Polar Biol (2007) 30:249–252
DOI 10.1007/s00300-006-0179-5

ORIGINAL PAPER

Subcutaneous clostridial infection in Adelie penguins in Hope Bay, Antarctica

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Received: 18 April 2006 / Revised: 8 June 2006 / Accepted: 19 June 2006 / Published online: 19 July 2006

Abstract During the 2000–2001 breeding season in Hope Bay, Antarctica, two adult Adelie penguins were found dead with lesions compatible with subcutaneous clostridial infection. *Clostridium cadaveris* was isolated from the musculature and the subcutaneous tissue of one of these two penguins, whereas *Clostridium sporogenes* was isolated from the subcutaneous tissue of the other penguin. *Escherichia coli* and *Staphylococcus* spp. were isolated from both animals. This is the first report of subcutaneous clostridial infection in Antarctic Adelie penguins.

Introduction

Subcutaneous clostridial infection is a poultry disease characterized by necrotizing areas on the skin and a severe inflammation of underlying tissues. This disease can be caused by *Clostridium perfringens* type A, or *Clostridium septicum*, either singly or in combination with *Staphylococcus* spp. and *Escherichia coli*. In poultry, pathogenesis of subcutaneous clostridial infection begins with a primary traumatic lesion of the skin (Ficken 1995). This wound is colonized by aerobic bacteria that consume oxygen, thus producing an adequate atmosphere for the development of anaerobic bacteria (Kölher et al. 1981).

In Hope Bay (63°24′S, 56°59′W) in the Northeast extreme of the Antarctic Peninsula, there is a large colony of Adelie penguins comprised of approximately 124,000 pairs (Myrcha et al. 1987). Among infectious diseases, avian cholera was described as affecting this penguin population. In addition, enteropathogens such as *Edwardsiella tarda* and *Salmonella Enteritidis* were isolated from Adelie penguin chicks in this area (Leotta et al. 2001).

The purpose of this study was to determine the cause of the death of two Adelie penguins in Hope Bay, Antarctica.

Methods

In Hope Bay, between November 2000 and January 2001, two sick adult Adelie penguins, a male (AP-1) and a female (AP-2), were seen. They evidenced difficulty in walking, and a general weakness and a loss of fear response to approaching humans. These birds were taken to the laboratory in Hope Bay, placed into cages of 1 m^3, and hydrated and fed with fish through a gastric tube. These animals were observed for 3 days, until their natural death. Necropsies were performed immediately thereafter. Skin, subcutaneous tissue, spleen, liver and muscle samples were fixed in 10% neutral buffered formalin and stained with haematoxylin and eosin. Those tissue samples were collected aseptically and submitted to bacteriological studies, where they were subjected to a gram stain and streaked on 5% sheep's blood agar and hektoen enteric agar (Difco Laboratories Incorporated, Cambridge, UK),

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incubated at 37°C for 24 h under aerobic conditions. These samples were simultaneously cultured on 5% sheep's blood agar (Difco) which were then incubated under anaerobic conditions at 37°C for 24 h. Identification of aerobes was carried out by means of standardized methodology (Koneman et al. 1999a). *E. coli* isolates were characterized at Servicio Fisiopatogenia (Instituto Nacional de Enfermedades Infecciosas, Argentina) by PCR using specific primers, with regard to the following virulence markers: attaching and effacement factor (*eae*) of enteropathogenic *E. coli* (EPEC); heat labile toxin (LT) and heat stable toxin (ST) of enterotoxigenic *E. coli* (ETEC); Shiga toxin (Stx1 and Stx2); enteroaggregative plasmid of enteroagreggative *E. coli* (EaggEC), as previously described (Chinen et al. 2002). *Staphylococcus* isolates were identified by biochemical tests according to Koneman et al. (1999b). Identification of anaerobes bacteria was carried out by gram stain and the following biochemical tests: H_2S (Laboratorios Britania, Buenos Aires, Argentina), indole (Laboratorios Britania), lecithinase (Difco), catalase (Waco Pure Chemical Industries, Osaka, Japan), gelatinase (Difco) and lipase production (Difco); meat and milk digestion (Difco), esculin (Difco) and starch hydrolyzation (Difco); motility (Difco); haemolysis (Laboratorios Britania); and acid production from glucose (ICN Biomedicals, OH, USA), mannitol (ICN), lactose (ICN), trehalose (ICN), maltose (ICN) and sucrose (ICN) according to Holdeman et al. (1997) and Koneman et al. (1999b). Toxin neutralization test (Holdeman 1997) for *Clostridium botulinum* group I was performed on anaerobes isolated from AP-1. Each isolate of anaerobic bacteria was experimentally inoculated into ten BALB/C specific pathogen-free (SPF) mice weighing from 18 to 20 g. A 0.5 ml bacterial suspension plus 10% calcium chloride were inoculated intramuscularly into the gluteal region.

Results

One of the Adelie penguins studied had small scars of approximately 2 cm in length on the incubator patch surrounded by incarnated feathers. An area of wide haemorrhage and oedema covering the pectoral region and both pelvic members was observed under the skin of both carcasses. Muscles were grey, and oedema and gas were observed in muscle fibres. AP-1 showed sticky and serosanguinolent synovial fluid inside knee joints. Both birds had hepatomegaly and splenomegaly. Necrotic areas, 2 mm in diameter, were observed in liver of AP-2. No macroscopic lesions were found in remaining organs. Histopathology results were oedema, emphysema, congestion, haemorrhage and great basophil rods in subcutaneous tissue. Necrosis, subhaemorrhagic myositis with fragmentation of muscle fibres and loss of striations in affected muscles were observed. Serohaemorrhagic exudates and cellular infiltration in dermis and epidermis were seen. In liver and spleen from AP-2 a clump of gram-negative rods was observed. Histopathological findings were coagulative necrosis with heterophil infiltration and lymphocytes in liver and perivascular haemorrhage and congestion in spleen.

In smears of affected muscles, a great number of gram-positive rods with oval subterminal and terminal spores along with a few gram-negative rods and gram-positive cocci were observed. In muscles and subcutaneous tissue from AP-1 and AP-2, two species of *Clostridium* were isolated. In AP-1, *Clostridium* isolated was characterized by gram stain showing straight rods with oval and terminal spores swelling the cells, producing H_2S, esculin, lipase, gelatinase and digesting meat and milk. In addition, this isolate fermented glucose, was motile, and produced haemolysis. The rest of the biochemical tests and toxin neutralization tests were negative. This isolate was identified as *C. sporogenes*. In AP-2, *Clostridium* isolated was characterized by gram stain showing straight rods with oval and subterminal spores distending the cells, producing H_2S, indole, gelatinase and digesting meat and milk. In addition, this isolate fermented glucose, was motile, and produced haemolysis. The rest of the biochemical tests were negative. This isolate was identified as *C. cadaveris*. In addition, *Staphylococcus* spp. and *E. coli* were also isolated in these samples from both animals. Gram-positive cocci with a negative coagulase, phosphatase negative, urease negative and mannitol positive were isolated and identified as *Staphylococcus* spp. All *E. coli* isolates were negative for virulence markers studied. Moreover, *E. tarda* was isolated from liver and spleen from AP-2.

All 20 experimentally inoculated mice showed necrosis, oedema and gas into muscles of femoral, pelvic and pectoral region. These mice died between days 3 and 4 postinoculation. *C. sporogenes* was reisolated pure from ten mice inoculated with strain isolated from AP-1. *C. cadaveris* was reisolated pure from ten mice inoculated with strain isolated from AP-2. Histopathological lesions were edema, emphysema, and congestion in muscles and subcutaneous tissue. Also, necrosis and myositis were observed.

Discussion

In this study we identified the cause of the death of two Adelie penguins in Hope Bay, Antarctica. We considered that both penguins died of subcutaneous clostridial infection as *C. sporogenes* and *C. cadaveris* were isolated. In addition, all mice showed lesions and died after being inoculated with clostridial strains. Our results do not support the conjecture that the *Staphylococcus* spp. and *E. coli* caused the death of the two Adelie penguins. *E. coli* did not carry the principal virulence factors, and *Staphylococcus* isolated was identified as neither *S. aureus* nor *S. epidemidis*. These microorganisms were considered the most relevant staphylococci pathogens for birds (Skeeles 1995).

Subcutaneous clostridial infection is widely described in poultry (Ficken 1995), but this is not so in penguins. However, serosanguinolent liquid and emphysema in subcutaneous tissue without loss of skin integrity were observed in broilers (Hofacre 1986). It is possible that in both birds there has been an interaction between aerobic and anaerobic bacteria. Aerobic microorganisms might have colonized the subcutaneous tissue through small wounds produced by predators, feathering or fights, and these microorganisms might have thus created an adequate atmosphere for clostridial colonization. Source of clostridial strains may be the soil of Hope Bay as *C. sporogenes* and *C. cadaveris* were found in the soil at Syowa Station, located in East Ongul Island in Lützow Holm Bay, Prince Herald coast of Antarctica (69°0′S, 39°30′W) (Miwa 1975). Anyway, there are no previous reports relating *C. sporogenes* and *C. cadaveris* to subcutaneous clostridial infection in birds. Apart from this, both clostridial species were associated with lesions in different animal species and human beings (Miskew el al. 1979; Gucalp et al. 1993; Peek et al. 2003).

Among *Enterobacteriaceae* potentially pathogenic for birds in Hope Bay, only *E. tarda* was isolated. This bacterium was associated with a case of chronic enteritis on rockhopper penguins (*Eudyptes chrysocome*) (Cook and Tappe 1985). It is possible that *E. tarda* behaved as an opportunistic agent taking advantage of the base disease. The Scientific Committee Antarctic Research (2001) (SCAR) suggested taking practical measures that might be implemented to detect aetiological agents, to determine the cause and to minimize the adverse effects of wildlife mortality and morbidity events in Antarctica (http://www.cep.aq). Virtually nothing is known of endemic diseases or non-indigenous diseases in Antarctic bird populations. In addition, it is likely that agents capable of causing diseases are present among Antarctic bird populations, but their expression in terms of diseased individuals has not been observed. However, with the data reported to date, it is not possible to determine whether a disease is exogenous or endemic in Antarctica. This is the first report relating *C. sporogenes* and *C. cadaveris* to subcutaneous infection in Adelie penguins in Antarctica. Subcutaneous clostridial infection could be considered an Antarctic endogenous illness.

Acknowledgments The authors would like to thank Instituto Antártico Argentino and Departamento de Biología, Dirección Nacional del Antártico, for providing support to field work in Antarctica, especially to N. R. Coria and D. Montalti. We thank Dr. M. Rivas, Servicio Fisiopatogenia, Instituto Nacional de Enfermedades Infecciosas–ANLIS "Dr. Carlos G. Malbrán", Argentina, for *E. coli* strains characterization. We are grateful to M. Perez Cometto for his collaboration in Hope Bay, and also to L. Isturiz for the grammatical revision.

References

Chinen I, Rivas M, Soriano V, Miliwebsky E, Galvez GF, Chillemi G, Baschkier A, Wang G, Caldeira R, Woodward DL, Rodgers FG (2002) *Escherichia coli* ehl1 gene-positive serotype O18ac:H31 associated with an outbreak of diarrhea in a neonatal nursery in Neuquén City, Argentina. J Clin Microbiol 40:1225–1229

Cook RA, Tappe JP (1985) Chronic enteritis associated with *Edwardsiella tarda* infection in rockhopper penguins. J Am Vet Med Assoc 187:1219–1220

Ficken MD (1995) In: Calnek BW (ed) Enfermedades de las Aves 1a ed. Springer Editorial El Manual Moderno, S.A. de C.V. México D.F. pp 324–328

Gucalp R, Motyl M, Carlisle P, Dutcher J, Fuks J, Wiernik PH (1993) *Clostridium cadaveris* bacteremia in the immunocompromised host. Med Pediatr Oncol 21(1):71–72

Hofacre CL, French JD, Page RK, Fletcher OJ (1986) Subcutaneous clostridial infection in broilers. Avian Dis 30:620–622

Holdeman LV, Cato EP, Moore WEC (1997) Anaerobe Laboratory Manual 4th ed Department of Anaerobic Microbiology. Virginia Polytechnic Institute and State University, Blacksburg, pp 79–105

Köhler B (1981) Infecciones e intoxicaciones por Clostridios (Tomo II, Capítulo 53) in Enfermedades Infecciosas de los animales domésticos, 1 edición. Editorial Acribia, Zaragoza, pp 191–228

Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC (1999a) *Enterobateriaceae*. In: Koneman (ed) Diagnóstico Microbiológico 5th ed. Editorial Médica Panamericana S.A. Springer, Buenos Aires, pp 171–250

Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC (1999b) Cocos grampositivos. In: Koneman (ed) Diagnóstico Microbiológico 5th ed. Editorial Médica Panamericana S.A. Springer, Buenos Aires, pp 527–562

Leotta GA, Vigo GB, Nievas VF, Nievas WD, Igal SU, Aguirre JI (2001) Prevalencia de *Edwardsiella tarda* en aves antárticas. III Congreso Argentino de Zoonosis, II Congreso La-

tinoamericano de Zoonosis. Facultad de Ciencias Veterinarias, Universidad de Buenos Aires

Miskew DB, Pinzur MS, Pankovich AM (1979) Clostridial myonecrosis in a patient undergoing oxacillin therapy for exacerbation of chronic foot ulcers and osteomyelitis. Clin Orthop 138:250–253

Miwa T (1975) Clostridia in soil of the Antarctica. Japan J Med Sci Biol 28(4):201–213

Myrcha A, Tatur A, Del Valle R (1987) Numbers of Adelie penguin breeding at Hope Bay and Seymour Island rookeries (West Antarctica) in 1985. Pol Polar Res 8:411–422

Peek SF, Semrad SD, Perkins GA (2003) Clostridial myonecrosis in horses (37 cases 1985–2000). Equine Vet J 35(1):86–92

Scientific Committee Antarctic Research (2001) Report on the open-ended Intersessional Contact Group on Diseases of Antarctic Wildlife. IV CEP Working Paper WP-11 Agenda Item 4d, Australia http://www.cep.aq

Skeeles JK (1995) In: Calnek BW (ed). Enfermedades de las Aves 1a ed. Springer, Editorial El Manual Moderno, S.A. de C.V. México D.F., pp 357–364