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Avian Cholera in a Southern Giant Petrel (*Macronectes giganteus*) from Antarctica

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ABSTRACT: A southern giant petrel (*Macronectes giganteus*) was found dead at Potter Peninsula, King George Island, South Shetland, Antarctica. The adult male was discovered approximately 48 hr after death. Macroscopic and microscopic lesions were compatible with avian cholera and the bacterium *Pasteurella multocida* subsp. *gallicida,* serotype A1 was isolated from lung, heart, liver, pericardial sac, and air sacs. In addition, *Escherichia coli* was isolated from pericardial sac and air sacs. This is the first known report of avian cholera in a southern giant petrel in Antarctica.

Key words: Antarctica, avian cholera, *Escherichia coli, Macronectes giganteus, Pasteurella multocida,* South Shetland Islands, southern giant petrel.

Avian cholera is an infectious disease caused by the bacterium *Pasteurella multocida.* The etiologic agent is a Gram negative, capsulated, non-motile rod. Transmission is thought to be either by inhalation of a bacteria-ladened aerosol (Simensen and Olson, 1980) or by drinking contaminated water or food (Botzler, 1991). The disease can be either acute or chronic in poultry, but typically is reported as only acute in wild birds (Wobeser, 1997). Morbidity and the mortality are variable and susceptibility depends on the affected avian species (Botzler, 1991). Avian cholera has been documented in over 100 wild bird species (Botzler, 1991) and several massive mortalities of birds associated with aquatic environments have been reported in waterfowl from the USA (Oddo et al., 1978; Wilson et al., 1995), Japan (Nakamine et al., 1992) and Canada (Wobeser et al., 1979); snow geese (*Chen caerulescens*) from the Arctic (Samuel et al., 1997) and penguins (*Eudyptes chrysocome*) from New Zealand (de Lisle et al., 1990). There are few reports on avian diseases in Antarctica (Leotta et al., 2002).

Southern giant petrels (*Procellariids*) are large and have a circumpolar distribution in the southern hemisphere. The world population is estimated to be 37,000 breeding pairs; their numbers decline during winter at all sites. Immature birds and some adults disperse widely, probably circumnavigating Antarctica (Marchant and Higgins, 1993). Partially migratory, some adults are resident at most sites of distribution, except in Antarctica where they are rare visitors during May and June (Mougin, 1968). At Potter Peninsula, King George Island, the population is approximately 75 breeding pairs (Aguirre, 1995).

During February 2000, an adult male southern giant petrel was found during routine surveillance, approximately 48 hr after its death at Potter Peninsula (62°15′S, 58°36′W), King George Island, South Shetland, Antarctica. No other birds were found dead in the site during the summer 2000.

Tissues collected at necropsy were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 3 μm, and stained with hematoxylin and eosin (HE) and periodic acid-Schiff (PAS).

Ectoparasites and skin lesions were not seen during field necropsy. The conjunctiva was hyperemic. Large quantities of mucus were in the pharynx and upper trachea. The bird was emaciated and the stomach was empty. Hyperemia and hemorrhage were observed on serosal surfaces of body cavity organs and peritoneal lining.

Fibrinous exudates involving the capsule of the liver, the mesentery, and the air sacs were observed. Histopathology of the capsule of liver and the air sacs showed heterophils, macrophages, lymphocytes, and few plasma cells. The pericardium contained an abundant yellow liquid and was strongly adhered to sternum and heart. Subepicardial petechial hemorrhages were found. Fibrinonecrotic pericarditis and epicarditis with coccobacilli, lymphocytes, and plasma cells were observed. Hemorrhages and heterophilic infiltration were seen in areas of acute focal necrosis in the myocardium. The lung was edematous and congested and perivascular hemorrhage and heterophils, macrophages, and lymphocytes were observed. Hepatomegaly and splenomegaly with petechiae and ecchymoses were evident. The liver had hemorrhage, congestion, heterophilic infiltration, and necrotic foci containing bacteria. The spleen was congested with perivascular hemorrhage and there were areas of acute focal necrosis around arterioles. The dura mater showed congestion with perivascular hemorrhage. There were no fungal mycelia seen in any organs.

Samples of heart, lung, liver, pericardial sac, and air sacs were taken immediately after necropsy for microbiology. They were streaked on blood agar plates (blood agar base, Merck Química, Buenos Aires, Argentina +5% defibrinated sheep blood), Hektoen enteric agar (Becton Dickinson France S.A., France), and Sabouraud agar (Merck Química). Bacterial media were incubated at 37 C during approximately 48 hr and fungal media were incubated both at 28 and 37 C for 10 days. Bacterial colonies were stained by Gram's stain and then inoculated into brain heart infusion broth (Difco Laboratories, Detroit, Michigan, USA) with 30% glycerol at −20 C. Identifications were based on Gram's stain, morphologic characteristics and biochemical tests (Fegan et al., 1995; Koneman et al., 1997). A direct immunofluorescence test (Chlamydia Test Direct IF, Bio Merieux Laboratory, Marcy L'Etoile, France) was used to evaluate tissues for presence of *Chlamydophila psittaci.* Isolation of *Mycoplasma* spp. was attempted by swabbing the tracheal mucus and placing the swab's contents into Frey's liquid and solid media (Frey et al., 1968) which were incubated at 37 C for 30 days. Samples tested for *C. psittaci*, *Mycoplasma* spp., and fungi were negative.

Escherichia coli was isolated on Hektoen enteric agar from samples of the pericardial sac and air sacs. The strains were characterized for 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/Stx2) of enterohemorrhagic *E. coli* (EHEC); enteroaggregative plasmid of enteroagreggative *E. coli* (EaggEC), by PCR using specific primers as previously described (Chinen et al., 2002). In addition, colony blot hybridizations were carried out to determine the bundle-forming pilus (BFP) of EPEC and the fimbriae Daa of diffuse adherence *E. coli* (DAEC), and an enzyme-linked immunosorbent assay was used to detect the presence of the invasin of enteroinvasive *E. coli* (Chinen et al., 2002). Results of all tests for *E. coli* virulence markers were negative.

Pasteurella multocida was isolated on the blood agar plate from lung, heart, liver, pericardial sac, and air sacs samples. Morphologic and biochemical characteristics were typical of *P. multocida* subsp. *gallicida* (Fegan et al., 1995; Koneman et al., 1997). A subculture of the isolate was confirmed as *P. multocida,* using the API20E system (BioMerieux, St. Louis, Missouri, USA) before serotyping. The capsular serotype of the *P. multocida* strain was determined by use of a multiplex PCR assay (Townsend et al., 2001) using primers from Integrated DNA Technologies (Coralville, Iowa, USA) and reagents from Amersham Biosciences Corp. (Piscataway, New Jersey, USA). Heat-stable antigens were serotyped by immunodiffusion ac-

cording to the method of Heddleston et al. (1972) using antisera from National Veterinary Services Laboratory (Ames, Iowa). The API20E biocode was 0140524, the most common biotype for avian cholera in the United States (US Geological Survey, National Wildlife Health Center, Madison, Wisconsin, USA; unpubl. data). Based on results of morphologic, biochemical, and serotyping characteristics, the isolate was determined to be a *P. multocida gallicida* serotype A1.

The microbiologic results and lesions suggested that the southern giant petrel died of septicemia due to avian cholera (Rhoades and Rimler, 1995; Morishita et al., 1997). The source of *E. coli* was probably the digestive system as a result of early postmortem changes. Even though we could not find virulence factors in the *E. coli* isolate, we do not discard the possibility of an associated infection along with *P. multocida.* Other virulence factors, such as type 1 (F1A) and P (F11) fimbriae, curli, aerobactin, K1 capsule, and temperature-sensitive hemagglutinin (Tsh) could also be tested.

A possible source of avian cholera for this southern giant petrel could be either the drinking water because *P. multocida* can survive in fresh water with 0.5% salt added (Bredy and Botzler, 1989), or a food source because the southern giant petrel is a carrion-eating bird that could have eaten diseased birds (Williams et al., 1987). There are a number of ambiguities in regard to the relationship between climatic conditions, nutritional stress, quality of drinking water, environmental contaminants, and avian cholera in wild birds (Botzler, 1991).

The type of fibrinous exudate involving the capsule of liver, air sacs, and pericardial sac seen in the southern giant petrel was similar to those reported in some waterfowl (Montgomery et al., 1979) and raptors (Morishita et al., 1997). Facial edema, sinusitis, fibrinosuppurative meningitis, and localized infections in joints, ear and cranial bones, such as was described in the chronic avian cholera in domestic birds (Rhoades and Rimler, 1995) were not observed. However, abscesses in esophagus were not observed as were previously described in *Buteo* hawks (Morishita et al., 1997).

In the southern giant petrel massive hemorrhages, hepatomegaly, splenomegaly, necrotic foci in the liver, liquid accumulation in the pericardial sac, and heterophilic infiltration in lungs and in other parenchymatous organs were observed as was described for acute avian cholera in domestic birds (Rhoades and Rimler, 1995).

Emaciation and the great quantity of fibrin in the body cavity suggested the southern giant petrel developed a subacute avian cholera. This is the first known report of a southern giant petrel dying from avian cholera in Antarctica and shows that this species is susceptible to *P. multocida* infection. Avian cholera could affect different species of Antarctic birds.

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