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Molecular Diagnosis of Beak and Feather Disease in Native Brazilian Psittacines

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■Keywords

BFDV, macaw, parrot, psittacine, psittacine beak and feather disease.

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ABSTRACT

The incidence of the psittacine beak and feather disease virus (BFDV) was investigated in Brazilian native parrots with normal feathering arriving at rescue and triage centers for wild animals (CETAS, IBAMA) in the state of Minas Gerais, Brazil. BFDV DNA was investigated by previously described PCR technique for the partial amplification of BFDV ORF-1 in DNA extracts from blood, cloacal swab or liver of psittacines. Some birds provided more than one sample. Nine species of psittacines were sampled between January 2009 and October 2010. Blood (n=46) or cloacal swab (n=128) samples were obtained from psittacines immediately upon arrival at the triage centers. Liver samples were collected from necropsied birds dead on arrival (n=167). All swab samples were negative, except for one *Ara ararauna* individual (n=3) which blood presented the BFDV DNA. On the other hand, 11 liver samples were positive for BFDV DNA, with a prevalence of 7.8% in *Amazona aestiva* (n=140). No BFDV DNA was detected in the liver of *Amazona amazonica* (n=11), *A. vinacea* (n=5), *A. rhodochorytha* (n=4), *Anodorhynchus hyacinthinus* (n=3), *Ara ararauna,* (n=3), *Aratinga leucophtalma* (n=2), *Guarouba guarouba* (n=1) and *Pionus maximiliani* (n=1). In most cases, alopecia was not associated with BFDV detection in liver, and liver histopathology was inconclusive. Although all cloacal swab samples were negative, a few psittacines (n=19) that died at CETAS-Belo Horizonte were retested, and 21% were detected as positive in liver. A group of psittacines (n=16) was clinically evaluated, and despite showing feather dystrophy, all birds were negative in the cloacal swabs, except for one, which blood sample was positive (*A. ararauna*). The obtained sequences of the BFDV strains BH 215 and BH 732 were deposited in the GenBank (JQ649409 and JQ649410). A 98% similarity with strain sequences described in Australia, Japan, and New Zealand was observed. It is possible that these strains arrived in Brazil through the legal and illegal trade of parrots. However, it was not possible to associate BFDV infection with the geographical origin of birds and no local marker was detected. The rates of detection, although similar to other studies, indicate the tendency of a high incidence of the disease, possibly associated with stress, and high bird density and wide transmission in captivity conditions.

INTRODUCTION

The highest global incidence of psittacine beak and feather disease (PBFD) is recorded in Australia (McOrist *et al.*, 1984; Pass and Perry, 1984; Raidal *et al.*, 1993), where at least one species is threatened of extinction. Infection by the PBFD virus (BFDV) is disseminated in more than 40 species of companion psittacines in Asia, Europe, Africa, and North and South America as a result of the international avian trade (Borthwick *et al.*, 2005; Rahaus et al., 2003). However,

species of the Cacatuidae family appear to be more susceptible (Bassami *et al.*, 2001). BFDV is a DNA virus of Circoviridae family with tropism for epithelial cells of several tissues, including skin and gastrointestinal mucosa (Latimer *et al.*, 1991). An acute form occurs in about 90% of wild cockatoos and results in hepatic necrosis, leucopenia, and greenish diarrhea in nestlings (Raidal and Cross, 1995). The chronic form results in progressive feather loss (alopecia), beak deformation, and predisposition to opportunistic infections (Pass and Perry, 1984). All psittacines may be susceptible, despite the morbidity differences possibly associated with host species and virus strains, and in Australia, PBFD is considered partially responsible for the successful predation of affected birds (Shearer *et al.*, 2008).

Transmission occurs by inhalation or ingestion, including cloacal drinking, of the infective virus exported from sloughed feather follicles, feces with bile, and mucus secretion from crop, with high concentrations of virus detected in such tissues, and will appear in skin and feather dander and feces (Ritchie, 1995; Ritchie *et al.*, 1990). Vertical transmission is suspected (Ritchie, 1995), similarly to chicken anemia virus and porcine circovirus 2. Diagnosis may be based on clinical signs (alopecia), history, histopathology (feather follicle biopsy) showing intranuclear inclusion bodies, and BFDV DNA detection in feathers, feces, or other tissues, such as blood, feather follicle, liver, and spleen, by PCR (Ypelaar *et al.*, 1999). The liver is considered a site of virus replication (Raidal *et al.*, 1993). Differential diagnosis should consider other causes of alopecia, such as polyomavirus, nutritional diseases, bacterial and fungal diseases, and psychogenic feather picking (Ritchie, 1995). In Brazil, the diagnosis of PBFD was first reported in 1997, in a *Cacatua alba* with alopecia (Werther *et al*., 1998). However, BFDV incidence and its clinical impact on infected Brazilian native avian species are yet unknown. This paper describes an investigation on the incidence of BFDV DNA in native Brazilian psittacines housed in triage centers and examines its possible association with clinical disease.

MATERIALS AND METHODS

Psittacines

Nine psittacine species rescued from illegal wildlife trade (n=170) were studied, and included 140 *Amazona aestiva,* 11 *Amazona amazonica*, five *Amazona vinacea*, four *Amazona rhodocorytha*, three *Anodorhynchus hyacinthinus*, three *Ara ararauna,* two *Aratinga leucophtalma,* one *Guarouba guarouba* and one *Pionus maximiliani.* The number of the rescued species evaluated was a direct function of trafficking preferences.

Sampling

Cloacal swabs, blood, and/or liver samples were collected between January 2009 and October 2010 at triage centers (Table 1). Cloacal swab (n=128) and blood (n=46) samples were collected upon bird arrival at the triage centers of Belo Horizonte and Uberaba, Minas Gerais, Brazil, avoiding contact with resident birds. Cloacal swabs were collected and immediately placed in microtubes with PBS (0.15M) and stored at -20ºC. Blood samples (0.5 mL) were collected by brachial vein puncture after physical restriction, using sterile disposable syringe and needle, and allowed to clot. The serum was separated and both serum and clot were frozen (-20ºC) until DNA extraction. Liver samples were collected from psittacines dead on arrival or which died at the triage center during the period of study (n=167). Both cloacal swab and liver samples were collected from 19 *A. aestiva* individuals. The clinical evaluation of 16 birds arriving at triage center was performed using a protocol based on literature (Rupley, 1999).

Table 1 – Occurrence of psittacine beak and feather disease virus (BFDV) as detected by PCR a.

Species	Total	Positive	Prevalence (%)
Amazona aestiva	160	11	6,8% (liver)
Amazona amazonica	11	0	0%
Amazona rhodochoryta	4	0	0%
Amazona vinacea	5	0	0%
Anodorhynchus hyacinthinus	3	0	0%
Ara ararauna	3	1	33,3% (blood)
Aratinga leucophtalma	2	0	0%
Guaruba guarouba	1	0	0%
Pionus maximiliani	1	0	0%
Total	190	12	6,3%

[a] BFDV VP1 specific PCR. [47]

DNA extraction

Liver DNA extraction was performed by sodium iodide (NaI) protein denaturation and silica DNA adsorption (Boom *et al.*, 1990; Vogelstein and Gillespie, 1979). Two hundred microliters of macerated liver tissue or blood clot were added to three volumes (600 µL) of saturated (6M) NaI (Synth, Diadema, SP, 09990-080, Brazil) and incubated at 55 °C for 15 min. After centrifugation (4,000 g/15 min), the supernatant was recovered and 50 µL of silicon dioxide (Sigma, Chemical Co., St, Louis, MO, USA) packed suspension were added, rapidly mixed in a vortex, and incubated in an end-over-end mixer for 10 min at room temperature. The mixture was centrifuged (14,000 g/30 s), the supernatant discarded by inversion, the silica pellet suspended in 1 mL NaI, vortexed, and centrifuged (14,000 g/30 s). The pellet was washed three times with ethanol wash buffer (ethanol 50%, 50mM Tris-HCl pH8,0, 10mM EDTA at pH 8.0), the ethanol supernatant was removed with pipette, 1 mL acetone was added to the pellet, and then vortexed and centrifuged (14,000 g/30 s). The pellet acetone residue was allowed to evaporate (55 °C/10 min) and the DNA adsorbed into silica eluted with 50 µL of TE (5 mM Tris-HCl at pH 8.0, 0.5 mM EDTA at pH 8.0), incubated at 55 °C/10 min, and centrifuged (14,000 g/30 s). The supernatant DNA was quantified, its purity verified, and then it was stored at -20 °C until use. Cloacal swab DNA was similarly extracted, except that 600µL NaI was added instead of 1 mL. Microtubes were vortexed, incubated at 55 °C/15 min, centrifuged (14,000 g/4 min), the swabs were removed and the silicon dioxide was added to supernatants, which were then treated as described above. Blood DNA extraction procedure was similar to that applied to liver DNA, except that 600 µL NaI were added to 50 µL unclotted samples. DNA concentration and purity were estimated in 1 µL of each extraction sample by spectrophotometry (NanoDrop ND-1000, Thermo Fisher Scientific, Wilmington, DE, USA) and frozen at -20 °C until tested.

PCR reaction and product evaluation

Previously described primer oligonucleotides (Table 2) (Ypelaar *et al.*, 1999), designed for amplifying a sequence of the VP1 region of open reading frame 1 (ORF-1), were employed (Life Technologies/Invitrogen, Vila Guarani, São Paulo, 04311-000, Brazil). The BFDV DNA positive control was extracted from the blood of clinically affected *Psittacula krameri* (rose-ringed parakeet) and tested positive elsewhere (Unigen, Santana, São Paulo, 02035-022, Brazil). The positive control DNA amplicon was sequenced and confirmed for identity to BFDV, and was denominated BH 27. PCR products (8µL with 2 µL 60% glycerol, 10% TBE, 10X bromophenol blue) were evaluated by agarose (1.5%) gel electrophoresis (100 V/20 min) in TBE 0.5 X (100mM Tris-base pH 8.3, 25mM EDTA and 50mM boric acid), stained with ethidium bromide (10mg/mL), and visualized under ultraviolet light (Pharmacia LKB MacroVue, Piscataway, NJ 08855, USA), for molecular mass estimation.

Sequencing

Sequencing was performed by the dideoxynucleotide method (Sanger *et al.*, 1997), in an automated capillary sequencer (ABI 310, Applied Biosystems, Bedford, MA, 01730, USA), after PCR reaction with Big Dye Terminator Mix and Save Money buffer (Applied Biosystems, USA), according to the manufacturer's instructions, using 1µL of the PCR product in a thermocycler (PTC-100, MJ Research Inc., USA). Labelled products were purified by precipitation with isopropanol and ethanol, homogenized in formamide, rapidly denatured at 95 °C/2 min, and the microtubes placed in flaked ice. The sequencing software employed was Sequencing Analyses version 5.2 (Applied Biosystems, USA).

Analyses of sequences

In order to perform electropherogram analysis of both sense and anti-sense sequences, the Bioedit software (Tamura *et al.*, 2011) was employed to evaluate the quality of bases (Hall, 1999). Database sequences were obtained at the National Center for Biotechnology Information (NCBI – http://www.ncbi.nlm.nih.gov) and aligned with Genbank sequences using ClustalX (Thompson *et al.*, 1997). The algorithms for nucleotide similarity or deduced amino acids were evaluated, respectively, using BLAST 2.0 (Basic Local Alignment Search Tool, BLASTn) or BLASTx (http://www.ncbi.nlm.nih.gov/BLAST/) Altschul *et al.*, 1997. The software ClustalW version 1.6 was utilized in the Molecular Evolutionary Genetics Analysis (MEGA 5.0/www.megasoftware.net) version 5.0 for Windows for nucleotide alignment or deducing amino acid sequences.

The phylogenetic analyses of VP1 encoding nucleotide sequences or deduced amino acids were performed by the neighbor-joining method, using MEGA 5.0 software, with topology confidence (bootstrap) of 1,000 oversampling, with the nucleotide substitution by Kimura 2 method (Kimura, 1980) or the amino acid JTT substitution model (Jones *et al.*, 1992).

Table 2 – Clinical evaluation, blood chemistry and molecular investigation of BFDV DNA by PCR in blood of Brazilian native psittacines with impaired feather coverage.

Bird No.	Species	PCR[a]	BC[b]	Ht%[c]	PP%[d]	Observations
01	A. aestiva	-	Good	61	6,2	Right eye blepharitis; head, around the eyes, and dorsal alopecia; abnormal facial pigmentation, with red spots; exposed right wing feather shafts.
02	Aratinga leucophtalma	-	Good	58	4,1	Absence of dorsal feathers; skin desquamation; exposed wing feather shafts.
03	A. aestiva	-	Good	50	4,7	Amorphous growing chest, dorsal and dorsal wing feathers.
04	Ara ararauna	+	Good	50	3,6	Caudal feather loss; wings with amorphous feathers and exposed feather shafts; absence of primary and secondary feathers on wings.
05	A. aestiva	-	Good	50	4,7	Absence of dorsal, chest and wing feathers; friable feathers, amorphous and abnormally colored feathers.
06	A. aestiva	-	Regular	41	4,8	Absence of caudal and wing feathers; amorphous, abnormal growth feathers and exposed feather shafts.
07	A. amazonica	-	Good	53	6,7	Amorphous and dark feathers; absence of chest feathers; amorphous tail feathers.
08	*Pionus maximiliani)*	-	Regular	47	5,8	Absence and amorphous caudal and head feathers.
09	A. aestiva	-	Good	54	4,6	Cervical air sac rupture; head and dorsal alopecia.
10	A. aestiva	-	Regular	51	5	Amorphous feathers; sinusitis; absence of feathers on dorsum and wings.
11	A. aestiva	-	Regular	62	6,8	Thoracic air sac rupture; few amorphous feathers on dorsum and tail.
12	A. aestiva	-	Good	48	4,0	Amorphous facial feathers; conjunctivitis; desquamation of beak; sinusitis.
13	Aratinga leucophtalma	-	Regular	46	3,4	Absence of head, tail and wing feathers.
14	A. aestiva	-	Bad	58	5,4	Absence of head feathers, amorphous sternum kell feathers; abnormally colored feathers.
15	A. aestiva	-	Regular	ND[e]	ND	Sinusitis; difficult breathing; amorphous feathers.
16	A. aestiva	-	Regular	54	7,0	Amorphous head and wing feathers; laceration on the head (dorsum).

[a]PCR polymerase chain reaction (blood) based on 717 bp ORF-1 sequence amplification. [47] [b]BC: body condition; [c]Ht% hematocrit; [d]PP% plasmatic protein; [e]ND: not done.

Histopathology

A few dead animals (n=38) were fully necropsied and liver samples were collected for PCR and histopathology. At necropsy, heart, kidney, liver, and spleen fragments were collected in buffered formaline at 10% for histopathology (H&E).

RESULTS AND DISCUSSION

A 717 base pair product was obtained from liver or blood samples (Fig. 1), which was compatible with the fragment described in literature (Ypelaar *et al.*, 1999).

Among birds with alopecia (n=16), only one was positive for BFDV in the blood and all tested cloacal swabs were negative (Table 2). The clinical evaluation of the 16 symptomatic psittacines with abnormal feathering is shown in Table 3. The clinical signs of feather abnormalities, despite being suggestive of PBFD (Rupley, 1999), were not accompanied by beak deformities. The successful confirmation of BFDV by PCR was obtained in only the blood sample of *Ara ararauna* individual, which cloacal swab, however, was tested negative. BFDV infection of New World psittacines may result in clinical disease (Phalen, 2006; Piçarra, 2009), reducing their chances of survival in the wild, as previously documented in New Zealand psittacines (New Zealand, 2012). However, efficient immune response may result in a latent subclinical infection, which may become clinically apparent only under stressful conditions (Dahlhausen & Radabaugh, 1997; Khalesi *et al.*, 2005; Phalen, 2006; Piçarra, 2009).

Out of the psittacines sampled at the triage centers 7.0% were positive for BFDV DNA, while 7.8% of *A. aestiva* individuals were positive (Table 2). All cloacal swabs, obtained both at the CETAS-Uberaba (n=18) and at CETAS-Belo Horizonte (n=110) were negative.

Out of the 46 blood samples tested, only one collected from an *A. ararauna* was positive. Nineteen birds that presented negative cloacal swab samples were retested, revealing 21% (4/19) positive liver samples, which suggests higher sensitivity of liver sampling. Previous studies showed 4% positivity in New World psittacines (genera *Amazona* and *Ara*) (Dahlhausen & Radabaugh, 1997), and infection indices are generally considered higher under captivity, which favors dissemination (Ritchie, 1995).

Only *Amazona aestiva* and *Ara ararauna* individuals were positive for BFDV (Fig. 1). Previous descriptions in *Amazona aestiva* (Huff *et al.*, 1988) and *Ara ararauna* (Piçarra, 2009) were documented. New World psittacines (*Amazona* sp., *Ara* sp.) present lower incidence of the disease compared with Old World species (*Eclectus* sp., *Agapornis* sp.) (Shearer *et al.*, 2008; Piçarra, 2009). Although BFDV was detected in *Amazona aestiva* individuals in the present study, the birds were asymptomatic, in agreement with a previous report (Piçarra, 2009). BFDV DNA was detected in a symptomatic Blue-and-Yellow macaw (Table 2). Although there are few reports available on the incidence of BFDV in Brazil, the virus has been described in other continents (Pass & Perry, 1984; Shearer *et al.*, 2008). In our study, swab and blood samples were collected at arrival of the birds at the triage centers, avoiding contact with resident birds.

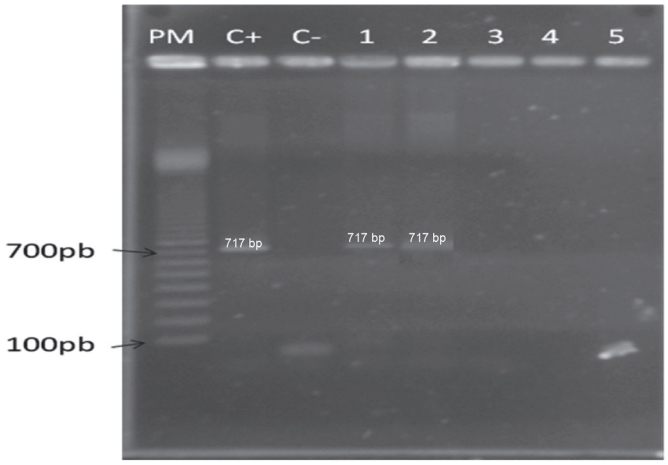

Figure 1 – Agarose gel eletrophoresis (1,5%) stained with ethidium bromide of BFDV VP1 ORF-1 amplicon. The partial sequence 717 pb bands detected in Amazona aestiva and Ara ararauna livers are shown. Lanes: PM molecular weight (100 bp); C+ positive control (from Psittacula krameri); C- negative control (from chicken liver DNA); Lanes 1, 2, 3, 4 and 5: psittacine field samples (liver); Lanes 1 (Amazona aestiva) and 2 (Ara ararauna): positive for the 717 bp BFDV DNA sequence; Lanes 3, 4 and 5: negative.

Out of the 15 birds symptomatic showing alopecia, only individual (*Ara ararauna*) was positive for BFDV by PCR. However, there may have been false negative BFDV results in truly positive, but nonviremic, birds. The negative results obtained in cloacal swabs may have been due to the presence of inhibitors in the DNA extract (Das *et al.*, 2009). Testing multiple tissue samples in individual is a strategy for diagnosis (Khalesi *et al.*, 2005; Piçarra, 2009).

The regular diagnosis and monitoring associated to biosecurity have enabled the reduction of prevalence the disease in the USA (Bert *et al.*, 2005), and may possibly reduce individual losses by PBFD also in Brazilian psittacines in captivity, triage centers and or in the wild. Quarantine should be employed when purchasing birds, and all birds, including those clinically normal, should be tested (Olsen & Speer, 2009).

The sequence (strain BH-27) extracted from the blood of the rose-ringed parakeet (positive control) resulted in a complete match with BFDV VP1 (ORF-1) sequences stored in the GenBank. Sequencing of purified PCR products of BH-215 and BH-732 strains, both extracted from A*mazona aestiva* liver, allowed BLAST (Altschul *et al.*, 1997) search for matches in the NCBI, which showed similarity only with BFDV published sequences.

The two local BFDV sequences of isolates BH-215 (Accession number JQ649409) and BH-732 (Accession number JQ649410), both from *Amazona aestiva*, and isolate BH-27 from *Psittacula krameri* (Accession number JQ649411) were evaluated. The analysis of the nucleotide sequences of BH-215 and BH-732 strains comparing the sequences available in the GenBank, showed identity only to BFDV.

The alignment (ClustalW in MEGA 5.0) of strain BH-215 with 11 published BFDV sequences revealed 19 nucleotide substitutions. However, a 98% homology (677/696 bp) was observed with the *Cacatua galerita* Australian strain AU-SCC1-WA-2000 (AF311302.1) (Massaro *et al.*, 2012), with 1181 bits score and no base insertion nor deletion. The analysis the 232 deduced amino acids sequences of strain BH-215 revealed 99% similarity with the strain BKS3ZA-86, with one substitution of threonine for alanine. This substitution could result from genomic mutation or polymerization error, although high-grade polymerase was employed.

The analysis of a 659 nucleotide sequence (nt 203-861) of the BH-732 strain revealed 18 nucleotide substitutions and also 98% homology (641/659 bp), but with the strain BKS3ZA_86 (GQ165758.1) of a New Zealand budgerigar (*Melopsittacus undulatus*) (Massaro *et al.*, 2012) with 1118 bit score. No insertion or deletion were detected. Also, the analysis showed 98% identity with BKS3ZA-86 strain (GQ165758-1), with positive substitution from aspartic to glutamic acid, although with 100% similarity of the 219 amino

acids sequence between residues 25 and 243. In this case, a similar property amino acid (polar, negative side chain) substitution, which apparently would not change protein properties, has enabled adaptation to mice lung for influenza virus hemagglutinin (Sakabe *et al.*, 2011). Isolate BH-732 was also very similar (98%) to the Japanese strain MU-JP1P (AB277746.1). Considering that circoviruses affecting other animal species have very high mutation and recombination rates, it would be necessary to study the complete genomes to understand interspecies transmission (Massaro *et al.*, 2012).

Considering that the demarcation threshold for strain sequence identity of >94%, and strain subtype sharing of >98% (Varsani *et al.*, 2011), the here described isolates BH-215 and BH-732 would belong to the same strain subtype as strains AU-SCC1-WA-2000 (AF311302.1), AU-Galah-WA-2000 (AF311298.1), BKS3ZA-86 (GQ165758-1) and MU-JP1P (AB277746). The control positive strain BH-27 (*Psittacula krameri*) was similar to the strain BFDV-J3 DE-PEG07-2004 (AY521237.1) found in an African grey parrot (*Psittacus erithacus*) found in Germany and distant from the local strains detected in *Amazona aestiva*.

A phylogenetic tree based on the nucleotide sequences of strains BH-27, BH-215 and BH-732 was constructed (Fig. 2) in order to evaluate their potential geographical association, as compared to geographically distinct strain sequences available in the GenBank. No geographical marker was detected for local isolates; hence, none was considered unique, but similar to the strains described in Asia and Oceania, in contrast with the strains characterized in New Zealand (Massaro *et al.*, 2012), which are unique. Isolate BH-215 was grouped with the *Cacatua galerita* strain AU-SCC1-WA-2000 and the *Eolophus roseicapillus* strain AU-Galah-WA-2000, both from Australia, whereas isolate BH-732 was grouped with the Japanese strain MU-JP1P and the New Zealand strain ZA-BKS3ZA_86-2008. The least related strains, as compared to BH-27, BH-215 and BH-732, were TW04-13 (Taiwan), BFDV-16 (Thailand), AFG4-ZA (South Africa), and Isolate 05-106 (Australia). The deduced amino acid tree topology (not shown) was in agreement to nucleotide sequences.

The *Amazona aestiva* individuals tested positive for BFDV DNA by PCR that died during this study were necropsied and examined by histopathology (n = 5). No histopathological findings could be directly associated to BFDV infection, including no intranuclear inclusion bodies. The liver of bird n. 15 presented multi-focal coalescent hepatocytes, lymphocytic proliferation, and blastic mytotic erythrocyte precursors. Bird n. 412 was a male, and presented cachexia, hepatomegaly, splenomegaly, and air sacculitis. Its liver histopathology (not shown) revealed plasmacytosis, macrophage

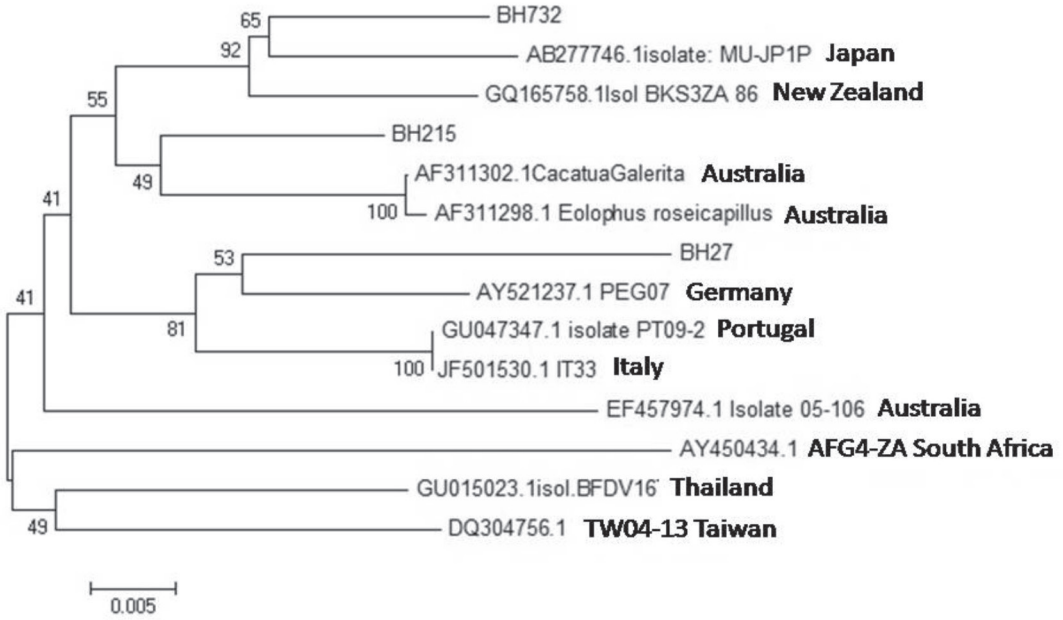

Figure 2 – Neighbor-joining dendrogram using the nucleotide substitution Kimura-2 parameter in Mega 5.0. Numbers represent Bootstrap values (1,000 oversamplings), in which >70 is considered significant. The local Amazona aestiva BFDV sequence, isolate BH-215 (Accession number JQ649409), was grouped with strains AU-SCC1-WA-2000 (AF311302) and AU-Galah-WA-2000 (AF311298.1), both from Australia, and isolate BH-732 (Accession number JQ649410), was grouped with strains MU-JP1P (AB277746.1), from Japan, and BKS3ZA-86 (GQ165758), from New Zealand. A local isolate BH-27 from Psittacula krameri (Accession number JQ649411), considered the positive control, as obtained from an exotic psittacine and tested in an independent laboratory, was grouped with strain PEG07.

hemosiderosis,. and a focal necrosis, which may also suggest Pacheco disease or *Chlamydophila psittaci* infection. However, no indication of intranuclear or intracytoplasmic inclusion bodies were found. The spleen presented eosinophilic areas with lymphoid depletion. The main gross lesions found in bird n. 732, a female, were cachexia, air sacculitis, hepatomegaly, splenomegaly, hemorrhagic gastroenteritis, and pale lungs. At histopathology, hemosiderosis and particularly periportal lymphocytosis and multifocal lympho-plasmacytic infiltrations associated with focal necrosis were found in the liver. The histology of bird n. 865, which presented hepatomegaly and air sacculitis, also showed hemosiderosis, with large hemosiderin deposits in the hepatocytes and splenocytes, liver multifocal lympho-plasmacytic infiltrations, proliferation of bile ducts, which indicates chronic hepatosis; however, no intranuclear or cytoplasmic inclusion bodies were found. Bird n. 885 had hemorrhagic gastroenteritis and splenomegaly, and presented moderate lymphocytic infiltration in the liver and a moderate population of spleen macrophages with hemosiderin, moderate lymphoid depletion and moderate lymphoid activation. In addition, megakaryocytes, which are platelet precursors, were visible, indicating extra-bone marrow erythropoiesis. The histopathological findings indicative of PBFD were previously described (Doneley, 2003), and included the demonstration of intranuclear inclusion bodies in bone marrow, hepatocytes, kidney and thymus (Pass & Perry, 1984).

No beak deformities were observed. Birds n. 412, 732, 865 and 885 did show hepatic lesions suggestive of virus infection, although not typical of BFDV and/or other DNA virus, except for bird n. 412 (Pacheco disease).

CONCLUSIONS

The silica extraction was the most successful method for BFDV detection by PCR in blood and liver samples; however, this method may need to be modified when testing cloacal swabs. The DNA of BFDV was detected in psittacines arriving at Belo Horizonte triage center. However, BFDV was not detected in birds presenting alopecia, suggesting other causes of feather dystrophy. Among the clinically affected birds, BFDV DNA was detected in the blood of only one *Ara ararauna* . No beak deformities were observed in any of the birds examined. Out of the tested psittacines, BFDV was detected only in *Amazona aestiva* and *Ara ararauna* individuals, which, in general, presented normal feathers and had died due to other conditions. The higher detection rate of BFDV in *A. aestiva* could be associated with the higher number of individuals available for testing relative to the other species. However, this hypothesis cannot be applied to *A. ararauna*, which sample was very small, or to *Amazona amazonica*, which comprised the second largest sample and all individuals were tested negative for BFDV. Moreover, BFDV detection could be related to a higher susceptibility to infection.

Histopathology of livers did not allow the demonstration of BFDV intranuclear inclusions bodies.

The genomic similarity of the tested isolates with reference strains from Asia and Oceania indicates the exotic origin of the BFDV strains detected in the present study, suggesting their introduction in Brazil by the legal or illegal trade of psittacines from those continents.

The partial VP1 gene sequence of BH-215 and BH-732 strains are the first published for Brazilian isolates. No sequence was unique or exclusive of a geographical location.

Due to mostly asymptomatic occurrence of the BFDV in psittacines, the establishment of strict biosecurity measures, including quarantine, is strongly recommended in wild bird rescue facilities.

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