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CASE REPORT

Fatal dual infection of avian polyomavirus and psittacine beak and feather disease virus in Chile

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ABSTRACT. A 6 week old Plum-headed parakeet (*Psittacula cyanocephala)* from a private bird collection in the Metropolitan Region of Chile died after presenting depression, ataxia, tremors of the head, subcutaneous hemorrhage and delayed crop emptying. Histological examination of liver tissue revealed intranuclear viral inclusion bodies and focal necrosis. Liver tissues and blood contained both avian polyomavirus (APV) and psittacine beak and feather disease (PBFD) viral nucleic acids (DNA), indicating dual viral infection. The purpose of this report is to describe the first case of concurrent psittacine beak and feather disease virus PBFDV and APV infection in a psittacine bird in captivity in Chile. PBFDV has not been reported until now in Chile.

Key words: avian polyomavirus, psittacine beak and feather disease.

RESUMEN. Una cotorra ciruela *(Psittacula cyanocephala)* de seis semanas de edad de una colección privada de aves en la Región Metropolitana de Chile, murió después de presentar depresión, ataxia, tremor de la cabeza, hemorragia subcutánea y enlentecimiento en el vaciamiento del buche. El examen histopatológico del tejido hepático reveló la presencia de cuerpos de inclusión intranucleares y necrosis focal. Hígado y sangre del ave contuvieron ácidos nucleicos virales de "avian polyomavirus" (APV) y "psittacine beak and feather disease virus" (PBFDV) indicando una infección viral dual. El propósito de esta comunicación es describir el primer caso de infección concurrente por APV y PBFDV en un ave psitácida en cautiverio en Chile. Hasta ahora, PBFDV no había sido reportado en Chile.

Palabras clave: virus polioma aviar, enfermedad del pico y pluma de psitácidas.

Avian polyomavirus (APV) disease or budgerigar fledgling disease and psittacine beak and feather disease (PBFD) are the most common recognised viral diseases of captive psittacine birds and both diseases have similar clinical manifestations (Bert *et al* 2005, Fungwitaya *et al* 2009). Co-infections of APV and psittacine beak and feather disease virus (PBFDV) have been revealed in China, Thailand, Poland, and other regions including Central America (Ramis *et al* 1998, Fungwitaya *et al* 2009, Piasecki and Wieliczko, 2010, Zhuang *et al* 2012, Dolz *et al* 2013). Concurrent APV and PBFDV infections have been reported in budgerigars, lovebirds, parrots, and several species of cockatoos (Latimer *et al* 1993).

Avian polyomaviurs and PBFDV cause serious and often fatal disease in parrots and other bird species (Katoh *et al* 2010). In this communication, we described the first case of APV and PBFDV co-infection in psittacine birds in captivity in Chile, based on clinical history, histopathological and biomolecular diagnosis.

A 6 week old Plum-headed Parakeet (*Psittacula cyanocephala,* order: Psittaciformes) of a private collection died on December 2013 after presenting depression, ataxia, tremors of the head, subcutaneous hemorrhage and delayed crop emptying. Feather abnormalities were not observed. Neither biochemical nor hematologic tests were performed. Gross changes at necropsy were haemorrhage under the skin, hepatomegaly, splenomegaly and pale kidneys. Sections of liver, spleen and kidney from the bird were submitted in 10% neutral-buffered formalin for histologic examination in Citovet. Blood and replicate liver tissue were stored at 5 °C for virologic analyses by polymerase chain reaction assays (PCR) in Bioingentech.

Histological examination of hematoxylin and eosin stained tissue sections revealed liver foci of necrosis and presence of intranuclear inclusion bodies in hepatocytes.

Viral DNA extraction from blood sample and liver tissue was performed using the APV Kit of Bioingentech. The protocol described by the manufacturer was used to purify the DNA from both samples. A Techne TC 4000 thermocycler was used for initial denaturing at 94 ºC for 3 minutes, then 30 cycles with 30 seconds for denaturation at 94 ºC, 30 seconds annealing at 57 ºC and 30 seconds extension at 72 ºC followed by a final extension step 5 minutes at 72 ºC. The PCR based on the specific primers of the *Agnoprotein 1a* gene (Based in GenBank sequence: AF241170.1) using the APV kit Bioingentech resulted in an amplification product of the expected size of nearly 500 bp, in the blood and liver tissue samples from the *Psittacula cyanocephala*. We also observed the presence of PBFDV infection using the PBFD kit Bioingentech based on the specific primers of the putative replication associated protein (*rep*) gene (Based in Genbank sequence: DQ384621.1). The expected size for PBFDV gene and internal control were 395 bp, and 140 bp, respectively. PCR products were separated on 1.0% agarose gel and stained with GelRed™. DNA bands were visualised by UV

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transiluminator (figure 1) Positive, negative, and internal controls of the kit, were used to validate the procedures.

The PCR products of blood sample obtained were purified using the Kit Wizard® SV gel and PCR clean-up system, (Promega). Finally, the purified PCR products were subjected to sequence analysis in order to verify that the bands corresponded to the correct genes. The PCR products were automatically sequenced in both directions using both primers at Pontifical Catholic University of Chile. Sequencing was done on an ABI PRISM® 3130 Applied Biosystems. For bioinformatics analysis of the sequences and alignments, Blast and ClustalW2 software were used and matched with the database. The sequences of each gene were rather conserved (98 to 99% similarity within each gene). Accession numbers of sequences presented in this paper are KU500898.1 and KU500899.1 (GenBank).

Neurologic disease has been associated with APV of nestling budgerigars, Moluccan and Ducorps'cockatoos (Schmidt *et al* 1987, Latimer *et al* 1996). The neurologic disease in the parakeet of this report could be associated with concurrent APV and PBFDV infection, which has been documented previously in psittaciform birds (Latimer *et al* 1996). PBFDV infection is often associated with clinical evidence of acquired immunodeficiency, leading to a variety of infections. The APV infection in this parakeet involved systemic lesions and inclusions were observed in the common target organ (liver). Because the cerebellum and brain were not submitted for examination, the origin of the body tremors could not be evaluated. Differential diagnosis in this dead bird included Chlamydiosis, liver disease, clotting disorders, bacterial septicemia, Pacheco's disease virus, adenovirus, reovirus and paramyxovirus infections.

Polyomavirus and PBFDV transmission may occur by both horizontal and vertical routes (Ritchie 1995). The source of infection of the parakeet that belonged to a private collection of parrots, was not determined. It is probably that both viruses are present and circulating in exotic psittacine birds in Chile. Effective prevention techniques for both pathogens are often not implemented in aviaries of psittacine birds in the country.

Very few reports exist about APV or PBFDV infections in psittacine birds in South America. The first known suggestions of APV infections by antibodies detection in South America were made in the United States on Sun Conures (*Aratinga solstitialis*) from Guyana exposed to birds from other areas, and in wild dusky-headed parakeets (*Aratinga weddellii)* from Peru by serosurveys (Complement Fixation and Virus Neutralization VN) (Clubb and Davis 1984, Gilardi *et al* 1995). On the other hand, PBFD was recognised in Brazil in a White Cockatoo, *Cacatua alba*[1]. The presence of PBFD viral nucleic acid was confirmed in this bird by *in situ* DNA hybridisation. PBFDV was also detected by real time PCR in 11/20 (55%) psittacine birds in Argentina (Origlia *et al* 2013). The first known suggestion of APV infections in Chile was made on 36 out of 100 psittacines in captivity with antibodies against APV found using the serum neutralisation test (González Hein 2006).

[1] Werther K, Durigon E, Raso T, Latimer K, Campagnoli R. 1998. Description of the First Case of Psittacine Beak and Feather Disease in Brazil. http://archive.is/0Tlki, accessed November 3, 2015.

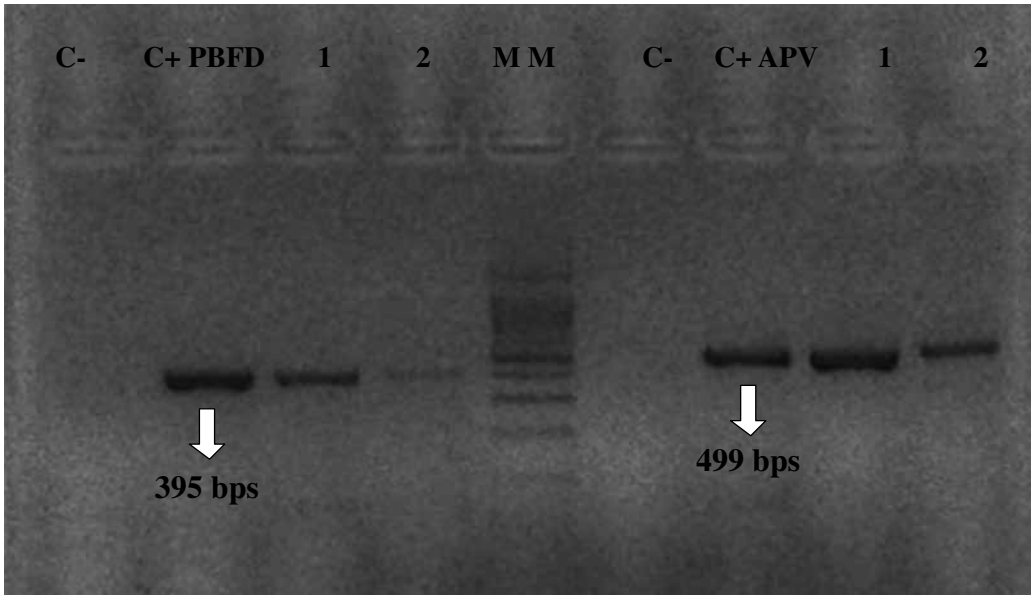

Figure 1. Gel electrophoresis of PCR products of PBFDV and APV. MM: molecular marker 100 bp DNA Ladder; C−: negative control; C+ PBFD: positive control to PBFDV and C+ APV: positive control to APV; 1: blood sample of bird positive to PBFDV and APV; 2 liver sample of bird positive to PBFDV and APV; 395 bps (PBFDV specific band) and 499 bps (APV specific band).

The dual infection of APV and PBFDV in a *Psittacula cyanocephala* in Chile for the first time showing the presence of both diseases such as in other countries, suggests that a survey designed to investigate the frequency of PBFDV and APV inside the population of captive psittacine birds and a monitoring program especially in breeding are crucial, as well as the isolation of infected birds. Moreover, a strict bio-security system should be established in order to control these diseases in Chile. Important measures for the prevention and control include to vaccinate susceptible adults and neonates, quarantine for 60 to 90 days if new birds must be added to the flock and the use of DNA probe to test the birds and the nursey environment.

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