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ORIGINAL PAPER

Cutaneous and diphtheritic avian poxvirus infection in a nestling Southern Giant Petrel (*Macronectes giganteus*) from Antarctica

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Abstract The Southern giant petrel (*Macronectes giganteus*) is declining over much of its range and currently is listed as vulnerable to extinction by the International Union for the Conservation of Nature (IUCN). Island-specific breeding colonies near Palmer Station, Antarctica, have been monitored for over 30 years, and because this population continues to increase, it is critically important to conservation. In austral summer 2004, six diseased giant petrel chicks were observed in four of these colonies. Diseased chicks were 6–9 weeks old and had multiple proliferative nodules on their bills and skin. One severely affected chick was found dead on the nest and was salvaged for necropsy. Histopathological examination of nodules from the dead chick revealed epithelial cell hyperplasia and hypertrophy with numerous eosinophilic intracytoplasmic inclusions (Böllinger bodies). A poxvirus was isolated from multiple nodules. Poxviral infection has not been reported in this species, and the reason for its emergence and its potential impact on the population are not yet known.

Keywords Avian poxvirus · Antarctica · Southern giant petrel · *Macronectes giganteus* · Pelagic seabird · Infectious disease

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Introduction

The Southern giant petrel (*Macronectes giganteus*) is a long-lived, large seabird with a circumpolar pelagic range in the Southern Ocean. Southern giant petrels nest in small colonies on Antarctic and sub-Antarctic islands, and nesting pairs raise a single chick each nesting season (Voisin 1988). This species is in decline globally (Patterson et al. (2007); Birdlife International 2006), and is currently listed as vulnerable to extinction by the International Union for the Conservation of Nature (IUCN). Threats to Southern giant petrel populations include drowning following entanglement in commercial longline fishing, reductions in southern elephant seal (*Mirounga leonina*) populations (an important source of carrion), and human disturbance (Birdlife International 2006). Reports of disease in this species are rare (Leotta et al. 2003).

Avian pox is a disease of worldwide distribution, which has affected approximately 278 species of wild and domestic birds representing 20 orders (van Riper and Forrester 2007). The causative agent is a double-stranded DNA virus of the family Poxviridae, subfamily Avipoxvirus. Avian poxvirus is specific to birds, but some strains infect a broad range of bird species. The virus may be mechanically transmitted by insect vectors such as mosquitoes, mites or ticks by direct contact with another infected bird, by contact with contaminated food, water, or surfaces, or by exposure to

air-borne particles. During recent years there has been an increase in reported cases of this disease, and cases in new bird species have occurred, suggesting that avian pox may be a re-emerging avian disease (Hansen 1999). Recent reports of avian pox in threatened and endangered bird species, such as the Galapagos mockingbird (*Nesominus parvulus*) (Vargas 1987; Thiel et al 2005), the Andean condor (*Vultur gryphus*) (Kim et al. 2003), the white-tailed laurel-pigeon (*Columba junoniae*) (Medina et al. 2004), the short-toed lark (*Calandrella rufescens*) and the Berthelot's pipit (*Anthus berthelotti*) (Smits et al. 2005), the houbara bustard (*Chlamydotis undulata macqueenii*) (Samour et al. 1996), and the Hawaiian goose (Kim and Tripathy 2006) are of particular concern. The introduction of the avian pox virus to the Hawaiian Islands has been shown to have had a negative effect on the population dynamics of the native forest birds of Hawaii (van Riper et al. 2002). Prior to our observation of avian pox in Southern giant petrels, there have been no reports of avian poxvirus infection in birds in the Antarctic and sub-Antarctic regions (van Riper and Forrester 2007).

Six Southern giant petrel chicks, each 6–9 weeks old, from four separate breeding colonies near Palmer Station, Anvers Island, Antarctica, (64°46′S, 64°04′W) were observed to have multiple prominent nodular masses on their bills during austral summer 2004. Three affected chicks were present on Stepping Stones Island (colony of 137 breeding pairs), one on Shortcut Island (52 breeding pairs), one on Norsel Point Island East (148 breeding pairs), and one on Humble Island (58 breeding pairs). Earlier in the breeding season, several adult giant petrels were observed to have small, oozing sores on the feathered skin around their bills, non-feathered skin around the gape, and occasionally near the eyes. Some also had a thick yellow discharge from the eyes. A severely affected chick (Fig. 1) observed earlier on Stepping Stone Island was later found dead on the nest and salvaged for necropsy. In the other five chicks, the lesions increased in size over the course of the summer, but erupted (burst) suddenly and concurrently with the onset of below freezing weather temperatures in autumn. The dried lesions then healed completely and the chicks, to the best of our knowledge, fledged without difficulty.

Methods and results

The salvaged chick was frozen and sent to the USGS National Wildlife Health Center in Madison, WI, USA, for complete diagnostic examination. The chick was in good nutritional condition with adequate fat reserves. Multiple small to large ($0.7 \times 0.6 \times 0.3$ cm to $4 \times 3 \times 2$ cm) smooth nodular growths were present on the maxillary and mandibular bills and the feathered skin of the head (Fig. 2). Larger nodules were ulcerated and oozed thick, greasy, yellow material; this same material coated much of the surface of the head. Most of the nodules were fleshy, tan, firm, mildly gritty or fibrous, and occasionally striated on cut surface. Larger, ulcerated nodules contained areas of cavitation and hemorrhage. Smaller, firm to rubbery, intact nodules with smooth surfaces were present on the scaled skin of the feet and digits. Additionally, a $2 \times 1.2 \times 1$ cm firm, tan, wet fleshy growth was present on the surface of the tongue, and two similar, smaller growths were present arising from the mucosa of the proximal crop. Most of the ileum was diffusely reddish-black with intraluminal hemorrhage, and the lungs were homogeneously dark red and wet (edema). Other organs were grossly unremarkable.

Tissue samples from all major organs and from each cutaneous and mucocutaneous nodule were fixed in 10% buffered formalin, embedded in paraffin, cut at 5 µm, and stained with hematoxylin and eosin for histological

Fig. 1 Southern giant petrel (*Macronectes giganteus*) chick infected with avian poxvirus on Stepping Stone Island. Note multiple proliferative nodules on the bill and fleshy growths on the tongue

Fig. 2 Southern giant petrel (*Macronectes giganteus*) chick at necropsy

examination. Samples of multiple cutaneous nodules, the lingual and ingluvial growths, ileum, lung, feather pulp, spleen, kidney, and cloacal and tracheal swabs were submitted for virus isolation. Samples of the ileum, lung, liver, spleen, kidney, and multiple cutaneous nodules were also submitted for aerobic and anaerobic bacterial cultures. The entire gastrointestinal tract was examined for the presence of parasites.

Histological examination of the cutaneous, lingual, and ingluvial nodules revealed diffuse, marked epithelial hyperplasia (endophytic and exophytic), hypertrophy and hyperkeratosis (Fig. 3a). Within each discrete nodule there was cytoplasmic swelling and vacuolation of keratinocytes, especially in the stratum spinosum, with degeneration and rupture of swollen keratinocytes. Many keratinocytes contained prominent, single or multiple, variably sized eosinophilic cytoplasmic inclusions (Böllinger bodies) (Fig. 3b, inset). The larger nodules had erosions and ulcerations of the epithelial surface, characterized by necrosis of keratinocytes, granulocytic infiltrates, and occasional colonies of coccobacilli. All nodules, regardless of size and location, consisted of proliferating and degenerate epithelial cells with variable numbers of Böllinger bodies, consistent with pox infection. Histological examination of the ileum was severely compromised due to the autolyzed state of the tissue; however, the presence of blood in the lumen was confirmed.

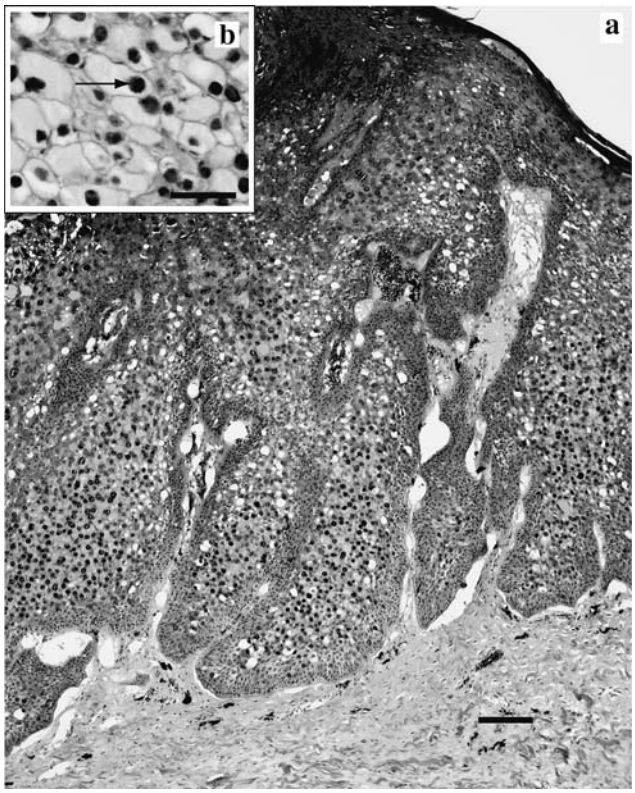

Fig. 3 **a** Growth from the proximal crop of the Southern giant petrel (*Macronectes giganteus*) chick. There is prominent epithelial hyperplasia. Keratinocytes in the stratum spinosum are swollen and vacuolated. *Bar* = 250 µm. **b** (*inset*). Many keratinocytes contain prominent eosinophilic cytoplasmic inclusions (Böllinger bodies) (*arrow*). *Bar* = 25 µm

Tissue samples for virus isolation from nodular lesions were homogenized in a glass Tenbrock homogenizer in BA-1 medium and inoculated in the chorioallantoic membrane (CAM) of day 13 embryonated chicken eggs. The eggs were incubated for 5 days at 99°F (37°C) and the CAM excised for examination under a microscope. White opaque pocks were observed on CAM inoculated with samples from the lingual and ingluvial growths and from nodules on the feet, the feathered skin of the head, and the bill. Characteristic brick-shaped pox virions were observed in materials prepared from the CAM by negative stained electron microscopy (data not shown). No other virus was isolated from submitted tissues.

Two regions of the Southern giant petrel pox DNA were amplified by PCR, sequenced, and compared to the homologous regions of fowlpox and canarypox, using the procedure described by Thiel et al. (2005). Southern giant petrel pox and fowlpox were most similar with 83 and 96% identity for the CFPV and CFP2 regions, respectively, compared to 62 and 77% identity between Southern giant petrel pox and canarypox for CFPV and CFP2, respectively. For the CFPV and CFP2 regions, fowlpox and canarypox are 59 and 76% identical, respectively. There are differences not only in the nucleotides but in the sequence lengths. The CFPV region DNA sequence for fowlpox is 49 nucleotides shorter than canarypox but Southern giant petrel pox is only 25 nucleotides shorter than canarypox. For the CFP2 sequence, Southern giant petrel pox is 3 nucleotides longer than canarypox, but only one nucleotide longer than fowlpox. The phylogenic trees produced using the two intergenic regions amplified by primer sets CFPV and CFP2 indicated that the Southern giant petrel pox is distinct from both fowlpox and canarypox, but is more closely related to fowlpox than to canarypox (Fig. 4).

The only bacterial isolate considered significant was *Clostridium perfringens*, which was isolated from the ileum and determined to be producing alpha toxin by matrix polymerase chain reaction (PCR).

Approximately 20 *Stegophorus* sp. nematodes were identified in the ventriculus and proventriculus. No pathological changes were associated with these nematodes, and no other internal or external parasites were seen, despite extensive examination.

Discussion

The immediate cause of death in this giant petrel chick was necrohemorrhagic enteritis due to intestinal infection with

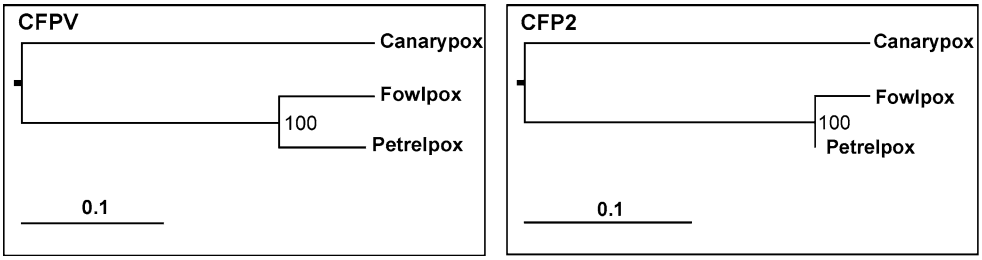

Fig. 4 Phylogenetic relationship among three avian pox strains. The unrooted trees were based on the DNA sequences for two regions of the three avian pox strains, amplified using primer sets CFPV and CFP2. Bootstrap values of 100% support for the trees are shown. The *bar* in the lower left shows the distance representing 0.1 substitutions per site

alpha toxin-producing *Clostridium perfringens*. Necrotic enteritis due to enterotoxemia resulting from infection with this bacterium is well-documented in domestic chickens and turkeys, where it most often occurs in 2–12 weeks old birds (Wages and Opengart 2003). *C. perfringens* is an obligate anaerobic bacterium which is ubiquitous in nature and may be part of the normal intestinal flora of healthy birds. Factors known to be involved in the pathogenesis of necrotic enteritis in poultry include dietary changes and intestinal coccidial infestation, which are believed to disrupt the normal balance of the intestinal flora. The pathogenesis of the disease in wild birds is poorly understood, although stresses of various kinds are likely to play a role (Wobeser 1997). The florid cutaneous and diphtheritic pox lesions seen in this chick may have served as significant stressors triggering clostridial overgrowth.

The effects of avian poxvirus are mild in some bird species and severe in others. The disease may result in cutaneous or diphtheritic lesions, or a mixture of the two, as seen in this giant petrel chick (Fig. 1). Rarely, virus infection may be systemic. Diphtheritic lesions are infrequently detected in wild bird avian pox infections (van Riper and Forrester 2007). Mortality rates are highest in birds with diphtheritic or systemic infections (Tripathy and Reed 2003).

Avian poxvirus infection in Southern giant petrels in Antarctica has not been previously reported, nor has it been reported for any other species in this region (van Riper and Forrester 2007). Because this is a unique event in Southern giant petrels, it is difficult to attribute causality, particularly since our adult study populations have foraging ranges that encompass latitudes as far north as the coasts of Chile and Argentina (~45°S; unpublished data). This potentially exposes these birds to many, if not all of the vectors that have been identified as possible pathways of disease introduction in Antarctic seabirds, including, in particular, poor food and waste management at research bases and private or government operated vessels, and sewage and slaughterhouse outflows near larger human population centers (Kerry et al. 1999). Although the Antarctic Treaty mandates a best practices approach to dealing with all forms of human waste, which is strictly observed in our study region, the extent to which these practices are enforced elsewhere is unknown, and obviously not at all applicable outside the Treaty area.

While we cannot pinpoint the actual source of infection in the adult giant petrel population, we are more confident that infection in the chicks resulted from parental feeding. This is based on the fact that lesions suggestive of mild pox infection were noted on adults prior to the onset of breeding. Moreover, some other possible vectors such as mosquitoes do not occur in Antarctica and no external parasites such as mites or ticks were found on the dead chick. Increased future monitoring for the presence of competent arthropod vectors on or near adult and nestling giant petrels would still be prudent, however, as their presence would suggest an additional mode of transmission within giant petrel colonies and also potentially to other Antarctic bird species. The Antarctic tick *Ixodes uriae*, for example, is ubiquitous in the area (Lee and Baust 1987) and commonly infests Adélie penguins (*Pygoscelis adéliae*), a seasonally important prey of Southern giant petrels. Both Humboldt (*Spheniscus humboldti*) and Jackass (*S. demersus*) penguins have been documented to be susceptible to poxvirus infection (Bolte et al. 1999), hence there is reason to believe that Adélie penguins may be equally vulnerable. A recent first report of suspected avian pox in Gentoo penguins (*Pygoscelis papua*) in the Falkland Islands with significant associated mortality (ProMED-mail 2006) underscores the urgency for increased surveillance.

The emergence of avian pox in Southern giant petrels and its potential impact in this population raise serious concerns. Avian pox represents another potential environmental stressor for a species already classified as vulnerable due mostly to incidental mortality from long-line fishing operations in the Southern Ocean (Birdlife International 2006).

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