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Salmonella in sub-Antarctica: low heterogeneity in salmonella serotypes in South Georgian seals and birds

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SUMMARY

The number of human visitors to Antarctica is increasing rapidly, and with it a risk of introducing infectious organisms to native animals. To study the occurrence of salmonella serotypes in sub-Antarctic wildlife, faecal samples were collected from gentoo penguins, macaroni penguins, gray-headed albatrosses, black-browed albatrosses and Antarctic fur seals on Bird Island in the South Georgian archipelago during the austral summer of 1996 and 1998. In 1996, *S. havana*, *S. typhimurium* and *S. enteritidis* were isolated from 7% of gentoo penguins and 4% of fur seals. In 1998, however, 22% of fur seals were found to be infected with *S. havana*, *S. enteritidis* and *S. newport*. All isolates, except one, showed identical pulsed-field gel electrophoresis-patterns within each serotype, irrespective of sampling year and animal reservoir. No significant antibiotic resistance was found. The very low heterogeneity in the salmonella isolates found could either indicate a high genetic adaptation of the bacteria to the environment or a recent introduction of salmonella into the area.

INTRODUCTION

In recent years, there has been an increase in the number of humans visiting Antarctica [1]. As a consequence, there is now a risk of humans introducing infectious organisms into the region, which could cause diseases that are new to the endemic animal species. For example, disposal of poultry waste at scientific stations in Antarctica is suggested to be a source of infection in penguins [2]. In addition, antibodies to Infectious Bursal Disease Virus and Newcastle Disease Virus, have been detected in a range of penguin species breeding close to Antarctic bases [2, 3]. Salmonella serotypes known to be human pathogens have also been found in penguins [4, 5]. The origin of these pathogens is unknown. However, human derived micro-organisms have been detected in sewage outlets and waste dumps from Antarctic stations [6, 7] and damage to the fauna has been caused by accidental pollution from sewage [8]. Low water temperature allows these bacteria to survive in the marine environment for long periods [9], where transfer to seabirds and seals could take place.

Salmonella is a cause of food borne disease in

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humans and domestic animals, even though some animals and humans can also be apparently healthy carriers. Wild birds are known to be carriers of the genus [10–15], yet, little is known about the frequency of disease in wild animal populations, although there are reports of severe salmonella infection in these animals [16–19]. The wide ranging behaviour of seabirds and marine mammals makes them not only likely to encounter pathogens associated with man, but also potential vectors of bacteria to remote areas such as the Antarctic region.

The aim of this study was to investigate the presence of salmonella in a population of sub-Antarctic seabirds and seals and to characterize further the isolates found in terms of serotype, phagetype and genotype.

METHODS

Sampling

Samples were collected from various seabird species and Antarctic fur seals (*Arctocephalus gazella*) on the sub-Antarctic island of Bird Island (54° 00′ S, 38° 02′ W), South Georgia. In February and March 1996, faecal swabs were taken from 40 pups of *A. gazella*, 30 adult gentoo penguins (*Pygoscelis papua*), 50 macaroni penguin chicks (*Eudyptes chrysolophus*), 50 black-browed albatross chicks (*Diomedea melanophrys*) and 50 grey-headed albatross chicks (*D. chrysostoma*) (Table 1). Sampling was repeated in February and March 1998, when swabs were collected from 206 Antarctic fur seal pups, 100 macaroni penguin chicks, 100 gray-headed and 40 black-browed albatross chicks (Table 1). Pups and chicks were caught by hand, and adult fur seals were captured using a standard noose pole [20]. Animals were sampled at two locations on Bird Island: Jordan Cove close to the research station and Johnson Cove situated two kilometres from the station. Faecal samples were collected using cotton wool swabs inserted into the rectum. Samples were stored in a charcoal transport medium (Transwab, BioDisc, Solna, Sweden) at 5–10 °C and transported to Sweden, where they were cultured within 3 weeks from the date collected.

Isolation and identification of bacteria

Each sample was enriched in selenite broth (Oxoid AB, Stockholm, Sweden) and incubated at 37 °C for 18–24 h. This was subcultured on xylin-lysine-desoxycholate agar and H_2S positive colonies were verified as salmonella by their reaction in fermentation tests. Serotyping was carried out according to the Kauffmann–White scheme and phage typing of *S. typhimurium* was performed according to Anderson [21] and *S. enteritidis* according to Ward [22].

Pulsed-field gel electrophoresis

Restriction enzyme digests for pulsed-field gel electrophoresis (PFGE) were performed with *Spe*I, *Bln*I and *Xba*I (Boehringer–Mannheim, GmbH, Germany). Each salmonella isolate was analysed with all three enzymes. The isolates were grown on blood agar at 37 °C for 18–20 h and 4 colonies were dispersed in 1 ml TEN-buffer (1 M NaCl, 10 mM Tris pH 8·0, 10 mM EDTA) and centrifuged at 6000 rpm. The bacteria were suspended in 250 μl lysis buffer (1 M NaCl, 10 mM Tris pH 8·0, 200 mM EDTA, 0·5% Sacrosyl, 0·2% Sodium deoxycholate) and embedded in 2% Low Melt Prep Agarose (Bio–Rad, Richmond, CA, USA) with 35 μl (20 mg/ml) lysozyme per agarose slice. The slices were incubated in 2·5 ml lysis buffer with 85 μl proteinase K (1 mg/ml final concentration) at 56 °C for 36 h, and then washed 6 times in 1 × TE-buffer. Half of each agarose slice was incubated for 18 h with 20 U of respective restriction enzyme in 100 μl enzyme buffer at 37 °C. One mm of each slice was run on a 1% agarose gel (Pulsefield Certified Agarose, Bio–Rad) in 10% PFGE buffer (Bio–Rad) at 10 °C, on an automated PFGE apparatus (Gene Path, Bio–Rad). Standard programmes for fragment sizes 50–400 kb (*Spe*I and *Xba*I) and 50–700 kb (*Bln*I) were used and a standard lambda DNA ladder (New England Biolabs Inc, MA, USA) was run alongside the samples. The gel was stained with 0·2% ethidium bromide, washed in tap water, and photographed using a DS34 Polaroid camera (Bio–Rad).

Analysis of clonality between isolates belonging to the same serotype was based on 30–35 restriction fragments per serotype. Fragments sized < 100 kb were excluded to minimize the effect of plasmid fragments. Gels were analysed visually except for *S. newport* isolates which were compared using GelCompar version 4.0 (Applied Maths, Kortrijk, Belgium). Polaroid photographs of macrorestriction profiles were scanned with a UMAX Vista-S6E scanner (UMAX Technologies Inc, CA, USA) and

Table 1. *Number of samples and* Salmonella *spp. isolates from faecal swabs of birds and seals on Bird Island (1996 and 1998)*

	1996		1998	
Species	No.	Salmonella isolates *n* (%)	No.	Salmonella isolates *n* (%)
Antarctic fur seal (*Arctocephalus gazella*)	40	2 (5)	206	45 (22)
Gentoo penguin (*Pygoscelis papua*)	30	2 (7)	0	
Macaroni penguin (*Eudyptes chrysolophus*)	50	0	100	0
Black-browed albatross (*Diomedea melanophrys*)	50	0	40	1 (2)
Grey-headed albatross (*Diomedea chrysostoma*)	50	0	100	0
Total	220	4 (2)	446	46 (10)

Table 2. *Distribution of serotype and reservoirs of the 50* salmonella *isolates from Bird Island*

		n				
Serotype	Phage type	1996	1998	Seals	Penguins	Albatrosses
S. havana	ND	2	15	16	1 (1996)	0
S. typhimurium	DT 150	1	0	1	0	0
S. enteritidis	PT 4, PT 4-like, PT 35	1	6	6	1 (1996)	0
S. newport	ND	0	24	23	0	1 (1998)
Salmonella spp. (not serotypeable)	ND	0	1	1	0	0
Total		4	46	47	2	1

digitalized using Adobe Photoshop 3.0.5 for Windows, and saved in TIFF format. Banding patterns of combined gels were compared by the UPGMA (Unweighted Pair Group Method with Arithmetic averages) clustering method using the Dice coefficient, according to the manufacturer's instruction. A band position tolerance of 1·2% was applied.

Antimicrobial susceptibilities

The susceptibility of isolates to sulfisoxazole, streptomycin, ciprofloxacin, gentamicin, ampicillin, trimethoprim, and chloramphenicol was determined by disk diffusion with paper discs on PDM agar (AB Biodisk, Solna, Sweden) according to the protocol from the Swedish Reference Group for Antibiotic and Resistance Methods (RAF-M) (http://ltkronoberg.se/ext/raf/raf.htm).

RESULTS

In the bacteriological survey of 1996, four salmonella isolates of three different serotypes were found (Table 1). *S. havana* was isolated from a fur seal pup and a gentoo penguin, *S. typhimurium* definitive type (DT) 150 from an Antarctic fur seal pup, and *S. enteritidis* phage type (PT) 4 from a gentoo penguin (Table 2). The survey of 1998 revealed a much higher incidence of salmonella, with 45 (22%) of the 206 fur seal pups positive for the organisms. Of these positive samples, 24 (52%) were *S. newport*, 15 (33%) *S. havana* and 6 (13%) *S. enteritidis*. One isolate was not serotypeable (Table 2). One of the 40 faecal samples from black-

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browed albatrosses was positive for *S. newport* but *Salmonella* spp. were not detected in samples from 100 macaroni penguins and 100 grey-headed albatrosses (Table 1).

All Bird Island salmonella isolates, except one *S. newport*, exhibited identical PFGE patterns within each serotype, irrespective of sampling year or reservoir. The exception was an isolate from an Antarctic fur seal that showed a slight difference in band pattern from the other sub-Antarctic *S. newport* isolates which by the criteria of Tenover and colleagues [23] was considered to be closely related to the other *S. newport* isolates. *S. havana* isolates from Bird Island, although from different animal reservoirs and different years, exhibited identical PFGE patterns with all three restriction enzymes. The PFGE pattern of all sub-Antarctic isolates of *S. enteritidis* was identical although one was PT 4, five PT 4-like and one PT 35.

All *Salmonella* spp. isolated from Bird Island seabirds and fur seals were susceptible to the antibiotics tested with the exception of a *S. newport* isolate which showed reduced susceptibility to streptomycin.

DISCUSSION

Salmonella spp. have been reported from other animals in the Antarctic region. On Ross Island, Antarctica, 12% of adelie penguins and 18% of south polar skuas (*Catharacta maccormicki*) were positive for *Salmonella* spp. [4]. In our study, 7% of gentoo penguins and 2% of black-browed albatross were found to be carriers of salmonella at different times, a result comparable with the Ross Island report.

There was a marked increase in prevalence of salmonella in the seal pup population from 5 to 22% between 1996 and 1998. At that time, there was also a change in seal breeding success as pup mortality prior to weaning was 20% in 1996 and 40% in 1998 (BAS unpublished data). These differences in mortality were probably due to poor feeding conditions for adult females. In 1998, there was a shortage of Antarctic krill (*Euphausia superba*) resulting in poor body condition of the pups. This may have made them more susceptible to infection, as has been reported for both animals and humans [24, 25], and promoted the spread of salmonella in the seal population. The extent to which salmonella infection in the seal population contributes to poor breeding success and high pup mortality requires further investigation. In addition, it is not clear if this increase in prevalence signifies a true epizootic outbreak as the seals were not specifically investigated for signs of salmonella disease. We found four serotypes, *S. havana*, *S. typhimurium*, *S. newport* and *S. enteritidis* in seabirds and seals on Bird Island. All are known pathogens in man, with *S. enteritidis* and *S. typhimurium* being among the most common cause of human salmonellosis [26, 27]. Most of the Bird Island *S. enteritidis* isolates belonged to PT 4, or were PT 4-like. PT 4 is one of the most common *S. enteritidis* phage types in human disease and has spread throughout the western world over the past 10 years [27–29]. *S. typhimurium* is endemic in several countries, and found in both domestic and wild animal populations. This serotype is also common in birds associated with urban activities, such as gulls, pigeons and sparrows [11, 15, 30] and together with *S. enteritidis* and *S. newport*, it has also been isolated from a variety of seal species around the world [16, 18, 31]. However, to our knowledge, prior to this report *S. havana* has not been isolated from seals. The correlation of these serotypes to human activities may indicate that the Bird Island isolates are of human origin and therefore introduced in this sensitive ecosystem.

PFGE analysis of the Bird Island salmonella isolates showed total identity in restriction patterns of DNA cut with three different restriction enzymes. The only exception was a *S. newport* isolate which was very closely related to other isolates of this serotype. According to our present knowledge of the interpretation of PFGE patterns [23, 32–34], this could indicate an ongoing epizootic in the seal population of Bird Island, with a rapid spread of salmonella in the dense seal pup populations on the island. PFGE is a sensitive tool for detection of subtle changes in the bacterial genome. Therefore, we surmise that the genetic identity found in salmonella from seals and birds on Bird Island, indicate that they are either subject to low selective pressure from the environment or have recently been introduced to the area. The behavior of sealpups and penguins, living closely together in colonies, promotes rapid spread of bacteria in these populations. *S. havana*, *S. enteritidis* and *S. newport* were found in two different reservoirs: Antarctic fur seals and gentoo penguins (*S. havana*, *S. enteritidis*) and Antarctic fur seals and black-browed albatrosses (*S. newport*). Despite this, isolates showed identical PFGE pattern within each serotype. This may indicate transmission of salmonella between different species and reservoirs in the region.

To compare differences in presumed exposure to human waste, seals on Bird Island were sampled in two different locations in 1998, 2 km apart. One of the sites was sampled both in 1996 and in 1998 and there was a significant rise in salmonella prevalence in seals at that site, from 5% in 1996 to 18% in 1998 ($P = 0{\cdot}026$ by χ^2 test). There was no significant difference in prevalence of salmonella between the two sites ($P = 0{\cdot}161$ by χ^2 test) since 26% and 18% of fur seal pups were colonized at the two sites respectively (data not shown). If the presence of salmonella in the seal population was associated with human activity on the island, we might have expected a higher rate of colonization close to the first site where sewage flows directly into the sea. The antibiogram for *S. typhimurium* isolates demonstrated full susceptibility to the seven antibiotics tested although one isolate showed reduced susceptibility to streptomycin. Exposure to antibiotics is very rare in the animal population on Bird Island, although antibiotics are occasionally used in the course of zoological studies.

If the *Salmonella* spp. in seals and birds on Bird Island have been recently introduced, their origin remains obscure. Since the human population on Bird Island is a maximum of eight persons, it would appear that the risk of human introduction of salmonella is relatively small. However, seabirds and Antarctic fur seals breeding on Bird Island are wide ranging and may pick up pathogens in contaminated waste at some distance from the island. In spite of this risk, only one black-browed albatross and no grey-headed albatrosses tested positive for salmonella, although both species of birds regularly winter in the Benguela current off South Africa [35]. There, these species might encounter salmonella and related organisms of human origin more regularly than Bird Island seals and penguins.

The question remains as to whether reduced breeding and the observed augmented pup mortality, is due to increased salmonella infection in the seals, or whether the deteriorating nutritional state of the seals, due to natural fluctuations in the numbers of krill, increase the secretion of salmonella from the seal gut. The demonstration of salmonella serotypes known to be pathogenic in man on Bird Island could indicate either an ongoing epizootic with salmonella strains recently introduced in the area, or that salmonella has been present on Bird Island long enough to become well adapted to the environment. The crucial question of whether or not expanding human activities in the Antarctic area could lead to the introduction of new and possibly devastating pathogens for Antarctic animal populations must be given more attention in future studies.

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