Psittacine Beak and Feather Disease in Three Captive Sulphur-Crested Cockatoos (Cacatua galerita) in Thailand

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ABSTRACT. Three sulphur-crested cockatoos (Cacatua galerita) were diagnosed as psittacine beak and feather disease (PBFD). Histopathology of the feather pulp and follicles showed intracytoplasmic botryoid clusters or granular inclusion bodies in epithelial cells and macrophages. Electron microscopy revealed multiple cytoplasmic clusters of electron dense viral particles corresponding to the inclusions. PBFD virus (circovirus) DNA-specific product was detected from formalin-fixed paraffin-embedded feathers by nested polymerase chain reaction (PCR) method.

KEY WORDS: circovirus, cockatoo, psittacine beak and feather disease (PBFD).

NOTE Pathology

The psittacine beak and feather disease (PBFD) was first described in various species of Australian cockatoos in early 1970s [9, 12]. Until the present, apparently, many species of psittacine birds were widely affected with PBFD [7, 15], and the infection has been becoming worldwide [6, 10, 11, 13]. The disease is caused by PBFD virus, which belongs to a new virus family named Circoviridae. The virions are small (14 to 17 nm in diameter), non-enveloped and icosahedral, and have circular single-stranded DNA (1.7 to 2.0 kb) [1, 11, 16]. Biological characters of the virus is similar to those of porcine circovirus (PCV), chicken anemia virus, pigeon circovirus, and some plants viruses [2, 8]. However, only PCV and PBFD were assigned to genus circovirus [11, 12]. Major clinical signs of PBFD were feather dystrophy and loss as well as immunosuppression [14, 16]. This report deals with the first onset of PBFD in three captive cockatoos in Thailand and the detection of the viral DNA sequences from the formalin-fixed paraffin-embedded (FFPE) tissues [4, 5] by nested polymerase chain reaction (PCR) method.

Feathers of three sulphur-crested cockatoos (Cacatua galerita) were brought to the Veterinary Diagnostic Center, Faculty of Veterinary Sciences, Chulalongkorn University, Thailand in December 1999. The cockatoos were between 5 and 6 months old and unidentified sex. They had been imported from Indonesia when they were very young. They had been 5 and 6 months old and unidentified sex. They had been imported from Indonesia when they were very young. They showed anorexia, feather loss, alopecia, itching, red skin, and many lacerated wounds caused by biting and/or scratching. The downs were also lost or rough. Further clinical and pathological examinations were not available. The feather samples were fixed in 10% buffered formalin and routinely processed. Four µm paraffin sections were stained with hematoxylin and cosin (HE), periodic acid-Schiff, Grocott’s methenamine silver, and Feulgen reagent. For electron microscopy, FFPE samples were deparaffinized in xylene, rehydrated through tap water, and then washed in 0.1 M phosphate buffer (PB, pH 7.4). The samples were refixed in 2% glutaraldehyde in PB, post-fixed in 1% osmium tetroxide and embedded in epoxy-resin. Ultra-thin sections were double-stained with uranyl acetate and lead citrate and observed with a transmission electron microscope (1200 EX, Nihon-Denshi, Tokyo, Japan).

Paraffin sections of 4 µm thickness were deparaffinized, desiccated and suspended in DNA extraction buffer (0.4 mg/ml proteinase K, 100 mmol/l NaCl, 10 mmol/l Tris-HCl (pH 8.0), 25 mmol/l EDTA (pH 8.0), and 0.5% sodium dodecyl sulfate). After incubation at 55°C for 24 hr, DNA was pelleted down in phenol-chloroform-iso amyl alcohol (25:24:1) in TE buffer (10 mM Tris-Cl and 1 mM EDTA, pH 8.0). Three µg of DNA samples was subjected to PCR amplification. The primers were selected according to the published sequences of PBFD virus genome (Gene bank no. AF 080560) [16]. The sets of primer designated as AF11 (5’-GAGATCGAATTGCCGCTTTCTC-3’) and AF12 (5’-GTAAACTCCAAAAAGGGCCC-3’) were used for the first PCR amplification, and AF21 (5’-CCCCCATTTGCAAGGCTACTTT-3’) and AF22 (5’-CATGCTTACG-TAGCTTCTGG-3’) were used for the nested PCR amplification, respectively. The PCR amplification was performed using reagents supplied with a commercial kit (Takara EX Taq, Takara, Shiga, Japan) and a PCR thermal cycler (Takara). The first PCR amplification was conducted at 35 cycles of denaturing (94°C for 1 min), annealing (58.6°C for 1 min) and extension (72°C for 1 min). The nested PCR amplification was conducted in the similar manner except that 30 cycles were used. The nested PCR products were electrophoresed on 2% agarose gel in Tris-borate EDTA buffer, stained with ethidium bromide, and visualized under a UV transilluminator, FAS III (Toyobo, Osaka, Japan).
Amphophilic intracytoplasmic botryoid inclusion bodies were observed in macrophages infiltrating in the feather pulp and follicle of all cases. Large clusters of granular inclusions were also detected in the cytoplasm of epithelial cells of the outer keratinized sheath. The intracytoplasmic inclusions were varied in size (5–25 µm), round, homogeneous in shape, magenta to dark purple in color (HE stain), and positive for Feulgen reaction (Fig. 1). Electron microscopy revealed multiple electron dense intracytoplasmic clusters of small virus particles (20 nm in diameter) in macrophages and epithelial cells (Figs. 2 and 3). The first PCR amplification did not produce any virus specific DNA products, while the nested PCR resulted in amplified products of specific DNA of 269 bp (Fig. 4).

Histopathology and electron microscopy demonstrated viral inclusion bodies in the feather cells as described in the previous reports [13]. The Feulgen stain clearly showed the DNA inclusions in those cells. The size and the shape of virus particles were identical to those of the circovirus by electron microscopy. PBFD virus-specific DNA (269 bp) was also detected by the nested PCR method from the FFPE feather samples. The present cases were, therefore, confirmed to be PBFD. Peracute or acute form of PBFD occurs in neonatal or young birds and chronic form in older ones [11, 15]. The present cases are 5 to 6 months old and considered chronic infection. The present report is the first onset of avian PBFD in Thailand.

The virus specific DNA could not be enough produced by the first PCR reaction in the present study, probably due to the partial degradation of target DNA during formalin fixa-
tion [3]. However, we finally succeeded to obtain specific PCR products by the nested PCR method that can increases the sensitivity 10- to 100-fold, compared with the single PCR [4]. Using the nested PCR method, retrospective detection of PBFD viral DNA from FFPE samples can be available.

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REFERENCES