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MINIREVIEW

Avian Bornavirus Associated with Fatal Disease in Psittacine Birds▽

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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 species, classical Borna disease virus (BDV), which is the causative agent of a progressive neurological disorder that affects primarily horses, sheep, and some other farm animals in central Europe. Although ABV and BDV share many biological features, there exist several interesting differences, which are discussed in this review.

BDV, THE PROTOTYPE MEMBER OF THE *BORNAVIRIDAE* FAMILY

Borna disease virus (BDV) is an enveloped virus with a nonsegmented negative-stranded RNA genome of approximately 8,900 bases (12). 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 (12, 70). BDV further replicates its genome using a highly unusual mechanism which results in genomic and antigenomic viral RNAs with trimmed 5′ ends that contain mono- rather than triphosphorylated 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 is only marginally impaired, as the virus replicates in a strictly noncytolytic 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 that cells persistently infected with BDV release only very few infectious viral particles into the culture supernatant and that the virus spreads mainly by cell-to-cell contact. This viral lifestyle raises questions regarding its mode of transmission in nature. Since there were surprisingly high viral titers 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, rats, and mice, has been successful (50, 66). BDV exhibits a high tropism for the central nervous system (CNS) in both natural and experimental hosts, where it can establish persistent, noncytolytic infections of neurons and astrocytes (76). The clinical symptoms of Borna disease can be either 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 provide protection 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 is correct, BDV-infected rats or mice that lack functional CD8 T cells remain healthy, although the virus actively replicates in many cells in the CNS (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

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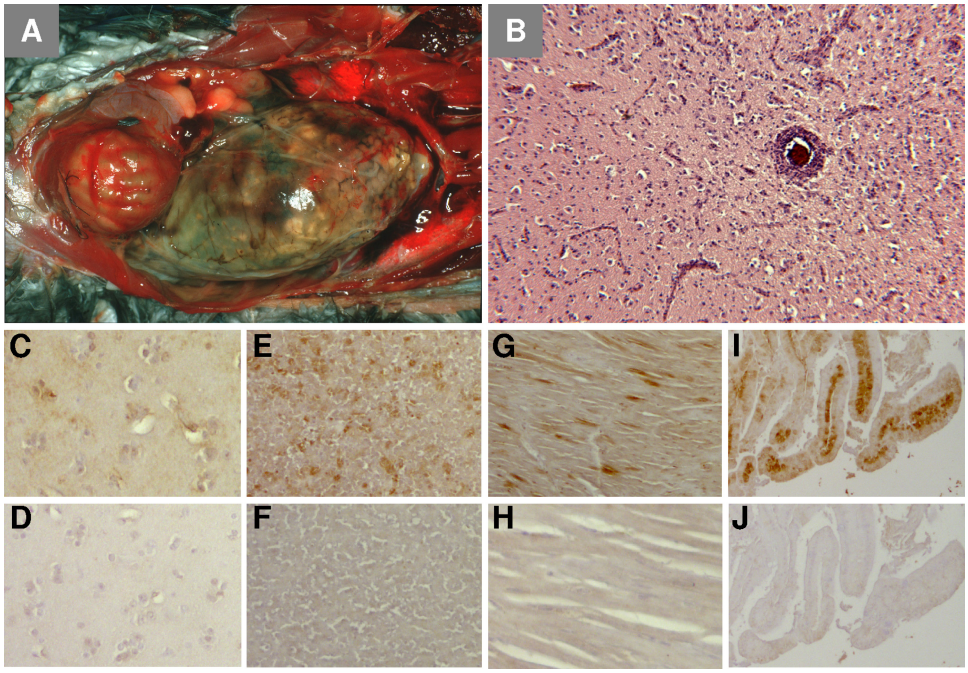

FIG. 1. Manifestation of PDD in parrots. (A) Severely enlarged proventriculus of an orange-cheeked parrot (*Pionites melanocephala*) with PDD. The muscular wall of the proventriculus is highly atrophic with ingesta shining through. Within the pylorus region, a rupture of the proventriculus wall which spontaneously occurred *intra vitam* is visible. (B) Perivascular lymphoplasmacytic infiltrates in the brain of a diseased white cockatoo. (C to 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 stained with either a cross-reactive rabbit antiserum against the P protein of BDV (C, E, G, and I) or preimmune serum (D, F, H, and J): brain (C and D), liver (E and F), heart (G and H), and intestine (I and J). Brown staining indicates specific staining of ABV antigens by the cross-reactive antiserum.

favor of such a link is rather weak. It is based mainly 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 has been 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 disease (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 as occurring 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, myenteric ganglioneuritis, and others (13, 31). Birds presenting 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. Central nervous system symptoms, such as seizure, ataxia, abnormal head movement, reduced proprioceptive skills, and motor deficit, can be observed with some but not all cases of PDD (31).

Clinical laboratory parameters are generally inconclusive in PDD and may simply reflect the gastrointestinal dysfunction (hypoproteinemia, hypoglycemia) or the presence of opportunistic infections (heterophilia) which are frequently associated with the disease (77). Consequently, *ante mortem* diagnostics rely 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 ingesta (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 postmortem 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 and may also be seen in conduction fibers of the heart, the adrenal

gland, the brain (Fig. 1B), and the 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 of 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 reported only for captive parrots in North America and Europe, but since then, the disease has been diagnosed for psittacines worldwide (13). It is assumed that intensive trading has contributed to spreading in the pet bird population (28). PDD has been reported for more than 50 species of *Psittaciformes* (8, 51, 81). African gray 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 in wild Canada geese (*Branta canadensis*) was reported (9), and cases of PDD-like clinical and pathological findings have been described for 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 in toucans, honeycreepers, weaver finches, and roseate spoonbills have also been reported (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 being fed liquid or semisolid diets and by having antimicrobials applied 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 can be transmitted experimentally (28), and the identification of viral particles in tissues and feces of birds affected by PDD.

The 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 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 the idea 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 (10, 28). Other viruses proposed as causative agents of PDD included coronavirus (23), equine encephalitis virus (20), and avian herpesvirus. 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 to 140 nm were identified in organs (23) and fresh feces (24) from PDD cases. Evidence for the transmissibility of PDD came from experiments in which 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 (ABVs). In one of the first studies, RNA extracted from parrots with PDD was hybridized to a panviral microarray that carried multiple cDNA probes from known viruses. This screening 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 tissues of parrots with PDD was reverse transcribed and directly subjected to high-throughput sequencing followed by searches 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 the 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 in psittacine birds have been discovered (40, 44, 62, 81). Further, one additional distinct genotype of ABV was identified in a canary suffering from PDD-like disease (82). Comparisons based mostly on incomplete genome sequences indicate that ABV strains from psittacine birds exhibit 50 to 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, namely, 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 represent only 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 polymer-

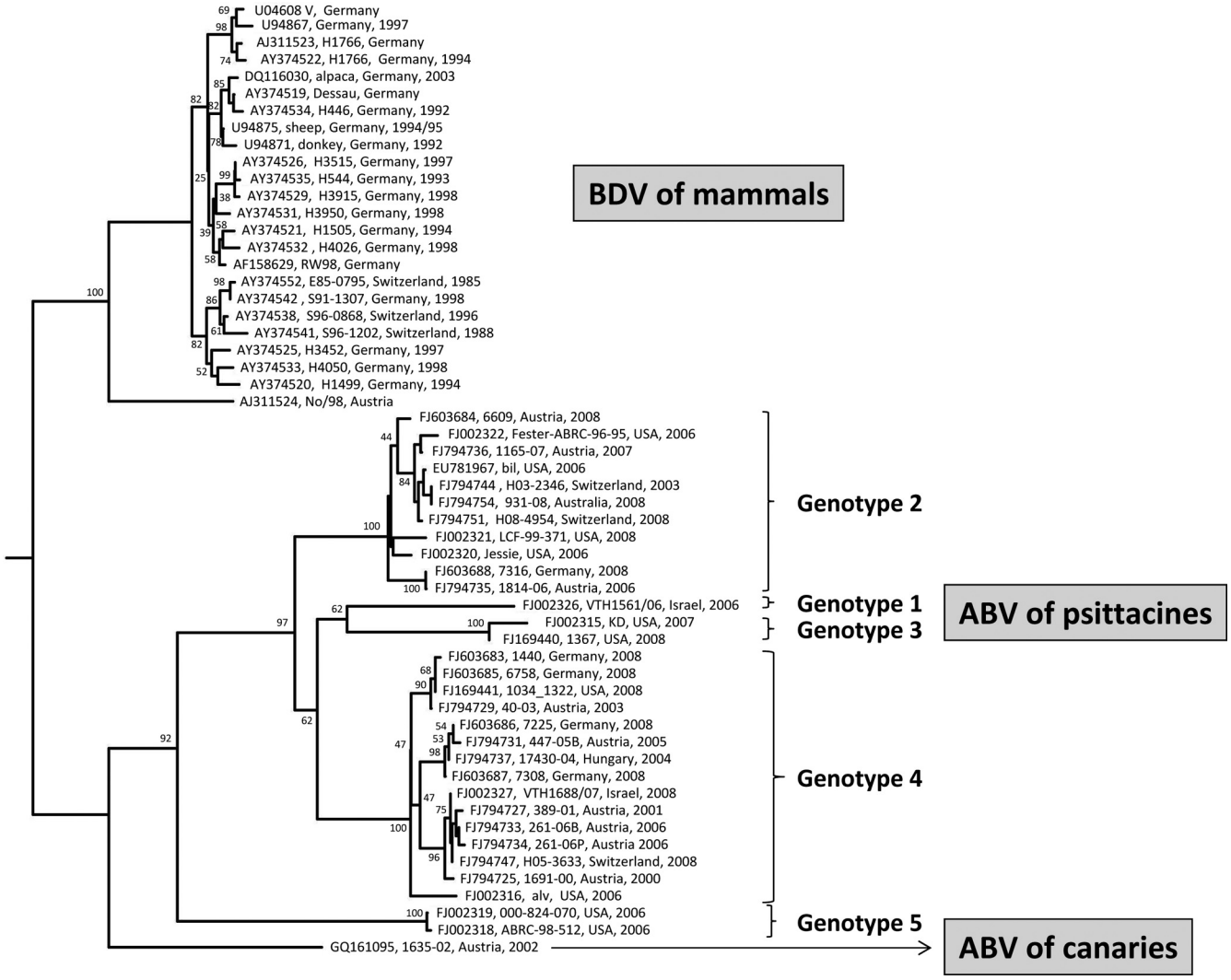

FIG. 2. Phylogenetic tree based on partial N gene sequences of bornaviruses from mammals and birds. Bootstrap values are given as percentages for the main nodes. Brackets illustrate the rationale for grouping known psittacine bornaviruses into five distinct genotypes. The GenBank accession number, strain name, and country and year of isolation are given.

ase cofactor 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 bicistronic 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 noncoding 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 gray parrots infected with ABV genotypes 2 and 4, respectively (62). Isolation attempts were successful only 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 noncytolytic, 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 with PDD starting 3 to 4 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

systems, gastrointestinal tract, kidney, heart, spleen, and pancreas. The presence of virus in the brain and myenteric ganglion was further confirmed by immunohistochemistry (19), but it 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 nonpsittacine 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 toward 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 biopsy or postmortem tissue samples for viral nucleic acids using reverse transcription-PCR (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. 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 reference 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 to BDV. Finally, as antisera recognizing conserved epitopes of ABV are now becoming available (55, 78), highly sensitive *intra vitam* or postmortem 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 of 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 that a paralytic syndrome in young ostriches from Israel has been reported (80); based on serological testing, this syndrome 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 symptomless persisting infections of parrots and other birds occur frequently and whether such persistently infected birds serve as a virus reservoir. Recent reports (11, 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 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 that from BDV.

Fourth, 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-cell response rather than neutralizing antibodies. To evaluate any candidate vaccines, simple and affordable animal models that mimic the hallmarks of the ABV-induced disease in parrots are required. 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.

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