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Occurrence of avian bornavirus infection in captive psittacines in various European countries and its association to proventricular dilation disease

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Abstract

A total of 1,442 live and 73 dead birds out of 215 bird collections in Spain, Germany, Italy, United Kingdom and Denmark were tested for avian bornavirus (ABV) infection by four different methods. The majority of the birds were psittacines belonging to 54 different genera of the order *Psittaciformes*. In total, 22.8% of the birds reacted positive for ABV in at least one of the tests. Combined testing of swabs from crop and cloaca, and serum for the diagnosis of ABV infection in alive birds revealed that virus shedding and antibody production coincided in only one fifth of the positive birds so that the examination of these three samples is recommended for reliable ABV diagnosis. By statistical analysis of this large number of samples, the ABV infection proved to be highly significant ($p < 0.001$) associated with histopathologically-confirmed proventricular dilatation disease (PDD) in dead birds as well as with clinically assumed PDD in live birds. However, ABV infection was also detected in psittacines without pathological lesions or clinical signs of PDD. Twelve non-psittacine birds belonging to the genera *Aburria*, *Ciconia*, *Geopelia*, *Leucopsar* and *Pavo* were tested negative for ABV infection. Within the order of *Psittaciformes*, birds belonging to 33 different genera reacted positive for ABV. In 16 of these psittacine genera, the ABV infection was demonstrated for the first time. This study emphasizes the widespread occurrence of clinically variable ABV infections in Europe by analysing a large number of specimens from a broad range of bird species in several assays.

Introduction

In 2008, two independent research groups in Israel and USA identified a group of novel non-segmented negative sense single-stranded RNA viruses by high throughput viral screens which were classified as four different genotypes of a new genus of the family *Bornaviridae* designated avian bornavirus (ABV) (Kistler et al., 2008; Honkavuori et al., 2008). The ABV RNA was detected in parrots which died from proventricular dilation disease (PDD). After these first publications, further reports of ABV infections in PDD affected psittacines originated from Germany (Enderlein et al., 2009; Lierz et al., 2009; Rinder et al. 2009), Austria, Switzerland, Hungary, Australia (Weissenböck et al., 2009a) and Canada (Raghav et al., 2010). Besides, a report on the detection of ABV genome in a canary (*Serinus canaria*) with enteric ganglioneuritis and encephalitis indicates that not only psittacine birds may be susceptible for ABV infection (Weissenböck et al., 2009b).

PDD is a progressive, often fatal disease of captive psittacine birds worldwide. The disease is characterized by lymphohistioplasmocytic infiltration of the ganglia of the central and peripheral nerve system leading to gastrointestinal dysfunction and associated wasting as well as neurological symptoms. PDD is one of the most threatening diseases for parrots including endangered psittacine species. It has been found in about 60 species belonging to 20 different psittacine genera (Lutz & Wilson, 1991; Vice, 1992; Gregory et al., 1994; Sullivan et al., 1997; Berhane et al., 2001; Lierz, 2005; Lublin et al., 2006, Lierz et al., 2010), but also in some non-psittacine bird species (Staeheli et al., 2010). Since its first description in the late 1970s several viruses (adenovirus, herpesvirus, polyomavirus, eastern and western equine encephalitis viruses, paramyxovirus types 1 and 3) (Mannl et al., 1987; Sullivan et al., 1997; Cazayoux Vice, 1992; Gregory, 1995; Gregory et al., 1998; Grund et al., 2002; Gough et al., 2006; Lublin et al., 2006; Orosz & Dahlhausen, 2007) have been proposed to be the aetiologic

agent of the disease but it was not proven for any of these viral agents. However, results of two first experimental infections of a small number of cockatiels (*Nymphicus hollandicus*) and Patagonian conures (*Cyanoliseus patagonis*) provided evidence for the aetiological role of ABV in the development of PDD (Gancz et al., 2009; Gray et al., 2010). Besides, ABV infections were also found in psittacines with no clinical signs of PDD (De Kloet & Dorrestein, 2009; Lierz et al., 2009; Villanueva et al., 2009).

Meanwhile, different diagnostic methods have been developed for the direct and indirect diagnosis of ABV. ABV RNA can be detected by RT-PCR using various primer sets. In PDD affected dead psittacine birds ABV RNA was demonstrated not only in the nervous system and the gastrointestinal tract but in nearly all other tissues (Kistler et al., 2008; Honkavuori et al., 2008; Lierz et al., 2009; Rinder et al., 2009; Villanueva et al., 2009; Enderlein et al., 2009; Gray et al., 2010; Kistler et al., 2010; Raghav et al., 2010). Further methods for the direct detection of ABV in dead birds as well as in crop biopsies are immunohistochemistry (Rinder et al., 2009; Ouyang et al., 2009; Weissenböck et al., 2009a; Herzog et al., 2010; Raghav et al., 2010), virus isolation in the quail cell lines CEC-32 and QM7 (Rinder et al., 2009, Herzog et al., 2010) or duck embryo fibroblasts (Gray et al., 2010), and in situ hybridization (Weissenböck et al., 2010). In live birds, ABV can be diagnosed directly by detection of ABV RNA using RT-PCR in faeces, swabs of crop and cloaca, and blood (Rinder et al., 2009; Lierz et al., 2009; Enderlein et al., 2009; Gray et al., 2010; Kistler et al., 2010). As serological methods for the demonstration of anti-ABV antibodies a Western blot assay (De Kloet & Dorrestein, 2009; Lierz et al., 2009; Villanueva et al., 2009), an ELISA (De Kloet & Dorrestein, 2009) and an indirect immunofluorescence assay (Gray et al., 2010; Herzog et al., 2010) have been developed.

In this study, analysis of the results of testing a high number of samples from psittacine and non-psittacine birds from Spain, Germany, Italy, United Kingdom and

Denmark for ABV shall provide further information on the suitability of different kinds of samples and the existing diagnostic tools for ABV detection as well as on the occurrence of ABV in different species and countries and its association with PDD.

Materials and Methods

Birds and samples. In total, 1,515 birds out of 215 different bird collections in Spain, Germany, Italy, United Kingdom and Denmark were included in the study (Table 1). The birds or different samples of them had been submitted by veterinarians, bird owners and breeders as well as zoos for diagnostic purposes in most cases without any or with poor anamnestic data concerning number and species of other birds in the flock. The examined birds belonged to 54 different genera of the order *Psittaciformes* and 5 genera of non-psittacine birds (Table 2). Seventy three of these birds with or without clinical suspicion of PDD were dead birds (42) or the organs of dead birds which were submitted freshly (20) or fixed in formalin or embedded in paraffin (11). From 1,442 live birds, sera (1,349), crop (347) and cloacal (381) swabs were received as either single or combined samples per bird. Sixty three of these live birds were suspected of PDD due to clinical signs like undigested feed in the faeces, loss of weight, proventricular dilatation in radiography and/or central nervous symptoms. From the other live birds, no clinical signs were reported, and samples of them were taken to detect subclinical ABV infections.

General examination. All dead birds were necropsied, and the brain, spinal cord, retina, *Nervus ischiadicus*, crop, proventriculus, gizzard, small and large intestine, heart, liver, kidney, pancreas, pectoral muscle, skin were collected. These as well as the submitted fresh

organ samples (same range of organs) were processed for histopathological evaluation (fixation in 10% buffered formalin, embedding in paraffin, preparation of 5 µm sections, staining with hematoxylin and eosin). Additionally, in dead birds parasitological, microbiological (blood, Gassner and Kimmig agar) and virological (3 passages in primary chicken embryo fibroblast and liver cell cultures as well as in SPF egg cultures, (Valo, Lohmann, Cuxhaven, Germany)) examinations were performed for the detection of pathogenic infectious agents by standard methods (Dufour-Zavala et al., 2008). The presence of *Chlamydophila* spp. were excluded by PCR (Sachse & Hotzel, 2003). All sera were tested for antibodies against paramyxovirus type 1 (PMV-1) by haemagglutination inhibition test (OIE, 2004).

Specific testing for avian bornavirus (ABV). *Reverse transcriptase PCR (RT-PCR).* Organs and swabs were tested for the presence of ABV RNA by quantitative real-time RT-PCR (TaqMan) with two different primer sets (Honkavuori et al., 2008). In the case of negative results in these PCRs, but positive results in other tests a further primer set for standard PCR was used to detect other strains of ABV (Enderlein et al., 2011).

Immunohistochemistry (IHC). The different organ samples of the dead psittacines were analysed immunohistochemically for the presence of ABV antigen by the avidin biotin complex (ABC) method using a polyclonal rabbit antibody directed against the phosphoprotein (p24) of the Borna disease virus (BDV) (Herden et al., 1999; Herzog et al., 2010).

Virus isolation. Organ samples were processed in infectivity assays according to the method described by Narayan et al. (1983). Briefly, tenfold dilutions of the organ homogenates (10%,

w/v) were prepared in GMEM medium (Gibco, Invitrogen, UK) plus 10% fetal bovine serum (FBS), mixed with equal volumes of freshly dispersed cells of the quail cell line CEC32 and incubated on chamber slides (Lab-Tek Products, Nunc, Roskilde, Denmark) for six days at 37°C. Virus replication was demonstrated by indirect immunofluorescence using polyclonal sera from experimentally BDV-infected rats cross-reacting with ABV (Herzog et al., 2010).

Immunofluorescence assay. The demonstration of anti-ABV serum antibodies (doubling dilutions of serum starting with a dilution of 1:10) were performed by an indirect immunofluorescence assay (IIFA) on persistently BDV-infected Madin-Darby canine kidney (MDCK) or ABV-infected CEC32 cells using a fluorescein isothiocyanate (FITC)-conjugated goat anti-bird IgG (Bethyl Laboratories, Montgomery, Texas) in a dilution of 1:50 according to Herzog et al. (2010).

Statistical analysis. The chi-square test was applied for analysis of differences between bird groups. With one degree of freedom, calculated values ≥ 10.83 were considered to be highly significant ($p < 0.001$) (Dixon, 1993).

Results

Post mortem diagnosis of PDD and ABV. In 37 out of 73 examined dead psittacines PDD was confirmed by histopathological detection of typical lesions of non-suppurative ganglioneuritis in various parts of the gastrointestinal tract often in combination with nonsuppurative encephalitis, neuritis, myelitis and myocarditis. The affected birds belonged to the bird genera *Ara* (n=10), *Psittacus* (7), *Cacatua* (6), *Amazona* (5), *Eclectus* (2),

Poicephalus (2), *Guaruba* (1), *Nandayus* (1), *Pionus* (1), *Barnardius* (1), and *Nymphicus* (1). All these 37 cases were positive for ABV in at least one test (Table 3): In 26 cases, ABV RNA and antigen were detected by PCR and immunohistochemistry, respectively. The ABV antigen was detected in brain, spinal cord, retina, proventriculus, gizzard, and intestine by the anti-phosphoprotein antibody. Additionally, ABV was isolated from brain, spinal cord and retina of 16 of these positive birds. Because of too small sample size or the condition of the tissues (beginning autolysis or formalin fixation/paraffin embedding), further 11 birds were only tested by RT-PCR or IHC, and were found to be positive for either ABV RNA (5) or antigen (6). Parasites, fungi, bacteria including chlamydia, and viruses other than ABV were not detected in any of the 37 birds.

The other 36 out of the 73 examined dead birds revealed no histopathological lesions typical for PDD (Table 3). In 29 of these birds neither ABV RNA nor antigen was detected by RT-PCR and immunohistological staining. Virus isolation attempts were performed in 15 of these cases with negative results. However, seven (19%) of the birds without PDD lesions reacted positive for ABV in RT-PCR (4), IHC (1) or both tests (2).

The calculated value in chi-square test of 49.45 indicates highly significant differences between the groups and a highly significant association of PDD with the ABV infection (Table 3).

Intra vitam diagnosis of ABV. From 1,442 live birds, 2,077 samples composed of crop and cloacal swabs and sera were received for direct and indirect detection of ABV. In RT-PCR, 86 of 347 crop swabs and 99 of 381 cloacal swabs were found to be positive for ABV RNA. Of the 1349 sera tested, 228 revealed anti-ABV antibodies in IIFA, while all sera were negative for antibodies against PMV-1 in the HI test. Table 4 demonstrates the results of testing for ABV RNA and/or antibodies with respect to the number of birds of which different

kinds and combinations of samples were examined. In total 299 (20.7%) of the live birds reacted positive for ABV. The percentage of positive birds was even higher in those cases where several kinds of samples were investigated. As presented in Table 4 the ABV infection rate revealed to be markedly higher (121/276 birds = 43.8%), when crop and cloacal swab and serum from a single bird were tested in combination. While only about one fifth (25/121) of these birds showed positive reactions in all three samples submitted, the samples of the other cases were only partly positive for ABV: about one third (45) reacted only indirectly positive for ABV by exhibiting specific antibodies, another third (35) was only positive for ABV RNA in both swabs and 16/121 reactors proved to be positive in only one swab in combination with the serum or even alone.

Table 5 demonstrates the relation of anamnestic data with regard to PDD to ABV detection in the tested live birds. Birds with clinical signs of PDD (63) were positive for ABV to 66.6%. From 1379 birds without clinical signs of PDD, 81.4% were found to be negative for ABV, but in 257 birds (18.6%) ABV was detected directly or indirectly. The chi-square value of 84.56 indicated that the differences between the groups were highly significant, and hence that ABV infection was associated with PDD at a high significance level.

Occurrence of ABV in countries and bird genera. ABV was detected in birds from all five European countries from where birds or samples had been submitted for testing (Table 1). The positive birds belonged to 33 of 54 different genera of the order *Psittaciformes* of which birds were examined (Table 2), and in 16 of them ABV infection was found for the first time. In total 22.8% of all the psittacines reacted positive for ABV. Considering those genera with more than 30 birds tested, substantial differences in the percentages of ABV infected psittacines were seen. While the groups of *Ara*, *Brotogeris* and *Eolophus* contained about 20% positive reactors, the psittacines of the genera *Aratinga* (4.5%), *Pyrrhura* (9.0%),

Psittacula (10%) and *Agapornis* (12.5%) revealed a notably lower infection rate. In contrast to this, higher percentages of ABV infected psittacines were observed in the genera *Poicephalus* (54.5%), *Psittacus* (46.5%), *Cacatua* (40.0%), *Eclectus* (36.2%) and *Amazona* (30.2%). The 12 non-psittacine birds included in this study were negative for ABV.

Discussion

In the present study, the results of testing a large number of different psittacine and some other bird species from various European countries on the occurrence of ABV infection were analysed and brought into relation with the available data on the PDD status in the tested birds. Additionally, the suitability of different specimens and test methods for ABV diagnostics were compared.

. In other studies for post mortem diagnosis of ABV infection (Rinder et al., 2009; Gray et al., 2010, Raghav et al., 2010), virus isolation, RT-PCR and/or IHC were performed only with freshly dead or euthanized birds or frozen organ samples. In this study, however, birds or samples of birds had been submitted for ABV diagnosis with different grades of freshness and different organ fixation. For this reason, organ samples of only 31 cases were suitable for virus isolation attempts without negative influence of toxic reactions and bacterial contamination in cell culture additionally to the use of RT-PCR and IHC. Organs of further 31 birds could be examined in RT-PCR and IHC, although autolysis in few cases hampered the IHC analysis due to high unspecific background staining. Tissue samples of 11 birds were already fixed in formalin or paraffin embedded and could be only processed immunohistochemically. These examinations indicated that RT-PCR and IHC are suitable test methods also for diagnostic material of different quality. To ensure optimal examination

results of dead birds, which have to be send over long distances to the diagnostic laboratory it is proposed to transport the organ samples in an RNA stabilization reagent or in formalin fixed status.

By intra vitam diagnostics for ABV about 20% of the nearly 1,500 birds were found to be positive for ABV RNA or displayed ABV-specific antibodies. The detection of more than 40% positive reactors in the group of birds with combined testing of three different samples, however, gave indication of a probably higher occurrence of ABV infection in the population of captive psittacines. As in this group only one of five birds revealed to be positive in all three samples, testing of only one kind of sample may imply a high risk of false negative results. The observation that the presence of ABV RNA in cloacal excretions and anti-ABV antibodies in serum did not always coincide has already been made by Villanueva et al. (2009) in a smaller number of birds. Possible explanations for seropositivity without detection of ABV RNA might be that i) the currently used RT-PCR are not able to detect all ABV genotypes (Enderlein et al., 2011) or that ii) the virus is only intermittently present in crop and/or cloaca which has been demonstrated for urofaeces of five ABV infected birds by daily testing over a period of 5 days (Raghav et al., 2010) or that iii) the virus was eliminated by the infected bird. ABV RNA detection in swabs of a bird without any humoral immune reaction might be due to sample taking at an early stage of infection or to an ABV infection which is able to hide from the immune system. Regarding these results, we strongly recommend the combined molecularbiological and serological testing of cloacal and crop swabs (eventually pooled) as well as serum for the diagnosis of ABV infection.

One further purpose of this study was to relate the results on the occurrence of ABV in dead and alive birds to their known status concerning signs of PDD. In post mortem examinations, other working groups (Kistler et al., 2008; Honkavuori et al., 2008; Lierz et al., 2009; Ouyang et al., 2009; Rinder et al. 2009, Weissenböck et al., 2009a; Raghav et al., 2010)

detected ABV infection in a high percentage (up to 100%) of the PDD affected birds by different test methods, but without including non-affected birds. Due to the large number of birds with and without PDD in our examinations it was now possible for the first time by statistical analysis to prove this association as highly significant in naturally infected birds. However, we also found ABV RNA or antigen in psittacines without typical lesions of PDD. Until now, this has only been observed in single birds by Lierz et al. (2009) and Raghav et al. (2010) and may be an indication for inapparent infection with a low pathogenic ABV strain or an early stage of infection, when PDD lesions have not yet been induced. Nevertheless, these results strongly underline the causative role of ABV for the development of PDD, which has also been shown in first successful experimental ABV infections (Gancz et al., 2009; Gray et al., 2010).

By calculation in chi-square test, the relation of PDD to ABV infection was also clearly demonstrated as highly significant in live psittacines. The lower percentage (67%) of ABV positive birds within the group of PDD suspected birds in comparison to dead birds with confirmed PDD reflects the current difficulties in *intra vitam* diagnosis of PDD. The clinical signs of PDD like loss of weight, indigested grains in faeces, dilated proventriculus sometimes in combination with central nervous symptoms can also be seen in other diseases of the gastrointestinal tract and/or the nervous system (Villanueva et al., 2009; Lierz et al., 2010). Thus, it seems likely that not all of the birds in this study with clinically suspected PDD really suffer from this disease. However, because of the now proven association of ABV infection with PDD it is in all likelihood that a psittacine showing PDD like symptoms and being ABV positive is affected by PDD and not another disease.

Although the predominant part of the clinically healthy birds was negative for ABV, in nearly 20% of them ABV was detected directly and/or indirectly. These results further support the findings of De Kloet & Dorresstein (2009), Lierz et al. (2009) and Villanueva et

al. (2009), who tested a limited number of apparently healthy birds in a few aviaries, and found some of them to be ABV positive. Whether these birds were still in the incubation phase of the disease or remained only virus carriers without developing manifestation of clinical disease, as it is known for mammalian Borna disease (Herden & Richt, 2009), can only be speculated at the present state of knowledge on ABV pathogenesis. It might be that similar immunopathological mechanisms like those which induce the outbreak of the Borna disease in mammals (Richt et al., 2007) may play an important role in the course of ABV infections. Rossi et al. (2008) suggest an autoimmune mechanism being involved in the development of clinical signs, as they found anti-ganglioside antibodies in PDD-affected birds and hypothesize that ABV might just serve as trigger for the development of the disease.

Concerning the occurrence of ABV in different countries ABV detections in captive psittacines have been reported from Israel, USA, Australia and Canada (Kistler et al., 2008; Honkavuori et al., 2008; Weissenböck et al., 2009; Raghav et al., 2010) as well as from various European countries like Germany (Enderlein et al., 2009; Lierz et al., 2009; Rinder et al. 2009), Austria, Switzerland and Hungary (Weissenböck et al., 2009a). In this study psittacines out of different bird collections in Spain, Italy, United Kingdom and Denmark were found to be positive for ABV, and the presence of ABV was further confirmed in German psittacines. Based on these findings it is likely that ABV is distributed worldwide in captive psittacine collections which may be due to the intense exchange and trade with these birds.

In total, about 20% of the 1,503 psittacines tested revealed signs of previous or ongoing ABV infection. These positive birds belong to 33 different psittacine genera (Table 2) and can be grouped as follows: i) 15 genera which are known to be affected by PDD, and ABV infection has been described in them before; they partly reveal remarkably high infection rates (e.g. *Ara*, *Amazona*, *Poicephalus*, *Psittacus*, *Eclectus*, *Cacatua*,) in the present

study; ii) five genera which are known to be susceptible to PDD, and in which ABV now has been detected for the first time, some of them with only low percentages of positive birds (e.g. *Agapornis*, *Anodorhynchus*, *Psittacula*); iii) one genus in which ABV has been reported before, but PDD is unknown so far, and iv) further 12 genera in which neither PDD nor ABV infection has been reported previously (e.g. *Bolborhynchus*, *Pyrrhura*, *Pionopsitta*), and now were demonstrated to be ABV positive. Further systematic epidemiological investigations and experimental infection trials are necessary to investigate whether the observed differences are only due to the non representative mixture of psittacine birds examined or are caused by differences of psittacine genera in susceptibility for ABV infections.

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Table 1. *Origin and number of tested birds*

Country	Number of bird collections	Number of birds	Number of ABV positive birds ^a
Spain	14	996	142
Germany	171	442	170
Italy	12	32	17
United Kingdom	16	16	5
Denmark	2	29	9
Total	215	1515	343

^a detection of avian bornavirus (ABV RNA, virus, antigen) and/or ABV specific antibodies

Table 2. Bird genera examined for ABV infection

Family ^a	Genus ^a	Present study			Previous reports on	
		Birds (n)	ABV positive birds ^b (n)	% ABV positive birds	PDD ^c	ABV
Micropsittacidae	Agapornis	56	7	12.5	Gregory et al., 1994	
Psittacidae	Forpus	17	0	0.0		
	Bolborhynchus	20	1	5.0		
	Anodorhynchus	23	3	13.0	Woerpel & Rosskopf, 1984	Weissenböck et al., 2009a
	Cyanopsitta	2	0	0.0	Heldstab et al, 1985	
	Ara	222	53	23.9	Ridgway & Gallerstein, 1983	Kistler et al., 2008
	Guaruba	7	1	14.3	Heldstab et al, 1985	Villanueva et al.,2009
	Nandayus	6	3	50.0	Phalen, 1986	Villanueva et al.,2009
	Rhynchopsitta	7	1	14.3	Graham, 1991	
	Aratinga	134	6	4.5	Ridgway & Gallerstein, 1983	Kistler et al., 2008
	Pyrrhura	167	15	9.0		
	Enicognathus	5	0	0.0	Berhane et al., 2001	
	Cyanoliseus	10	3	30.0	Cazayoux Vice, 1992	
	Brotogeris	52	10	19.2	Gregory et al., 1994	
	Pionopsitta	12	1	8.3		
	Amazona	179	54	30.2	Graham, 1984	Honkavuori et al., 2009
	Deroptyus	8	2	25.0	Gregory et al., 1994	Weissenböck et al., 2009a
	Pionus	25	6	24.0	Gregory et al., 1994	Kistler et al., 2008
	Triclaria	4	0	0.0		
	Pionites	7	0	0.0	Reavill & Schmidt, 2007	
	Poicephalus	33	18	54.5	Suedmeyer, 1992	Shivaprasad et al., 2009
Psittacus	101	47	46.5	Ridgway & Gallerstein, 1983	Kistler et al., 2008	

	Coracopsis	7	3	42.9	Gregory et al., 1994	Weissenböck et al., 2009a
Psittaculidae	Psittacula	30	3	10.0	Gregory et al., 1994	
	Psittirichas	2	1	50.0		
	Eclectus	47	17	36.2	Graham, 1984	Weissenböck et al., 2009a
	Psittinus	3	1	33.3		
Polytelidae	Aprosmictus	3	0	0.0		
	Alisterus	6	3	50.0		
	Polytelis	3	1	33.3		
Loriidae					Reavill & Schmidt, 2007	
	Lorius	8	0	0.0		
	Neopsittacus	1	0	0.0		
	Oreopsittacus	2	0	0.0		
	Trichoglossus	8	2	25.0		
	Eos	3	2	66.7		
	Chalcopsitta	2	1	50.0		
	Psittaculirostris	3	0	0.0		
	Lathamus	4	3	75.0		
Platycteridae	Purpureicephalus	5	1	20.0		
	Barnadius	5	0	0.0		
	Psephotus	3	0	0.0		
	Platycerus	10	0	0.0		
	Eunymphicus	3	0	0.0		
	Cyanoramphus	1	0	0.0		Weissenböck et al., 2009a
	Neophema	14	0	0.0		
	Neopsephotus	2	0	0.0		
Strigopidae	Strigops	18	0	0.0		
Cacatuidae	Probosciger	6	2	33.3		Kistler et al., 2008

	Calyptorhynchus	2	0	0.0		
	Callocephalon	4	0	0.0		
	Eolophus	32	6	18.8	Lublin et al., 2006	Kistler et al., 2008
	Cacatua	140	56	40.0	Ridgway & Gallerstein, 1983	Kistler et al., 2008
	Nymphicus	6	2	33.3	Graham, 1984	Ouyang et al., 2009
Nestoridae	Nestor	4	0	0.0	Grund et al., 2002	
Unknown	Unknown	19	8	42.1		
Psttaciiformes	Total	1503	343	22.8		
Cracidae	Aburria	2	0	0.0		
Ciconidae	Ciconia	4	0	0.0		
Columbidae	Geopelia	1	0	0.0		
Sturnidae	Leucopsar	4	0	0.0		
Phasianidae	Pavo	1	0	0.0		
Non-Psittaciiformes	Total	12	0	0.0		
Total		1515				

^a according to Wolters, 1982

^b detection of avian bornavirus (ABV RNA, virus, antigen) and/or ABV specific antibodies

^c proventricular dilatation disease

Table 3. Association of PDD status and ABV detection in dead birds

PDD	ABV		Total number of birds	% ABV positive birds
	positive ^a	negative		
positive ^b	37	0	37	100
negative	7	29	36	19
Total	44	29	73	

Chi-square value: 49.45*

^a ABV positive: detection of ABV (RNA, virus, antigen) and/or ABV specific antibodies

^b PDD positive: pathohistological lesions typical for PDD

* highly significant (p<0.001)

Table 4. *Detection of ABV RNA and/or antibodies in swabs and sera of live birds*

Sample(s)/ bird	Number of birds (samples)	Sample(s) +	Number of positive birds with						Total number of positive birds	% posi- tive birds
			Swab(s)-/ S+	Swab(s)+/ S-	only CrS+	CrS+/S+	only CIS+	CIS+/S+		
CrS	1 (1)	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0	0
CIS	24 (24)	7	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	7	29.2
CLS, CrS	68 (136)	16	n.a.	n.a.	n.a.	n.a.	1	n.a.	17	23.9
CIS, CrS, S	276 (828)	25	45	35	5	3	3	5	121	43.8
CIS, S	13 (26)	4	1	3	n.a.	n.a.	n.a.	n.a.	8	61.5
CrS, S	2 (4)	1	0	1	n.a.	n.a.	n.a.	n.a.	2	100.0
S	1,058 (1,058)	144	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	144	13.6
Total	1,442 (2,077)	197	46	39	5	3	4	5	299	20.7

+ = positive CIS = Cloacal swab
 - = negative CrS = Crop swab
 n.a. = not applicable S = Serum

Table 5. Association of PDD status and ABV detection in live birds

PDD	ABV		Total number of birds	% ABV positive birds
	positive ^a	negative		
+ ? ^b	42	21	63	67
- ? ^c	257	1122	1379	19
Total	299	1143	1442	

Chi-square value: 84.56*

^a ABV positive: detection of ABV (RNA, virus, antigen) and/or ABV specific antibodies
^b + ? : tentative diagnosis PDD: clinical signs like indigested feed, loss of weight, PDD in radiography, CNS disorder
^c - ? : no report on clinical signs of PDD
* highly significant (p<0.001)