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ORIGINAL PAPER

Enteropathogenic *Escherichia coli* (EPEC) in Antarctic fur seals *Arctocephalus gazella*

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Abstract Rectal swabs were collected from Antarctic fur seal pups *Arctocephalus gazella* at Cape Shirreff, South Shetland Islands, and analyzed for the presence of anthropogenic pathogens. Two of the 33 pups tested positive for enteropathogenic *Escherichia coli* (EPEC). These samples are the first records of EPEC in Antarctic wildlife and suggest that more needs to be done to protect the Antarctic fauna from exotic anthropogenic pathogens.

Introduction

There is growing international concern about harmful pathogens being introduced accidentally into Antarctica by human activities. Such microbial pollution could result in infectious disease outbreaks and high mortality among sensitive, colonial-breeding Antarctic wildlife. Surveys in the region have already detected well-known pathogens such as *Salmonella enteritidis* Phage Type 4, *S. typhimurium*, *Campylobacter jejuni*, and *Pasteurella multocida*, some of which could have been introduced there by humans (Olsen et al. 1996; Palmgren et al. 2000; Broman et al. 2000; de Lisle et al. 1990). In the present paper, we report the discovery of enteropathogenic *Echerichia coli* (EPEC) in Antarctic fur seals *Arctocephalus gazella*.

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Methods

Our study site was a fur seal breeding colony at "Lobería" beach, 2 km from the Chilean research station "Dr. Guillermo Mann" on the east coast of Cape Shirreff (62°27'30" S, 60°47'17" W), Livingston Island, South Shetland Islands, just off the Antarctic Peninsula. There we sampled fur seal pups for *Salmonella* spp., *Campylobacter* spp., and enteropathogenic *E. coli*.

Thirty-three pups were trapped in the breeding colony and sampled individually by rectal swabbing. To minimize the possibility of infecting the samples ourselves, gloves were used and each sterile vial was not opened until the moment of cloacal swabbing. After insertion of the swab into the cloaca, each swab was immediately put back into its airtight vial with culturing medium and the cap securely closed. Vials were not opened again until they were analyzed in the lab. Samples were also stored in the charcoal transport medium (Transwab, BioDisc, Solna, Sweden) and kept at refrigerator temperature until cultured at the Laboratory of Enteropathogens, Faculty of Medicine, University of Chile, Santiago. Each sample was streaked onto MacConkey agar, an enteropathogenic selective substrate for *E. coli*, and cultivated at 37°C for 24 h. *E. coli* was identified

using colony morphology and a standard conventional biochemical test for the identification of Gram-negative bacteria (Isenberg 1995).

There was substantial *E. coli* growth in the direct culture of all fecal samples, except for one of the 33 samples that required enriched nutrient broth. From each plate, 10 lactose-fermenting colonies exhibiting *E. coli* morphology were tested in a multiplex polymerase chain reaction (PCR) assay designed to detect simultaneously the presence of enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), and enteropathogenic *E. coli* (EPEC). Specific primers to detect virulence genes: *stx1*, *stx2* for EHEC, *eae* and *bfp* for EPEC, and *stII* and *lt* for ETEC were used as described in Vidal et al. (2004). Diarrheagenic *E. coli* reference strains 933J (*stx1*, *stx2*, and *eae*), 2348/69 (*eae*), and H10407 (*st* and *lt*) were included as positive controls and an *E. coli* ATCC 25922 strain as a negative control.

Results

Two (6%) of the samples showed positive amplification of the *eae* gene, indicative of the presence of EPEC. In these two fur seals, five of ten colonies and three of ten colonies were *eae* positive. The primers used to detect the *eae* gene were designed to consider sequences of most described variants (Vidal et al. 2002). The EPEC strains could not be typed with EPEC commercial polyvalent antiserum (antiserum O and H, polyvalent A: O26, O55, O111, O119; Polyvalent B: O114, O125, O142, O158; and polyvalent C: O86, O126, O127, O128; Probac, Sao Paulo, Brasil). All 33 samples were negative for *Salmonella* and *Campylobacter*.

Discussion

Our results are the first report of EPEC in Antarctic wildlife and in pinnipeds. As an anthropogenic pathogen, EPEC infects humans, pets, and domestic livestock. It is a common cause of diarrhea in developing countries, especially in children under 2 years of age and can at times be fatal (Oswald et al. 2000; Krause et al. 2005; Pakpynio et al. 2002; Nakazato et al. 2004; Stephan et al. 2004).

How was this pathogen introduced into the seemingly pristine and remote wilderness of Antarctica? We can think of several hypothetical routes of introduction. Direct spread of EPEC could, for example, occur with contaminated food or sewage from land-based operations or ships and fishing vessels that frequent waters around the Antarctic Peninsula. Waste dumped from fishing vessels operating near Cape Shirreff is a greater problem than earlier recognized. Plastic litter and food products are found regularly along the shore (Torres and Jorquera 1992; Torres and Jorquera 1994; Torres et al. 1997).

An increasing number of humans visit Antarctica for professional and recreational reasons. In a previous study, we did not detect any human-associated microorganisms in wildlife exposed to frequent tourist visits (Bonnedahl et al. 2005); however, we did find human-associated *Salmonella* and *Campylobacter jejuni* in wildlife breeding close to scientific bases (Olsen et al. 1996; Palmgren et al. 2000; Broman et al. 2000). The Antarctic treaty regulates treatment of human waste and sewage, but requires sewage treatment only for human populations of more than 30 persons. Scientists and tourists may also come in direct contact with the Antarctic wildlife. In doing so, they may unconsciously transport pathogens from infected to uninfected animal populations via equipment, clothes, or hands. For example, fecal pathogens can be transferred by footwear that has been in contact with fecal material from an infected animal population.

Another source of infection could be seabirds breeding in proximity to fur seal colonies. Many seabirds feed in enriched waters outside sewage outlets (Ferns and Mudge 2000) and then return to their nests near the fur seals (P. Prince, personal communication). Infected birds may also forage and defecate in fur seal colonies (Oelke and Steiniger 1973).

Individual fur seals and seabirds may also wander to lower latitudes near South America, where they could pick up EPEC. Then, if the infection lasts long enough and they return to the Antarctic with it, they could introduce the microorganism there.

Numerous investigators have emphasized the strong association between carriage of the *eae* gene and the capacity of EPEC strains to cause severe human disease (Jerse et al. 1991; Bower et al. 1989). However, we do not know if Antarctic fur seals infected with EPEC experience higher mortality, nor do we know if EPEC causes mortality among any Antarctic wildlife species, as is reported for pets and domestic livestock (Krause et al. 2005; Nakazato et al. 2004; Pakpynio et al. 2002; Stephan et al. 2004). Nevertheless, the discovery of EPEC in this remote, relatively pristine wilderness is distressing, for it suggests that the nations of the world are still not doing enough to protect Antarctic wildlife from exotic pathogens.

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