Case Report: An Avian Pox Outbreak in Captive Psittacine Birds in Chile

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Abstract
An outbreak of avian pox in a psittacine bird colony was recognized in Chile. Although 12 psittacine birds of different genera were in contiguous cages within the same building, clinical signs and mortality were restricted to the *Agapornis*, *Neophema*, *Polytelis*, and *Platycercus* genera. Fifty of 188 psittacine birds were affected with clinical signs; the disease was usually self-limiting, but 11 birds died. In the affected birds, cutaneous lesions were usually observed on the featherless parts of the face and legs. A strain of avian poxvirus was isolated from cutaneous lesions harvested from *Polytelis swainsonii* by inoculation onto the chorioallantoic membrane of chicken embryos. Intracytoplasmatic inclusion bodies were visualized by light microscopy in the infected chorioallantoic membranes and also in infected psittacine tissues. Diagnosis of this pox outbreak was made by clinical signs, epidemiology, histopathology, virus isolation, and serological identification. Birds that exhibited clinical signs were treated. As far as we know, this is the first report of avian pox in *Polytelis swainsonii* and *P. alexandrae*. Copyright 2008 Elsevier Inc. All rights reserved.

Key words: avian pox; avian poxviruses; psittacine birds; Psittaciforme; *Polytelis swainsonii*; *Polytelis alexandrae*

Avian pox (AP) is an infectious disease of worldwide distribution that affects commercial poultry (e.g., chickens, turkeys), domestic pets, and free-living birds of many species. The etiologic agent, avian pox virus (APV), is a member of the genus *Avipoxivirus* of the family Poxviridae. About 232 avian species in 23 orders have been reported to have acquired a natural pox infection, including the Psittaciforme order. Species of this order that have been affected clinically and/or pathologically in different locations of the world are: *Amazona* spp., *Agapornis* spp., *Ara* spp., *Psephotus* spp., *Psittacula* spp., *Pionus* spp., *Loriculus vernalis*, *Deropogtyus accipitrinus*, *Pionites melanocephalus*, *Anodorhynchus hyacinthinus*, *Anatinga solstitialis*, *Eupsittula canicularis*, *Enicognathus leptorhynchus*, *Brotogeris pyrrhoptera*, *Aprosmictus erythropterus*, *Platycercus eximius*, *Melopsittacus undulatus*, and *Nymphicus hollandicus*. Lories (*Neopsittacus* spp.) also have been affected by APV.

APV is transmitted within a captive population through direct transmission, contact with contamin
nated objects, and vector transmission (e.g., mosquitoes, midges, flies), which are the primary sources of disease exposure.\(^5\) In a contaminated environment, the aerosol generated by feathers and dried scabs containing poxvirus particles provides a source for both cutaneous and respiratory infections.\(^2\) Very small lesions, such as slight abrasions during transport or minor trauma from cages, allow entrance of the virus into the skin; however, the APV can penetrate intact epithelium.\(^4\) AP disease in psittacine birds has 2 forms of presentation: 1) the cutaneous form characterized by the formation of papules followed by the development of vesicles that, after rupture, become erosions and scabs;\(^6\) and 2) the diphtheric or mucosal form that can induce pneumonia or air sacculitis.\(^7\) Psittacine birds also develop clinical signs associated with APV infection that present as systemic and oncogenic entities.\(^8\) Most birds infected with APV are mildly affected and rarely die; however, when infections involve the eyelids or mucous membranes of the oral or respiratory cavities, mortality rates are higher.\(^9\)

Olmos and others described a report of pox in parrots (\textit{Amazona aestiva}) in Mexico (Central America), whereas Lawrence and Petrak reported natural APV infections in both hyacinth macaws and green-winged macaw in South America.\(^10,11\) APV infection in South American parrots, particularly \textit{Amazona} spp., and \textit{Ara} spp., has also been reported.\(^12\) Until now, there are no reports about psittacine birds that have been affected by APV infections in Chile. This study is the first description of a natural AP outbreak in a captive exotic psittacine collection in Chile, which affected \textit{Agapornis} spp., \textit{Neophema} spp., \textit{Polytelis} spp., and \textit{Platycercus} spp.

**Case History**

An epizootic occurred in an aviary located in the Metropolitan Region, Chile, between March 20, 2004 and the end of June 2004, affecting many psittacine birds that presented with cutaneous lesions around the head and featherless regions of the legs. Blepharitis and serous conjunctivitis similar to those described in AP were also observed.\(^12\) The aviary had a population of 188 psittacine birds represented by the following genera: \textit{Agapornis}, \textit{Aratinga}, \textit{Cyanoramphus}, \textit{Melopsittacus}, \textit{Neophema}, \textit{Nymphicus}, \textit{Polytelis}, \textit{Platycercus}, \textit{Psittacula}, \textit{Psophotus}, \textit{Pyrrhura}, and \textit{Trichoglossus}. Each genus was lodged in collective contiguous cages within the same building. There were also 85 birds from the Galliforme, Anseriforme, and Passeriforme orders on the same farm. These birds were housed in other buildings.

A group of 25 young lovebirds (\textit{Agapornis} spp.) were introduced into this aviary. Seven days later, some lovebirds showed conjunctivitis manifested by a clear, serous discharge from the eyes accompanied by ocular congestion and weakness. A week later, other birds, such as \textit{Neophema} spp., \textit{Platycercus} spp., and \textit{Polytelis} spp., kept in neighboring cages showed similar clinical signs. \textit{Polytelis} \textit{swainsonii} and \textit{P. alexandrae} also developed papules at the commissure of the mouth, the margins of the cere, and around the external nares (Fig 1). Birds identified with chronic ocular lesions had thin eyelids that became adhered because of conjunctival irritation and inflammation associated with the disease. Within the aviary, birds of the genera \textit{Aratinga}, \textit{Cyanoramphus}, \textit{Melopsittacus}, \textit{Nymphicus}, \textit{Psophotus}, \textit{Psittacula}, \textit{Pyrrhura}, and \textit{Trichoglossus} kept in other neighboring cages did not show any clinical signs associated with AP.

**Physical and Laboratory Evaluation**

**Physical Examination**

Each bird in the aviary was captured and examined for AP lesions.\(^9\) The location of pox-like hyperplastic lesions on legs or feet, eyelids, and cere were recorded. In psittacine birds, lesions compatible with AP and the severity of infection were ranked according to the following scoring method: light (1 body lesion), moderate (2 body lesions), or heavy (3 or more body lesions, or 1 lesion on the head).\(^9\) Psittacine birds with clinical signs were isolated, and birds that died were collected for postmortem examination.
Laboratory Evaluation

**Samples for Viral Isolation.** Samples of cutaneous lesions were obtained from sick superb parrots for viral isolation. Lesions in this parrot species were of a size that permitted sampling with minimal health risk to the bird. A small portion of affected tissue was excised with a sterile scalpel, placed into an Eppendorf vial, and frozen for later analysis in the Diseases Diagnostic Laboratory for Wild and Pet Birds, Department of Animal Pathology, University of Chile. Virus isolation was conducted by inoculating specific pathogen-free (SPF) chicken embryos (CE) on the chorioallantoic membrane (CAM). Viral isolates (homogenized CAMs) were identified by a double immunodiffusion test (DIT) with an anti-AP hyperimmune serum obtained from experimentally inoculated chickens. Conjunctival tissue samples of some sick birds and CAMs from CEs inoculated for viral isolation were fixed in 10% formalin and stained with hematoxylin and eosin for histopathological examination.

**Samples for Serology.** Blood samples from 37 psittacine birds representing 6 different species with clinical signs were collected to measure anti-AP antibodies through DIT. Positive and negative control sera obtained from chickens were used. Two antigens were pooled and used in the DIT test obtained from: 1) suspension of APV-infected CAMs after a third passage with fowlpox vaccine (Diftosec CT, DCEP25 modified strain; Merial, Lyon, France) that was inoculated in 10-day-old SPF-CE, and 2) suspension of infected CAM after a third passage, with APV isolated from psittacine (superb parrot) cutaneous lesions.

Diagnosis

**Clinical Examination**

Clinical examination of this bird collection allowed for the identification of 50 individuals with nodular lesions, on the head, including eyelids, periloral regions, cere, nares, rhinotheca, commissure of beak, and oral cavity; on the legs including the digits and tarsometatarsus; and the percloacal zone (only 2 cases; Neophema boukii). At the beginning, nodular cutaneous lesions involving the eyelids and cere were present in psittacine birds (Fig 2), and, after some weeks, these lesions showed inflammation at the base and became hemorrhagic with the formation of scabs and local epithelial hyperplasia (Fig 3). These lesions caused blepharosynechia in some severe cases. Of the 50 psittacine birds that developed clinical poxvirus infections, 90% were young birds (5-12 months old) and 10% were adults (2-4 years old). Birds affected with clinical signs included 10 masked lovebirds (*Agapornis personatus*), 4 Fischer’s lovebirds (*Agapornis fischeri*), 22 rosy-faced lovebirds (*Agapornis roseicollis*), 4 princess parrots (*Polytelis alexandrae*), 5 superb parrots (*Polytelis swainsonii*), 3 eastern Rosellas (*Platycercus eximius*), and 2 Bourke’s parrots (*Neophema bourkii*).

Most of the affected birds, 39 of 50, had AP lesions on the head. AP lesions on the legs or feet were found in 24 of 50 and on the eyelids in 27 of 50. Very few pox lesions were noted on the percloacal area (n = 2) and the diphtheritic form was also rare (n = 4) (Table 1).

In accordance with the intensity of the skin pox lesions, most species had a preponderance of heavy infections (n = 40), whereas 10 psittacine birds had light infections. Young and adult budgerigars and
cockatiels and the remainder of adult psittacine birds (18 months and older) did not show AP signs.

Viral Isolation

The inoculated samples on chorioallantoic membranes of CEs produced generalized thickening, edema, and focal white-opaque pocks as were described for AP lesions.\textsuperscript{13} Homogenates of these affected CAMs demonstrated the presence of AP virus antigen detected by DIT by means of AP antibodies prepared in experimentally infected chickens. Conjunctival samples of both clinically affected and dead lovebirds, and infected CAMs that were collected in 10% neutral buffered formalin for histopathological examination, showed cytoplasmic inclusion bodies (Bollinger bodies) in the cytoplasm of epithelial cells (Fig 4).

Serology

Among 37 psittacine sera samples collected from clinically ill birds, 7 were positive for precipitating antibodies anti-APV. Using DIT, sera of 1 Eastern rosella, 2 superb parrots, and 4 rosy-faced lovebirds showed a precipitating line within 48 and 72 hours.

Treatment

Treatment consisted of the administration of vitamin A in the form of water-soluble supplemental vitamins (Solvit 15, 1 g/1 L; Centrovet Laboratory, Santiago, Chile). This treatment was repeated for 3 weeks each month. Also, systemic antibiotic treatment with enrofloxacin (Enromic 10% oral solution, 2 mL/1 L drinking water; Farquimica, Santiago, Chile) was applied continuously for 10 or 14 days in older birds, and trimethoprim sulfamethoxazole (Cotrimoxazol, 48 mg/120 mL drinking water; Mintlab, Santiago, Chile) in younger birds\textsuperscript{15} Nystatin (Nistoral available as a suspension of 100,000 IU/mL for oral administration; Chile Laboratory, Santiago, Chile) 1 mL/300 g was administered every 12 hours for 7 days.\textsuperscript{17} Lesions around the eyes were treated by cleaning the wounds and applying safe topical antimicrobial agents.\textsuperscript{6} Some superb parrots with alopecia of the head due to a secondary dermatophyte infection were treated with Clotrimazol (Canesten, atomizer solution; Bayer, Leverkusen, Germany) administered topically every 24 hours. Quarantine and general hygiene procedures were used as an integral part of the management and control of this AP outbreak.

Discussion

Clinical signs, epidemiology, macroscopic and microscopic pathological findings, virus isolation, and serological identification confirmed APV as the etiologic agent of this outbreak in Chile. The characteristics of the temporal and spatial distribution of this infection have been previously described in other AP outbreaks.\textsuperscript{2,12}

In accordance with our epidemiological approach, the recent arrival of an infected group of
Agapornis spp. to the aviary could have introduced APV to the remaining psittacine bird population. The presence of mosquitoes possibly facilitated AP dissemination in this captive group of birds.

This AP outbreak affected 27% of this psittacine collection with clinical signs. In contrast to the findings of van Riper and others, the majority of lesions in this Chilean epornitic occurred on the head, and most of the psittacine birds had severe infections. AP lesions were usually observed on the eyelids or periorbital zones. In contrast to the findings of Smits and others, young birds 1 year of age or younger, in this case, were more likely to have pox lesions than adult birds.

Of the 18 species of Psittaciforme in the Chilean aviary, mostly young psittacine birds (90% of affected birds) belonging to 7 species of *Agapornis*, *Neophema*, *Platycercus*, and *Polytelis* were affected with clinical signs, whereas 10% of the sick birds were ≥2 years old, suggesting an APV susceptibility related with age as previously reported. Other psittacine birds such as budgerigars and species of the Cacatuidae (*Nymphicus hollandicus*) and Loriinae (*Trichoglossus* spp.) families did not present with clinical signs during the outbreak. A similar situation occurred in an epornitic of AP where only lovebirds were affected by APV but not cockatiels or finches. In this case from Chile, none of gallinaceous birds, canaries, finches, or swans were affected by the AP outbreak.

Psittacines considered most susceptible to APV infection include Amazons, pionus, macaws, conures, parakeets, and lovebirds; cockatoos and cockatiels appear to be more resistant to infection. Of the 50 birds with clinical signs of AP, 11 died during this outbreak (2 *Neophema bourkii*, 1 *Polytelis alexandrae*, 3 *Platycercus eximius*, 3 *Agapornis roseicollis*, and 2 *Agapornis personatus*). The mortality by AP could have been higher if control steps to avoid further exposure and infection were not taken. Morbidity and mortality rates in lovebirds with AP may reach 75% of the at-risk population. In another epornitic of AP that occurred in rosy-faced lovebirds, there was an increased risk of mortality in young birds (150/400, 37.5%) compared with breeders (5/160, 3.1%).

In this study, the conventional laboratory procedures for APV confirmed the diagnosis of APV but did not allow for differentiating virus species. Polymerase chain reaction in combination with restriction enzyme analysis and the sequencing of the amplified fragment is a rapid and effective diagnostic system for detection and differentiation of genomes of most APV, and is a new approach to refine epidemiologic studies of APV infections in the future. Studies of cross-protection need to be performed to investigate any immunological correlation between the poxvirus isolated from superb parrots in this study and other strains isolated from the Psittaciforme order, such as agapornipox and other characterized avipoxvirus species.

**Summary**

During the 3-month period of the epornitic, AP affected 50 psittacine birds representing 7 species (*Agapornis* spp., *Neophema bourkii*, *Platycercus eximius*, and *Polytelis* spp.) at a Chilean aviary. Other birds maintained in the same aviary that were kept in neighboring pens (e.g., Psittaciforme, Passeriforme, Galliforme, Anseriforme) did not show any clinical signs. Lesions were limited to nonfeathered skin and consisted of epithelial cell hyperplasia, and secondary inflammatory changes; intracytoplasmic eosinophilic inclusion bodies were also detected by light microscopy. When CEs were inoculated with material from eyelid and cere scabs, histological lesions of AP developed on the chorioallantoic membrane of SPF CEs. The disease was usually self-limiting in birds, but 11 animals died at the aviary described in this case report. This is the first report on the occurrence of an AP outbreak in captive psittacines in Chile and is also the first report on poxvirus infection in the superb parrot (*Polytelis swainsonii*) and princess parrot (*Polytelis alexandrae*).

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**References**