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Case Report

First report of Psittacid Avipoxvirus in *Agapornis* in Mexico

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Abstract

A postmortem study was performed on two lovebirds (*Agapornis fischeri* and *Agapornis personatus*) that had scabs in the periocular region and on the eyelid, as well as serous blepharitis. Microscopically, the eyelids showed ulcers, necrosis and serocellular crusts, severe hyperplasia of keratinocytes with eosinophilic intracytoplasmic inclusion bodies (Bollinger's bodies), bacterial colonies of gram-positive coccoid morphology and PAS-positive septate and 45° branching hyphae. The microbiological study identified the colonies as *Staphylococcus* spp. and *Aspergillus fumigatus*, respectively. Using molecular techniques, avian pox clade C was identified on the eyelid. This is the first report in Mexico of a case of avian pox in parrots associated with clade C *Avipoxvirus*.

Key words: Avian pox, psittacine, Bollinger´s bodies, scabs.

Introduction

Avian pox is a cosmopolitan viral disease that affects domestic (chickens, turkeys and quails), ornamental (canaries, pigeons and parrots) and wild birds (34). The etiological agent is a virus of the genus *Avipoxvirus* that belongs to the subfamily *Chordopoxvirinae* and the family *Poxviridae*. Approximately 232 of the 9,000 known bird species have naturally acquired infections with some species of *Avipoxvirus* (33,34). The names of the different species of *Avipoxvirus* are based on the species that they affect (23). The International Committee on Taxonomy of Viruses (ICTV) considers the genus *Avipoxvirus* to comprise twelve species of viruses (http://ictvonline.org/) (viruses of the canary, flamingo, chicken, pigeon, junco, mynah, penguin, quail, parrot, turkey, sparrow and starling). Phylogenetic analysis based on polymorphism of the P4b (major central protein) gene and DNA polymerase gene grouped avipoxviruses into three major clades: A (fowlpox-like virus), B (canarypox-like virus) and C (psittacinepox-like virus). (19, 21). Two additional clades have also been proposed (D and E): clade D has been detected in Japanese quail (*Coturnix japonica*) in Italy, and clade E viruses have been detected in turkeys (*Meleagris gallopavo*) in Hungary, hens in Mozambique and chickens in Brazil (27, 2, 22, 21). *Avipoxvirus* infections in domestic birds are very well documented (3), but, there are few cases that report psittacine poxvirus in parrots worldwide, likewise, this is the first description in Mexico.

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Case description

On August 19, 2019, two dead lovebirds (*Agapornis fischeri* and *Agapornis personatus*) were sent to the diagnostic and research laboratory in bird diseases of the Department of Medicine and Animal Husbandry of the FMVZ-UNAM for necropsy and histopathology study. The birds were between 1 and 3 years of age respectively and were referred because they presented scabs in the periocular region and on the eyelid, as well as serous blepharitis with palpebral adhesions. The birds came from a breeding ground for exotic Psittaciformes and Passerines located in the municipality of Sahuayo, Michoacán. The aviary has a population of 3,800 lovebirds (*Agapornis fischeri* and *Agapornis personatus*), and the total number of affected birds was 339 (8.9%), of which 198 (58.4%) had died. The birds' diet was based on a mixture of seeds: birdseed, white millet and rooster wheat.

In the postmortem examination, the *Agapornis fischeri* had a weight of 33 grams and a good body condition, and the *Agapornis personatus* had a weight of 38 grams. On external inspection, it was observed that the periocular ring and the eyelid showed moderate areas of thickening due to the presence of brown and yellow scabs (2/2 birds) (Fig. 1A).

In the region of the base of the beak and inside the nostrils, moderate dark brown crusts were observed (1/2 birds). The proventriculus was slightly increased in size (1/2 birds). The ureters presented abundant urates (2/2 birds). The remaining body parts and systems did not show significant macroscopic changes in the two birds. Samples of the eyes and eyelid, liver, spleen and kidney were collected for histopathological study. The tissues were fixed in formalin buffered with 10% phosphate for 24 hours and processed with the routine technique of dehydration, inclusion in paraffin and H&E, Gram and PAS staining (25).

Microscopically, the eyelids presented extensive areas of ulceration and necrosis of the epidermis covered by serocellular crusts and interspersed with bacterial colonies of gram-positive coccoid morphology and PAS-positive, septate and 45° branching hyphae (2/2 birds) measuring 5x10 µm. Similarly, severe keratinocyte hyperplasia was observed, and eosinophilic intracytoplasmic inclusion bodies (Bollinger's bodies) were observed (Figs. 1B, C and D).

In the palpebral conjunctiva, large aggregates of inflammatory cells mainly composed of lymphocytes that extended to the palpebral folia and bulbar conjunctiva were detected. In the iris and ciliary bodies, moderate aggregates of inflammatory cells composed of heterophils were observed in 1 of 2 birds. The corneas showed areas of ulceration and extensive aggregates of coccoid bacterial colonies and heterophile in 1 of 2 birds (Figs. 1E and F).

The beak showed areas of necrosis of the keratin layers with hyperplasia of epithelial cells and eosinophilic intracytoplasmic inclusion bodies in the cytoplasm of some cells in 1 of 2 birds. The liver and spleen presented aggregates of macrophages with ocher-brown pigment in the cytoplasm (interpreted as iron) in both birds; this same pigment was observed in the cytoplasm of some epithelial cells of the renal tubules in 2 of 2 birds. The mucosa of the proventriculus exhibited multifocal hemorrhages in 1 of 2 birds.

Eye and eyelid samples were sent for microbiological isolation, under aseptic conditions, the eye was washed with sterile physiological saline solution (SSF), and fragments of the ocular tissue were deposited into two tubes with 5 ml of Sabouraud dextrose broth (Bioxón® BD) supplemented with chloramphenicol and seeded in culture media, blood agar (Bioxon® BD) and MacConkey agar (Bioxon® BD). The results showed the isolation of *Aspergillus fumigatus* and *Staphylococcus* spp.

The viral particles in the eye and eyelid were identified through the extraction and purification of the DNA of the macerated eye and eyelid using the DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's specifications. Subsequently, using the initiators and methods described (17, 18), two fragments of the *Avipoxvirus* genome were amplified with endpoint PCR using the QIAGEN® Taq PCR Master Mix Kit (Qiagen, Hilden, Germany). The PCR products were purified and sequenced by the chain termination method using the BigDye™ Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA). The consensus sequences of the two sequenced segments of the *Avipoxvirus* genome were analyzed using MEGA version 11 (30) against 98 DNA polymerase gen sequences and 98 sequences of the *Avipoxvirus* 4b protein gen available in GenBank. A sequence of 913 nt was obtained from the DNA polymerase of *Avipoxvirus* that presented 100% homology with the sequences collected from *Amazonas ochrocephala* in New York in 1980 (KC017925) and *Amazonas* spp. (KC017849) in the UK without a specific collection date (Fig. 2). The 580 nt sequence of the 4b protein showed 100% homology with KC018069 (*Amazona ochrocephala*, New York, 1980), AM050383 (*Amazona* spp., UK, without a specific collection date), AM050382 (*Ara* spp., UK, without a specific collection date), KY748236 (*Psephotus haematonotus*, Chile, 2016), KY748237 (*Barnardius zonarius*, Chile, 2016). On the other hand, there are 3 sequences from exotic psittacines in Brazil (MG601779, MG601780 and KT187552) and 1 from Germany (AY530311) that differ by up to 2 nucleotides from our sequence (Fig. 3).

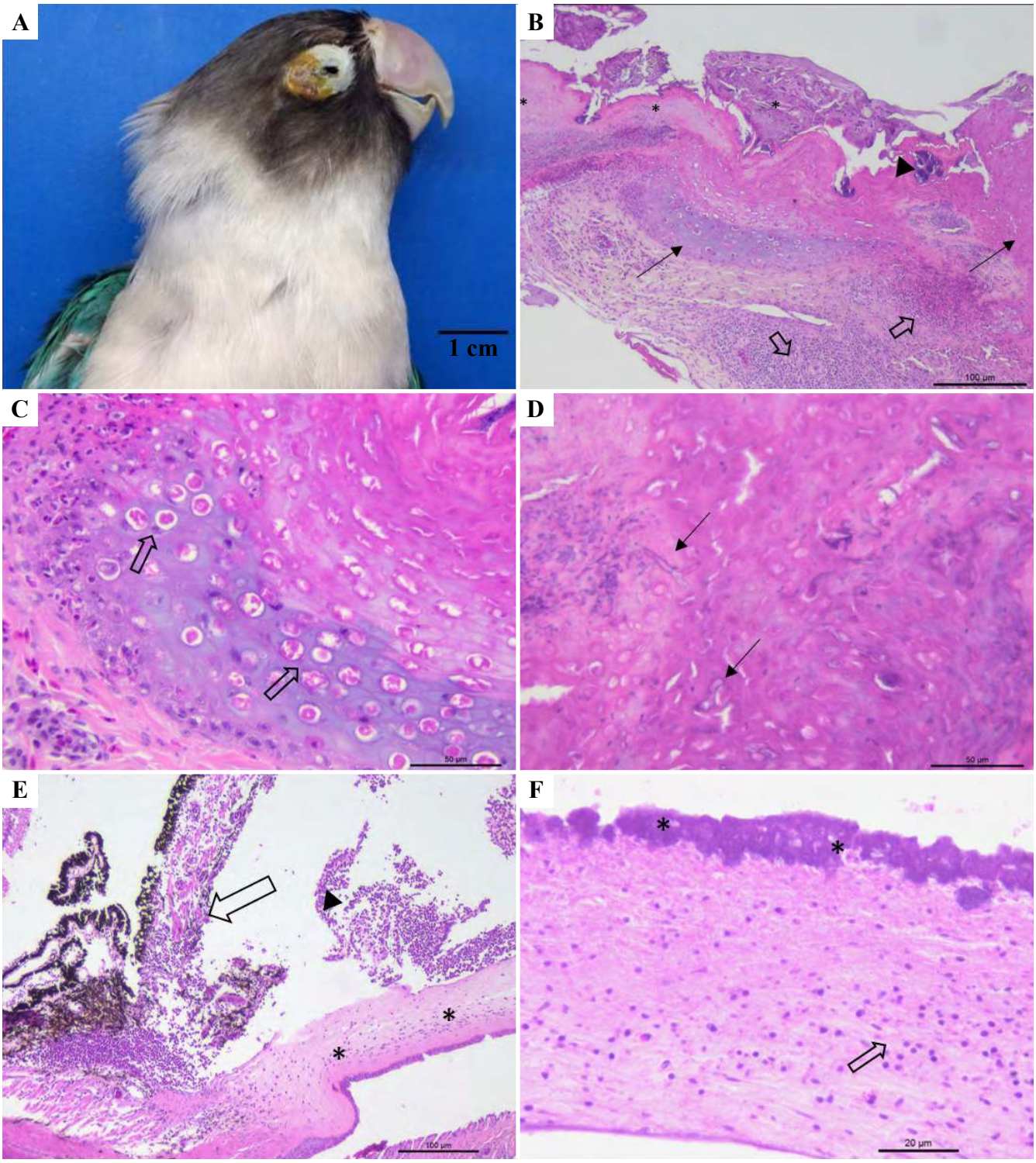

Figure 1. *Agapornis personatus*. **(A)** The periocular ring and the eyelid showed moderate crustal lesions. **(B)** Photomicrograph of the eyelid. Severe epidermal hyperplasia (arrows), scabs (asterisks) and intralesional coccoid bacterial colonies (arrowhead) were present. In the palpebral conjunctiva, moderate aggregates of lymphocytes and heterophils (arrow silhouettes) were detected. H&E staining, 100 µm bar. **(C)** Photomicrograph of the eyelid. Epithelial cells exhibited eosinophilic intracytoplasmic inclusion bodies (Bollinger´s bodies) (arrow silhouettes). H&E staining, 50 µm bar. **(D)** Photomicrograph of the eyelid. Zones of epithelial hyperplasia and necrosis with the presence of fungal structures consistent with septate hyphae (arrows) located within the lesion. H&E staining, 50 µm bar. **(E)** Photomicrograph of the eye. The iris and ciliary bodies were infiltrated by inflammatory cells composed of heterophils (arrow silhouette), and an inflammatory reaction was observed in the anterior chamber of the eye (arrowhead) and in the thickness of the cornea (asterisks). H&E staining, 100 µm bar. **(F)** Photomicrograph of the cornea. Presence of abundant bacterial colonies of coccoid morphology (asterisks) and aggregates of inflammatory cells in the stroma (arrow silhouette). H&E staining, 20 µm bar.

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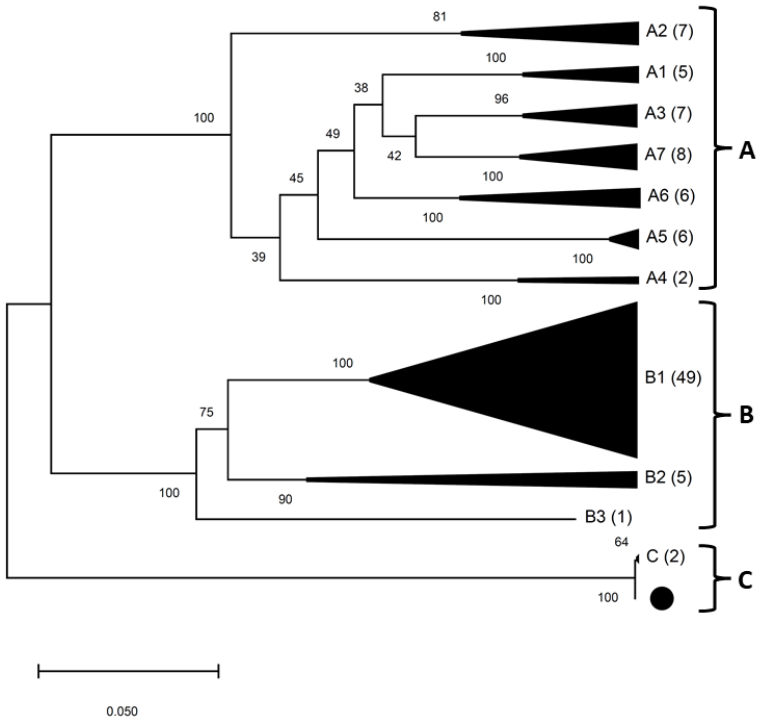

Figure 2. Phylogenetic inferences of the partial sequence of DNA polymerase. The letters A, B and C indicate the three groups of *Avipoxvirus* that were identified. Subgroups are indicated by the letters A or B followed by a number. The number of sequences in each collapsed branch is indicated in parentheses. The position of the 913 nt sequence obtained in this study is indicated with a black circle. For each branch, the bootstrap values are indicated.

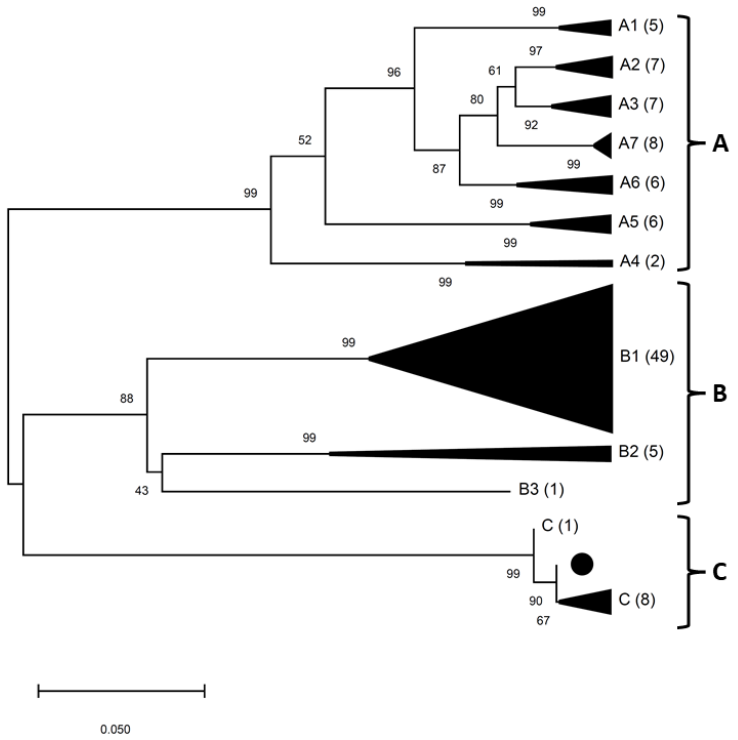

Figure 3. Phylogenetic inferences of the partial sequence of protein 4b. The letters A, B and C indicate the three groups of Avipoxvirus that were identified. Subgroups are indicated by the letters A or B followed by a number. The number of sequences contained in each collapsed branch is indicated in parentheses. The position of the 580 nt sequence obtained in this study is indicated with a black circle. In each branch, the bootstrap values are indicated.

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Discussion

The macroscopic and histological examinations of the lesions in the lovebirds indicated cutaneous *Avipoxvirus* infection and coinfection with *Staphylococcus* spp. and *Aspergillus fumigatus*. The PCR and phylogenetic analysis identified the virus as a psittacinepox-like virus belonging to clade C. Most of the phylogenetic analysis of Avipoxvirus are grouped near to bird-specific species (Clade A) or a range of different bird species (Clade B), while Clade C seems to be so far specific for psittacine regardless the geographic origin (13). In addition, the natural reservoir-host and complete genome for psittacinepox-like virus remains elusive.

It has been documented that parrot manifest the disease in four ways: cutaneous, diphtheritic, systemic and pseudoneoplastic (16, 35). These four presentations have also been described in passerines (1). In both parrots and other birds, the cutaneous presentation is the most common, as was the case in the present study. Coinfections have been reported in parrots with avian pox, with *Candida* sp. as a secondary agent (35, 8). Additionally, *Reza et al*, in 2013 (26), described a simultaneous infection of avian pox and *Aspergillus* sp., just like we observed. Therefore, we must consider that avian pox may predispose to secondary fungal infections by *Aspergillus* sp., causing higher mortality in affected birds. The finding of *Staphylococcus* spp. in the present case report indicates a secondary pathogen that might be due to avian pox primary infection as well.

Cutaneous lesions due to avian pox occur in areas of skin without feathers, such as the face, the periocular region, the base of the beak and the legs, depending on the species of parrot affected. The lesions are characterized by the formation of papules, vesicles, pustules and scabs that can spread to the eyelid, causing blepharoconjunctivitis (6), as occurred in the present case. Additionally, in the present study, the replication of Avipoxvirus in the cornea was not observed, as has been rarely described in parrots and other species (35), but a bacterial infection was found.

The clinical history record mention that the aviary that experienced this outbreak of avian pox is located in an area near other breeding sites of Psittaciformes and passerines, with a history of skin lesions in the eye and eyelid region during summer which favors the likelihood that the disease being endemic (17). Outbreaks of avian pox in psittacine birds occur frequently in breeding place and aviaries where the elimination or excretion of the virus through scabs, skin scales and feathers can favor transmission through small skin wounds. Mosquitoes also participate in transmission as mechanical vectors; therefore, it is important to establish programs for the control of these insects, particularly during rainy seasons and in hot and humid climates, as was a factor in this case (7, 14). Cases of avian pox that were self-limited to cutaneous presentation have been reported in African gray parrots (*Psittacus erithacus*) and roselas (*Platycercus elegans*) and this may be influenced by age, immunological status, the virulence of the virus, secondary infections, route of exposure, nutritional deficiencies, and aviaries with a high population density, poor air quality and poor cleaning and disinfection of facilities (31, 29).

Avian pox in parrots is serious and can cause mortality; for example, one case reported a morbidity of 30% and mortality of 5% in lovebirds (12, 10). In the present case, the morbidity rate was 8.9%, with a mortality of 58.4% in a 5-month period. In another study, it was reported an atypical outbreak of avian pox in lovebirds with cutaneous, diphtheritic, systemic and pseudoneoplastic presentation and a mortality rate of 37.5% for young birds and 3% for breeders (35).

The present case of avian pox in lovebirds may be a risk for the 22 species of endemic or resident parrots in Mexico that have special protection or are in danger of extinction. It may be a factor that increases the risk of species extinction (8, 28) because parrots of the genus *Amazona* are particularly susceptible to infection and can develop severe outbreaks of avian pox in its diphtheritic presentation, leading to high mortality (4).

Additionally, the present case may help in assessing the risk for different species of susceptible exotic parrots, such as the African gray parrot (*Psittacus erithacus*) and the monjita (*Myiopsitta monachus*) (20, 15, 11, 32, 36) or very susceptible parrots, such as *Agapornis*, *Platycercus*, *Polytelis* and *Neophema* (35, 14, 5, 24), which are found in Mexico.

On the other hand, the psittacinepox virus can infect broilers and laying hens vaccinated with against the fowlpox virus, which can cause economic losses for producers (4). To the best of our knowledge Fowlpoxvirus outbreaks or cases are uncommon in Mexico, probably due poultry vaccination, but, companion or ornamental birds like chickens, canaries and psittacine are more likely to be susceptible due lack of vaccination, as well as backyard turkeys (9).

The lack of information about avian poxvirus surveillance in Mexico does not allow to identify the possible origin of this case, but this report encourage to do molecular surveillance on Fowl poxvirus, Canarypox virus and psittacine poxvirus in poultry, companion, and ornamental bird.

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Conflict of Interest

The authors declare no competing interests.

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