cuéllar et al., 2019; Johansson et al., 2018). In this study we evaluated the occurrence of three *Campylobacter* species and *Salmonella* serovars and their genetic diversity in Sub-Antarctic islands.

By direct PCR detection from cloacal swabs, the overall *Campylobacter* prevalence we obtained in the 2020 sampling was 16,67%, but we failed to identify *Campylobacter* species. This might be because of the limit of detection of the species-specific PCRs or because the species tested were not present in the samples. The *Campylobacter* prevalence we have found is lower than that found in previous years by culture methods, especially in Amsterdam Is. where a prevalence up to 30% was found (unpublished data) while this year it was 9.7%. The *Campylobacter* prevalence obtained in Subantarctic skuas and Kelp gulls are in line with previous studies in the region (Cerdà-Cuéllar et al., 2019; Johansson et al., 2018; Leotta et al., 2006). The higher presence of these pathogens in these species seems to be influenced by their feeding habits, since as predators and sometimes scavengers they are more exposed to enteric bacteria than species with granivorous or insectivorous habits (Waldenström et al., 2002).

A total of 54 isolates from previous years have been identified at the species level, of which 22 are *C. lari*, 30 are *C. jejuni* and 2 are *C. coli*, with at least one species present in each of the sampled localities (King George Is., Gough Is., Crozet Is., Kerguelen Is., Amsterdam Is. and Falkland Is.). It is noteworthy to mention that *C. jejuni*, which is the major cause of foodborne diarrhoeal illness in humans worldwide (WHO, 2020), has been isolated from all the locations except Falkland Islands. Although this is not the first

time that *C. jejuni* has been reported in the Southern Ocean (Broman et al., 2000; Cerdà-Cuéllar et al., 2019; Johansson et al., 2018), many studies have failed to isolate it (Bonnedahl et al., 2005; García-Peña et al., 2017) while *C. lari* has been shown to be much more widespread in the fauna inhabiting these regions (Bonnedahl et al., 2005; Cerdà-Cuéllar et al., 2019; Johansson et al., 2018; Leotta et al., 2006).

The genetic diversity assessed by *flaA*-RFLP showed, in most cases, identical profiles in isolates from the same individual. A possible explanation may be because strains were selected due to their greater ability to survive prolonged transport or might be a result of highly host-adapted strains outcompeting and displacing less well adapted strains. To further deepen in the genetic analysis, PFGE was performed selecting strains from each individual with different *flaA*-RFLP profiles and also those that could not be analysed by *flaA*-RFLP.

Both molecular typing techniques showed a wide genetic diversity among *Campylobacter* isolates from different individuals, results that agree with previous studies carried on in the same regions (Cerdà-Cuéllar et al., 2019; García-Peña et al., 2010). Also, the widespread distribution of *C. lari* and its high genetic diversity suggests that these strains have been circulating for a long time in the studied region, although there are no conclusions about the origin of this colonisation (Johansson et al., 2018). On the other hand, while novel MLST sequence types have been found for *C. lari* strains in former studies, suggesting that those strains may be indigenous to the area (García-Peña et al., 2017; Johansson et al., 2018), for *C. jejuni,* genotypes exclusively reported in humans and livestock have been found as an evidence of a possible reverse zoonosis (Cerdà-Cuéllar et al., 2019).

Despite the diversity obtained with our isolates, when incorporating data from previous years we observed nearly identical *C. lari* isolates from different localities and samplings conducted 8 to 10 years apart, which suggests an endemic circulation of *C. lari* strains and certain connectivity among the Southern Ocean, as previously described (Cerdà-Cuéllar et al., 2019; García-Peña et al., 2010). These results highlight the role of seabirds as disseminators of enteric pathogens during their foraging and migration movements, facilitating the rapid spread of new pathogens throughout the Southern Ocean. This can

lead to mass mortality of the wildlife population such as that reported in New Zealand due to *Salmonella* serovar Typhimurium (Alley et al., 2002). Besides, the presence of same *C. lari* and *C. jejuni* strains in different bird species indicate that there might not be host specificity, at least within the seabird species studied.

The occurrence of *Salmonella* in wild birds has been extensively studied in both healthy and sick birds (Benskin et al., 2009) with higher occurrence in birds from urban areas rather than non-urban areas (Iveson et al., 2009). The overall *Salmonella* prevalence obtained (6,67%) in our study area is similar to that obtained in previous years in the region by our group (unpublished data) and is also consistent with the low number of *Salmonella* isolates detected in other studies in the Southern Ocean, suggesting that probably it is not an indigenous bacterium of this region (Cerdà-Cuéllar et al., 2019; Iveson et al., 2009; Palmgren et al., 2000; Vigo et al., 2011).

Our isolates belonged to 4 different serovars: Newport, Enteritidis, Typhimurium and Chincol, of which three of them (Enteritidis, Typhimurium and Newport) are among the five most commonly isolated serovars in human outbreaks in Europe and USA (EFSA & ECDC, 2018; Tack et al., 2019) and also, typically found in farm animals such as poultry or pork (Aung et al., 2020). Previous works that have evaluated *Salmonella* occurrence in wild birds have mainly identified the presence of serovars Typhimurium and Enteritidis in birds associated with urban areas (Jurado-Tarifa et al., 2016; Kaczorek-Łukowska et al., 2020; Moré et al., 2017; Refsum et al., 2002). Although much less frequently, these serovars have also been isolated in both, birds and mammals, inhabiting Antarctic and Sub-Antarctic regions (Cerdà-Cuéllar et al., 2019; Dougnac et al., 2015; Fenwick et al., 2004; Iveson et al., 2009; Olsen et al., 1996; Palmgren et al., 2000; Retamal et al., 2017; Vigo et al., 2011). The source of introduction of *Salmonella* into the Southern Ocean is still unknown, some studies have hypothesized a human origin with evidences of multidrug-resistant strains (Retamal et al., 2017) or the presence of strains with a sequence type with a worldwide distribution (Cerdà-Cuéllar et al., 2019).

Genetic diversity of *Salmonella* isolates depends largely on serovars. We found a low or no clonality among isolates from the same serovar, so that, different individuals sampled

in different localities and in some instances, belonging also to different bird species, had the same PFGE profile. These results, as suggested previously, may indicate an epidemiological link between different individuals due to transfer of the strains or a common source of infection (Moré et al., 2017). However, our results should be interpreted with caution as the analysis could not be done with the Fingerprinting software and, moreover, the high clonality of these *Salmonella* serovars requires a further analysis with a secondary enzyme (BlnI) to confirm the results.

For a more in-depth evaluation of the obtained results, further PFGE analysis with the secondary restriction enzyme for both *Campylobacter* and *Salmonella* are needed. Furthermore, for a better understanding of the origin of these isolates, complementary analyses such as MLST and antimicrobial susceptibility testing will be performed in the future. MLST will provide us information on whether the strains of this study have been previously isolated from other hosts or locations. In addition, assessment of the antimicrobial susceptibility of the isolated strains can provide important information on their potential anthropogenic origin.

The finding of certain *Salmonella s*erovars such as Typhimurium and Enteritidis in these pristine regions, suggests, as for certain *C. jejuni* genotypes, a reverse zoonosis. On the contrary, for *C. lari* the results suggest an endemic circulation and certain connectivity among the different locations of the Southern Ocean which may be caused by foraging or migratory movements of birds (Vigo et al., 2011). Future studies with an interdisciplinary approach are needed to determine whether human activity has led to the entry of new pathogens into these remote areas and how these may affect wildlife population.

7. Conclusions

1. In the Southern Ocean, *Campylobacter* is more prevalent than *Salmonella*.

2. Molecular typing techniques *flaA*-RFLP, ERIC-PCR and PFGE have demonstrated their usefulness in order to study the genetic diversity of *Campylobacter* and *Salmonella* from seabirds' isolates, providing insight into the epidemiology of these zoonotic agents. However further tests are needed, such as PFGE with a second enzyme, for a more in-depth analysis of the similarity among isolates.

3. *Campylobacter* isolates have shown high genetic diversity, but also, we found the same pulsotype in different localities, suggesting a certain connectivity among the Southern Ocean.

4. No host specificity was observed for *Campylobacter* isolates.

5. The presence of *Salmonella* serovars commonly isolated in humans, particularly ser. Enteritidis and Typhimurium, suggest a reverse zoonosis.

8. References

Alley, M. R., Connolly, J. H., Fenwick, S. G., Mackereth, G. F., Leyland, M. J., Rogers, L. E., Haycock, M., Nicol, C., & Reed, C. E. M. (2002). An epidemic of salmonellosis caused by *Salmonella* Typhimurium DT160 in wild birds and humans in New Zealand. *New Zealand Veterinary Journal*, *50*(5), 170–176. https://doi.org/10.1080/00480169.2002.36306

Altizer, S., Ostfeld, R. S., Johnson, P. T. J., Kutz, S., & Harvell, C. D. (2013). Climate change and infectious diseases: From evidence to a predictive framework. *Science*, *341*(6145), 514–519. https://doi.org/10.1126/science.1239401

Antilles, N., Sanglas, A., & Cerdà-Cuéllar, M. (2015). Free-living Waterfowl as a Source of Zoonotic Bacteria in a Dense Wild Bird Population Area in Northeastern Spain. *Transboundary and Emerging Diseases*, *62*(5), 516–521. https://doi.org/10.1111/tbed.12169

Ash, A., Lymbery, A., Lemon, J., Vitali, S., & Thompson, R. C. A. (2010). Molecular epidemiology of *Giardia duodenalis* in an endangered carnivore - The African painted dog. *Veterinary Parasitology, ELSEVIER*, *174*(3–4), 206–212. https://doi.org/10.1016/j.vetpar.2010.08.034

Aung, K. T., Khor, W. C., Octavia, S., Ye, A., Leo, J., Chan, P. P., Lim, G., Wong, W. K., Tan, B. Z. Y., Schlundt, J., Dalsgaard, A., Ng, L. C., & Lin, Y. N. (2020). Distribution of *Salmonella* serovars in humans, foods, farm animals and environment, companion and wildlife animals in Singapore. *International Journal of Environmental Research and Public Health*, *17*(16), 1–13. https://doi.org/10.3390/ijerph17165774

Baker, J., Barton, M. D., & Lanser, J. (1999). *Campylobacter* species in cats and dogs in South Australia. *Australian Veterinary Journal*, *77*(10), 662–666. https://doi.org/10.1111/j.1751-0813.1999.tb13159.x

Benskin, C. M. W. H., Wilson, K., Jones, K., & Hartley, I. R. (2009). Bacterial pathogens in wild birds: A review of the frequency and effects of infection. *Biological Reviews*, *84*(3), 349–373. https://doi.org/10.1111/j.1469-185X.2008.00076.x

Bonnedahl, J., Broman, T., Waldenström, J., Palmgren, H., Niskanen, T., Olsen, B., & Lundberg, C. (2005). In search of human-associated bacterial pathogens in antarctic wildlife: Report from six penguin colonies regularly visited by tourists. *Ambio*, *34*(6),

430–439. https://doi.org/10.1579/0044-7447-34.6.430

Borghello, P., Torres, D. S., Montalti, D., & Ibañez, A. E. (2019). Diet of the Brown Skua (*Stercorarius antarcticus lonnbergi)* at Hope Bay, Antarctic Peninsula: differences between breeders and non-breeders. *Polar Biology*, *42*(2), 385–394. https://doi.org/10.1007/s00300-018-2429-8

Brenner, F. W., Villar, R. G., Angulo, F. J., Tauxe, R., & Swaminathan, B. (2000). *Salmonella* Nomenclature. *Clinical Microbiology*, *38*(7), 2465–2467.

Broman, T., Bergström, S., On, S. L. W., Palmgren, H., McCafferty, D. J., Sellin, M., & Olsen, B. (2000). Isolation and characterization of *Campylobacter jejuni* subsp. *jejuni* from macaroni penguins (Eudyptes chrysolophus) in the subantarctic region. *Applied and Environmental Microbiology*, *66*(1), 449–452. https://doi.org/10.1128/AEM.66.1.449-452.2000

Carneiro, A. P. B., Manica, A., Clay, T. A., Silk, J. R. D., King, M., & Phillips, R. A. (2016). Consistency in migration strategies and habitat preferences of brown skuas over two winters, a decade apart. *Marine Ecology Progress Series*, *553*(July), 267–281. https://doi.org/10.3354/meps11781

Cerdà-Cuéllar, M., Moré, E., Ayats, T., Aguilera, M., Muñoz-González, S., Antilles, N., Ryan, P. G., & González-Solís, J. (2019). Do humans spread zoonotic enteric bacteria in Antarctica? *Science of the Total Environment*, *654*, 190–196. https://doi.org/10.1016/j.scitotenv.2018.10.272

Chlebicz, A., & Śliżewska, K. (2018). Campylobacteriosis, Salmonellosis, Yersiniosis, and Listeriosis as Zoonotic Foodborne Diseases: A Review. *International Journal of Environmental Research and Public Health*, *15*(5), 1–28. https://doi.org/10.3390/ijerph15050863

Coetzee, B. W. T., & Chown, S. L. (2016). A meta-analysis of human disturbance impacts on Antarctic wildlife. *Biological Reviews of the Cambridge Philosophical Society*, *91*(3), 578–596. https://doi.org/10.1111/brv.12184

Daczkowska-Kozon, E., & Brzostek-Nowakowska, J. (2001). *Campylobacter* spp. in waters of three main Western Pomerania water bodies. *International Journal of Hygiene and Environmental Health*, *203*(5–6), 435–443. https://doi.org/10.1078/1438-4639-00048

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2000). Emerging infectious diseases of wildlife - Threats to biodiversity and human health. *Science*, *287*(5452), 443–449. https://doi.org/10.1126/science.287.5452.443

Dougnac, C., Pardo, C., Meza, K., Arredondo, C., Blank, O., Abalos, P., Vidal, R., Fernandez, A., Fredes, F., & Retamal, P. (2015). Detection of *Salmonella enterica* in Magellanic penguins (*Spheniscus magellanicus*) of Chilean Patagonia: Evidences of inter-species transmission. *Epidemiology and Infection*, *143*(6), 1187–1193. https://doi.org/10.1017/S0950268814002052

Dunkley, K. D., Callaway, T. R., Chalova, V. I., McReynolds, J. L., Hume, M. E., Dunkley, C. S., Kubena, L. F., Nisbet, D. J., & Ricke, S. C. (2009). Foodborne *Salmonella* ecology in the avian gastrointestinal tract. *Anaerobe*, *15*(1–2), 26–35. https://doi.org/10.1016/j.anaerobe.2008.05.007

Eberle, K. N., & Kiess, A. S. (2012). Phenotypic and genotypic methods for typing *Campylobacter jejuni* and *Campylobacter coli* in poultry. *Poultry Science*, *91*(1), 255–264. https://doi.org/10.3382/ps.2011-01414

Epps, S. V. R., Harvey, R. B., Hume, M. E., Phillips, T. D., Anderson, R. C., & Nisbet, D. J. (2013). Foodborne *Campylobacter*: Infections, metabolism, pathogenesis and reservoirs. *International Journal of Environmental Research and Public Health*, *10*(12), 6292–6304. https://doi.org/10.3390/ijerph10126292

European Food Safety Authority. (2014). *Campylobacter*. *EFSA explains zoonotic diseases.* https://doi.org/10.2805/59450

European Food Safety Authority & European Centre for Disease Prevention and Control. (2018). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA Journal,* 16(12):5500, 262 pp. https://doi.org/10.2903/j.efsa.2018.5500

European Food Safety Authority, & European Centre for Disease Prevention and Control. (2021). The European Union One Health 2019 Zoonoses Report. *EFSA Journal,* 19(2):6406, 286 pp. https://doi.org/10.2903/j.efsa.2021.6406

Fakhr, M. K., Nolan, L. K., & Logue, C. M. (2005). Multilocus sequence typing lacks the discriminatory ability of pulsed-field gel electrophoresis for typing *Salmonella enterica* serovar Typhimurium. *Journal of Clinical Microbiology*, *43*(5), 2215–2219.

https://doi.org/10.1128/JCM.43.5.2215-2219.2005

Fenwick, S. G., Duignan, P. J., Nicol, C. M., Leyland, M. J., & Hunter, J. E. B. (2004). A comparison of *Salmonella* serotypes isolated from New Zealand sea lions and feral pigs on the Auckland Island by Pulsed-field gel electrophoresis. *Journal of Wildlife Diseases*, *40*(3), 566–570. https://doi.org/10.7589/0090-3558-40.3.566

Frenot, Y., Chown, S. L., Whinam, J., Selkirk, P. M., Convey, P., Skotnicki, M., & Bergstrom, D. M. (2005). Biological invasions in the Antarctic: Extent, impacts and implications. *Biological Reviews of the Cambridge Philosophical Society*, *80*(1), 45–72. https://doi.org/10.1017/S1464793104006542

García-Peña, F. J., Llorente, M. T., Serrano, T., Ruano, M. J., Belliure, J., Benzal, J., Herrera-León, S., Vidal, V., D'Amico, V., Pérez-Boto, D., & Barbosa, A. (2017). Isolation of *Campylobacter* spp. from Three Species of Antarctic Penguins in Different Geographic Locations. *EcoHealth*, *14*(1), 78–87. https://doi.org/10.1007/s10393-016-1203-z

García-Peña, F. J., Pérez-Boto, D., Jiménez, C., Miguel, E. S., Echeita, A., Rengifo-Herrera, C., García-Párraga, D., Ortega-Mora, L. M., & Pedraza-Díaz, S. (2010). Isolation and characterization of *Campylobacter* spp. from Antarctic fur seals (*Arctocephalus gazella)* at Deception Island, Antarctica. *Applied and Environmental Microbiology*, *76*(17), 6013–6016. https://doi.org/10.1128/AEM.00316-10

Gebreyes, W. A., Dupouy-Camet, J., Newport, M. J., Oliveira, C. J. B., Schlesinger, L. S., Saif, Y. M., Kariuki, S., Saif, L. J., Saville, W., Wittum, T., Hoet, A., Quessy, S., Kazwala, R., Tekola, B., Shryock, T., Bisesi, M., Patchanee, P., Boonmar, S., & King, L. J. (2014). The Global One Health Paradigm: Challenges and Opportunities for Tackling Infectious Diseases at the Human, Animal, and Environment Interface in Low-Resource Settings. *PLoS Neglected Tropical Diseases*, *8*(11). https://doi.org/10.1371/journal.pntd.0003257

Giannella, R. A. (1996). *Salmonella*. In S. Baron (Ed.), *Medical Microbiology* (4th ed.). University of Texas Medical Branch at Galveston.

Grimont, P. & Weill, F. X. (2007). Antigenic formulae of the *Salmonella* servovars. World Health Organitzation Collaborating Center for Reference and Research on *Salmonella*, Institut Pasteur, Paris. Available: http://www.pasteur.fr/ip/portal/ac-

tion/WebdriveActionEvent/oid/01s-000036

Hald, B., Skov, M. N., Nielsen, E. M., Rahbek, C., Madsen, J. J., Wainø, M., Chriél, M., Nordentoft, S., Baggesen, D. L., & Madsen, M. (2016). *Campylobacter jejuni* and *Campylobacter coli* in wild birds on Danish livestock farms. *Acta Veterinaria Scandinavica*, *58*(1), 1–10. https://doi.org/10.1186/s13028-016-0192-9

Harrington, C. S., Moran, L., Ridley, A. M., Newell, D. G., & Madden, R. H. (2003). Inter-laboratory evaluation of three flagellin PCR/RFLP methods for typing *Campylobacter jejuni* and *C. coli*: The CAMPYNET experience. *Journal of Applied Microbiology*, *95*(6), 1321–1333. https://doi.org/10.1046/j.1365-2672.2003.02101.x

Harris, L. J., Farber, J. N., Beuchat, L. R., Parish, M. E., Suslow, T. V., Garrett, E. H., & Busta, F. F. (2003). Outbreaks associated with fresh produce: Incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety*, *2*(1), 78–141. https://doi.org/10.1111/j.1541-4337.2003.tb00031.x

Iglesias-Torrens, Y., Miró, E., Guirado, P., Llovet, T., Muñoz, C., Cerdà-Cuéllar, M., Madrid, C., Balsalobre, C., & Navarro, F. (2018). Population structure, antimicrobial resistance, and virulence-associated genes in *Campylobacter jejuni* isolated from three ecological niches: Gastroenteritis patients, broilers, and wild birds. *Frontiers in Microbiology*, *9*(1676), 1–13. https://doi.org/10.3389/fmicb.2018.01676

Iveson, J. B., Shellam, G. R., Bradshaw, S. D., Smith, D. W., Mackenzie, J. S., & Mofflin, R. G. (2009). *Salmonella* infections in Antarctic fauna and island populations of wildlife exposed to human activities in coastal areas of Australia. *Epidemiology and Infection*, *137*(6), 858–870. https://doi.org/10.1017/S0950268808001222

Johansson, H., Ellström, P., Artursson, K., Berg, C., Bonnedahl, J., Hansson, I., Hernandez, J., Lopez-martín, J., Medina-Vogel, G., Moreno, L., Olsen, B., Engvall, E. O., Skarin, H., Troell, K., Waldenstro, J., Agren, J., & González-Acuña, D. (2018). Characterization of *Campylobacter* spp. isolated from wild birds in the Antarctic and Sub-Antarctic. *Plos One*, *13*(11), 1–12.

Jurado-Tarifa, E., Torralbo, A., Borge, C., Cerdà-Cuéllar, M., Ayats, T., Carbonero, A., & García-Bocanegra, I. (2016). Genetic diversity and antimicrobial resistance of

Campylobacter and *Salmonella* strains isolated from decoys and raptors. *Comparative Immunology, Microbiology and Infectious Diseases*, *48*, 14–21. https://doi.org/10.1016/j.cimid.2016.07.003

Kaakoush, N. O., Castaño-Rodríguez, N., Mitchell, H. M., & Man, S. M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Reviews*, *28*(3), 687–720. https://doi.org/10.1128/CMR.00006-15

Kaczorek-Łukowska, E., Sowińska, P., Franaszek, A., Dziewulska, D., Małaczewska, J., & Stenzel, T. (2020). Can domestic pigeon be a potential carrier of zoonotic *Salmonella*? *Transboundary and Emerging Diseases*, *March*, 1–13. https://doi.org/10.1111/tbed.13891

Keller, J. I., Gregory Shriver, W., Waldenström, J., Griekspoor, P., & Olsen, B. (2011). Prevalence of *Campylobacter* in wild birds of the mid-atlantic region, USA. *Journal of Wildlife Diseases*, *47*(3), 750–754. https://doi.org/10.7589/0090-3558-47.3.750

Klena, J. D., Parker, C. T., Knibb, K., Claire Ibbitt, J., Devane, P. M. L., Horn, S. T., Miller, W. G., & Konkel, M. E. (2004). Differentiation of *Campylobacter coli, Campylobacter jejuni, Campylobacter lari,* and *Campylobacter upsaliensis* by a multiplex PCR developed from the nucleotide sequence of the lipid A gene *lpxA*. *Journal of Clinical Microbiology*, *42*(12), 5549–5557. https://doi.org/10.1128/JCM.42.12.5549-5557.2004

Lane D.J. (1991). 16S/23S rRNA sequencing. In E. Stackebrandt and M. Goodfellow (Ed.), *Nucleic acid techniques in bacterial systematics* (pp. 115 - 117). John Wiley and Sons, New York.

Leotta, G., Vigo, G., & Giacoboni, G. (2006). Isolation of *Campylobacter lari* from seabirds in Hope Bay, Antarctica. *Polish Polar Research*, *27*(4), 303–308.

Lin, T., Lin, L., & Zhang, F. (2014). Review on Molecular Typing Methods of Pathogens. *Open Journal of Medical Microbiology*, *04*(03), 147–152. https://doi.org/10.4236/ojmm.2014.43017

Linton, D., Owen, R. J., & Stanley, J. (1996). Rapid identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals. *Research in Microbiology*, *147*(9), 707–718. https://doi.org/10.1016/S0923-2508(97)85118-2

Llarena, A., Taboada, E., & Rossi, M. (2017). Whole-Genome Sequencing in Epidemiology of *Campylobacter jejuni* Infections. *Journal of Clinical Microbiology*, *55*(5), 1269–1275.

Mackenzie, J. S., & Jeggo, M. (2019). The one health approach: Why is it so important? *Tropical Medicine and Infectious Disease*, *4*(2), 5–8. https://doi.org/10.3390/tropicalmed4020088

Man, S. M. (2011). The clinical importance of emerging *Campylobacter* species. *Nature Reviews Gastroenterology and Hepatology*, *8*(12), 669–685. https://doi.org/10.1038/nrgastro.2011.191

Messenger, A. M., Barnes, A. N., & Gray, G. C. (2014). Reverse zoonotic disease transmission (Zooanthroponosis): A systematic review of seldom-documented human biological threats to animals. *PLoS ONE*, *9*(2), 1–9. https://doi.org/10.1371/journal.pone.0089055

Moore, J. E., Corcoran, D., Dooley, J. S. G., Fanning, S., Lucey, B., Matsuda, M., McDowell, D. A., Mégraud, F., Millar, C. B., O'Mahony, R., O'Riordan, L., O'Rourke, M., Rao, J. R., Rooney, P. J., Sails, A., & Whyte, P. (2005). *Campylobacter*. *Veterinary Research*, *36*(5–6), 351–382. https://doi.org/10.1051/vetres

Moré, E., Ayats, T., Ryan, P. G., Naicker, P. R., Keddy, K. H., Gaglio, D., Witteveen, M., & Cerdà-Cuéllar, M. (2017). Seabirds (*Laridae*) as a source of *Campylobacter* spp., *Salmonella* spp. and antimicrobial resistance in South Africa. *Environmental Microbiology*, *19*(10), 4164–4176. https://doi.org/10.1111/1462-2920.13874

Munro, K. J., & Burg, T. M. (2017). A review of historical and contemporary processes affecting population genetic structure of Southern Ocean seabirds. *Emu - Austral Ornithology 117*(1) 4–18. https://doi.org/10.1080/01584197.2016.1271988

Nachamkin, I., Bohachick, K., & Patton, C. M. (1993). Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. *Journal of Clinical Microbiology*, *31*(6), 1531–1536. https://doi.org/10.1128/jcm.31.6.1531-1536.1993

Nachamkin, I., Ung, H., & Patton, C. M. (1996). Analysis of HL and O serotypes of *Campylobacter* strains by the flagellin gene typing system. *Journal of Clinical Microbiology*, *34*(2), 277–281. https://doi.org/10.1128/jcm.34.2.277-281.1996

Nair, D. V. T., & Kollanoor Johny, A. (2019). *Salmonella* in Poultry Meat Production In K. Venkitanarayanan, S. Thakur, & S. C. Ricke (Eds.), *Food Safety in Poultry Meat Production* (pp. 1–24). Springer. https://doi.org/10.1007/978-3-030-05011-5_1

Nebola, M., & Steinhauserova, I. (2006). PFGE and PCR/RFLP typing of *Campylobacter jejuni* strains from poultry. *British Poultry Science*, *47*(4), 456–461. https://doi.org/10.1080/00071660600829159

Newell, D. G., & Fearnley, C. (2003). Sources of *Campylobacter* Colonization in Broiler Chickens. *Applied and Environmental Microbiology Journal*, *69*(8), 4343–4351. https://doi.org/10.1128/AEM.69.8.4343

Olsen, B., Bergström, S., McCafferty, D. J., Sellin, M., & Wiström, J. (1996). *Salmonella enteritidis* in antarctica: zoonosis in man or humanosis in penguins? *Lancet*, *348*, 1319–1320. https://doi.org/10.1016/s0140-6736(05)65806-0

Painset, A., Day, M., Doumith, M., Rigby, J., Jenkins, C., Grant, K., Dallman, T. J., Godbole, G., & Swift, C. (2020). Comparison of phenotypic and WGS-derived antimicrobial resistance profiles of *Campylobacter jejuni* and *Campylobacter coli* isolated from cases of diarrhoeal disease in England and Wales, 2015-16. *Journal of Antimicrobial Chemotherapy*, *75*(4), 883–889. https://doi.org/10.1093/jac/dkz539

Palmgren, H., McCafferty, D., Aspán, A., Broman, T., Sellin, M., Wollin, R., Bergström, S., & Olsen, B. (2000). *Salmonella* in sub-Antarctica: Low heterogeneity in *Salmonella* serotypes in South Georgian seals and birds. *Epidemiology and Infection*, *125*(2), 257–262. https://doi.org/10.1017/S0950268899004586

Polo, F., Figueras, M. J., Inza, I., Sala, J., Fleisher, J. M., & Guarro, J. (1998). Relationship between presence of *Salmonella* and indicators of faecal pollution in aquatic habitats. *FEMS Microbiology Letters*, *160*(2), 253–256. https://doi.org/10.1016/S0378-1097(98)00040-8

Pui, C. F., Wong, W. C., Chai, L. C., Tunung, R., Jeyaletchumi, P., Noor Hidayah, M. S., Ubong, A., Farinazleen, M. G., Cheah, Y. K., & Son, R. (2011). *Salmonella*: A foodborne pathogen. *International Food Research Journal*, *18*, 465–473.

Quessy, S., & Messier, S. (1992). Prevalence of *Salmonella* spp., *Campylobacter* spp. and *Listeria* spp. in ring-billed gulls (*Larus delawarensis*). *Journal of Wildlife Diseases, 28*(4), 526–531.

Rahn, K., De Grandis, S. A., Clarke, R. C., McEwen, S. A., Galán, J. E., Ginocchio, C., Curtiss III, R., & Gyles, C. L. (1992). Amplification of invA gene of *Salmonella* by polymerase chain reaction (PCR) as a specific method for detection of *Salmonellae*. *Molecular and Cellular Probes*, *6*, 271–279.

Ramos, A. P., Leonhard, S. E., Halstead, S. K., Cuba, M. A., Castañeda, C. C., Dioses, J. A., Tipismana, M. A., Abanto, J. T., Llanos, A., Gourlay, D., Grogl, M., Ramos, M., Rojas, J. D., Meza, R., Puiu, D., Sherman, R. M., Salzberg, S. L., Simner, P. J., Willison, H. J., … Pardo, C. A. (2021). Guillain-Barré Syndrome Outbreak in Peru 2019 Associated With *Campylobacter jejuni* Infection. *Neurology(R) Neuroimmunology & Neuroinflammation*, *8*(2), 1–12. https://doi.org/10.1212/NXI.0000000000000952

Ramos, R., Cerdà-Cuéllar, M., Ramírez, F., Jover, L., & Ruiz, X. (2010). Influence of Refuse Sites on the Prevalence of *Campylobacter* spp. and *Salmonella* Serovars in Seagulls. *Applied and Environmental Microbiology*, *76*(9), 3052–3056. https://doi.org/10.1128/AEM.02524-09

Refsum, T., Handeland, K., Baggesen, D. L., Holstad, G., & Kapperud, G. (2002). *Salmonellae* in avian wildlife in Norway from 1969 to 2000. *Applied and Environmental Microbiology*, *68*(11), 5595–5599. https://doi.org/10.1128/AEM.68.11.5595-5599.2002

Retamal, P., Llanos-Soto, S., Salas, L. M., López, J., Vianna, J., Hernández, J., Medina-Vogel, G., Castañeda, F., Fresno, M., & González-Acuña, D. (2017). Isolation of drug-resistant *Salmonella enterica* serovar enteritidis strains in gentoo penguins from Antarctica. *Polar Biology*, *40*(12), 2531–2536. https://doi.org/10.1007/s00300-017-2163-7

Ruiz-Palacios, G. M. (2007). The health burden of *Campylobacter* infection and the impact of antimicrobial resistance: Playing chicken. *Clinical Infectious Diseases*, *44*(5), 701–703. https://doi.org/10.1086/509936

Sabat, A. J., Budimir, A., Nashev, D., Sá-Leão, R., van Dijl, J. M., Laurent, F., Grundmann, H., & Friedrich, A. W. (2013). Overview of molecular typing methods for outbreak detection and epidemiological surveillance. *Eurosurveillance*, *18*(4), 1–15. https://doi.org/10.2807/ese.18.04.20380-en

Saxena, M. K., Singh, V. P., Lakhcharua, B. D., Taj, G., & Sharma, B. (2002). Strain

differentiation of Indian isolates of *Salmonella* by ERIC-PCR. *Research in Veterinary Science*, *73*(3), 313–314. https://doi.org/10.1016/S0034-5288(02)00030-9

Secundo de Souza, A. I., De reitas Neto, O. C., Alves Batista, D. F., Celis Estupinan, A. L. del P., De Almeida, A. M., Barrow, P. A., & Berchieri, A. (2015). ERIC PCR genotyping of field isolates of *Salmonella enterica* subsp. *enterica* serovar Gallinarum biovars Gallinarum and Pullorum. *Avian Pathology*, *44*(6), 475–479. https://doi.org/https://doi.org/10.1080/03079457.2015.1086975

Silva, J., Leite, D., Fernandes, M., Mena, C., Gibbs, P. A., & Teixeira, P. (2011). *Campylobacter* spp. As a foodborne pathogen: A review. *Frontiers in Microbiology*, *2*(SEP), 1–12. https://doi.org/10.3389/fmicb.2011.00200

Stevens, M. P., Humphrey, T. J., & Maskell, D. J. (2009). Molecular insights into farm animal and zoonotic *Salmonella* infections. *Philosophical Transactions of the Royal Society B*, *364*(1530), 2708–2723. https://doi.org/10.1098/rstb.2009.0094

Su, L. H., & Chiu, C. H. (2007). *Salmonella*: Clinical importance and evolution of nomenclature. *Chang Gung Medical Journal*, *30*(3), 210–219.

Su, L. H., Chiu, C. H., Chu, C., & Ou, J. T. (2004). Antimicrobial resistance in nontyphoid *Salmonella* serotypes: A global challenge. *Clinical Infectious Diseases*, *39*(4), 546–551. https://doi.org/10.1086/422726

Swanenburg, M., Urlings, H. A. P., Keuzenkamp, D. A., & Snijders, J. M. A. (1998). Validation of ERIC PCR as a tool in epidemiologic research of *Salmonella* in slaughter pigs. *Journal of Industrial Microbiology and Biotechnology*, *21*(3), 141–144. https://doi.org/10.1038/sj.jim.2900568

Szczepanska, B., Andrzejewska, M., Spica, D., & Klawe, J. J. (2017). Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from children and environmental sources in urban and suburban areas. *BMC Microbiology*, *17*(1), 1–9. https://doi.org/10.1186/s12866-017-0991-9

Taboada, E. N., Clark, C. G., Sproston, E. L., & Carrillo, C. D. (2013). Current methods for molecular typing of *Campylobacter* species. *Journal of Microbiological Methods*, *95*(1), 24–31. https://doi.org/10.1016/j.mimet.2013.07.007

Tack, D. M., Marder, E. P., Griffin, P. M., Cieslak, P. R., Dunn, J., Hurd, S., Scallan, E., Lathrop, S., Muse, A., Ryan, P., Smith, K., Tobin-D'Angelo, M., Vugia, D. J., Holt, K.

G., Wolpert, B. J., Tauxe, R., & Geissler, A. L. (2019). Preliminary incidence and trends of infections with pathogens transmitted commonly through food — Foodborne Diseases Active Surveillance Network, 10 U.S. sites, 2015–2018. In *Morbidity and Mortality Weekly Report.* Centers for Disease Control and Prevention *68*(16). https://doi.org/10.1111/ajt.15412

Taylor, A. J., Lappi, V., Wolfgang, W. J., Lapierre, P., Palumbo, M. J., Medus, C., & Boxrud, D. (2015). Characterization of foodborne outbreaks of *Salmonella enterica* serovar enteritidis with whole-genome sequencing single nucleotide polymorphism-based analysis for surveillance and outbreak detection. *Journal of Clinical Microbiology*, *53*(10), 3334–3340. https://doi.org/10.1128/JCM.01280-15

Taylor, L. H., Latham, S. M., & Woolhouse, M. E. J. (2001). Risk factors for human disease emergence. *Philosophical Transactions of the Royal Society B*, *356*(1411), 983–989. https://doi.org/10.1098/rstb.2001.0888

Versalovic, J., Koeuth, T., & Lupski, R. (1991). Distribution of repetitive DNA sequences in eubacteria and application to finerpriting of bacterial enomes. *Nucleic Acids Research*, *19*(24), 6823–6831. https://doi.org/10.1093/nar/19.24.6823

Vigo, G. B., Leotta, G. A., Caffer, M. I., Salve, A., Binsztein, N., & Pichel, M. (2011). Isolation and characterization of *Salmonella enterica* from Antarctic wildlife. *Polar Biology*, *34*(5), 675–681. https://doi.org/10.1007/s00300-010-0923-8

Waldenström, J., Broman, T., Carlsson, I., Hasselquist, D., Achterberg, R. P., Wagenaar, J. A., & Olsen, B. (2002). Prevalence of *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* in Different Ecological Guilds and Taxa of Migrating Birds. *Applied and Environmental Microbiology Journal*, *68*(12), 5911–5917. https://doi.org/10.21825/vdt.v83i4.16642

Whiley, H., van den Akker, B., Giglio, S., & Bentham, R. (2013). The role of environmental reservoirs in human campylobacteriosis. *International Journal of Environmental Research and Public Health*, *10*(11), 5886–5907. https://doi.org/10.3390/ijerph10115886

Woods, R., Jones, H. I., Watts, J., Miller, G. D., & Shellam, G. R. (2009). Diseases of Antarctic Seabirds. In K. R. Kerry & M. Riddle (Eds.), *Health of Antarctic Wildlife: A Challenge for Science and Policy* (pp. 35–55). Springer Berlin Heidelberg.

https://doi.org/10.1007/978-3-540-93923-8_3

World Health Organization. (2020). *Campylobacter*. Availiable: https://www.who.int/news-room/fact-sheets/detail/campylobacter