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SHORT NOTE

Prevalence of *Edwardsiella tarda* in Antarctic wildlife

Gerardo A. Leotta · Pablo Piñeyro · Soledad Serena · Germán B. Vigo

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Abstract For many years, the Antarctic region has been isolated from human activity. However, there is little data available regarding endemic and exotic diseases. The purpose of this work was to determine the prevalence of *Edwardsiella tarda* in Antarctic wildlife, including birds, mammals and fish. During the summer of 2000 and 2002 in the Potter Peninsula, and during the summer of 2001 and 2003 in Hope Bay, a total of 1,805 faecal samples from Antarctic animals and 50 infertile eggs of Adelie penguins (*Pygoscelis adeliae*) were collected in order to isolate *E. tarda*. The classic *Edwardsiella tarda* was isolated from 281 (15.1%) of the 1,855 Antarctic wildlife samples. This is the first report of *E. tarda* isolation from southern giant petrels (*Macronectes giganteus*), brown skuas (*Stercorarius lonnbergi*), south polar skuas (*Stercorarius maccormicki*), kelp gulls (*Larus dominicanus*), greater sheathbills (*Chionis albus*), chinstrap penguins (*Pygoscelis antarctica*), eggs of Adelie penguins and Weddell seals (*Leptonychotes weddelli*). None of the evaluated animals showed clinical signs of disease. Our results suggest that *E. tarda* is a common bacterium amongst Antarctic birds and mammals.

G. A. Leotta
Laboratorio de Diagnóstico e Investigaciones Bacteriológicas, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, CC: 296, La Plata (1900), Buenos Aires, Argentina

P. Piñeyro
Cátedra de Patología Especial, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, CC: 296, La Plata (1900), Buenos Aires, Argentina

S. Serena
Cátedra de Virología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, CC: 296, La Plata (1900), Buenos Aires, Argentina

G. B. Vigo (✉)
Cátedra de Microbiología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, CC: 296, La Plata (1900), Buenos Aires, Argentina
e-mail: gvigo@fcv.unlp.edu.ar

Introduction

Due to the geographical isolation of the Antarctic region, the contact between the Antarctic fauna and bacterial pathogens is limited. However, the presence of humans has significantly increased as a result of tourism and scientific expeditions (Bonnedahl et al. 2005). Given the limited data available regarding endemic and exotic diseases, it is rather difficult to determine the true origin of current diseases (Leotta et al. 2006a; Nievas et al. 2007). *Edwardsiella tarda* is considered a common inhabitant of the normal intestinal flora in aquatic animals (White 1984); however, under certain circumstances, this bacterium can cause intestinal and extra-intestinal diseases and wound infections in reptiles, amphibians and terrestrial endotherms, including humans (Bockemuhl et al. 1971; Sakazaki and Tamura 1992; Janda and Abbott 1993; Baya et al. 1997). This bacterium is also considered a common opportunistic agent in sick or injured marine wildlife (Coles et al. 1978). In Antarctic and sub-Antarctic animals, two cases associated with *E. tarda* have been reported. The first case was in Rockhopper penguins (*Eudyptes crestatus*) with chronic enteritis (Cook and Tappe 1985), and the second case, in an Adelie penguin (*Pygoscelis adeliae*) with a subcutaneous clostridial infection (Nievas et al. 2007). In addition, only two reports describe *E. tarda* in Antarctic wildlife (Zunino et al. 1985). However, the prevalence of *E. tarda* in Antarctic

wildlife has never been reported. The purpose of this work was to determine the prevalence of *Edwardsiella tarda* in Antarctic wildlife, including birds, mammals and fish.

Methods

Sampling was carried out in Potter Peninsula, located on King George Island, South Shetland Islands (62°15′S, 58°36′W), and in the Hope Bay area, located on the tip of the Antarctic Peninsula (63°24′S, 56°59′W). During the spring and summer seasons, these are breeding areas for some seabirds (Hahn et al. 1998; Leotta et al. 2006a), and occasionally for Antarctic sea mammals. One of the main fish found in these regions is *Nothotenidae*, which is one of the food sources of both mammals and birds. A total of 1,855 samples were collected from Antarctic animals in the summer of 2000 and 2002 (Potter Peninsula), and 2001 and 2003 (Hope Bay). Non-probabilistic sampling was carried out in animals that were separated from their colonies for mere convenience. Breeding areas for brown skuas (*Stercorarius* lonnbergi), south polar skuas (*Stercorarius maccormicki*), kelp gulls (*Larus dominicanus*), south giant petrels (*Macronectes giganteus*), Adelie penguins, gentoo penguins (*Pygoscelis papua*), chinstrap penguins (*Pygoscelis antarctica*), and greater sheathbills (*Chionis albus*) were identified. The population was calculated using a census carried out by Hahn et al. (1998) and Leotta et al. (2006a). A total of 1,622 seabirds were captured, clinically evaluated by experienced veterinarians, and sampled with cloacal swabs, which were conserved on Stuart transport media (Difco Laboratories Incorporated, Cambridge, UK) at 4°C. Fifty infertile eggs of Adelie penguins were collected from the nest after the incubation period, they were washed with distilled water and neutral detergent, dried and immersed into alcohol 70° for 1 h. Then they were dried and the content was streaked onto hektoen agar (Difco) and incubated at 37°C for 48 h. In addition, 161 Antarctic fur seals (*Arctocephalus gazella*) and Weddell seals (*Leptonychotes weddelli*) were observed but not captured, and immediately after defecation an aliquot of fresh faeces was collected in sterile bags (Nasco's Whirl-pak, Network International Technologies, Buenos Aires, Argentina) and conserved at 4°C. Finally, a total of 22 fishes (*Nothotenia coriiceps*) were captured, sacrificed, necropsied, and an aliquot of intestinal contents was collected in sterile bags (Nasco's Whirl-pak) and conserved at 4°C. All samples of birds, mammals and fish were processed between 2 and 12 h after being collected. Each sample was plated directly onto hektoen enteric agar (Difco), and incubated at 37°C for 48 h. The suspected blue-green colonies with black centres were subcultured onto trypticase soy agar (Difco) and incubated at 37°C for 24 h. Pure colonies were subsequently inoculated into brain heart infusion broth (Difco) with 30% of glycerol and stored at −20°C. All suspected *E. tarda* colonies were subjected to a complete phenotypic characterisation. The Gram stain of the isolates was determined, and the following biochemical tests were carried out according to the manufacturer's directions: catalase (Waco Pure Chemical Industries, Osaka, Japan), oxidase (Laboratorios Britania, Buenos Aires, Argentina), β-galactosidase (Laboratorios Britania), indole (Laboratorios Britania), methyl red (Difco), Voges-Proskauer (Difco), citrate (Difco), lysine (Difco), urea (Difco), malonate (Difco), ornitine (Difco), sulfhydric acid (tri sugar iron, Difco), glucose (ICN Biomedicals, Aurora, Ohio, USA), lactose (ICN), dulcitol (ICN), salicine (ICN), raffinose (ICN), sorbitol (ICN), arabinose (ICN), maltose (ICN), xylose (ICN), trehalose (ICN), and sucrose (ICN), according to the method described by Koneman et al. (1999). A statistical analysis of data was performed using Statgraphics Centurion XV version 15.2.05 (StatPoint Inc., Herdon, Virginia, USA).

Results

The classic *Edwardsiella tarda* was isolated from 281 (15.1%) of 1,855 Antarctic wildlife samples obtained from southern giant petrels, brown skuas, greater sheathbills, Adelie penguins, gentoo penguins and chinstrap penguins from Potter Peninsula in 2000 and 2002. *E. tarda* could not be isolated in 54 faecal samples from Antarctic fur seals, 22 samples from fish and 32 samples from south polar skuas in 2002. *E. tarda* was isolated from brown skuas, kelp gulls, greater sheathbills, Adelie penguins and their eggs, chinstrap penguins and Weddell seals from Hope Bay in 2001 and 2003. However, in the summer of 2003, *E. tarda* could not be isolated from the cloacal samples from 22 gentoo penguins. In all the birds sampled in both places, no clinical signs of disease were observed. In 15 fishes, no pathological lesions were observed, but in the intestine from seven fishes, endoparasites were observed. Animal species, seasons, geographical regions, identification of Antarctic wildlife population sampled, and the prevalence of *E. tarda* are shown in Table 1. As a result of the non-probabilistic sampling method used, the positive rate observed amongst seasons, areas and species did not exhibit a normal distribution. There were no statistical differences amongst seasons and/or geographic areas of the positive samples obtained ($P = 0.24$, Kruskal Wallis test) ($P = 0.07$, Kolmogorov–Smirnov test). Due to the lack of positive results amongst the fish evaluated, only the differences amongst the mean of positive results in birds and mammals were analysed, where no statistical differences were observed ($P = 0.93$, Kolmogorov–Smirnov test).

Table 1 Antarctic wildlife species, geographical region, animal population, season, number of analyzed samples and prevalence of *E. tarda*

Species	Geographical region	Population	Year	Samples			95% CI
				N	Positive	Prevalence	
Southern giant petrels *Macronectes giganteus*	Potter Peninsula	150	2000	90	20	22.2	13.07–31.36
			2002	48	16	33.3	18.85–47.71
Brown skuas *Stercorarius lonnbergi*	Hope Bay	75	2001	14	2	14.3	1.77–42.81
	Hope Bay	75	2003	36	10	27.8	11.75–43.79
South polar skuas *Stercorarius maccormicki*	Potter Peninsula	166	2000	66	9	13.6	4.6–22.67
	Potter Peninsula	166	2002	32	0	0.0	–
Kelp gulls *Larus dominicanus*	Hope Bay	213	2001	50	18	36.0	21.69–50.3
			2003	54	23	42.6	28.47–56.7
Greater sheathbills *Chionis albus*	Hope Bay	87	2001	50	19	38	23.54–52.45
	Potter Peninsula	12	2002	6	4	66.6	22.27–95.67
	Hope Bay	87	2003	22	4	18.2	5.18–40.28
Adelie penguins *Pygoscelis adeliae*	Potter Peninsula	29,108	2000	50	2	4.0	0.48–13.71
	Hope Bay	247,218	2001	755	88	11.7	9.3–14.01
			2003	144	14	9.7	4.53–14.9
Adelie penguin eggs	Hope Bay	125,000	2001	50	2	4.0	0.48–13.71
Gentoo penguins *Pygoscelis papua*	Potter Peninsula	4650	2000	50	1	2.0	0.05–10.64
	Hope Bay	690	2001	100	16	16.0	8.31–23.68
	Potter Peninsula	4650	2002	33	5	15.1	15.15–31.89
	Hope Bay	690	2003	22	0	0.0	–
Chinstrap penguins *Pygoscelis antarctica*	Potter Peninsula	530	2002	39	5	12.8	4.29–27.43
Antarctic fur seals *Arctocephalus gazella*	Potter Peninsula	Unknown	2002	32	0	0.0	–
Weddell seals *Leptonychotes weddelli*	Hope Bay	Unknown	2003	90	23	25.5	15.98–35.12
Nothotenia coriiceps	Potter Peninsula	Unknown	2002	22	0	0.0	–
Total			2000–2003	1855	281	15.1	13.49–16.8

Discussion

In this study we isolated *E. tarda* in 15.1% of Antarctic wildlife animals sampled in two places of Antarctica, Hope Bay and Potter Peninsula. We considered that *E. tarda* is a common bacterium in the faeces of Antarctic birds and mammals, because no significant differences were found with regard to the areas and seasons investigated. This is supported by the fact that there are no significant differences in the distribution of positive rates obtained within the species evaluated. However, *E. tarda* was described as an opportunistic bacterial pathogen and has been commonly isolated from different healthy, sick and moribund fish, birds and reptiles in several ecosystems around the world (White and Simpson 1973; Trust and Bartlett 1974; Baya et al. 1997). Antarctic birds and mammals eat fish, particularly *Nothotenia coriiceps*. We considered that the trophic chain could be one of the links in *E. tarda* transmission. However, none of the 22 *N. coriiceps* samples was positive for *E. tarda*. The negative results, though inconclusive, could be due to the small number of fish sampled.

Several enteropathogens such as *Campylobacter lari*, *Campylobacter jejuni*, *Salmonella* spp., and enteropathogenic *Escherichia coli*, were isolated from Antarctic animals (Oelke and Steiniger 1973; Bonnedahl et al. 2005; Leotta et al. 2006a, b). The known prevalence of *E. tarda* in Antarctic animals is an important precedent for the future control and surveillance of populations of mammals, birds and fishes, since this enteropathogen could cause diseases in these animals, which could be an indicator of the health of the ecosystem.

This is the first report of *E. tarda* isolation from southern giant petrels, skuas, kelp gulls, greater sheathbills, chinstrap penguins, eggs of Adelie penguins, and Weddell seals. However, further investigations are necessary in order to determine the role of *E. tarda* as a pathogen of Antarctic wildlife fauna.

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