

*Case report*

## IS PBF D SIMPTOMATOLOGY SPECIES SPECIFIC RATHER THEN STRAIN SPECIFIC? – A CASE OF 8 LOVEBIRDS

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Eight lovebirds of both sexes and different age were admitted showing alterations in behaviour and apathy. During the initial examination delamination of the beak was noted in all birds and discrete areas of alopecia in three of eight birds. Based on clinical experience, molecular diagnostic on PBF D, sequencing of obtained amplicons, and histopathological examination were performed. All birds tested positive on the presence of PBF D virus, despite the fact they did not exhibit expected clinical signs for PBF D. Sequencing results showed 100% match with sequences previously isolated from parrots with PBF D exhibiting classical PBF D manifestation. Histopathological examination showed similar findings as in previously described cases of PBF D in parrots. Other studies on psittacine birds correlate clinical manifestations and nucleotide variations with geographic localization. Our results indicate that the clinical manifestation of the disease is more dependent on bird species than on the genetic variation of the virus or the geographical distribution.

**Key words:** histopathology, parrots, PBF D, PCR, species specific symptoms

### INTRODUCTION

Beak and feather disease (PBF D) is the most common, globally spread disease of wild parrots. It is a highly contagious viral disease of many bird species, primarily those of the Psittaciformes order and has been described in over 60 parrot species, but all are believed to be susceptible [1]. This disease is the greatest threat to endangered species of parrots and is likely to be a factor that will lead to their disappearance [2]. The virus was considered to express tropism solely to Psittaciformes, but research also identified infected individuals of some other orders - *Ninox strenua* (Strigiformes) and *Merops*

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*ornatus* (Coraciformes) [3,4]. The disease is manifested by the rapid death of young birds, dystrophy and loss of feathers in older birds, deformation of the beak and strong immunosuppression [2,5,6]. However, do we always have to expect the same manifestation of the disease?

## CASE PRESENTATION

In the period between 1<sup>st</sup> January 2015 and 31<sup>st</sup> December 2018, eight lovebirds were admitted to the Teaching Hospital for Small Animals at the Faculty of Veterinary Medicine, University of Belgrade. The birds were of different age and of both sexes (gender was determined as described in [7]). The primary reason for their admission was alterations in behaviour or apathy. Owners were uncertain when the symptoms first appeared or how long they were in that condition. All birds were in good condition, they were all able to fly and had normal food and water intake. By clinical examination of five birds, the presence of delamination and black spots on the beak were observed (Figure 1).



**Figure 1.** Delamination and necrotic areas on the beak

In three birds apart from changes on the beak, an alopecic area up to 1 cm in diameter on the upper back area was perceived. Also, in those birds, in that area only, a small number of deformed feathers was noted. Other symptoms were not present. Blood and serum parameters were not altered in the observed animals. Sterile swabs and skin scrapes were taken from alopecic areas. Results showed the absence of parasites, bacteria and fungi. Based on changes on the beak and deformed feathers that were observed, a tentative diagnosis of PBFDF was established.

Feathers (both altered and unaltered) were sampled from all birds and molecular diagnostics of the presence of beak and feather disease virus (BFDV) nucleic acid was done using the methodology described by Vučićević et al. [8]. The obtained PCR products were directly sequenced in two directions using the BigDye® Terminator method in an ABI 3730XL automatic DNA sequencer (Macrogen Europe, The Netherlands). Sequence similarity analysis was performed using the BioEdit version 7.2.5 and Clustal W software.

In one case, after diagnosis was established, the owner had euthanised the diseased bird and we were allowed to perform a necropsy. After the necropsy of bird, feathers and organs with macroscopic changes were sampled and fixed in 10% neutral buffered formalin. The tissue for histopathological evaluation was processed in automatic tissue processor LEICA TP1020, embedded in paraffin, and cut at 4 µm. Initial sections were stained with hematoxylin and eosin (HE) and analyzed by light microscope (BX51, Olympus Optical, Japan). Digital images were made using an optical microscope Olympus BX51 with digital camera Olympus Color View III.

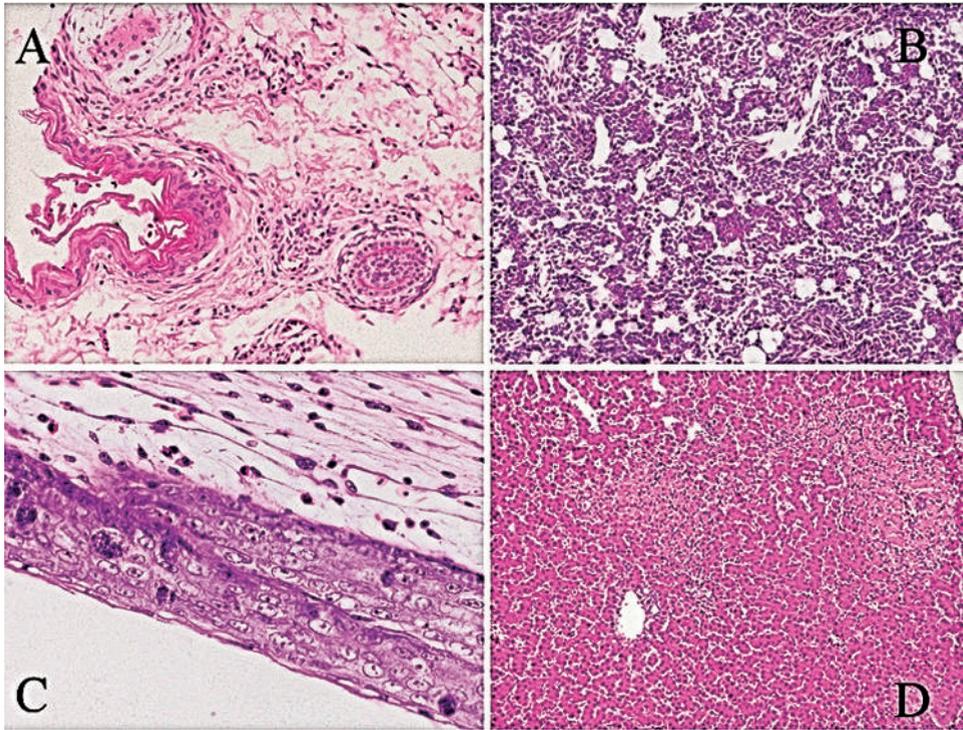
PCR amplification proved the presence of BFDV nucleic acid in all examined samples. Sequencing of amplicates showed a 100% match with sequences previously isolated from cockatoos with PBFD [8].



**Figure 2.** Bleeding in newly grown feather

Ultrastructurally, hyperkeratotic changes were present on the epithelium surface (Figure 3a). Multifocal to diffuse necrosis and intracellular edema were observed in the keratinocytes, particularly in the basal epithelium layer. The necrotic changes were also visible in the feather pulp with an intense infiltrate of inflammatory cells, predominantly composed of heterophils, plasma cells, macrophages and lymphocytes (Figure 3b). Numerous macrophages containing intracytoplasmic inclusions were observed within the affected epidermis and feather pulp (Figure 3c), while intranuclear inclusions were

present (Figure 3d). Non-suppurative inflammatory cells (macrophages, lymphocytes and plasma cells) infiltrated the tissue around the feather follicles – perifolliculitis.



**Figure 3.** Microscopic changes in the skin, feather epithel and intestine in PBFD: **a)** Skin, Parrot, hyperkeratotic deposits on surface of the skin and perifolliculitis, HE, 400x; **b)** Feather pulp, Parrot, feather pulp with necrotic changes and intense infiltrate of inflammatory cells, predominantly heterophils, HE, 400x; **c)** Feather epithel, Parrot, Macrophages in epithel with numerous cytoplasmic inclusion (arrow), HE, 600x; **d)** Liver, Parrot, Focal necrosis, HE, 200x

Histologically, the liver had foci of coagulative necrosis that could be seen as individual necrotic cells to disseminated areas of necrosis surrounded by a mononuclear inflammatory infiltrate. The histopathological examination of the lymphoid tissue revealed large number of apoptotic cells, as well as atrophy. Inclusion bodies were observed in the cytoplasm of some spleen and intestinal macrophages. The lamina propria of the small intestine was markedly infiltrated with macrophages and lymphocytes.

## DISCUSSION

The histopathological investigation demonstrated intranuclear inclusion bodies in keratinocytes of the feather follicles as described in previous reports [8,9]. The presence of intranuclear inclusions suggests that the virus is epitheliotropic and has a particular predilection for rapidly dividing cells such as those within the basal layer of the epithelium. The inclusions were also observed in the cytoplasm of macrophages, but the mechanism of its infection is still unclear and is possible that the virus uses a similar mechanism for cellular entry into macrophages as that observed for porcine circovirus by Misinzo et al. [10]. Macrophages with intracytoplasmic inclusions could be found in the feather epidermis, pulp, spleen, intestine and other organs and are probably infected during the removal of cell epithelial detritus containing the virus [11]. Entering the macrophages, Circovirus can endanger their antigen-presenting function and microbicide activity. In addition to suppression of macrophage function, the virus could also directly impair immunity by cell necrosis and consequent lymphatic tissue depletion [12]. Immunosuppression leading to secondary bacterial infection could be the explanation of the presence of heterophils in the feather pulp. Histopathological examination of the liver was comparable with previous findings that revealed the presence of extensive hepatocellular necrosis [13].

Why is the clinical manifestation of PBFD of those examined lovebirds quite different from the one described in cockatoos [8], although the obtained viral nucleotide sequences from both species were identical and all the birds lived in the same territory? Also, the symptoms were different from those previously found in lovebirds by other authors who examined lovebirds with feather dystrophy, elongated beak and color changes [14-16].

There is no definite answer. However, it was long considered that there are no genomic or antigenic variations between strains of BFDV in different parrot species [17], in other words, it was believed that the virus is highly conserved. However, Bassami et al. [18] established the presence of significant variations not only in nucleotide composition but also in the size of the viral genome. Eight new isolates from different parrot species in Australia were studied and it was found that the size of the genome varied from 1992 to 2018 nucleotides with a similarity of only 84-97% with previously studied BFDV genomes in Australia. Studies of ORF C1 similarity were also conducted, and showed 80-90% similarity with previously published sequences [18] with only three ORF (ORF C1, ORF V1 and ORF 5) present in all studied isolates. The role and the importance of mutations for viral pathogenicity and virulence are unknown. Numerous subsequent studies, including those conducted on Serbian territory [8], indicated the existence of differences in genome and/or studied nucleotide sequences. However, alterations in the viral characteristics and disease manifestations could not be determined. Furthermore, there is no correlation between changes in the genome and regional distribution, antigenic and physicochemical properties of the virus.

The focus of primary studies was the correlation of nucleotide variations with geographical locations of isolates. However, later studies suggested that isolates should be classified in a different manner, namely according to the host they were isolated from [19-24]. This assumption was confirmed by Shearer *et al.* [25], who found the existence of serological and antigenic differences of isolates taken from species *Nymphicus hollandicus* compared with the known ones obtained from other species regardless to the geographical origin of samples. This issue remains contentious, since there is evidence that South African isolates have genetically diverged from those found in other parts of the world and formed a separate genetic group [26]. Everything stated above indicates that relation between isolates, host species and pathogenicity/virulence is an exceedingly complex one [20].

Species-specific characteristics of the immune system are stated as one of the reasons for this. Selective pressure of the immune system on the virus could lead to genetic drifts [23,27]. Furthermore, a single-stranded DNA molecule does not form a double helix in a way it occurs in double-stranded DNA and therefore lacks protein which occurs as a consequence of the existence of such formation. Consequently, nucleotides of the single-stranded DNA molecule are more susceptible to deamination, cytosine and adenine are particularly prone to this which leads towards C → T and A → G transitions, respectively. Deamination can often be the result of external factors which the circovirus is exposed to [23]. It can be concluded that the PBFD virus, like other single-stranded DNA viruses, have a higher rate of mutation compared to double-stranded DNA viruses due to the absence of the double helix. On the other hand, viral repair mechanisms can be more effective in single-stranded molecules.

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### **Authors' contributions**

VM designed the paper, did clinical examination of patients and wrote the manuscript; VI carried out necropsies and histopathological investigation; DM and AN helped to draft the manuscript; RM participated in the sequence alignment; RR and SZ made substantial contribution to the conception, acquisition and interpretation of data.

### **Declaration of conflicting interests**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## **DA LI JE SIMPTOMATOLOGIJA PBFĐ VIŠE ZAVISNA OD VRSTE DOMAĆINA NEGO OD SOJA VIRUSA? – SLUČAJ 8 ROZENKOLISA**

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Osam rozenkolisa oba pola različite starosti su primljeni na pregled zbog uočenih promena u ponašanju i apatije. Tokom inicijalnog pregleda kod svih ptica je uočena delaminacija kljuna, a kod tri jedinke bila su prisutna i diskretna polja alopecije. Na osnovu kliničkog iskustva postavljena je sumnja na oboljenje bolesti kljuna i perja te je urađena molekularna dijagnostika na prisutvo virusa bolesti kljuna i perja, sekvencioniranje dobijenih amplifikata i patohistološko ispitivanje. Rezultati ispitivanja su potvrdili prisustvo virusa u uzorcima od svih ispitivanih jedinki, poklapanje nukleotidnih sekvenci od 100% sa uzorcima od drugih ptica sa drugačijom kliničkom slikom, a patohistološke promene su identične onima opisanim kod bolesti kljuna i perja. Studije brojnih autora povezivale su kliničku manifestaciju bolesti sa nukleotidnim varijacijama ili geografskom lokalizacijom. Međutim, opisani rezultati bi mogli da ukazuju da je kliničko ispoljavanje bolesti više zavisno od vrste domaćina nego od genotipa virusa koji bolest izaziva i od teritorije na kojoj se bolest ispoljava.