Psittacine beak and feather disease in Iran, molecular and histopathologic detection

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Abstract: **Psittacine beak and feather disease (PBFD) is a major viral disease in wild and captive psittaciformes all around the world. The disease was suspected in a 7 years old lesser sulphur-crested cockatoos (*Cacatua sulphurea*) with a minor feather loss at the back of neck and head. The bird was comprehensively examined by macroscopic pathology, histopathology and polymerase chain reaction (PCR). Marked intracellular edema of the keratinocytes and necrosis were evident in histopathological observation of dystrophic feather follicles. Numerous macrophages with cytoplasmic inclusions "botryoid" and Prevasculitis were also present in the dermis. Histopathologically, the feather lesions and inclusions were typical of PBFD. The presence of psittacine beak and feather disease virus (BFDV) DNA was confirmed by PCR. This is the first documented report of the occurrence of the PBFD in Iran.**

Key words: PBFD, BFDV, avian circovirus, Iran, lesser sulfur-crested cockatoo.

Introduction

Psittacine beak and feather disease (PBFD) is the most commonly recognized viral disease of wild and captive Psittaciformes (Pass and Perry, 1984). The disease has worldwide distribution (Pass and Perry, 1984; Pass *et al.*, 1994; Sanada *et al.*, 1999; Doneley, 2003; Albertyn *et al.*, 2004; Khalesi *et al.*, 2005). In most species the disease is characterized by chronic progressive, symmetrical feather dystrophy and occasional beak deformity (Pass and Perry, 1984; Todd, 2000). Due to various susceptibilities, the clinical presentation of the disease may vary greatly. Generally, PBFD is a disease of young ages, but older ages may also develop disease (Woods and Latimer, 2003). In most instances, the feather changes, the beak, and more rarely, the nails lesions, occur symmetrically. Based on clinical presentations, peracute, acute and chronic forms of PBFD have been described. These different forms of the disease appears to be influenced by the age (Todd, 2000; Woods and Latimer, 2003). Based on the condition of the disease some birds may die shortly after showing the first indication of malformed feathers, others may live in a featherless state for several years. Most birds with PBFD die from a secondary infection within several months of the first clinical signs of disease.

The causative agent of the disease, psittacine beak and feather disease virus (BFDV), is non-enveloped with an icosahedral symmetry, and 14-16 nm in diameter with a circular single-stranded DNA (ssDNA) genome. The complete nucleotide sequence of the virus was revealed that the size of the genome is 1993 nucleotides (Bassami *et al.*, 1998). In another report the size of the genome of an isolate was 2018 nucleotides (Bassami *et al.*, 2001).

Case report

A 7-year-old lesser sulphur-crested cockatoos

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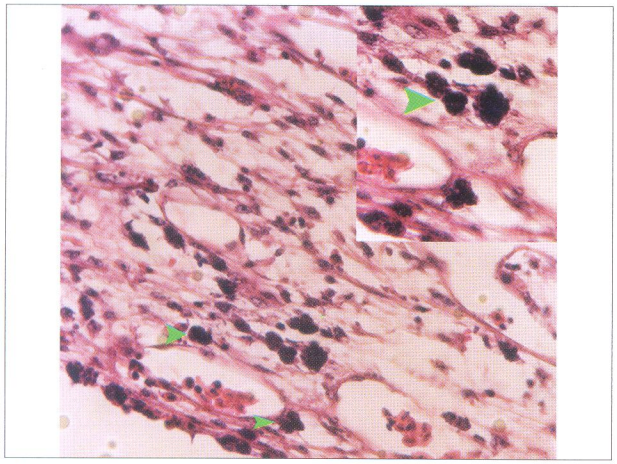

Figure 1. Feather follicle dermis: Large inclusions are seen in the cytoplasm of macrophages (H&E, x200, Top right: x400).

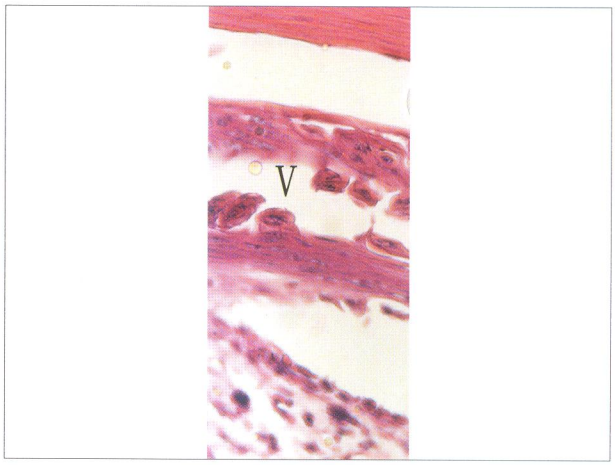

Figure 2. Feather follicle epidermis: round, acantholytic epithelial cells have formed and are detached in the lumen of the vesicle (H&E, x400).

(*Cacatua sulphurea*), weighing 350g, which had been imported from Pakistan 6 years before, was presented to our clinic for examination due to graduate and progressive feather dystrophy and loss. Feather lesions were first noticed 7 months prior to refereing to the clinic. After taking the history, a comprehensive clinical examination was undertaken. A blood sample was collected for hematological and parasitological examinations. Radiographs were taken. An abnormal feather from the affected bird was collected for polymerase chain reaction (PCR). As a negative control for PCR a normal feather from a healthy African grey parrot was also provided. An additional abnormal feather from the affected bird, in formalin 10%, was submitted to the laboratory for histopathological examinations.

In initial examination, the bird had abnormal feathers over the head and neck without any beak deformations. The bird's behavior, appetite, and feces appeared normal. The bird was anesthetized with Ketamin and Xylazine for a detailed clinical examination, radiography, blood cell counts (CBC), and skin and feather follicle biopsy. Feather abnormalities included retained feather sheaths, clubbed and deformed feather. No blood was found within feather shafts. Partial loss of crest and tail feathers also was observed. The beak appeared normal. The radiographs revealed mild renalomegaly and abundant grit within the gizzard. The caudal thoraco-abdominal air sacs were slightly thickened. Wet smears of the cloacal and oral cavity were normal after Gimsa and Gram staining. There were not any parasites in droppings.

Microscopic feature in dystrophic feather follicles consisted of intercellular attachment of epidermal keratinocytes that had lost their cohesions ends were characterized by marked intracellular edema of the keratinocytes (ballooning degeneration) and necrosis. Consequently intraepidermal (supra basal) vesicles formed between the keratinocytes. In the dermis, numerous macrophages with cytoplasmic inclusions "botryoid" and prevesiculitis were present (Figs. 1, 2, and 3). Sections of feathers often contained a necrotic pulp with an intense infiltrate of heterophils. Portions of viable feather epithelium exhibited necrosis of basal epithelial cells. Some of these cells also contained glassy basophilic nuclear inclusions. In addition, macrophages within the basal layers of the feather epithelium contained multiple, globular, basophilic, cytoplasmic inclusions (Fig. 1). Histopathologically, the feather lesions and inclusions were typical of PBFD.

DNA extraction was carried out as described previously (Sambrook and Russell, 2001) with some minor modifications. To extract the DNA, the tip of the feather sample was cut with a sterile fine tipped scissors and transferred into a labeled 1.5 ml microcentrifuge tube. A feather tip was collected, cut and digested with proteinase K (10 mM Tris-HC1, pH 8.0, 2 mM EDTA, 10 mM NaC1, 1% SDS, 10 mg/ml

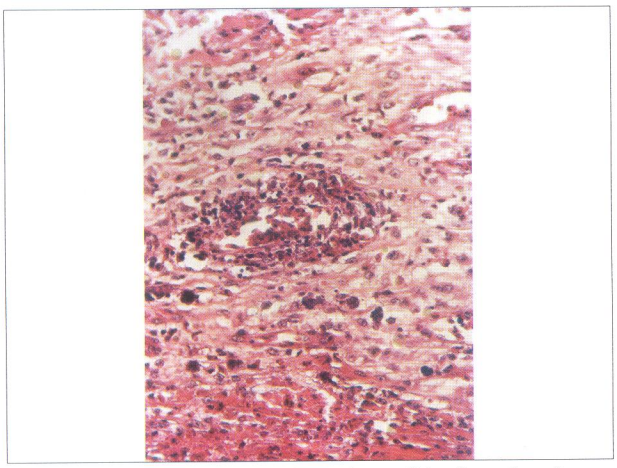

Figure 3. Feather follicle dermis: Perivasculitis of venuleand some macrophages with inclusion bodies (H&E, x200).

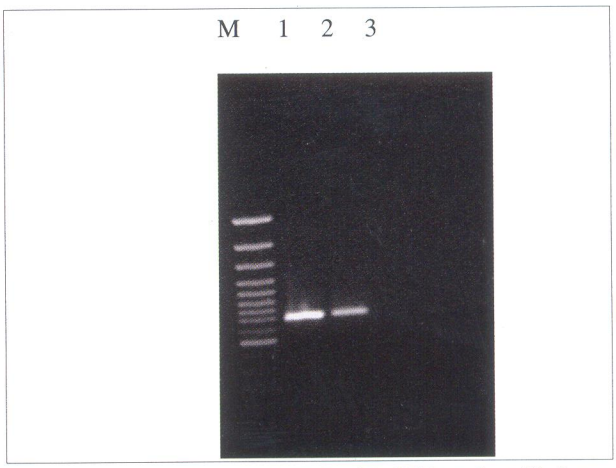

Figure 4. Agarose gel electrophoresis of the PCR product. The Lanes illustrate molecular weight marker (M), positive samples with the 717 bp band (1 and 2), and the negative control (3).

DTT, 0.5 mg/ml proteinase K). The digestion was performed in a total volume of 400 µl, at 37°C for 2 h. The DNA was extracted twice with an equal volume of phenol/chloroform and once with chloroform (Sambrook and Russell, 2001). The DNA content of the aqueous phase was precipitated with 95% ethanol and 0.3 M Sodium acetate. The precipitated DNA was washed with 70% ethanol. The washed DNA was resuspended in 30 µl TE (10 mM Tris-HC1, pH 8.0, 1 mM EDTA), and stored in -20°C until used for PCR assay. PCR primers used in this study were: 5´-AACCCTACAGACGGCGAG-3´ and 5´-GTCACAGTCCTCCTTGTACC-3´ as forward and reverse primers, respectively (Bassami, 2000; Bassami *et al.*, 2001). The predicted size for these primers were a PCR product with a size of 717 bp. The selected primers were examined against genomic databases using blast N program to check the specificity of the primers. The typical PCR reactions were carried out in a 25-µl volume consisted of final concentrations of 1× PCR buffer, 200 µM dNTP, 0.5 µM of each primer, 2 mM $MgCl_2$, 2 µl of DNA template, and one unit Taq DNA polymerase. Ultrapure water was added to reach to a total volume of 25 µl. The PCR reactions were carried out on a Bio-Rad thermocycler at 93°C for 30 s, 55°C for 45 s, and 72°C for 2 min for 35 cycles. An initial denaturation of 5 min at 93°C and a final extension of 10 min at 72°C were also included in the PCR cycles. The PCR product were electrophoresed in a 1.5% agarose gel, stained with ethidium bromide, and visualized by a transilluminator under the ultraviolet light. As the processed sample was too much less, quality of the extracted DNA was not evaluated by agarose gel electrophoresis. Due to same reason, quantity of the DNA was also not quantified by spectrophotometer. When the agarose gel was analyzed, a PCR product with a predicted size of 717 bp was detected. No PCR product was obtained in the negative control reactions (Fig. 4).

Discussion

According to the results of gross pathology, histopathology and PCR assay a diagnosis of PBFD was made. From the epidemiological point of view, the exact source of the bird was not identified. Based on the information provided by the owner, the bird had been imported from Pakistan 6 years before. There were no more data about the history of the bird, including the place of hatch and the rearing condition. After importing to Iran, the bird had been kept as a pet in an isolated environment. Virus transmission probably occurs predominantly by horizontal spread but vertical transmission by carrier birds may also contribute to this matter (Woods and Latimer, 2003). Virus infectivity probably persists in contaminated nests for many months or years. As the transmission of the disease is commonly via the aerosol exposure to contaminated materials, it may be concluded whatever had happened was associated with the breeding period of first year of life in Pakistan.

Although the chance of infection in an isolated environment is hardly possible, the possibility of transmission via contaminated material entered to the captivity place by the owner could not be ruled out. In Australia, the major land of psittaciformes, infection to beak and feather disease virus (BFDV) is widespread (Pass and Perry, 1984; Raidal *et al.*, 1993; Pass *et al.*, 1994; Doneley, 2003; Khalesi *et al.*, 2005). The infection has been distributed in captive birds all around the world (Sanada *et al.*, 1999; Rahaus and Wolff, 2003; Ritchie *et al.*, 2003; Albertyn *et al.*, 2004). In the case of this study the bird might had been latently infected and due to this fact, in spite of captivity during the last 6 years, the chronic form of the disease has occurred. The hematological profile of the disease did not reveal something significant; although hematological changes in PBFD, itself, are highly variable. This fact is depending on the length and severity of disease and presence or absence of secondary infections. No anemia was found in hematological profile of the bird. A minor increase in the number of monocytes (4% monocytosis) may or may not be associated to the direct effect of chronic pattern of the viral disease.

Slight thickness of the caudal thoraco-abdominal air sacs could be the result of immunosuppression caused by the virus. The cause of nephromegaly seen in the radiograph is not clear. It may not be related to the disease. The beak deformity was not observed in this case. The lack of abnormal behaviour may rule out the involvement of feather-pecking etiology of the disease. Although most birds in chronic cases have some difficulty in eating, losing weight is expected but in this case there was no sign of weight loss. In PBFD, secondary disease problems commonly exist (Todd, 2000). These include bacterial, fungal and viral infections. No complete study was conducted to examine the involvement of viral, bacterial and fungal agents. Due to feather dystrophy clinical diagnosis of the disease was not difficult. Histopathological examination of feather and skin biopsy was comparable with previous findings (Pass and Perry, 1984; Gerlach, 1994) and clearly demonstrated that the disease is, undoubtedly, PBFD. Additionally, PCR was employed to confirm the presence of the BFDV DNA as has been reported previously (Bassami *et al.*, 1998; Ypelaar *et al.*, 1999; Bassami *et al.*, 2001; Ritchie *et al.*, 2003).

PBFD is a highly contagious and debilitating disease. Until an effective vaccine is developed, diligent screening and management programs must be put in place. BFDV detection by PCR is a valuable component of these programs, but it must be used with clinical observations and DNA specific tissue testing to provide the most comprehensive screening. The ability to detect carrier states is still in question. The possibility of genetic variations of the circovirus is becoming more apparent and may explain some of the inconsistencies of PBFD PCR testing of lories. This is the first documented report of the occurrence of the PBFD in Iran.

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بیماری پر و منقار طوطی در ایران: جستجوی ملکولی و هیستوپاتولوژیکی

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چکیده

بیماری پر ومنقار طوطی (PBFD) یکی از مهمترین بیماریهای ویروسی طوطی سانان دربند یا وحشی در سراسر دنیا می‌باشد. یک قطعه طوطی (Cacatua sulphurea) هفت ساله با نشانی پر ریزی جزئی در عقب گردن و سر به کلینیک ما ارجاع شد و از نظر ابتلا به این بیماری مظنون واقع شد. پرنده بطور گسترده‌ای از نظر نشانیهای بالینی، آسیب شناسی، و تشخیص ملکولی مورد مطالعه قرار گرفت. در بررسی‌های هیستوپاتولوژیک فولیکول‌های دیستروفه شده پر، نکروز و ادم مشخص بین سلولی کراتینوسیت‌ها قابل مشاهده بودند. تعداد زیادی ماکروفاژ دارای گنجیدگی‌های سیتوپلاسمیک (botryoid) و التهاب عروقی و بافتهای اطراف عروقی در درم وجود داشتند. از نظر آسیب شناسی ریزبینی وجود جراحات پر و گنجیدگی‌ها تایید کننده بیماری PBFD بود. وجود DNA ویروس عامل بیماری نیز توسط آزمایش PCR مورد تایید قرار گرفت. این اولین گزارش مستند از وجود این بیماری در ایران است.

واژه‌های کلیدی: PBFD، BFDV، Lesser sulfur-crested cockatoo، ایران، طوطی.

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