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Avian Pox in Magellanic Penguins (*Spheniscus magellanicus*)

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ABSTRACT: Avian pox is an enveloped double-stranded DNA virus that is mechanically transmitted via arthropod vectors or mucosal membrane contact with infectious particles or birds. Magellanic Penguins (*Spheniscus magellanicus*) from two colonies (Punta Tombo and Cabo Dos Bahías) in Argentina showed sporadic, nonepidemic signs of avian pox during five and two of 29 breeding seasons (1982–2010), respectively. In Magellanic Penguins, avian pox expresses externally as wart-like lesions around the beak, flippers, cloaca, feet, and eyes. Fleas (*Parapsyllus longicornis*) are the most likely arthropod vectors at these colonies. Three chicks with cutaneous pox-like lesions were positive for *Avipoxvirus* and revealed phylogenetic proximity with an *Avipoxvirus* found in Black-browed Albatross (*Thalassarche melanophrys*) from the Falkland Islands in 1987. This proximity suggests a long-term circulation of seabird *Avipoxviruses* in the southwest Atlantic. Avian pox outbreaks in these colonies primarily affected chicks, often resulted in death, and were not associated with handling, rainfall, or temperature.

Key words: Argentina, avian pox, Magellanic penguin, *Spheniscus magellanicus*.

Avian pox, caused by an *Avipoxvirus* in the *Poxviridae* family, is an enveloped double-stranded DNA virus that is mechanically transmitted via arthropod vectors or mucosal membrane, broken skin, or abraded skin contact with infected particles or individuals (Hansen, 1999). Poxvirus infection has been reported for at least 278 of the approximately 9,800 bird species (Van Riper and Forrester, 2007), but all avian species are likely susceptible (Karstad, 1971).

In the most common form, cutaneous, wart-like growths occur around the eyes, beak, or unfeathered skin (Hansen, 1999). Infected birds are often emaciated, and

although pox infection itself is rarely fatal, secondary bacterial or fungal infections are common and cause mortality (Hansen, 1999). The rarer form, diphtheritic pox, has a higher case fatality ratio and manifests as raised, yellow plaques in the mouth, throat, trachea, and lungs, causing difficulty breathing or swallowing (Hansen, 1999). Mortality from avian pox depends on infection type, species infected, and location of growths (Hansen, 1999; Van Riper et al., 2002). Avian pox prevalence often coincides with weather-induced increases in vector populations. For example, Hawaiian bird populations have higher infection rates during seasons of high precipitation when breeding conditions for mosquitoes are better (Van Riper et al., 2002; Young and VanderWerf, 2008).

The most commonly reported forms of *Avipoxvirus* might be fowlpox and canarypox viruses (Stannard et al., 1998); however, the actual number of strains remains unknown (Smits et al., 2005). Avian pox harms many wild bird populations and continues to be discovered in new species (Bolte et al., 1999). Little is known about the prevalence of the disease in wild seabird populations (Young and VanderWerf, 2008; Parker, 2009).

Avipoxvirus has been documented in three penguin species—African (*Spheniscus demersus*; Stannard et al., 1998), Humboldt (*Spheniscus humboldti*; Landowska-Plazewska and Plazewski, 1968 as cited by Bolte et al., 1999), and Gentoo (*Pygoscelis papua*; Munro, 2007), but more species are affected. In 2006 an avian pox outbreak affected five Gentoo Penguin colonies in the Falkland Islands (Islas Malvinas), but

nearby Magellanic Penguin (*Spheniscus magellanicus*) colonies showed no signs of infection (Munro, 2007). However, in 2000 and 2001 a few Rockhopper Penguin (*Eudyptes chrysocome*) chicks and one Magellanic Penguin chick (in separate colonies in the Falkland Islands) had wart-like growths characteristic of avian pox (Van Buren, unpubl. data). Additionally, Galapagos Penguins (*Spheniscus mendiculus*) in the El Niño years of 1972 and 1998 had pox-like lesions on their beaks with juveniles more frequently infected than adults (Boersma, 1977, unpubl. data).

Here, we document Magellanic Penguins with pox-like lesions at two colonies in Chubut, Argentina: Punta Tombo (44°2.7'S, 65°13.4'W) and Cabo Dos Bahías (45°0.5'S, 65°37.2'W, Fig. 1). The colonies are in a temperate desert where the penguins nest under bushes or in burrows. We have studied Magellanic Penguins at Punta Tombo since 1982, handling 200–4,000 chicks, up to 530 juveniles, and 200–3,000 adults yearly. We visited Cabo Dos Bahías one to seven times per year handling up to 77 birds annually. To track noninfected chicks (all years) and infected chicks (starting in 1997), we marked individuals' flippers with tape until their feet were greater than 9 cm long. We then pierced foot webbings with numbered small-animal ear tags (No. 1005–3, 2×10 mm; National Band and Tag Company, Newport, Kentucky, USA). We banded chicks that reached fledging age and weight (>1.8 kg) with numbered stainless-steel flipper bands (Lambournes-Porzana, East Sussex, United Kingdom). We washed our hands and disinfected equipment, clothing, and boots in 10% bleach for 20 min after handling infected chicks.

With the use of simple linear regression, we investigated presence or absence of presumptive avian pox from 1983 to 2009 at Punta Tombo with annual temperature (average minimum and average maximum temperature [C]) and total precipitation (mm) from October through January (chick hatching through fledging), as they are known factors that affect ectoparasite



FIGURE 1. Location of two Magellanic Penguin (*Spheniscus magellanicus*) colonies in Argentina (Punta Tombo and Cabo Dos Bahías) where we observed pox-like infections. Also, the Falkland Islands (Islas Malvinas) location of pox-infected Black-browed Albatross (*Dyomedea melanophrys*) from 1987.

abundance and thus may correlate with avian pox prevalence (Krasnov et al., 2001). We looked for observational bias with the use of simple linear regression to model presumptive avian pox prevalence as a function of number of chicks handled per year. We calculated prevalence at Punta Tombo as the number of infected chicks divided by total number handled per year. For all chicks in a bounded area in 1997, we compared 1) percent of infected to percent of noninfected chicks that fledged and 2) adult resighting of those fledged chicks with the use of a χ^2 goodness-of-fit test. All statistical tests were done with R version 2.13.0.

We collected tissue samples from three necropsied chicks with pox-like lesions at Cabo Dos Bahías in 2007. We preserved tissue samples in 10% formalin for histopathologic examination and stored a subset of samples in liquid nitrogen for culture, viral isolation, and molecular diagnosis. Centro Diagnostico Veterinario (a

private diagnostic laboratory, Conde 4799, Buenos Aires, Argentina) analyzed the samples histopathologically and Wildlife Conservation Society pathologist, Denise McAloose (Bronx Zoo, New York), did confirmatory image analysis.

The National Institute of Agricultural Technology (Castelar, Argentina) used ionic interchange columns to extract nucleic acids from a maceration of lesion scabs for molecular diagnosis (QIAamp DNA Mini Kit, QIAGEN, Valencia, California, USA). Polymerase chain reaction (PCR) reactions and primers for the pox virus 4b core protein were identical to those described by Luschow et al. (2004) and Lee and Lee (1997). We used DNA from an *Avipoxvirus* isolated from turkeys as a positive control and DNA from a nonrelated avian virus (chicken anemia virus) as a negative control. We sequenced directly from PCR fragments with the use of the BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems) following the manufacturer's instructions. We generated the consensus nucleotide sequences of the viruses with the use of Megalign (DNASTAR, Madison, Wisconsin, USA) and then performed phylogenetic analyses with additional virus sequence data available in GenBank. We then assembled sequences and edited with Lasergene 8.1 (DNASTAR) and used BioEdit 7 for alignment and residue analysis. We constructed neighbor-joining (NJ) trees with the use of PAUP* 4.0. We calculated estimates of the phylogenies by performing 1,000 NJ bootstrap replicates and used TREEVIEW to create the phylogenetic trees.

We encountered 108 Magellanic Penguins with pox-like lesions in five and two of the last 29 breeding seasons (1982–2010) at Punta Tombo (1983, 1984, 1985, 1997, and 2007) and Cabo Dos Bahías (2004 and 2007), respectively. Mean prevalence was 0.002 (SE=0.001, $n=28$ yr) infected chicks to every handled chick. We did not find any observational bias (i.e.,

there was no correlation between infected chicks and number of chicks handled yearly ($F_{(1,25)}=2.54$, $P=0.12$). Birds with suspected avian pox had nodular lesions in feathered and unfeathered areas on flippers, feet, neck, eyes, beak, and surrounding area, under and on the tongue, and around and inside the cloaca. Chicks were often in poor condition (Fig. 2). Fifteen of 77 (20%) infected chicks developed lesions in their eyes. Of those, seven (47%) became blind in at least one eye. None of the infected chicks' parents exhibited symptoms, however; one 3-yr-old female had bare patches with wart-like lesions at the base of each flipper.

Of the 107 chicks with pox-like lesions, 18 died, the fates of 87 are unknown, and we humanely euthanized one severely infected chick. Thirty-four percent (30 of 87) of the unknown chicks likely fledged; however, survival at sea is unknown and infected fledglings are likely more susceptible to starvation and predation. The remaining 57 chicks were not revisited or disappeared at a time or body condition not conducive to survival. Whether the 18 chicks who died on land succumbed to *Avipoxvirus* infection, secondary infections, or unrelated causes is unknown.

Histopathology of tissues from two of the three sampled chicks showed unspecific inflammatory lesions in the lungs, kidneys, heart, spleen, and digestive tract. All sampled chicks had dermatitis, epidermal hyperplasia, and eosinophilic intracytoplasmic inclusions consistent with an *Avipoxvirus* infection. We positively identified poxviruses by PCR from the collected samples ($n=3$, JN615018 GenBank accession number). Phylogenetic analysis from these three sample amplicons revealed they were identical and are highly similar to an *Avipoxvirus* from a Black-browed Albatross (*Thalassarche melanophrys*) in the Falkland Islands in 1987 (AM050392 GenBank accession number).

In 1997, the outbreak was in a bounded area where we observed and measured all

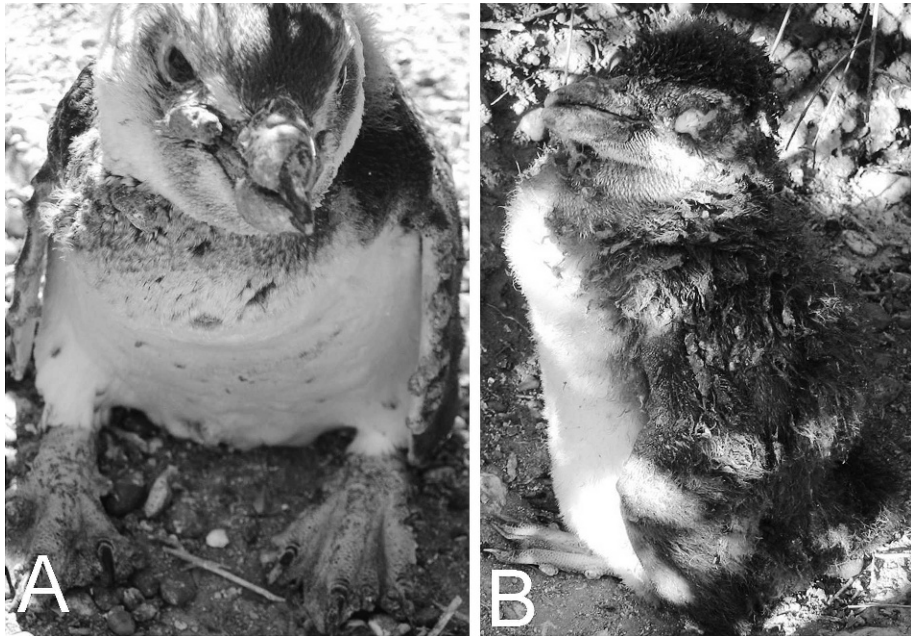


FIGURE 2. Two Magellanic Penguin (*Spheniscus magellanicus*) chicks with pox-like lesions: (a) chick's bill double the normal size with large lesions over most of body and (b) chick blinded by lesions and subsequent secondary infection in both eyes.

chicks from hatching until fledging or death. Of 130 chicks that hatched in this area, 10 showed evidence of poxvirus infection. The percent of chicks that died or went missing was similar for infected (67%) and noninfected (62%) chicks ($\chi^2=0.05$, $df=1$, $P=0.81$). Our adult resight rate was similar for infected (0 resighted of 3 fledged) and noninfected (2 of 48) chicks that fledged with flipper bands ($\chi^2=1.37$, $df=1$, $P=0.24$). We have not resighted any of the seven banded infected chicks that fledged in 2007. In 2008, 2009, and 2010 the adult female that previously exhibited pox-like symptoms returned to the colony in healthy condition.

Many infected chicks occurred in clusters, while some were isolated cases. One cluster occurred near a tourist walkway and in close proximity to many other penguins, suggesting the possibility of transmission between colonies by tourists. Chicks huddle together, allopreen, and feed competitively allowing for direct transmission of the virus; however,

isolated infected individuals and distance between clusters suggests vector transmission may occur.

Two species of ectoparasites affect Magellanic Penguins. The most common is the flea (*Parapsyllus longicornis*, Carnegie Museum B-98582, B-98581, B-99243), which infests most adults, chicks, and nests and may be a mechanical vector (Smits et al., 2005). The second ectoparasite, the mosquito, is rarely observed. *Parapsyllus longicornis* are confined to birds and are most commonly found on seabirds, including Black-browed Albatross (Murray et al., 2003). Flea survival and abundance is affected by humidity and temperature, but unlike mosquitoes, fleas do not need standing water for reproduction (Krasnov et al., 2001). We did not collect data on ectoparasite abundance, so we investigated the effect of weather condition on infection presence. For all years, average minimum (9.9 C, SE=0.15) and maximum temperature (23.7 C, SE=0.33) showed little variation

while total precipitation (78.3 mm, SE=9.93) did vary. We found annual average minimum ($F_{(1,25)}=2.39$, $P=0.13$) and maximum ($F_{(1,25)}=0.53$, $P=0.47$) temperature and total annual precipitation ($F_{(1,24)}=3.28$, $P=0.08$) for all years were not significant covariates in explaining infection presence.

Avian pox in Magellanic Penguins is spatially and temporally variable. Infection is more common in chicks than adults suggesting that adults may have acquired immunity or a stronger immune system. Phylogenetic proximity of the *Avipoxvirus* found in Magellanic Penguins at Cabo Dos Bahías with that found in Black-browed Albatross suggests long-term circulation of seabird avipoxviruses in the Southwest Atlantic. Further study is needed to understand the transmission mechanism and the unpredictable nature of outbreaks.

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