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OUTBREAKS OF AVIAN CHOLERA IN HOPE BAY, ANTARCTICA

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ABSTRACT: During austral summers 1999–2000 and 2000–01, two outbreaks of avian cholera occurred in the Hope Bay area (63°24′S, 56°59′W), located on the tip of the Antarctic Peninsula. Eighty-six dead birds were found: five kelp gulls (*Larus dominicanus*), 36 skuas (*Stercorarius* sp.), and 45 Adelie penguins (*Pygoscelis adeliae*). The carcasses were studied using clinical, pathological, and microbiological criteria. Water samples from ponds where birds were settled and samples from 90 healthy birds also were analyzed during the second outbreak. *Pasteurella multocida* isolates were identified by biochemical tests, capsular type, somatic serotype, and susceptibility to nine antibiotics. Molecular subtyping was performed by *Apa*I and *Sma*I pulsed-field gel electrophoresis (PFGE) and enterobacterial repetitive intergenic consensus (ERIC-PCR). In February 2000, mortality in skuas was 16% and 2% in kelp gulls. In the 2000–01 breeding season, mortality in south polar skuas was 47%, 24% in brown skuas, 1.4% in kelp gulls, and 0.01% in Adelie penguins. All birds had lesions of avian cholera. In kelp gulls the presentation was chronic, whereas skuas and penguins suffered subacute and acute disease, respectively. Fifty-five isolates recovered from dead birds and one from water were identified as *P. multocida gallicida*, type A:1. The strains presented a unique molecular pattern by PFGE and ERIC-PCR. A possible hypothesis to explain the origin of the outbreaks was that nonbreeder kelp gulls carried *P. multocida gallicida* to Hope Bay, and avian cholera was transmitted through water to skuas and penguins. This study reports avian cholera in new bird species, their potential role in the transmission of the disease, and the different responses of these species to the disease.

Key words: Adelie penguin, Antarctica, avian cholera, ERIC-PCR, kelp gull, *Pasteurella multocida*, PFGE, skua.

INTRODUCTION

Avian cholera is an infectious disease caused by *Pasteurella multocida*. Transmission is thought to be either by inhalation of a bacteria-ladened aerosol (Simensen and Olson, 1980) or by consumption of contaminated water or food (Botzler, 1991). The disease can be either acute or chronic in poultry, but in wild birds the predominant clinical presentation is acute (Wobeser, 1997). Morbidity and mortality are variable, and susceptibility is species dependent. Avian cholera has been documented in over 100 different wild bird species and in North America epizootics, killing thousands of waterfowl annually (Botzler, 1991). Mortality due to avian cholera also has been reported in Japan (Nakamine et al., 1992), Canada (Wobeser et al., 1979), New Zealand (de Lisle et al., 1990), the Arctic (Samuel et al., 1997), and Denmark (Pedersen et al., 2003). Mortality of brown skuas (Parmelee et al., 1979) and kelp gulls (Kaschula and Truter, 1951) has been reported previously.

Two potential reservoirs for *P. multocida* in waterfowl populations have been suggested: carrier birds and wetland sites. *P. multocida* occasionally has been isolated from healthy waterfowl (Vaught et al., 1967; Korschgen et al., 1978), and a recent study showed evidence that birds serve as a reservoir for avian cholera (Samuel et al., 2005).

The Hope Bay area, located on the tip of the Antarctic Peninsula, encompasses Base Antártica Esperanza, the northernmost continental Antarctic Station (63°24′S, 56°59′W). During spring and summer seasons, Adelie penguins (*Pygoscelis adeliae*), gentoo penguins (*Pygoscelis papua*), kelp gulls (*Larus dominicanus*), brown skuas (*Stercorarius antarctica lonn-*

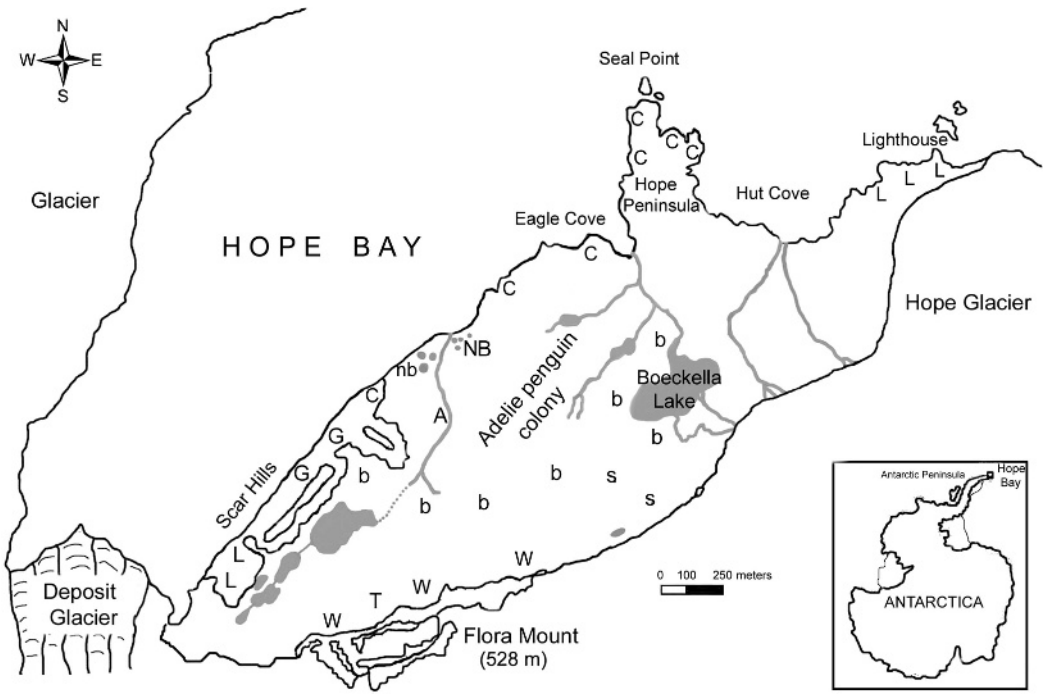

FIGURE 1. Location of birds in Hope Bay during the 2000–01 breeding season: (s) nesting areas of south polar skuas, (b) nesting areas of brown skuas, (L) nesting areas of kelp gulls, (NB) settlement of nonbreeder skuas and kelp gulls, (C) nesting areas of snowy sheathbills, (G) gentoo penguin colony, (T) nesting area of Antarctic terns, (W) nesting areas of Wilson's storm petrels, (nb) settlement of nonbreeders skuas and kelp gulls during the 1999–2000 breeding season.

bergi), south polar skuas (*Stercorarius maccormicki*), snowy sheathbills (*Chionis albus*), Antarctic terns (*Sterna vittata*), and Wilson's storm petrels (*Oceanites oceanicus*) are breeding in Hope Bay; and six species—southern giant petrels (*Macronectes giganteus*), southern fulmars (*Fulmarus glacialoides*), cape petrels (*Daption capense*), snow petrels (*Pagodroma nivea*), black-bellied storm petrels (*Fregetta tropica*), and imperial shags (*Phalacrocorax albiventer*)—are visiting the area (Coria and Montalti, 1993). This study describes two outbreaks of avian cholera among wild birds in Hope Bay during the 1999–2000 and 2000–01 breeding seasons.

MATERIALS AND METHODS

Study areas and populations

Hope Bay (63° 24′S, 56° 59′W) is separated from Bransfield, Joinville, and Dundee Islands by the Antarctic Strait. In general, the surroundings of the bay are covered by glaciers except for its southern face, which has an ice-free area of approximately 10 km^2. The southwestern limit is the Deposit Glacier, and the northeastern margin is defined by a lighthouse. There are two coves in the abovementioned area, Hut Cove and Eagle Cove, separated by Seal Point. Hope Bay is characterized by the presence of 12 water bodies, mostly of glacial origin, the most important of which is Boeckella Lake (Fig. 1).

On 15 February 2000, during the 1999–2000 breeding season and an avian cholera outbreak, a population census of skuas and kelp gulls was conducted by direct counting. During the 2000–01 breeding season (between November 2000 and February 2001), breeding areas for brown skuas, south polar skuas, kelp gulls, and snowy sheathbills were identified (Fig. 1), and active nests with at least one egg were marked. Population census was performed again by direct counting. Four transects between 1,000 and 3,000 m^2 each were established, and breeding areas were surveyed during walks along these transects at seven-day intervals.

Egg-laying and hatching data were recorded, and a population census of nonbreeding south polar skuas, brown skuas, and kelp gulls was performed by direct counting. In addition, walks through the four transects were carried out each day to record mortality, and six additional transects of 1,500 m^2 were established through the Adelie penguin colony.

Sample collection and histopathology

Carcasses collected during the 1999–2000 breeding season were frozen and sent to Laboratorio de Diagnóstico e Investigaciones Bacteriológicas (LADIB; Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina) for microbiological and pathological studies. Dead birds involved in the 2001 outbreak were collected and identified; they were necropsied at Hope Bay. Tissue samples were collected for microbiologic and histopathologic studies. Samples from trachea, lung, air sacs, pericardiac sacs, esophagus, stomach, intestine, heart, spleen, brain, liver, and kidney from carcasses taken during the two outbreaks were fixed in 10% buffered formalin for histopathologic studies. After embedding in paraffin, the samples were sectioned (3 μm) and stained with hematoxylin and eosin and periodic acid–Schiff (PAS).

Microbiological studies

Samples from lung, liver, spleen, and heart from carcasses collected during February 2000 were streaked on a blood agar plate (blood agar base, Difco, Becton Dickinson, Le Pont de Claix, France, with 5% defibrinated sheep blood) and incubated at 37 C for 48 hr. Samples from blood, liver, spleen, lung, air sacs, pericardiac sacs, and pharynx from all birds collected during 2001 were streaked on a blood agar plate, *P. multocida* selective agar PMSA (Moore et al., 1994) and hektoen enteric agar (Becton Dickinson); all plates were incubated at 37 C for 48 hr. For enterobacteria isolation, samples from cloaca swabs were streaked on hektoen enteric agar and incubated at 37 C for 48 hr. Bacterial colonies were stored at −20 C in brain-heart infusion (Difco Laboratories, Detroit, Michigan, USA) with 30% glycerol and sent to LADIB for identification. Samples from intestine, lung, and trachea were stored at −20 C for isolation and identification of *Campylobacter* spp. (Lior, 1984; Giacoboni et al., 1993) and *Mycoplasma* spp., as well as for detection of *Chlamydophila psittaci* (Leotta et al., 2003).

For fungus isolation, samples from trachea were streaked in duplicate on Sabouraud dextrose agar containing yeast extract and chloramphenicol (Difco, Becton Dickinson), and incubated at 28 and 37 C for 10 days. *Thelebolus microsporus* was identified as previously described (Leotta et al., 2002; de Hoog et al., 2005), and yeasts were identified by colony and cell morphology and by physiologic and biochemistry tests (Fell and Statzell-Tallman, 1998a, b).

Between January and February 2001, samples from pharyngeal swabs were collected from 90 healthy birds (30 Adelie penguins, 30 skuas, and 30 snowy sheathbills). These samples were streaked on PMSA and incubated at 37 C for 48 hr.

Between 19 January and 13 February 2001, 30 surface water samples from 10 lakes and ponds were collected. Water samples (250 ml) were collected at three different times from each of six lakes and four minor ponds. For each water sample, temperature and pH were measured in situ with a Hanna HI8314 (Hanna Instruments Argentina, Buenos Aires, Argentina). In addition, for each water sample, salinity was measured with a Hanna HI8033 (Hanna Instrument Argentina). One hundred microliters from each water sample were streaked on a blood agar plate and on PMSA in duplicate and incubated at 37 C for 48 hr.

Characterization of *Pasteurella multocida* isolates

Isolates were identified by morphologic and biochemistry characteristics (Fegan et al., 1995; Koneman et al., 1999). The capsular serotype was determined by a multiplex PCR assay (primers from Integrated DNA Technologies, Coralville, Iowa, USA, and reagents from Amersham Biosciences Corp., Piscataway, New Jersey, USA) (Townsend et al., 2001). Heat-stable antigens were serotyped by immunodiffusion according to the method of Heddleston et al. (1972) using antisera from the National Veterinary Services Laboratory (Ames, Iowa, USA). All isolates were serotyped by M. J. Wolcott at the USGS–National Wildlife Health Center, Madison, Wisconsin, USA.

The susceptibility of *P. multocida* strains to ampicillin, ceftiofur, cephalothin, enrofloxacin, florfenicol, gentamicin, streptomycin, tetracycline, and tiamulin was established. The minimum inhibitory concentration was determined by the broth microdilution test, and growth inhibition was evaluated according to the National Committee for Clinical Laboratory Standards for *P. multocida* (NCCLS M31-A2 and M7), with the exception of gentamicin (according to other non-*Enterobacteriaceae*

values), ampicillin, tetracycline (according to *Enterobacteriaceae* values), tiamulin (according to *Actinobacillus pleuropneumoniae* values), and streptomycin (according to NORM/NORM-VET 2002 for *Escherichia coli* values).

Molecular subtyping of *Pasteurella multocida* isolates

All isolates of *P. multocida* from both outbreaks were subtyped by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) and pulsed-field gel electrophoresis (PFGE). For molecular comparison, six *P. multocida* strains were included: *P. multocida multocida* type A:1 (X-73), *P. multocida gallicida* type A:14 (NADC P-2225), *P. multocida gallicida* type A:1 from Australia poultry (donated by Dr. P. Blackall), *P. multocida gallicida* type A:1, and *P. multocida septica* type A:1 from Argentina poultry, and one strain isolated from a southern giant petrel during February 2000 in the South Shetland Islands at 200 km from Hope Bay (Leotta et al., 2003).

The ERIC-PCR was carried out essentially as described by Amonsin et al. (2002). One milliliter of *P. multocida* culture grown overnight in brain-heart infusion at 37 C (OD_{620}:0.5 ml of culture) was used for genomic DNA extraction and purification by the Wizard™ kit (Promega, Madison, Wisconsin, USA). Oligonucleotide primers ERIC1R (5′ ATGTAAGCTCCTGGGGATTCAC) and ERIC2 (5′ AAGTAAGTGACTGGGGTGAGCG) were used to generate DNA fingerprints. The fragments obtained were separated by electrophoresis in 2% agarose gels containing ethidium bromide (2 μg/ml). Electrophoresis was carried out in a 1X tris-acetate-EDTA buffer for 3.5 hr at 80 V; 1 kb DNA Ladder (Promega) as a fragment size marker was used.

The PFGE was carried out essentially as described by Gunawardana et al. (2000). Briefly, the plugs of agarose containing DNA were digested with 40U of *Apa*I (Promega). Lambda Ladder PFGE Marker (New England BioLabs, Beverly, Massachusetts, USA) as fragment size marker was used. The fragments were separated in a 1.2% agarose gel (Pulsed Field Certified Agarose, BioRad, Hercules, California, USA) in 0.5X tris-borate-EDTA buffer at 14 C in a contour CHEF-DR III system (BioRad). The run time was 22 hr, with a constant voltage of 170 V, using a linear pulse ramp of 1–30 sec. The staining was carried out with 0.5 μg/ml of aqueous ethidium bromide solution (BioRad). As a second enzyme, 40U of *Sma*I (Promega) was used for restriction with a linear pulse ramp of 0.5–40 sec for 22.5 hr (Pedersen et al., 2003). *Salmonella* Braenderup CDC H-9812 was included as fragment size marker to analyze the patterns generated with *Sma*I PFGE.

The gel images of PFGE and ERIC-PCR fingerprints obtained by Kodak Digital Science 1D™ were analyzed using BioNumerics version 3.5 (Applied Maths, Kortrijk, Belgium). The relationship among the patterns was estimated by the proportions of shared bands applying the Dice coefficient with a 1.5% band position tolerance, and a dendrogram based on the UPGMA method was generated.

RESULTS

Bird populations

On 15 February 2000, 100 kelp gulls and 84 skuas were counted. Kelp gulls were breeding in the Scar Hills and lighthouse areas, and skuas were breeding between Boeckella Lake and Flora Mount; nonbreeder kelp gulls and skuas were grouped around three ponds (Fig. 1). During austral summer 2000–01, nine breeding bird species were recorded in Hope Bay: kelp gull, south polar skua, brown skua, snowy sheathbill, Wilson's storm petrel, antarctic tern, gentoo penguin, and Adelie penguin. The distribution of these species is shown in Figure. 1. Population estimates from direct counts for kelp gulls, south polar skuas, brown skuas, and snowy sheathbills collected in the 2000–01 breeding season are shown in Tables 1 and 2. Antarctic tern and Wilson's storm petrel colonies in Flora Mount were observed.

Avian cholera outbreak descriptions

In February 2000 the mortality rate for nonbreeder kelp gulls was 2% (2/100), and for nonbreeder skuas 16% (13/84). They were found dead around ponds where nonbreeder flying birds were settled. Between 10 January and 7 February 2001, three nonbreeder kelp gulls, representing 2% of the population, were found dead. On 17 January nonbreeder skuas started to die, and 23 skua carcasses were

TABLE 1. Data on population sizes of south polar skuas, brown skuas, kelp gulls, and snowy sheathbills in the Hope Bay area during the 2000–2001 breeding season.

Species	Active nest	Egg[a]			Chick[b]			Breeding adult[c]			Nonbreeding[d]			Total population
		Mean	SE	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range	
South polar skua	2	1[b]	0.5[b]	0–3[b]	0[c]	0[c]	0[c]	4	0.2	2–4	17	1.3	10–22	21
Brown skua	7	2	0.9	0–8	1	0.6	0–5	14	0.2	12–14	39	2.9	21–58	54
Kelp gull	24	28	3.5	5–38	20	2.7	7–32	48	2.8	38–65	145	5.2	115–160	213
Snowy sheathbill	18	26	4.3	3–45	22	2.5	5–29	36	1	32–40	29	1	25–33	87

SE = standard error.

[a] Monitored from 1 November to 31 December 2000.

[b] Monitored from 29 November 2000 to 31 January 2001.

[c] Monitored from 1 November 2000 to 28 February 2001.

[d] Monitored from 1 January to 28 February 2001.

collected until 20 February. Of these carcasses, 10 were south polar skuas, and 13 were brown skuas; the mortality for the south polar skua population was 47%, and for brown skuas it was 24% (Table 3). All birds affected by avian cholera were found around ponds.

Between 22 January and 6 February, 40 adults and five chick Adelie penguins were found dead or dying. The estimated mortality was 0.01% among 123,859 pairs of Adelie penguins (Myrcha et al., 1987). Dead penguins had been breeding around the ponds used by both skuas and kelp gulls. No other bird species was found dead in the area.

Pathology

In the 1999–2000 breeding season, two kelp gulls and eight brown skuas were studied. At necropsy, no ectoparasites or skin lesions were seen. Fibrin deposits over coelomic serous membranes, liver capsule, and pericardium of two kelp gulls and seven brown skuas were seen. Autolytic changes and damage due to freezing severely hampered the histopathologic evaluation of the tissues. Severe congestion, edema, and fibrin in the pulmonary parenchyma of all birds were observed. Lesions observed in skuas and kelp gulls were compatible with subacute avian cholera.

During the 2000–01 breeding season, 71 birds were studied. Among them, four south polar skuas, two brown skuas, and 14 Adelie penguins were found dying. The skuas had polydipsia, tremors, and dyspnea. Stertors, nostrils and mouth filled with abundant secretions, inability to fly

TABLE 2. Data on population sizes of nonbreeding south polar skuas, brown skuas, kelp gulls, and snowy sheathbills in Hope Bay area.

Species	Nonbreeding birds					
	1 November–31 December 2000			1 January–28 February 2001		
	Mean	SE	Range	Mean	SE	Range
South polar skua	5	0.5	4–9	17	1.3	10–22
Brown skua	42	4.1	32–58	39	2.9	29–45
Kelp gull	38	0.7	26–46	145	5.2	115–158
Snowy sheathbill	25	1	22–30	29	1	26–37

SE = Standard error.

TABLE 3. Lesions observed and number of *P. multocida* isolates from south polar skuas, brown skuas, kelp gulls, and Adelie penguins dying of avian cholera in Hope Bay during a 2001 outbreak.

Lesions and *P. multocida* isolated	South polar skuas ($n=10$)	Brown skuas ($n=13$)	Kelp gulls ($n=3$)	Adelie penguins ($n=45$)
General hemorrhages	10	13	0	45
Fibrin deposit over coelomic serous membranes	8	12	0	0
Pulmonary congestion	9	11	0	0
Pulmonary edema	9	11	0	0
Pulmonary fibrin	8	11	0	0
Fibrinonecrotic pericarditis	7	10	0	0
Myocarditis	7	10	0	0
Digestive tract with passive hyperemia	10	13	0	45
Hepatomegaly and splenomegaly	9	11	0	6
Hepatomegaly and splenomegaly with necrotic areas	0	0	0	44
Edema and hemorrhages in the head	0	0	2	0
Hyperemia in the meninges	0	6	0	29
P. multocida strains	10	13	2	28

and uncoordination, general weakness, and loss of a fear response to approaching humans were also seen. Moreover, they had a body temperature above 43 C. The penguins were observed with nervous signs, tremors, incoordination, and opisthotonos.

Field necropsies of 23 skuas, three kelp gulls, and 45 Adelie penguins were performed. In all birds the conjunctiva was hyperemic. Ectoparasites were not seen. The stomach was empty, except for penguins, which contained a normal amount of krill. Typical lesions of avian cholera were observed; large quantities of viscous mucus in the pharynx and hemorrhagic serosal surfaces of the coelomic cavity organs and peritoneal lining were observed. Predominant lesions observed in kelp gulls, skuas, and Adelie penguins are shown in Table 3.

Lesions observed in kelp gulls were compatible with chronic avian cholera. In these birds the outstanding gross lesion was exudate and edema in the air spaces of head with hemorrhages and congestion in skeletal muscles surrounding the skull. In one carcass no lesions of avian cholera were observed.

In skuas the principal lesions were compatible with subacute avian cholera. Fibrinous exudate involving the liver capsule, mesentery, air sacs, and pericardium with petechial hemorrhage and heterophilic infiltration in areas of acute focal necrosis was observed. In addition, these birds had respiratory tract edema, congestion, and perivascular hemorrhage with heterophils, macrophages, and lymphocytes.

In Adelie penguins the lesions were compatible with acute avian cholera. The principal lesions were hepatomegaly with multiple small necrotic areas and splenomegaly with petechiae and ecchymoses. The livers had multiple foci of coagulative necrosis and heterophilic infiltration. Hyperemia in the meninges was the only abnormality observed in the nervous system.

Isolation results

Pasteurella multocida was isolated from the lung of two skuas during the 2000 outbreak and from 53 birds during the 2001 outbreak. The number of isolates for each species is shown in Table 3. The two *P. multocida* isolates from kelp gulls were recovered from the mouth. Isolates from south polar skuas and brown skuas were primarily from lung. For Adelie penguins, *P. multocida* 27 isolates were recovered from liver and one from lung.

All isolates were gram-negative rods and produced indole, reduced nitrate, and were oxidase and catalase positive. Hemolysis production, MacConkey growth, and urease activity were negative. The isolates fermented glucose, mannitol, arabinose, sorbitol, dulcitol, mannose, and sucrose but not lactose, trehalose, maltose, galactose, and xylose. These isolates were sensitive to ampicillin, ceftiofur, cephalothin, enrofloxacin, florfenicol, gentamicin, streptomycin, tetracycline, and tiamulin. All 55 isolates were characterized as *P. multocida gallicida*, capsular type A, and somatic serotype 1.

Samples from 90 healthy birds were negative for *P. multocida*. *Pasteurella multocida gallicida* serotype A 1 was recovered from one of three water samples. The temperature, pH, and salinity of this pond were 5 C, 7, and 8.73 g/l, respectively. The pond was being used by nonbreeding skuas and kelp gulls.

During the 2000 outbreak, *Rhodotorula minuta* was isolated from cloaca and lung samples of three skuas. *Cryptococcus albidus* were recovered from cloaca samples of two skuas, and *Campylobacter lari* biotype I from the intestinal content of two skuas and one kelp gull. During the 2001 outbreak, *T. microsporus* from the trachea of one skua and three kelp gulls was isolated. An *E. coli* characterized as enteropathogenic *E. coli* was recovered from the pharynx of two skuas. All samples from both outbreaks were negative for *Salmonella* spp., *C. psittaci*, and *Mycoplasma* spp.

Molecular subtyping of *Pasteurella multocida* isolates

Sixty-two isolates (56 from Hope Bay, one from a southern giant petrel, and five additional isolates) were included in this study. Using ERIC-PCR, five different patterns were detected, with 10–13 separated bands between 0.2 and 1.2 kb. The 56 isolates of *P. multocida gallicida* type A:1 recovered during the two avian cholera outbreaks in Hope Bay and the strain isolated from a southern giant petrel from the South Shetland Islands were identical using ERIC-PCR, and the pattern had 10 bands. The other *P. multocida* studied yielded four different patterns. *Pasteurella multocida multocida* type A:1 (NADC X-73) and *P. multocida gallicida* type A:14 (NADC P-2225) shared the same pattern. This pattern was closely related to the pattern from the Antarctic strains with 94.9% similarity and one band difference. Three different patterns were obtained with the *P. multocida gallicida* type A:1 strain from Australia and *P. multocida septica* type A:1 and *P. multocida gallicida* type A:1 strains from Argentina; patterns for these had 81.6%, 80.0%, and 73.9% similarity to Antarctic strains, respectively (Fig. 2).

Using *Apa*I PFGE, isolates yielded six patterns with 9–14 defined and well-separated bands between 40 and 400 kb. The strains recovered from Antarctica, including the isolate from a southern giant petrel, were indistinguishable by *Apa*I PFGE, showing a pattern with 11 bands. The other *P. multocida* strains were genetically diverse showing five polymorphic *Apa*I PFGE patterns, different from the Antarctic pattern. The NADC X-73 and NADC P-2225 strains showed patterns with a two-band difference and 91.6% similarity. These strains were genetically related to Antarctic strains and had 82.5% similarity and a four-band difference. By *Apa*I PFGE, the *P. multocida gallicida* type A:1 strain from Australia and the *P. m. septica* type A:1 and *P. multocida gallicida* type A:1 strains from Argentina were only 46.9% similar to Antarctic strains (Fig. 2). The results obtained by *Apa*I were confirmed by *Sma*I PFGE.

DISCUSSION

The possibility of disease introduction into Antarctic wildlife has been recognized since the start of the Antarctic Treaty in 1962 (Kerry et al., 1999), and precautions

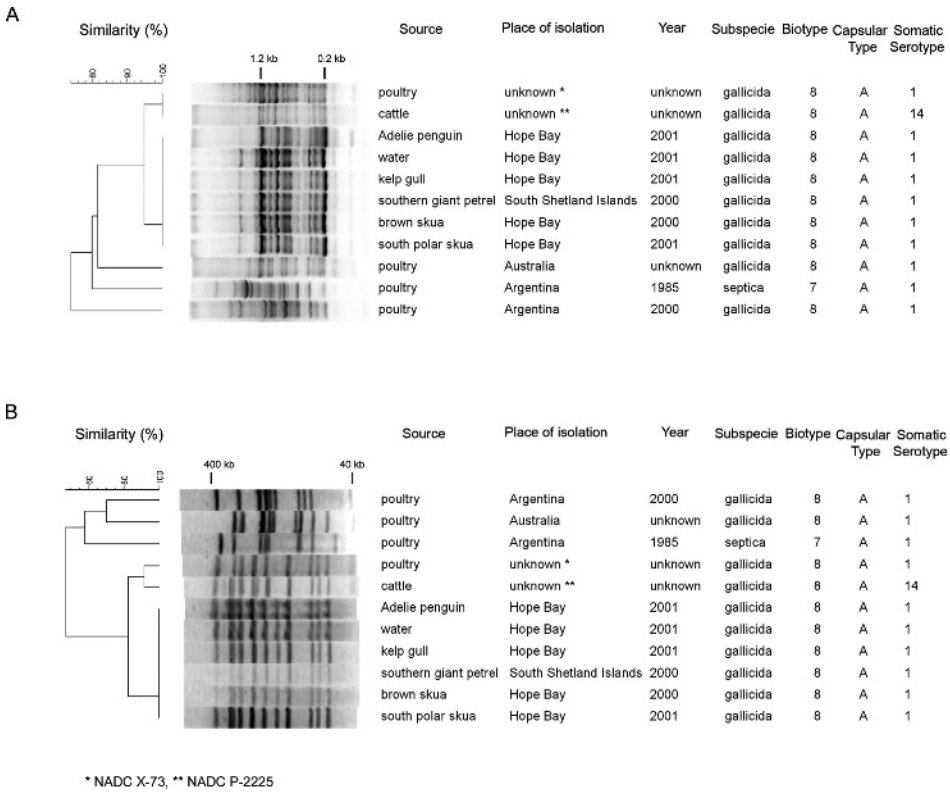

FIGURE 2. Dendrograms of *P. multocida* strains and sources, year of isolation, and phenotypic characteristics: (A) ERIC-PCR dendrogram; (B) *Apa*I-PFGE dendrogram.

to prevent the introduction of microorganisms into the region south of 60°S latitude are prescribed by the Protocol on Environmental Protection to the Antarctic Treaty of 1998. However, this issue has received limited attention, and very little is known about endemic or nonindigenous diseases in Antarctic bird populations (Kerry et al., 1999).

There have been two documented events of bacterial diseases that resulted in mortality in Antarctic birds. The first occurrence of avian cholera was in brown skuas reported on Livingston Island in the Antarctic Peninsula region; *P. multocida* A:1 was isolated (Parmelee et al., 1979). The second event, in February 2000, occurred in the South Shetland Islands; *P. multocida* A:1 was isolated from a dead southern giant petrel (Leotta et al., 2003).

In the past two decades, three bird mortality events were reported in Hope Bay. In 1981 and 1990, 12 skuas and 38 brown skuas were found dead, respectively (Montalti et al., 1996). The third mortality event occurred in February 1997, including 16 brown skuas and seven south polar skuas (Leotta et al., 2002). Unfortunately, the cause for this mortality was not determined.

During the *P. multocida* outbreaks in the 1999–2000 and 2000–01 breeding seasons in Hope Bay, avian cholera was diagnosed as the primary cause of mortality among birds. In wild birds this disease is predominantly described as acute (Wobeser, 1997). However, in Hope Bay apparent differences in susceptibility were observed between affected species. The chronic form of avian cholera was observed in kelp gulls, the subacute form in skuas, and the acute form in Adelie

penguins. Most of the mortality was associated with skuas, mainly the south polar skua; Adelie penguins were least affected. However, the potential impact of avian cholera on the Adelie penguin population could not be established because these birds started their migration in the first days of February.

The isolation of *P. multocida gallicida* type A:1 from carcasses reinforced the clinical diagnosis. In addition, all the Antarctic strains showed near-identical phenotypic and genotypic characteristics and were indistinguishable by ERIC-PCR and by *Apa*I and *Sma*I PFGE, confirming these outbreaks. According to Tenover's definition (Tenover et al., 1995), both outbreaks were due to a unique clone. It is interesting that all isolates of *P. multocida gallicida* recovered from wild birds of Hope Bay and the isolate from a southern giant petrel found dead in February 2000 in the South Shetland Islands have an indistinguishable molecular pattern; it is probable they share a common origin. The southern giant petrel might have acquired the infection eating dead or dying animals in Hope Bay and then died in the South Shetland Islands, as these birds are able to fly more than 400 km per day (Marchant and Higgins, 1993). Unfortunately, it was not possible to compare the isolates from Hope Bay with the former isolates from Livingston Island (Parmelee et al., 1979).

The unique molecular patterns obtained by *Apa*I and *Sma*I PFGE and ERIC-PCR with *P. multocida gallicida* A:1 strains from Antarctica were different from the patterns obtained with the five *P. multocida gallicida* A:1 strains of different origin, in spite of belonging to the same subspecies and serotype. However, it may be informative to compare the Hope Bay isolates with *P. multocida* strains isolated from other outbreaks involving wild birds and to utilize newer and more advanced molecular epidemiological techniques, such as multilocus sequence typing or multilocus variable-number tandem repeat analysis. The fact that all isolates were indistinguishable irrespective of bird species and the year of isolation could indicate a remarkable genetic stability and persistence of this strain. A similar situation was described in Denmark, where *P. multocida* strains recovered from wild birds in 1996 and 2003 were indistinguishable based on the same molecular techniques use in this study (Pedersen et al., 2003).

In contrast to other avian cholera outbreaks in wildfowl populations (Botzler, 1991), it is difficult to ascertain the possible origin of avian cholera in Hope Bay because of geographic isolation and the absence of domestic animals or invertebrate vectors. In addition, *P. multocida* has never been reported from Antarctic marine mammals. The extreme climatic conditions do not enhance environmental survival of *P. multocida*, and the failure to isolate *P. multocida* from healthy birds does not provide evidence for the presence of carriers.

Kelp gulls appeared to be more resistant to the disease. During the 1999–2000 breeding season, kelp gulls had lesions compatible with subacute avian cholera; in the 2000–01 breeding season lesions were compatible with chronic avian cholera. One possible hypothesis to explain both outbreaks is that nonbreeder kelp gulls carried *P. multocida gallicida* to Hope Bay. It is possible that the kelp gulls acquired the infection during the 1999–2000 breeding season and maintained the infection either in individual birds or in the population through bird-to-bird transmission according to Wobeser's hypothesis (1992). One hundred and seven nonbreeder kelp gulls arrived 15 days before the onset of the 2001 outbreak, and they may have served as carriers (Rosen and Bischoff, 1950; Korschgen et al., 1978; Titche, 1979).

Once *P. multocida* was introduced to Hope Bay, we believe that contaminated water provided the primary means for spread to skuas and penguins. Although some water bodies in Hope Bay can be considered typical lakes, since they do not

freeze to the bottom and are generally free of ice in summer, some shallow water bodies have permanent ice cover all year (Izaguirre et al., 1998). During the 2001 outbreak, *P. multocida gallicida* also was recovered from the water of a pond that both nonbreeding skuas and Adelie penguins drink from. This pond had the optimal physical and chemical conditions as well as pH, temperature, and salinity for *P. multocida* survival (Bredy and Botzler, 1989). In addition, only birds around these ponds were affected.

Other microoorganisms including *R. minuta*, *C. albidus*, *T. microsporus*, *C. lari* biotype I, and EPEC were isolated from some birds with avian cholera. As these microorganisms do not cause lesions similar to those of avian cholera, and they have never been reported as pathogens for wild birds, we consider that these birds could be asymptomatic carriers of these organisms in the Antarctic ecosystem.

We believe that avian cholera is not endemic in Antarctic and that *P. multocida* is introduced by migratory birds. All environmental isolates were restricted to one pool where the birds were settled, and to date the existence of an environmental reservoir has not been demonstrated. Currently it is not possible to take measures to resolve outbreaks by avian cholera in Antarctica because of international legislation (Antarctic Treaty). However, it is possible to prevent the introduction of microorganisms potentially affecting Antarctic birds populations as a consequence of human presence in Antarctica. To achieve this, we must know what diseases are present and understand their epidemiology in Antarctic wildlife.

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