Avian bornavirus associated with fatal disease in psittacine birds

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ABSTRACT

Thanks to new technologies which enable rapid and unbiased screening for viral nucleic acids in clinical specimens, an impressive number of previously unknown viruses have recently been discovered. Two research groups independently identified a novel negative strand RNA virus, now designated avian bornavirus (ABV), in parrots with proventricular dilatation disease (PDD), a severe lymphoplasmacytic ganglioneuritis of the gastrointestinal tract of psittacine birds that is frequently accompanied by encephalomyelitis. Since its discovery, ABV has been detected worldwide in many captive parrots and in one canary with PDD. ABV induced a PDD-like disease in experimentally infected cockatiels, strongly suggesting that ABV is highly pathogenic in psittacine birds. Until the discovery of ABV, the Bornaviridae family consisted of a single genus, classical Borna disease virus (BDV) which is the causative agent of a progressive neurological disorder that primarily affects horses, sheep, and some other farm animals in central Europe. Although ABV and BDV share many biological features, several interesting differences exist which will be discussed in this review article.

BDV, the prototype member of the Bornaviridae family

BDV is an enveloped virus with a non-segmented negative stranded RNA genome of approximately 8,900 bases (11). Unlike other viruses in the order Mononegavirales, BDV uses the nuclear compartment of the host cells for transcription and replication. This strategy enables the virus to assess the cellular splicing machinery for generating some of its mRNAs (11, 70). BDV further replicates its genome using a highly unusual mechanism which results in genomic and anti-genomic viral RNAs with trimmed 5’ ends that contain mono- rather than tri-phosphorylated terminal nucleotides (71, 72). Since such molecules are not recognized by RIG-I (41, 58), a cytosolic RNA sensor that triggers host innate immune responses, the seemingly complicated mode of replication employed by BDV may represent a smart viral evasion strategy (34). Another interesting feature of BDV is that the functionality of infected cells is not or only marginally impaired as the virus replicates in a strictly non-cytolytic manner. Since viral products are abundantly present in infected cells, the virus must actively suppress apoptosis. Recent work suggests that the viral accessory protein X may serve this function (59). Finally, it is of interest to note that cells persistently infected with BDV release only very few infectious viral particles into the culture supernatant, and that the virus mainly spreads by cell-to-cell contact. This viral life-style raises questions regarding its mode of
transmission in nature. Since surprisingly high viral titers were found in the urine of persistently infected rats (68), efficient release of BDV may be restricted to some specialized cell types in the kidney or urinary tract.

Natural BDV infections are most frequently seen in horses and sheep. Nevertheless, experimental infection of other mammals such as rabbits, rat and mice has been successful (50, 66). BDV exhibits a high tropism for the CNS in both natural and experimental hosts where it can establish persistent, non-cytolytic infections of neurons and astrocytes (76). The clinical symptoms of Borna disease can either be mild or severe, presumably reflecting the fact that the clinical picture mirrors immunopathological events rather than viral activity (75).

In fact, Borna disease is always accompanied by substantial immune cell infiltration of the CNS. Work with rats and mice revealed that CD8 T cells recognizing viral antigen play a key role in both antiviral defense and virus-triggered disease. If induced by immunization, virus-specific CD8 T cells can protect from infection (36, 38). However, once the virus has infected a substantial number of cells in the CNS, antiviral CD8 T cells are harmful as they attack infected cells and cause severe meningoencephalitis (37). As expected if this scenario was correct, BDV-infected rats or mice that lack functional CD8 T cells remain healthy, although many cells in the CNS are actively replicating virus (35).

The epidemiology of BDV is not well understood (74). There are no firm data which would indicate that the virus is transmitted by contact among horses and sheep. The observed distribution of distinct BDV genotypes in Europe is best explained by assuming that horses and sheep acquire the virus from unknown local reservoirs (16, 74). In fact, a recent study identified an infected insectivore on a farm with occasional cases of Borna disease (39). Since the viruses from the insectivore and diseased horses were almost identical, it seems likely that the horses acquired the infection from infected insectivores which presumably contaminated the feed.

A possible association of BDV with neuropsychiatric disorders in humans is fiercely debated (6, 15, 48). The evidence in favor of such a link is rather weak. It is mainly based on the fact that human sera frequently contain low levels of antibodies that can recognize BDV antigens, and that such antibodies are found more frequently in patients with neurological disorders than in healthy individuals (1, 5, 67). The detection of viral nucleic acids or even infectious virus in patient specimens was reported by many groups (7, 53). However, it is likely that most if not all of these studies were flawed by contamination of patient samples with either laboratory viruses or PCR amplification products (15). Furthermore, the specificity of
antibody-based assay systems which were used to detect viral antigen in plasma of patients (6) has been questioned (83).

**Proventricular dilatation disease of psittacine birds**

Proventricular dilatation diseases (PDD) is a fatal disease of mainly psittacine birds which was initially reported as a unique disease entity in macaws in the late 1970s (51) but subsequently also described in a growing number of other parrots (31). Even though the disease is now commonly referred to as PDD, several synonyms have been used in the literature, including macaw wasting syndrome, proventricular dilatation syndrome, neuropathic gastric dilatation of psittaciforms or myenteric ganglioneuritis, and others (13, 31). Birds presented with PDD frequently show weight loss associated with reduced appetite or polyphagia and various degrees of gastrointestinal dysfunction (51). Regurgitation, undigested seeds in the feces, impactation of the proventriculus and diarrhea are commonly reported clinical features. Affected birds may also show abdominal enlargement, muscle atrophy, weakness and polyuria to different degrees. Symptoms of the central nervous system such as seizure, ataxia, abnormal head movement, reduced proprioceptive skills, and motor deficit can be observed in some but not all cases of PDD (31).

Clinical laboratory parameters are generally inconclusive in PDD and may simply reflect the gastrointestinal dysfunction (hypoproteinemia, hypoglycaemia) or the presence of opportunistic infections (heterophilia) which are frequently associated with the disease (77). Consequently, ante mortem diagnostics relay on contrast radiographic procedures and the histopathological examination of biopsy specimens. Contrast radiography is routinely applied to diagnose the dilatation of the proventriculus, as well as duodenum descendens and extended transit times of ingestions (61). In rare cases even spontaneous ruptures of the dilated proventriculus with ingesta filling the caudal air sac group may be observed. As expected, the typical findings in post mortem examination are dilatation of the esophagus, proventriculus, ventriculus or small intestine and atrophy of the proventricular muscle (Fig. 1A) and pectoral muscles which is a consequence of malnutrition (42). A highly characteristic feature of PDD is the presence of lymphoplasmacytic infiltrates in the enteric nerve plexuses of the proventriculus and ventriculus and less frequently of the esophagus, crop and duodenum (64). However, lymphocytic infiltrates are not restricted to the neural tissue of the gastrointestinal tract but may also be seen in conduction fibers of the heart the adrenal gland and in the brain (Fig. 1B) and spinal cord. The pons, medulla and midbrain are most frequently affected, showing perivascular cuffing, lymphoplasmacytic encephalitis and
myelitis (21, 31). Several independent studies have shown that lymphoplasmacytic infiltrates 
in the ventriculus and proventriculus are highly characteristic for PDD (42) and may therefore 
be used to confirm a presumptive diagnosis of PDD by histopathological examination of 
biopsy samples (14, 30).

Initially, PDD was only reported in captive parrots in North America and Europe but since 
then the disease has been diagnosed in psittacines worldwide (13). It is assumed that intensive 
trading has contributed to spreading in the pet bird population (28). PDD has been reported in 
more than 50 species of psittaciformes (8, 51, 81). African grey parrots, blue and gold 
macaws, cockatoos and Amazon parrots seem to be most frequently affected (69) but, as 
Gregory and colleagues pointed out, this may reflect a population bias rather than a species 
predisposition (31). Interestingly, PDD does not seem to be restricted to psittacine birds.

Proventricular dilatation associated with a nonsuppurative encephalitis and ganglioneuritis 
was reported in wild Canada geese (Branta Canadensis) (9) and cases of PDD-like clinical 
and pathological findings were described in a canary (Serinus canaria), a greenfinch 
(Carduelis chloris), a long-wattled umbrella bird (Cephalopterus penduliger), a bearded 
barbet (Lybius dubius) (56) and a falcon (Falco peregrinus) (73). Suggestive lesions were also 
reported in toucans, honey-creepers, weaver finches and roseate spoonbills (29).

Prognosis of PDD affected birds is poor and a specific treatment is not available to date. Birds 
can survive for months to years if treated symptomatically by feeding liquid or semisolid 
diets, and by applying antimicrobials to control secondary infections (22, 28, 77). Even 
though the etiology of PDD was unclear until recently, isolation of affected birds was 
recommended (31, 64). This suggestion was originally based on the observation of PDD 
outbreaks in aviaries (57), the demonstration that PDD could be transmitted experimentally 
(28), and the identification of viral particles in tissues and feces of birds affected by PDD.

First evidence for a viral etiology of PDD came from transmission electron microscopy 
studies more than 20 years ago, demonstrating inclusion bodies and enveloped virus-like 
particles of 30 to 250 nm in size in the myenteric plexus and celiac ganglion of affected birds 
(51). This study and subsequent work (32, 33) proposed that a paramyxovirus might be the 
causative agent of PDD. However, this assumption was not supported by serological studies 
which failed to demonstrate paramyxovirus-specific antibodies in diseased birds (12, 28).

Other viruses proposed as causative agents of PDD included coronavirus (23), equine 
encephalitis virus (20) and avian herpes virus. Polyomavirus, adeno-like viruses, 
enteroviruses, reoviruses and avian encephalitis virus (63) have likewise been discussed as 
possible etiological agents of PDD. Enveloped virus-like particles of 80-140 nm were
identified in organs (23) and fresh feces (24) from PDD cases. Evidence for the transmissibility of PDD came from experiments where organ extracts from diseased birds containing the described viral particles were injected into healthy birds. All birds receiving the tissue homogenates developed clinical symptoms and showed histopathological lesions consistent with PDD (28). Since attempts to isolate the potential viral agent were unsuccessful at the time, it was proposed that PDD might represent an autoimmune disease triggered by virus infection (25) or gangliosides (65).

Discovery and preliminary characterization of ABV

Cutting edge technology that enables fast and unbiased searches for viruses in clinical specimens was initially employed to identify avian bornaviruses. In a first study, RNA extracted from parrots with PDD was hybridized to a pan-viral microarray that carried multiple cDNA probes from known viruses. This screen yielded evidence for the presence of a BDV-like virus in birds with PDD, which was confirmed by high throughput sequencing (44). In a second independent study, RNA extracted from tissue of parrots with PDD was reverse transcribed and directly subjected to high throughput sequencing followed by searching for sequence similarities to known viruses. Again, genetic material with similarity to BDV was identified (40). A limited epidemiological survey indicated that ABV was present in many but not all parrots with PDD and that ABV was absent in healthy animals (44). Subsequent studies from Europe yielded a similar picture: ABV was detected in most but not all parrots with clinically suspected PDD (49, 62, 81). The significance of this latter result is unclear: additional as yet undiscovered ABV strains with divergent genomes may exist. Alternatively, PDD-like illness in parrots may be induced by ABV as well as by other unrelated viruses that remain to be discovered. Currently, we are unable to distinguish between these possibilities.

To date, at least five distinct ABV genotypes were discovered in psittacine birds (40, 44, 62, 81). Further, one additional distinct genotype of ABV was identified in a canary suffering from PDD-like disease (82). Comparisons mostly based on incomplete genome sequences indicate that ABV strains from psittacine birds exhibit 50-90% identity. The similarity of the ABV strain from the canary is even less pronounced (Fig. 2). Thus, the genetic variability of ABV is much greater than the variability observed among BDV strains of mammals (15, 81, 82). The reasons for this striking difference remain unknown. If the above-discussed assumption is correct that BDV is not transmitted among horses and sheep but rather is introduced into these animals by insectivores which contaminate the feed, it remains possible
that the currently known BDV strains do not reflect the complete genetic repertoire of this virus. Rather, the currently known BDV strains might only represent a small fraction of virus variants which are able to cross the insectivore/horse and insectivore/sheep species barriers, respectively. This hypothesis implies that genetically distinct strains of BDV might exist in wild-living insectivores and possibly other natural hosts of BDV that have not yet been discovered simply because they were not successfully transmitted to farm animals.

Ultra high throughput sequencing was utilized to recover the first complete viral genome sequence from one of the ABV-positive PDD cases. Analysis revealed a genome organization similar to that of BDV (44). In ABV and BDV, the first transcription unit codes for the nucleoprotein N, the second transcription unit codes for regulatory protein X and polymerase co-factor P, and the third transcription unit codes for matrix protein M, surface glycoprotein G and polymerase L. In both ABV and BDV, X and P are synthesized from overlapping reading frames of a single bi-cistronic mRNA, and the primary transcript of the third transcription unit is processed by splicing. Further, a high degree of sequence conservation was found in the terminal non-coding and intergenic regions of the viral genomes. The only notable difference between the two viral genomes is that the region between the N and X genes is substantially enlarged in BDV (62). This region contains elements that control X protein synthesis in BDV-infected cells (60, 79). The absence of these elements in ABV suggests that fine-tuning of X protein synthesis may be achieved by other means in ABV-infected cells.

Virus was isolated from organs of two grey parrots infected with ABV genotypes 2 and 4, respectively (62). Isolation attempts were only successful if avian rather than mammalian cell lines were used. Interestingly, both ABV strains readily grew in cell lines derived from quails and chickens, suggesting that ABV has a high preference for avian cells but that its host range may not be restricted to psittacine and canary birds. The properties of ABV in cells from quails and chickens resembled those of BDV in mammalian cells. The ABV infection was non-cytolytic, and the viruses seemed to spread mainly by cell-to-cell contact (62). Further, the N and P antigens of ABV accumulated in the nuclei of infected cells.

Experimental transmission of ABV was recently achieved by simultaneous inoculation of brain homogenate from a confirmed ABV genotype 4 positive PDD case through the parenteral and mucosal routes into cockatiels (*Nymphicus hollandicus*) (19). Two out of three birds developed clinical signs typically seen in PDD starting three to four weeks after infection, and all birds showed the characteristic lymphoplasmacytic infiltrates in the myenteric ganglia and variable degrees of lesions in brain and spinal cord. Viral RNA was found in numerous tissues including the peripheral and central nervous system, the
gastrointestinal tract as well as kidney, heart, spleen and pancreas. Presence of virus was further confirmed by immunohistochemistry (19) in brain and myenteric ganglion, but did not show the widespread distribution previously observed in naturally infected parrots (62).

More recently, cultured ABV genotype 4 was shown to induce PDD-like symptoms in experimentally infected parrots (26), formally fulfilling Koch’s postulates and providing final proof that ABV can cause PDD in parrots. A first attempt to develop a non-psittacine animal model for PDD through intramuscular and mucosal inoculation of cultured ABV genotype 4 into ducks has failed (27), although successful initial infection was demonstrated by PCR and serological techniques.

Perspectives

The discovery of ABV as causative agent of PDD represents a first important step towards a rational approach to fight this devastating disease of parrots. Several problems deserve our special attention in the near future.

First, ABV infections are currently monitored by analyzing biopsies or post mortem tissue samples for viral nucleic acids using RT-PCR. However, presently used primer sets can probably not detect all circulating ABV strains. It is further unclear if the PCR assay has sufficient sensitivity to detect ABV infections before clinical symptoms have developed. Serological assays might be superior. However, it should be noted that serological assays do not work very well for diagnosing BDV infections of mammals as antibody titers of infected horses and sheep are notoriously low (reviewed in (1)). However, since ABV shows a less restricted organ tropism than BDV (Fig. 1), and since ABV antigen is abundantly present in many organs of infected birds (18, 62, 81, 82) it remains possible that the immune response to ABV is more robust than the immune response of BDV. Finally, as antisera recognizing conserved epitopes of ABV are now getting available (55, 78), highly sensitive intra-vitam or post-mortem detection of ABV antigen in tissue samples should soon be feasible.

Second, currently available epidemiological data suggest that ABV may be found in psittacine birds from most parts of the world. However, we do not yet have a good sense for the true extent of the virus distribution and of the medical problems the virus may cause globally. The recent detection of ABV in a diseased canary (82) demonstrates that the host range of ABV is not restricted to psittacine birds. In this context it is of interest to note that a paralytic syndrome has been reported in young ostriches from Israel (80) which, based on serological testing, was suggested to be the consequence of infection with BDV (2). The syndrome could be transferred to naïve birds by intramuscular injection or oral application of brain
homogenates derived from diseased animals. From today’s point of view, it seems that ABV rather than BDV might have caused the disease in the ostriches.

Third, it is of great interest to know whether symptom-less persisting infections of parrots and other birds occur frequently, and whether such persistently infected birds serve as virus reservoir. Recent reports (10, 45) suggest that this is a likely scenario. Further, the routes of virus transmission must be studied. It should be noted that viral nucleic acid was found in feces of diseased birds (62), but it remains unknown whether the virus in feces remained infectious. It is of interest to note that PCR analysis of fecal samples from wild birds in Sweden suggested the presence of BDV in a wide range of apparently healthy avian species (4). This observation requires reassessment with PCR primers that can clearly distinguish between genetic material from ABV and BDV.

Forth, as PDD has now been recognized as representing a virus-triggered disease, it might be possible to develop a protective vaccine. From previous vaccine studies aimed at preventing BDV-induced disease in rats and mice (17, 36, 38, 47, 54), we would predict that an effective vaccine might need to induce a robust antiviral CD8 T cells response rather than neutralizing antibodies. To evaluate any candidate vaccines, simple and affordable animal models are required that mimic the hallmarks of the ABV-induced disease in parrots. Animal models will also play a critical role for the evaluation of therapeutic approaches with antiviral substances. It was reported that BDV shows a high degree of sensitivity to ribavirin (43, 46, 52) and AraC (3) which are used to treat viral infections and cancer in humans. These drugs might be used to treat diseased birds or to block virus transmission in affected breeding colonies.

Acknowledgments:
We thank Helga Gerlach, Antje Reuter, Rüdiger Korbel, Sonja Kothlow and Urs Schneider for carefully reading the manuscript.
LITERATURE CITED:


FIGURE LEGENDS

Figure 1: Manifestation of PDD in parrots. (A) Severely enlarged proventriculus of an orange-cheeked parrot (*Pionites melanocephala*) with PDD. The muscular wall of the proventriculus is highly atrophic with ingesta shining through. Within the pylorus region a rupture of the proventriculus wall which spontaneously occurred *intra vitam* is visible. (B) Perivascular lymphoplasmacytic infiltrates in the brain of a diseased white cockatoo. (C-J) ABV antigen in various organs of an *Amazona ventralis* with PDD. Consecutive sections of paraffin-embedded organs of the diseased parrot infected with ABV genotype 2 strain #6609 (62) were either stained with a cross-reactive rabbit antiserum against the P protein of BDV (C, E, G, I) or pre-immune serum (D, F, H, J). Brown stain indicates specific staining of ABV antigens by the cross-reactive antiserum.

Figure 2: Phylogenetic tree based on partial N gene sequences of bornaviruses from mammals and birds. Bootstrap values are given as percentages for the main nodes. Brackets illustrate the rationale for grouping known psittacine bornaviruses into five distinct genotypes.