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Short Communication

Survey of bornaviruses in pet psittacines in Brazil reveals a novel parrot bornavirus

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ABSTRACT

Avian bornaviruses are the causative agents of proventricular dilatation disease (PDD), a fatal neurological disease considered to be a major threat to psittacine bird populations. We performed a survey of the presence of avian bornaviruses and PDD in pet psittacines in Brazil and also studied PDD's clinical presentation as well as the genomic variability of the viruses. Samples from 112 psittacines with clinical signs compatible with PDD were collected and tested for the presence of bornaviruses. We found 32 birds (28.6%) positive for bornaviruses using reverse transcriptase polymerase chain reaction (RT-PCR). Twenty-one (65.6%) of the 32 bornavirus-positive birds presented neurological signs, seven (21.9%) presented undigested seeds in feces, four (12.5%) showed proventricular dilatation, six (18.8%) regurgitation, three (9.4%) feather plucking and three (9.4%) sudden death. The results confirm that avian bornaviruses are present in pet psittacines in Brazil, and sequence analysis identified a distinct virus, named parrot bornavirus 8 (PaBV-8).

1. Introduction

The *Bornaviridae* family is comprised of enveloped, negative sense single-stranded and non-segmented RNA viruses, which infect mammals, birds and reptiles (Staeheli et al., 2010; Kuhn et al., 2014). Recently, a revised taxonomy was established for this virus family, which classifies the known bornavirus "genotypes" into new viral species and defines new virus names (Kuhn et al., 2014). Bornaviruses of avian origin were first discovered in psittacines in 2008 and to date seven parrot bornaviruses (PaBV-1 to -7) are known (Honkavuori et al., 2008; Kistler et al., 2008; Weissenböck et al., 2009a; Rubbenstroth et al., 2012). Further avian bornaviruses were identified in waterfowl (Payne et al., 2011) and songbirds (Weissenböck et al., 2009b; Rubbenstroth et al., 2013, 2014a).

In psittacines, bornaviruses were confirmed to be the etiological agents of proventricular dilation disease (PDD) (Payne et al., 2011; Piepenbring et al., 2012; Rubbenstroth et al., 2014b), a lethal neurological disorder discovered in the late seventies in North America and Europe (Honkavuori et al., 2008; Staeheli et al., 2010). Bornavirus infection affects autonomic, central and peripheral nervous systems and the histopathology of PDD is characterized by lymphoplasmacytic infiltration in the autonomic ganglia and neuritis and encephalomyelitis (Piepenbring et al., 2012). Therefore, clinical signs can vary between gastrointestinal and neurological origin. The most common neurological signs are depression, convulsions, ataxia, blindness and incoordination (Berhane et al., 2010), and

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the usual gastrointestinal signs are crop stasis, regurgitation, poor appetite and undigested seeds in stool (Villanueva et al., 2010). Death usually occurs due to starvation or organ failure. Proventricular dilatation occurs in the majority of diagnosed cases (Staeheli et al., 2010).

The genetic variability as well as unidentified virus variants are major challenges to the diagnosis of bornaviruses in birds. At present, their detection is based mainly on reverse transcriptase polymerase chain reaction (RT-PCR) assays (Honkavuori et al., 2008; Kistler et al., 2008; Rubbenstroth et al., 2013).

This study aimed to understand the epidemiology of avian bornaviruses in Brazil and investigate the genetic variability as well as the clinical presentation of this disease.

2. Material and methods

2.1. Origin of field samples

Between July 2012 and October 2013, 112 psittacines presenting PDD-like signs (Table 1) were tested for the presence of bornaviruses. The psittacines originated from four clinics in São Paulo and two bird breeding facilities, one in São Paulo and one in Rio de Janeiro; several of the birds were confiscated from illegal trade, but were in captivity for several years as pet or in breeding facilities. Clinical signs included for the selection of the tested birds were neurological signs, undigested seed in feces, regurgitation, lethargy and sudden death, and proventricular dilatation after macroscopic examination or X-ray diagnosis. Depending on the owner's approval, feces, cloacal swabs, feathers, blood and/or organs (at post mortem examination) were collected. Collected organs from post mortem examination included liver, intestine, brain, kidney, proventriculus and/or eye. All samples were analyzed by RT-PCR at the Laboratory of Avian Diseases, University of São Paulo.

2.2. RNA extractions

Viral RNA was extracted from all samples via phenol/chloroform extraction. Briefly, 500 μl of GIT phenol (pH 4), 50 μl of acetate sodium and 4 μl of betamercaptoetanol were added to 250 μl of the sample. The solution was left at room temperature for 5 min before 200 μl of chloroform was added; the mixture was placed in the refrigerator at 4 °C for 15 min and then centrifuged at 12,000 × *g* at 4 °C. The supernatant (approximately 700 μl) was transferred to a new tube containing 750 μl of isopropanol, incubated at −20 °C for 20 min and then centrifuged at 1200 × *g* for 10 min. The supernatant was discarded, and the pellet was washed with 1 ml of ethanol and centrifuged again at 12,000 × *g* for 10 min. The resulting pellet was dissolved in 20 μl of DPEC water for 10 min at 56 °C and stored at −20 °C. Feather samples were treated with proteinase K for 4 h at 56 °C prior to extraction.

2.3. ABV detection by RT-PCR

For RT-PCR, two previously published bornavirus-specific universal oligonucleotide primer pairs were used (Weissenböck et al., 2009a) which target either the

Table 1
Bornavirus, PBFD and APV detection in pet psittacines in Brazil.

Birds and Species		Birds examined $n = 112$	Bornavirus-positive $n = 32$	Bornaviruses	Concurrent infection
Amazona aestiva	Blue fronted amazon	43	22	PaBV-8 $n = 6$[a]	PBFDV $n = 6$[a]
Aratinga jandaya	Jandaya parakeet	2	0	N	NC
Ara ararauna	Blue and yellow macaw	3	0	N	NC
Triclaria malachitacea	Blue-bellied parrot	1	0	N	NC
Aprosmictus erythropterus	Red-winged parrot	1	0	N	NC
Platycercus icterotis	Western rosella	4	0	N	NC
Psephotus haematonotus	Red-rumped parrot	4	0	N	NC
Neophema bourkii	Bourke's parrot	1	0	N	NC
Barnardius zonarius	Australian ring neck	2	1	ND	NC
Forpus coelestis	Pacific parrolet	1	0	N	NC
Platycercus eximius	Eastern rosella	2	0	N	NC
Alisterus scapularis	Australian ring parrot	1	0	N	NC
Neophema splendida	Scarlet-chested parrot	2	0	N	NC
Nymphicus hollandicus	Cockatiel	15	3	ND	PBFDV $n = 1$[a]
Psittacus erithacus	African gray parrot	3	1	ND	PBFDV/APV $n = 1$[a]
Amazona amazonica	Orange-winged parrot	1	1	ND	NC
Melopsittacus undulatus	Budgerigar	14	0	N	NC
Aratinga leucophthalma	White-eyed parakeet	1	0	N	NC
Eclectus roratus	Eclectus parrot	1	0	N	NC
Agapornis personatus	Yellow-collared lovebird	3	0	N	NC
Guarouba guarouba	Golden parakeet	4	2	PaBV-8 $n = 1$[a]	NC
Pyrrhura frontalis	Maroon-bellied parakeet	1	0	N	NC
Cacatua alba	White cockatoo	2	2	PaBV-4 $n = 1$[a]; PaBV-8 $n = 1$[a]	NC

NC: No concurrent infection; ND: Not determined; N: Negative diagnosis for avian bornaviruses, PBFDV or APV; PBFDV: psittacine beak and feather disease virus; APV: avian polyomavirus.

[a] Number of birds positives in these topics for avian bornaviruses or PBFDV or PBFDV/APV.