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ORIGINAL PAPER

Health evaluation of wild gentoo penguins (*Pygoscelis papua*) in the Antarctic Peninsula

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Abstract Historically wildlife conservation was based on habitat protection and exploitation control. Only recently have diseases been considered an important issue. However, pathogens are usually described during or after disease outbreaks, but to determine which pathogens may be emerging, surveys of wildlife health are critical in a given time. This study deals with the health status of gentoo penguins *Pygoscelis papua* in two localities at the Antarctica Peninsula and one at Ardley Island off the South Shetland Islands. Cloacal swaps, fresh fecal samples, ectoparasites, and blood smears were collected. We examined and dissected 14 penguin corpses found dead. Fecal samples were positive for *Campylobacter*, *Escherichia coli* and in the carcasses four endoparasitic species were found: *Diphyllobothrium* sp. and *Parorchites zederi*, *Corynosoma shackletoni* and *Stegophorus adeliae*. The tick *Ixodes uriae* occurred in five of the examined penguins, and the louse *Austrogoniodes gressitti* on six birds. From the colony grounds, we collected 1,184 *I. uriae*. We recorded antibiotic-resistant bacteria, such as *E. coli*, in ecosystems where gentoo penguins breed. Cloacal samples (300) were negative for *Chlamydia*, as well as for *Salmonella*, *Campylobacter*, *E. coli*, Newcastle and Influenza viruses.

Keywords Health · Gentoo penguins · Antarctica · Pathogens · Endoparasites · Ectoparasites

Introduction

Penguins represent about 90 % of the bird biomass in the Southern Ocean, where they breed on the Antarctic continent and subantarctic islands (Williams 1990; Williams and Rothery 1990). Gentoo penguins *Pygoscelis papua* have a circumpolar distribution, usually nesting in small colonies on subantarctic islands and the Antarctic Peninsula (Bost and Jouventin 1990; del Hoyo et al. 1992; Woehler and Poncet 1993). Since 2000, this penguin species has been listed as near threatened (IUCN 2012; Bird

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life 2013), and knowledge about its diseases is scarce (Barbosa and Palacios 2009).

Pathogens are important in ecosystems because of their effects on host populations (Grenfell and Dobson 1995). As documented in more than 65 case studies, for many bird species the effects of parasites on life history traits include reductions in the clutch, brood, and offspring sizes (Møller 1997). High levels of parasitism are detrimental to at least some penguins in Antarctica (Fraser and Patterson 1997). Through research of wildlife diseases in natural environments, it is possible to detect the biotic and abiotic parameters that control the health condition of animal populations, as well as to assess the effect of one population on others of the same or of different species, including humans. Understanding the ecological factors that may facilitate transmission of infectious diseases is both important and urgent as there are significant implications for the future conservation of species (Daszak et al. 2000; Smith et al. 2009).

The human presence on the Antarctic continent and the subantarctic islands has increased steadily (Tin et al. 2009), increasing the risk of introducing exotic pathogens to wildlife (Curry et al. 2002). In addition, climate change associated with pollution caused directly or indirectly by human activities may increase the incidence of wildlife diseases (Gilbert et al. 2008) and may cause the establishment of new pathogens that could not survive in the past.

Published information about diseases and parasites of Antarctic birds is scarce and fragmented (Kerry and Riddle 2009). *Campylobacter jejuni* isolated from Macaroni penguin (*Eudyptes chrysolophus*) chicks may have been a recent introduction (Broman et al. 2000). Similarly, various strains of *Salmonella* in seals and seabirds examined at South Georgia may have been introduced there recently, unless these bacteria managed to adapt well to the subantarctic environment (Palmgren et al. 2000). Both cases are good examples of studies showing the possible emergence of infectious diseases in terrestrial and marine fauna in the subantarctic region, revealing that microorganisms infect wildlife probably as a consequence of human activity. Therefore, monitoring wild animal populations in Antarctica is an essential component in programs to control infectious diseases (Leotta et al. 2001). Also, it is relevant to study species that may serve as environmental indicators and to acquire baseline knowledge to measure anthropogenic activities that could affect the conservation status of marine wildlife in Antarctica. This paper provides baseline information on health indexes, including bacteria, viruses, endo and ectoparasites, and body condition of gentoo penguins in three localities of the Antarctic Peninsula that have the highest level of human occupation and visitation in Antarctica.

Materials and methods

Localities and study period

This study was carried out at three localities at the Antarctic Peninsula (Fig. 1) during the southern summers of 2011 (18 January–1 March) and 2012 (12 January–20 February) with authorization from the Chilean Antarctic Institute (INACH). To detect differences among localities, 100 clinically healthy birds (adults and chicks) were captured from each locality: Base Gabriel González Videla (GGV) (64°49′S, 62°51′W), Base Bernardo O'Higgins (BBO) (63°19′15″S, 57°51′01″W)—both situated in the Antarctic Peninsula—and Ardley Island (AI) (62°12′57″S, 58°57′35″W) at the South Shetland Islands (Fig. 1).

Number of samples

Before collecting samples, a population census of each penguin colony was made by direct counts of all observed penguins. The census was made by three observers separated approximately 50 m from each other and following the same pathway. We repeated the counting until the difference between observers was equal or <5 %. The adult population comprised of 6,200 individuals at GGV, 680 at BBO, and 1,406 at AI. The sample size required to estimate prevalence with a confidence interval of 95 % was determined by $n = 1.96^2\ P\text{exp}\ (1\text{-}P\text{exp})/d^2$, where n = required sample size; $P\text{exp}$ = expected prevalence; and d = desired absolute precision. Thus, for an estimated prevalence of infection of 50 % and a given margin of error of 10 % in a theoretically infinite population, the sample size is 96 (Thrusfield 2005), and 48 birds for an estimated prevalence of infection of 15 %. Therefore, our goal was to sample 50–100 penguins at each locality giving a total of 200 adults and 100 chicks examined.

Capture and handling of penguins, collection and storage of samples

At each locality, penguins were captured away from the nest using a bird net to avoid disturbance of breeding birds. Animal handling followed the CCAMLR Ecosystem Monitoring Program Standard Methods (Commission for the Conservation of Antarctic Marine Living Resources 2004). Each penguin was captured manually and restrained for approximately 6 min while it was weighed to the nearest gram, and its total length was measured by the same person. After collecting samples and taking measurements, birds were immediately released.

Schulte-Hostedde et al. (2005) and Ardia (2005) found that ordinary least squares residuals of mass on a linear measure of size usually showed higher correlations with the

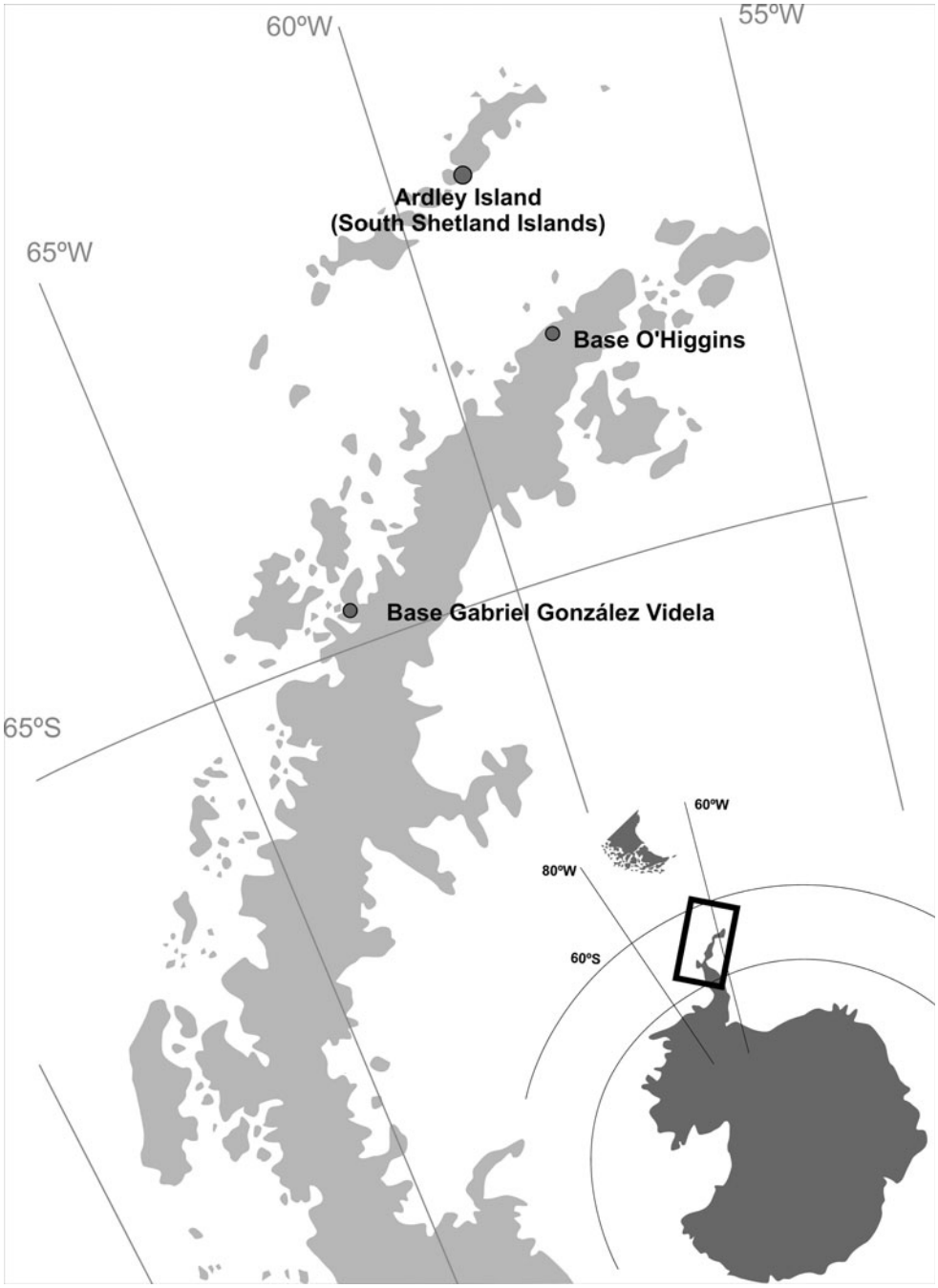

Fig. 1 Map of the Antarctic Peninsula showing the location of the three study areas, Base Gabriel González Videla, Base Bernardo O'Higgins, and Ardley Island

absolute size of fat stores (in g) and were also highly correlated with absolute values of lean dry mass (Schulte-Hostedde et al. 2005). To assess body condition, a linear regression of body weight against body length was performed in JMP 7®, and the residual of this analysis was used as the index of body condition (Brown 1996). Blood smears and cloacal swabs were collected from each penguin for laboratory analysis, and the plumage was searched for ectoparasites. Samples were preserved at −80 °C and sent in dry ice to the Clinical Laboratory of the Faculty of Veterinary Sciences, Universidad de Concepción (Chillán, Chile), where they were stored for approximately 2 months.

To detect blood parasites, we collected a drop of blood with 23G needles from the external metatarsal or brachial vein (Ash and Orihel 1987; Bennett 1970; Sergent et al. 2004). We air-dried and fixed smears in 100 % ethanol immediately after obtaining each sample. The smears were stained with 3 % Giemsa for 20 min and later examined at the Faculty of Veterinary Sciences, Universidad de Concepción (Chillán, Chile).

Also, 400 fresh fecal samples were collected (100 in GGV, 150 in OH, and 150 in AI). The samples were collected with cotton swabs (sterile tipped applicators) inserted in freshly deposited penguins feces on the surface of colony.

Detection of pathogens

RNA was extracted from the swabs and identified to species and stage. RNA was then converted into cDNA and analyzed by polymerase chain reaction (PCR) and sequenced for different pathogens. The samples were transported on dry ice to the Section for Zoonotic Ecology and Epidemiology, Department of Medical Sciences, Uppsala University and in the Linnaeus University, Kalmar, Sweden, where the PCR for the pathogens was analyzed. The Swedish National Board of Health and Welfare granted the permissions.

Bacteria

Chlamydia

In this study, we used what is believed to be a real-time PCR specific for the group of *Chlamydia psittaci*, *Chlamydia pneumoniae* and *Chlamydia pecorum*, amplifying a part of the 23S rRNA gene to screen for *C. psittaci* as previously described (Herrmann et al. 2000). Briefly, DNA was extracted from 300 cloacal swabs from gentoo penguins using a QIAamp tissue kit on a M48 robot (Qiagen, Hilden, Germany). The PCR was run on a LightCycler 2.0 (Roche Diagnostics, Basel, Switzerland). Material from an isolate of *C. psittaci* DC5 was used to evaluate the real-time PCR detection. In each PCR run a positive control with DC5 was included as well as a negative control of water.

Campylobacter

Campylobacter spp. Samples were cultured by methods previously described (Griekspoor et al. 2009). Each sample was plated on selective blood-free, charcoal-based agar plates and incubated at 42 °C under micro-aerophilic conditions for about 48 h. If the bacteria were positive for catalase and oxidase and exhibited an inability to grow in oxygen-rich environments, they were observed under the light microscope. If Gram-negative gull-shaped rods were observed, the bacteria were considered to be *Campylobacter* and were then further isolated on blood agar plates incubated at 42 °C for 24–48 h.

Salmonella

We applied routine procedures to culture putative *Salmonella* isolates, with enrichment in Rappaport–Vassiliadis broth and subsequent injection into xylose lysine deoxycholate (XLD) agar. On this medium, most *Salmonella enterica* serotypes appear as transparent colonies with black centers. If colonies with growth characteristics of *Salmonella* were observed, a full phenotypic identification was performed by using standard biochemical and serologic tests.

Escherichia coli

All water, cloacal, and fresh feces samples were cultured on Uriselect 4 plates (Bio-Rad Laboratories Ltd., Hemel Hempstead, UK) and the isolates were identified by conventional biochemical tests. To measure strain susceptibility, 11 antibiotics were used: tetracycline 30 μg/disk, ampicillin 10 μg/disk, streptomycin 10 μg/disk, chloramfenicol 30 μg/disk, nalidixic acid 30 μg/disk, cefadroxil 30 μg/disk, fosfomycin 50 μg/disk, tigecycline 15 μg/disk, sulfamethoxazole/trimethoprim 25 μg/disk, nitrofurantoin 100 μg/disk, and mecillinam μg/disk (Perry et al. 2003). To isolate *E. coli* for characterization, the penguin samples were cultivated and the strains were identified by conventional biochemical tests. Randomly chosen *E. coli* isolates ($n = 31$) from penguin samples were used to determine profiles of antibiotic susceptibility using the EUCAST disk diffusion method to receive an overall picture of resistance in the material. The panel included 11 antibiotic disks. The *E. coli* strain ATCC25922 was used as control.

Staphylococcus spp.

Typical colony morphology in *Uriselect* 4 plates was used. The putative *Staphylococcus spp.*were isolated and frozen for further characterization.

Viruses

We made separate RNA and DNA extractions of 300 cloacal samples from gentoo penguins to screen for viruses. The samples were pooled (3 samples in each well) and were frozen at −80 °C in 96-well formats.

Influenza

Four samples from gentoo penguins were pooled in each well; 100 μl of each sample was added to the 96-well plate, and PBS saline was added to bring the volume to a total of 500 μl. Both a positive (H5 low pathogenic isolate collected from a duck at Ottenby) and a negative control were added to the plate. RNA was extracted using the Roche MagNA Pure system following the manufacturer's directions, by which the virus is lysed and the RNA binds to magnetic beads. The RNA–bead complex was washed in two solutions prior to elution. Samples were screened

following a reverse transcriptase real-time PCR approach using a TaqMan probe to amplify a short region of the matrix (M) segment. A Cycle threshold (Ct) < 35 is deemed positive. If detection occurred, the samples from the positive pool were re-extracted and re-screened individually to determine specific information regarding the positive sample.

Avian paramyxovirus

Newcastle disease was searched for by using the real-time PCR (RT-PCR) method directed at conserved genes. RNA was isolated from the original samples by using the MagAttact Virus Mini M48 Kit in the M48 automated extraction robot, Qiagen. If detected, samples from the positive pool were re-extracted and re-screened individually to determine the specific information regarding the positive sample.

Metazoan parasites

Endoparasites

With the aim of detecting endoparasites, 14 gentoo penguin carcasses of birds which had died of natural causes were collected at the three localities (all adults: six in BBO, five in GGV, and three in AD). Their gastrointestinal tracts were removed and placed in plastic bags with 70 % ethanol until autopsies were performed and endoparasites extracted. As a complement to determining endoparasite prevalence, we collected 50 fecal samples off the ground in each locality ($n = 150$). Each sample was stored in 70 % ethanol and was later processed in the laboratory by the flotation and sedimentation methods (Ueno and Gonçalves 1998). Blood smears were also examined for hemoparasites by searching 300 samples under a compound microscope at 100× magnification.

Ectoparasites

Each live penguin was examined for external parasites for 4 min, and the collected ectoparasites—ticks and lice—were placed in individual vials with ethanol 70 %. Estimates of tick abundance are most often generated through the collection of ticks from underneath stones. Rocks were turned over, and all ticks on each rock and on the underlying soil were sampled for 10 min (Frenot et al. 2001), for a period of 3 h at each locality, totaling 540 min of sampling effort. All ticks were stored in vials with 70 % alcohol and later identified to species level at their determined developmental stages. Tick density was expressed as the number of ticks found per unit of searching time. To compare the density of ticks among the different sampling localities, we calculated the tick abundance index ($TAI = TR \times 100/t$, with TR = number of collected ticks, and t = sampling period in minutes), which is equivalent to the number of collected ticks divided by the sampling effort (t) and expressed in ticks numbers collected in 100 sampling minutes. The lice were slide-mounted following the technique described by Palma (1978) to allow their identification to species.

Meteorological data

Records of monthly average temperature, humidity, and precipitation were obtained from the meteorological stations at the Bases Gabriel González Videla, Bernardo O'Higgins, and Bellingshausen at King George Island, South Shetland Islands.

Statistical analysis

The tick abundance indexes (TAIs) were subjected to logarithmic transformation [ln (TAI + 1)] for statistical analyses. To compare the prevalence, abundance, and intensity among the three localities studied, we used the program *Quantitative Parasitology*, version 2.0 (Reiczigel and Rózsa 2001). The body condition and weight of penguins were submitted to the Shapiro–Wilk goodness-of-fit test. The weight ($W = 0.992$; $p = 0.385$), body condition ($W = 0.990$; $p = 0.213$), and humidity ($W = 0.692$; $p = 0.06$) followed a normal distribution, and hence, they were submitted to a two-tailed test. Values for temperature ($W = 0.952$; $p = 0.003$) and precipitation ($W = 0.521$; $p = 0.001$) did not follow a normal distribution, and hence, the nonparametric Kruskal–Wallis test was used for them. For other statistical analyses, we used JMP 7 (SAS Institute, Inc. 2007).

Results

Body condition

Among the 300 live penguins studied, 200 were adults and 100 chicks. All birds appeared to be in good condition. Body weights and body condition indexes for adults and chicks are given in Table 1. Positive values indicate a high weight for the size of the bird (good body condition), while negative values reflect a low weight for the size of the bird (low body condition) (Peig and Green 2009; Labocha and Hayes 2012). The weight of penguins at GGV was significantly lower than those at BBO and AI ($DF = 188$; $t = 1.97$; $p < 0.05$). However, the body condition did not differ among penguins from the three localities ($DF = 183$; $t = 1.97$; $p > 0.05$) (Table 1).

Meteorological data

The monthly average temperature reported at all sampled localities was significantly different (DF = 2, $\chi^2 = 24.596$, $p < 0.05$). The lowest temperature was reported at BBO (0.35 ± 1.03), while the highest was at GGV (1.73 ± 0.62; Table 2). Humidity was high in both localities and there were no significant differences between them in terms of level of humidity (DF = 57; $t = 2.00$; $p > 0.05$). With regard to precipitation, levels were highest at AI and were significantly different only to those at GGV (DF = 2; $\chi^2 = 13.194$; $p < 0.05$, Table 2). It should be mentioned that the substrate where the penguins nest at BBO has poor drainage. Consequently, snow and rain form loam under the stones, becoming an unsuitable habitat for the ticks to survive.

Pathogens

The 300 cloacal samples analyzed were negative for *Chlamydia*, *Salmonella*, *Campylobacter*, *E. coli*, Newcastle and Influenza viruses. However, 267 (89 %) of the cloacal samples contained *Staphylococcus spp.*that were selected for a comparative study with *Staphylococcus* of human origin. The culture of fresh environmental feces ($n = 400$) provided a total of 26 *Campylobacter* samples that were frozen for further analysis by PCR (MLST). These cultures also provided 38 samples of Enterobacteriaceae, which were subsequently characterized phenotypically by biochemical tests confirming the species *Escherichia coli* in 32 of them. Only one *E. coli* isolate from a penguin at BBO had a resistant phenotype, in this case to chloramphenicol.

Endoparasites

All sampled penguins tested negative for blood parasites. Four helminth species were collected from five (35.7 %) of the 14 autopsied dead penguins: *Diphyllobothrium* sp. and *Parorchites zederi* (Cestoda), *Corynosoma shackletoni* (Acanthocephala) and *Stegophorus adeliae* (Nematoda) (Table 3). One fecal sample contained a strobilus of *P. zederi*, while all the others ($n = 299$) had no parasites or eggs of any species.

Ectoparasites

We collected ticks of the species *Ixodes uriae* (Acari: Ixodidae) from five (1.6 %) of the 300 penguins examined. The five tick-positive penguins were all from GGV. However, near the nesting colonies, we collected 1,184 ticks (larvae, nymphs and adults) under stones with good drainage and high humidity (532 were from GGV and 652 from AI, but none were found in BBO). We found no significant differences between the abundance index of ticks in both tick-positive localities (DF = 1; $t = 4.48$; $p > 0.05$, Table 4). We also collected four males and five females of the chewing louse *Austrogoniodes gressitti* (Phthiraptera: Philopteridae) from the plumage of six (2 %) of the 300 penguins examined, 4 from GGV, 1 from BBO, and 1 from AD. Due to the small number of lice collected, a statistical analysis was not performed.

Discussion

Body condition

The recorded weights of penguins from BBO and AI were within reported ranges for *Pygoscelis papua* in different

Table 1 Body condition parameters of *Pygoscelis papua* in the three study localities

	Base G.G. Videla	Base Bernardo O'Higgins		Ardley Island	
	Adults ($n = 100$)	Chicks ($n = 50$)	Adults ($n = 50$)	Chicks ($n = 50$)	Adults ($n = 50$)
Body weight (kg) (mean ± SD)	4.94 ± 0.50	5.98 ± 1.33	6.23 ± 0.84	5.41 ± 1.28	6.02 ± 0.70
Body condition index (mean ± SD)	0.02 ± 0.54	0.14 ± 0.88	−0.21 ± 0.81	0.10 ± 0.91	−0.99 ± 0.65

Table 2 Meteorological data of the three study localities for the study period (January–February 2011)

Locality	Coordinates	Altitude (m)	Average temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Precipitation (mm)	Relative humidity (%)
Base Gabriel González Videla	64°49′S; 62°51′O	23	1.73 ± 0.62	3.27 ± 0.98	0.33 ± 0.68	0.13 ± 049	
Base Bernardo O'Higgins	63°19′S; 57°51′O	10	0.35 ± 1.03	1.74 ± 1.02	−1.17 ± 2.07	0.83 ± 1.93	84.7 ± 11.25
Ardley Island	62°19′S; 57°51′O	10	0.90 ± 0.94	2.16 ± 0.95	0.26 ± 1.07	1.15 ± 1.68	80.4 ± 10.90

Table 3 Number of sampled *Pygoscelis papua* and pathogen percentage of positive samples in each of the three study localities

Localities	Base G.G. Videla		Base Bernardo O'Higgins		Ardley Island	
Pathogens	*n*	Positive *n* (%)	*n*	Positive *n* (%)	*n*	Positive *n* (%)
Campylobacter	100	6 (6)	150	12 (8)	150	8 (5.3)
Escherichia coli	100	8 (8)	150	11 (7.3)	150	13 (8.7)
Parorchites zederi	5	1 (20)	6	2 (33.3)	3	0
Diphyllobothrium sp.	5	0	6	0	3	1 (33.3)
Corinosoma shackletoni	5	2 (40)	6	2 (33.3)	3	0
Stegophorus adeliae	5	2 (40)	6	1 (16.7)	3	1 (33.3)
Austrogoniodes gresitti	100	4	100	1	100	1
Ixodes uriae	100	5	100	0	100	0

Environmental fecal samples were used for bacteria (cloacal samples were negative)

Table 4 Number and abundance index (TAI) of *Ixodes uriae* collected in two of the study localities

Developmental stages	Base G.G.Videla		Ardley Island	
	Number	TAI[a]	Number	TAI[a]
Adults	208	38.52	420	77.78
Nymphs	244	45.19	132	24.44
Larvae	80	14.81	100	18.52

[a] Number of ticks collected in 100 min of sampling

colonies of King George Island, South Shetland Islands (Cuervo et al. 2009; Najle et al. 2006; Renner et al. 1998). However, the weights of adult penguins at GGV were lower than those of gentoo penguins at the northern edge of their range: male body weight 7.0 ± 0.7 kg, female: 6.6 ± 0.6 kg at East Falkland Island (51°42′S, 57°51′W) and 7.2 ± 0.6 kg at Bird Island (54°1′S, 38°3′W), South Georgia (Davis et al. 1989; Otley et al. 2005). Based on these data, it would seem that the weight and size of the penguins decrease from northern to southern populations. Genetic differences between the populations or less food availability could explain the lower body mass of adult penguins at GGV with respect to the penguins of the other studied localities. However, more investigation is needed to unveil the causes of specific differences in the body mass of the penguins.

Microorganisms

With the exception of *Campylobacter* and *Escherichia coli*, cloacal samples did not contain any other bacteria or viruses. Specifically, tests were negative for *Salmonella*, *Chlamydia* and for paramyxovirus and avian influenza virus. A similar study by Moore and Cameron (1969) at Bird Island reported antibodies for *Chlamydia* in various penguin species (*P. papua*, *P. adeliae*, *Aptenodytes forsteri*, *Eudyptes crestatus*), indicating that bacteria may be present in some penguins or that they were exposed to the pathogen. At the emperor penguin (*A. forsteri*) colony at Auster (67° 23′S, 63° 57′E), Cameron (1968) reported evidence of *Chlamydia* in a dead 3–4-week-old emperor penguin chick. This was the first evidence for this pathogen among birds in Antarctica. However, it is important to mention that our diagnosis is based on PCR and not on serological tests. Thus, our study assessed the prevalence of a pathogen at a given moment related to the time a pathogen survives in the host. For example, detecting antibodies for *Chlamydia* using serological diagnostic tests is evidence of induced immunity by an early infection of less measurable time. Also, swabs of the conjunctiva, pharynxes and cloacae should be taken in future studies to detect potential pathogens in these organs.

Bacteria of the genus *Campylobacter* are commensal in the gastrointestinal tract of many domestic animals including poultry, as well as in wild birds, that do not necessarily exhibit clinical symptoms. *Escherichia coli* is present in the commensal flora of humans, mammals, and birds. The prevalence of enterobacteria in the swabs of gentoo penguins in our study is generally low. The isolation of viable *E. coli* from only 32 (8 %) samples and of *Campylobacter* from 26 (6.5 %) out of a total of 400 fresh fecal samples collected may indicate a low prevalence of both microorganisms. However, these values are based on molecular analysis, so the percentages are from individuals that actually carry the pathogen. Had we used serological methods, the prevalence would have been higher. Only one

E. coli isolate from one penguin from BBO had a resistant phenotype, in this case to chloramphenicol.

Among the water samples, three isolates of *E. coli* were resistant to at least one antibiotic, and several were resistant to two or more antibiotics. The most frequently observed resistance was to ampicillin, found in 11 isolates, followed by tetracycline (6 isolates), streptomycin (4 isolates), and trimethoprim-sulfamethoxazole (4 isolates). One *E. coli* isolate was resistant to nalidixic acid. Hernández et al. (2012) isolated *E. coli* bacteria with ESBL (Extended Sppectrum β-Lactamase) type CTX-M resistance from water samples collected close to research stations in Antarctica. The presence of CTX-M-producing bacteria in Antarctic seawater and the finding of a high prevalence of CTX-M in certain wild bird populations need to be carefully evaluated. An important question is whether human-associated bacteria found close to areas of human activities infect Antarctic wildlife. None of the sampled penguins were positive for ESBL producing bacteria, and only one *E. coli* isolated from the feces collected in the colony exhibited antibiotic resistance, indicating that at the time of sampling, human-associated enterobacteria were uncommon in the sampled penguins. The risk of a bird being contaminated with *E. coli* harboring ESBL genes varies with its proximity to environments affected by human activity (Hernández et al. 2012).

Blood parasites

Most reports of infestations by blood parasites in penguins are from birds in captivity (Fix et al. 1988), and there are only two reports of *Plasmodium spp.* in Antarctic and subantarctic seabirds, but not from penguins (Bennett et al. 1993; Vogelnest 2000). The absence of blood parasites in our samples from wild gentoo penguins contrasts with the finding of malaria in jackass penguins (*Spheniscus demersus*; Graczyk et al. 1995). Because protozoans causing malaria are not always found in circulating erythrocytes of infested animals, a lack of malaria evidence in blood smears does not indicate a lack of infestation (Graczyk et al. 1995). Jones (1988) examined 143 smears of four species of penguins (including gentoo penguins) but did not find blood parasites. All records from penguins in the Antarctic or subantarctic have been negative (Jones and Shellam 1999) because the usual vectors, mosquitoes, are not present in Antarctica (Merino et al. 1997). However, gentoo penguins have been infected with *Plasmodium spp.* in captivity (Bishop and Bennett 1992; Fix et al. 1988).

Endoparasites

The low prevalence of parasites and the lack of eggs in the fecal samples contrast with the higher prevalence of intestinal parasites we found in the dissected penguins. Fecal samples from 44 Adelie penguins were examined for coccidian parasites in Cape Bird, Ross Island, but all the samples were negative for oocytes (Miller et al. 1993). All helminth endoparasites found in our study have been reported previously. Acanthocephalans are rarely found in marine birds (Ranum and Wharton 1996), but the *Corynosoma* sp. and *C. shackletoni* were discovered in gentoo penguins (Hoberg 1986; McKenna 1998). The nematodes *Stegophorus macronectes*, *Contracaecum heardi,* and *Stomachus* sp. were isolated from gentoo penguins (Mawson 1953). Our finding of *S. adeliae* represents the second species of *Stegophorus* from these penguins. The cestodes *Tetrabothrius pauliani*, the nematodes *Streptocara* sp., *Contracaecum* sp., *Ascaridia* sp., and an unidentified species of *Tetrabothrius* were also found in gentoo penguins (Prudhoe 1969; Fredes et al. 2006, 2007). The cestode *P. zederi* is probably a common endoparasite in *P. papua* since it has been recorded in several different studies (Ippen et al. 1981; Cielecka et al. 1992; Georgiev et al. 1996), as well as in chinstrap penguins, *Pygoscelis antarctica* at Deception Island (Vidal et al. 2012). In addition, three other gastrointestinal parasites were retrieved from chinstrap penguin samples: the cestode *Tetrabothrius pauliani*, an unidentified species of *Corynosoma,* and *S. macronectes* (Vidal et al. 2012). The impact of endoparasites on the health of Antarctic seabirds populations is as yet unknown (Woods et al. 2009).

Ectoparasites

The only tick species found in this study was *Ixodes uriae*, a species with a bipolar distribution, which parasitizes a wide range of host groups and species (Heath 1977). In their study of *I. uriae* distribution along the Antarctic Peninsula, Barbosa et al. (2011) did not find ticks on the penguins themselves, but at their nesting sites. Woods et al. (2009) consider *I. uriae* the most important of all ectoparasites of seabirds in terms of its impact on host health. A hyperinfestation of *I. uriae* probably caused the death of adult king penguins (*Aptenodytes patagonicus*) (Gauthier-Clerc et al. 1998). This tick is a vector for *Borrelia* spirochetes affecting seabirds (Olsen et al. 1995), and antibodies of *B. burgdorferi* were detected in 14 % of tick-infested adult king penguins (Gauthier-Clerc et al. 1998). *Ixodes uriae* forms large aggregations in and around nesting sites, especially under big stones with good drainage that are free of floods during the snow melt in spring and the summer rain (Lee and Baust 1987; Frenot et al. 2001; Benoit et al. 2007, 2008). In our study, ticks were found in large groups of different sizes under stones with high relative humidity and located close to nests. However, the stones where we found ticks were different in size in contrast to Lee and Baust's (1987) findings, who suggest that *I. uriae* is found under big

stones only. We did not find any ticks at BBO, where the temperatures are lowest (Table 3), although *I. uriae* is one of the arthropods with the widest thermal tolerance, from −30 °C to +40 °C (Lee and Baust 1987). Therefore, the absence of *I. uriae* in BBO has to be explained by other factors, possibly relating to the substrate, e.g., soil without drainage, in contrast to that in AI and GGV. Due to the low prevalence (1.6 %) and abundance (6.0) of ticks found on penguin bodies, we could not correlate parasite load with host age (adults and chicks) and host body condition. *Ixodes uriae* spends most of the time outside the host, only feeding on the host for short periods (Frenot et al. 2001). When penguins come to breed in spring, ticks are attracted to them by the uric acid present in the guano (Benoit et al. 2008). The reproductive season of gentoo penguins begins in October and lasts until the end of February (Renner et al. 1998; Quintana and Cirelli 2000). Hence, the beginning of spring is the period when more ticks are expected to be found on gentoo penguins and that number probably decreases as the host breeding season advances, at least on adults, because chicks remain on land for longer periods. The very low prevalence (1.6 %) of ticks detected in the 300 penguins examined agrees with this scenario because our sampling was done toward the end of the penguins' breeding season. Furthermore, the ticks were found between 18 and 20 January and the five parasitized birds were all adults.

The chewing louse *Austrogoniodes gressitti* was originally described from *P. papua* at South Georgia and *P. antarctica* at Anvers Island, Antarctica, by Clay (1967). It was further listed by Clay and Moreby (1970) and Murray et al. (1990) from the same hosts and recorded by Banks et al. (2006) from *P. papua* in the Falkland Islands, but it has not yet been found on any other host species. This louse is most likely host specific to these two species of *Pygoscelis*. The samples collected in this study represent material from three localities in addition to those mentioned above. Palma and Horning (2002) recorded the chewing louse *Naubates prioni* from a gentoo penguin but they regarded it as the result of straggling or contamination from a petrel. In general, chewing lice do not appear to cause ill health to the hosts and are not known to transmit infectious diseases among penguins (Woods et al. 2009), but cases of lice as intermediate hosts for organisms that cause disease have been reported (e.g. Bartlett 1993; Cohen et al. 1991; Clayton et al. 2008). However, all lice can cause irritation, discomfort, and reduce host fitness, especially when they are present in large numbers (Booth et al. 1993).

Another ectoparasite reported from gentoo penguins in the Falkland Islands is the flea *Parapsyllus longicornis alginus*, (see de Meillon 1952), now regarded as a junior synonym of *Parapsyllus longicornis*. We have not been able to find any further record of fleas from gentoo penguins.

Conclusions

Diseases affecting wildlife are an important cause of biodiversity loss in many areas of the world (Daszak and Cunningham 2002). Captive Antarctic birds have exhibited symptoms of a variety of diseases known in other wildlife populations; therefore, they are susceptible to these diseases (Kerry and Riddle 2009). Many reports on the presence of pathogens in Antarctic wildlife are available, but most are neither systematic studies nor have evaluated the health of the animals (see review in Barbosa and Palacios 2009). This paper provides the first comprehensive data set on the health status of free-ranging populations of gentoo penguins in Antarctica.

Despite being infected with *Campylobacter*, *E. coli* and six different species of metazoan parasites, the penguins sampled were in good condition and apparently healthy. However, our finding of some antibiotic resistance in *E. coli* in the absence of antibiotic usage implies that resistant bacteria have been introduced and transferred to penguins by human activities. Knowledge about pathogens and parasites of gentoo penguins at the Antarctic Peninsula, including information on the role played by other factors—environment, climate, diet, human activities—is essential to assess the potential survival and conservation of the species (Smith et al. 2009). Data presented here are not only showing the current health status of some populations but, also, provide a reference point for future evaluations and for comparisons with other populations of the same and closely related penguin species.

An increase in human activities and their environmental impact on Antarctica will likely continue (Curry et al. 2002; Lynch et al. 2010). Therefore, further health surveys of other gentoo penguin colonies and other bird species, including procedures for maintaining and coordinating serum and specimen banks from Antarctic wildlife, are essential for the long-term management of those species. However, that knowledge will not be sufficient to assist in the survival of Antarctic wildlife without a firm commitment to reduce the impact of human activities, especially in regard to the dissemination of potentially dangerous pathogens and hazardous pollutants into the Antarctic environment.

An Environmental Protocol for the protection of the Antarctic environment, which came into force in 1998 (Bastmeijer 2003), requires that solid waste be removed, but it still allows the discharge into the sea of sewage and food waste, including potentially dangerous bacteria, viruses, detergents, solvents, metals, nitrates, and phosphates, which have significant implications on the health of the Antarctic ecosystem. More strict regulations are needed (1) to control the number of people visiting and working in the continent, (2) to ensure the safe processing and removal

of all human-related waste solid and liquid, organic and inorganic, and (3) to implement a continuous monitoring of the environment to detect pathogens and pollutants before they become widespread in the ecosystem.

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