A Review of DNA Viral Infections in Psittacine Birds

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(Received 12 January 2010/Accepted 2 April 2010/Published online in J-STAGE 16 April 2010)

ABSTRACT. To date, several DNA viral infections have been reported in psittacine birds. Psittacine beak and feather disease (PBFD) is characterized by symmetric feather dystrophy and loss and development of beak deformities. PBFD is caused by beak and feather virus, which belongs to the Circoviridae, and is the most important infection in psittacine birds worldwide. Avian polyomavirus infection causes acute death, abdominal distention, and feather abnormalities. Pacheco’s disease (PD), which is caused by psittacid herpesvirus type 1, is an acute lethal disease without a prodrome. Psittacine adenovirus infections are described as having a clinical progression similar to PD. The clinical changes in psittacine poxvirus-infected birds include serious ocular discharge, rhinitis, and conjunctivitis, followed by the appearance of ulcers on the medial canthi of the eyes. Internal papillomatosis of parrots (IPP) is a tumor disease characterized by progressive development of papillomas in the oral and cloacal mucosa. IPP has been suggested to be caused by papillomavirus or herpesvirus. However, information about these diseases is limited. Here we review the etiology, clinical features, pathology, epidemiology, and diagnosis of these DNA viruses.

KEY WORDS: DNA virus, psittacine bird, review.

In recent years, various psittacine birds have been popular and kept as pets in Japan as well as other countries. Avian medicine for poultry has made significant progress over the last several decades. However, there are few studies with regard to avian medicine for psittacine birds. Today, infectious diseases, especially viral infectious diseases, are the most common clinical problems in captive psittacine birds because of their association with acute death and difficulties in treatment and control. Many viral infections in psittacine birds have been reported, including DNA virus: psittacine beak and feather disease (PBFD) [73], avian polyomavirus infection (APV) [5], psittacid herpesvirus infection (PsHV) [92], psittacine adenovirus infection (PsAdV) [81], poxvirus infection [58], and papillomavirus infection [14] and RNA virus: reovirus infection [106], coronavirus infection [26], paramyxovirus infection [29], influenzavirus infection [76], and bornavirus infection [50]. However, information about these diseases is limited.

The objectives of this review are DNA viral infections in psittacine birds with respect to (I) etiology, (II) clinical features, (III) pathology, (IV) epidemiology, and (V) diagnosis about each DNA virus disease in psittacine birds. Table 1 is summary of each viral infection in this review.

DNA VIRUS INFECTIONS IN PSITTAECINE BIRDS

Psittacine beak and feather disease (Circovirus infection): This chronic disease is characterized by symmetric feather dystrophy and loss, development of beak deformities, and eventual death. It was first observed in various species of Australian cockatoos in the early 1970s [73]. Because of the characteristic feather loss and abnormal beak associated with this disease, the disease was named Psittacine beak and feather disease (PBFD).

PBFD is found in many countries, including Australia [48], Germany [78], Italy [6], New Zealand [86], South Africa [31], Taiwan [18, 36], Thailand [49], and the U.S.A. [18]. Reported viral DNA positive rates in recent years are 23.0% in Australia [48], 40.4% in Germany [78], 8.0% in Italy [6], 41.2% in Taiwan [36], and 3.5 to 4.0% in the U.S.A. [18]. In Japan, PBFD has been reported in many psittacine species [70], with an overall positive rate of 18.5% [89]. At present, PBFD is the most important viral infection in psittacine birds in Japan as well as other countries.

Beak and feather disease virus (BFDV), the causative agent of PBFD, belongs to the family Circoviridae. The BFDV virion is isosahedral and nonenveloped with a diameter of 14–17 nm [84]. It carries a single-stranded ambisense circular DNA with a complete genome size of approximately 2 kb [84]. BFDV genome DNA has two major open reading frames (ORFs), which encode a replication associated protein (ORF V1 or Rep) and a capsid protein (ORF C1 or CP) [2, 3, 65]. A third ORF (ORF V2) has been suggested, although its function is unknown.

PBFD has been confirmed in over 60 species of both free-ranging and captive psittacine birds [48, 101]. This disease has peracute, acute, and chronic forms. Sudden death occurs in peracute and acute forms. The chronic form is characterized by progressive symmetric feather dystrophy and loss and by beak deformities [73]. Feather dystrophy is due to necrosis and hyperplasia of epithelial cells [6, 104]. Beak deformities are not always present and seem to occur in specific species or are dependent on other factors [84].
Histopathological examinations have revealed basophilic intranuclear and intracytoplasmic inclusion bodies within infected feather epithelial cells or macrophages (Fig. 1A) [37, 73]. This disease does not always cause death. BFDV-induced immunosuppression causes secondary infections, which are the cause of death in most infected birds [84].

In the case of progressive feather loss, PBFD should be suspected. However, visible feather changes grossly similar to those caused by BFDV can be induced by any number of factors, including APV, PsAdV, trauma, bacterial folliculitis, malnutrition, endocrine abnormalities, and drug reactions to penicillins and cephalosporins. Specific assays for BFDV infection have been developed. These include in situ hybridization [79], hemagglutination and hemagglutination inhibition [83], electron microscopy [84], PCR [70, 109], and real-time PCR [45, 82]. Among them, PCR is a very sensitive, specific, and rapid tool for detection of viruses.

*Avian polyomavirus infection*: APV infection causes acute death, abdominal distention, and feather abnormalities known as “French molt” in fledging and young budgerigars [34]. It also causes a loss of down feathers on the back and abdomen, filoplumes on the head and neck, and subcutaneous hemorrhage of nesting budgerigars [5, 16]. APV infections have also been detected in other psittacine bird species and in other avian orders. In these cases, APV causes clinical signs similar to those observed in budgerigars. However, the degree of susceptibility for and severity of the diseases seems to be dependent on the species infected [19]. Pathologic examinations have found hydropericardium, enlarged heart, swollen liver, congested kidneys, and hemorrhage within the body cavities [5, 16]. Histopathological findings have revealed large and slightly basophilic nuclear inclusion bodies in various tissues, especially in the spleen, liver, and kidneys (Fig. 1B) [5, 16].

This disease has been observed in Canada [5], China [51], Australia [71], Germany [95], Slovakia [55], Italy [6], and Taiwan [36]. In Italy and Taiwan, the viral DNA positive rates were 0.8% and 15.2%, respectively. In Japan, APV infections have been reported in several bird species, such as budgerigar, black-headed caique (*Pionites melanocephala*) and eclectus parrot (*Eclectus roratus*) [34, 47], with an overall DNA positive rate of 2.7% [69].

Polyomaviruses are widely distributed among mammalian and avian species. To date, four polyomaviruses in birds are known, namely avian polyomavirus (APV), goose hemorrhagic polyomavirus (GHPV), finch polyomavirus (FPyV) and crow polyomavirus (CPyV) [9, 30, 43]. In the early 1980s, the first bird polyomavirus, which was isolated from budgerigars (*Melopsittacus undulatus*) [16], was then designated as budgerigar fledgling disease polyomavirus by the International Committee on Taxonomy of Viruses (ICTV) [5, 9]. However, it is now termed APV because of its broad host range [40]. The other three bird polyomaviruses have not been reported in psittacine birds.

The APV virion is icosahedral and nonenveloped with a diameter 45–50 nm [9]. The APV genome is a circular double-stranded DNA, 4,981 bp in size, and forms a chromosome-like structure with cellular histones [88]. Functionally, the APV genome can be divided into early and late gene-coding regions. The early region encodes both large tumor antigen and a small tumor antigen by analogy with mammalian polyomaviruses. The late region in APV encodes a major structural protein, VP1, and three minor structural proteins, VP2, VP3, and VP4 [41]. The outer shell of the virion is composed of VP1. The three minor proteins are also present in the viral capsid [41]. A single amino acid at position 221 in VP2 has been reported to be a key element for propagating in cell cultures derived from several avian species [47, 94]. VP4 is a structural protein specific to polyomaviruses isolated from avian species and has multiple functions such as interaction with VP1 and double-stranded DNA and induction of apoptosis [42].
Clinical changes that are suggestive of APV infections include acute death or development of feather abnormalities. However, these changes can also be associated with other infectious and non-infectious diseases. Recently, APV has been identified by immunofluorescent antibody staining [28], in situ hybridization [79], electron microscopy [34], virus-neutralization test [74], PCR [70, 75], and real-time PCR [45].

Psittacid herpesvirus infection: In 1929, Brazilian veterinarian Pacheco encountered an outbreak of acute, fatal hepatitis with intranuclear inclusion bodies in psittacine birds in Brazil. This syndrome became known as Pacheco’s disease (PD). In 1975, an avian herpesvirus, later named psittacid herpesvirus type 1 (PsHV-1), was confirmed to cause PD [92]. The usual clinical history for a bird of normal appearance is to be found dead in its enclosure without a prodrome. If still alive, clinical signs may include depression, anorexia, diarrhea, tremors, and instability [91, 92]. Because the virus kills the birds very rapidly, affected birds may show no gross lesion but may show abnormal changes in the liver, spleen, kidneys, and intestines. Histopathological findings associated with PD include necrotizing lesions in many organs, as well as hemorrhage and congestion of the liver, spleen, and kidneys. Intranuclear inclusion bodies (Cowdry type A) are most commonly found in the liver, but have also been demonstrated in the kidneys, spleen, pancreas, and small intestines (Fig. 1C) [105]. PD has been observed in the U.S.A. [60, 92], UK [25], Spain [22], Kenya [44], South Africa [35], and Japan [105].

PsHV-1 has also been suggested to have an etiological role in the development of tumors, as specific PsHV-1 genome sequences have been repeatedly detected in mucosal papillomas from parrots [39, 64, 96]. Recently, another type of psittacid herpesvirus, PsHV-2, has been identified in three African grey parrots. However, the prevalence and pathogenicity of PsHV-2 is, as yet, unclear [97,
The adenovirus genome is 163,025 bp in length and contains 73 predicted ORFs [100]. PsHV-1 is closely related to infectious laryngotracheitis virus but distinct from Marek’s disease virus and herpesvirus of turkey. Thus it is proposed to belong to the Iltovirus genus. PsHV-1 has been classified into 4 genotypes on the basis of the UL16 gene sequence. These genotypes show distinct biological characteristics and the potential to cause PD [102]. On the other hand, PsHV-1 genotypes 1, 2, and 3, but not 4 have been found in mucosal papillomas [96]. The genotypes detected in PD-affected birds are biased according to bird species and their geographic origins. Therefore, specific PsHV-1 genotypes may be the cause of PD in certain species. Amazon parrots are those most commonly diagnosed with PD and have been identified with all 4 PsHV-1 genotypes. The 4 PsHV-1 genotypes were also found among birds from the Pacific region such as cockatiels and cockatoos. PsHV-1 genotypes found in African grey parrots include genotypes 2, 3, and 4, but not genotype 1. In the case of macaws, genotype 4 has been commonly found, while genotype 3 is rarely found and genotypes 1 and 2 have not been found [102].

PD should be suspected in any bird that dies suddenly without clinical signs. However, bacterial hepatitis, lead poisoning, APV, PsAdV, and reoviruses can all cause clinical and gross changes similar to those noted with PD. Additionally, the intranuclear inclusion bodies induced by PsHV-1 can appear under the microscope similar to those caused by APV and PsAdV. Thus, confirmation that a bird has died as yet, unclear.

Psittacine adenovirus infection: Adenovirus infections in psittacine birds have been associated with depression, anorexia, diarrhea, and cloacal hemorrhage [56, 62, 72]. Adeno-virus outbreaks have also been described as having a clinical progression similar to PsHV-1 infection, in which normal-appearing birds are found dead in their enclosures [59, 90]. Gross lesions associated with adenovirus infections in various birds include hepatomegaly, splenomegaly, dilatation of the duodenum and proventriculus, swollen kidneys as well as edema, congestion, and hemorrhage of the lungs [23, 79, 80]. Enlarged friable livers may be hemorrhagic, pale or mottled. Liver lesions are the most consistent gross change in infected birds. Basophilic intranuclear inclusion bodies are routinely seen in association with necrosis in the liver and spleen (Fig. 1D) [62].

Adenoviruses (AdVs) have been isolated from a wide range of vertebrates including mammals, birds, reptiles, amphibians, and fishes [4]. The classification of adenoviruses has been recently revised [4] and avian adenoviruses are now classified into three genera: Aviadenovirus, Atadenovirus, and Siadenovirus. Most of the avian adenoviruses that have been characterized are classified into the genus Aviadenovirus.

The adenovirus genome is a linear, double-stranded DNA and is estimated to be 25–45 kbp in length. The Aviadenovirus fowl adenovirus CELO strain, is 43,804 bp in length, TAdV-3 26,263 bp in length, and EDSV 33,213 bp in length [11, 32, 77]. The adenovirus is nonenveloped and has an icosahedral capsid with a diameter of 70 nm. The capsid is made up of 2 types of capsomeres: 12 vertex capsomeres composed of a fiber attachment protein and its penton base, and 240 hexons [15]. The hexon protein is the major viral capsid protein of the adenovirus [66] and consists of 2 functional components: the conserved pedestal regions P1 and P2, and the variable loops L1-L4 [1, 87]. L1, L2, and L4 are located at the surface of the hexon protein and interact with the immune response of the host. The hexon protein is known, therefore, to possess family-, genus-, species-, and type-specific determinants [15, 54]. The hexon gene has been used for the phylogenetic study of adenoviruses [17, 52].

Adenovirus infections in psittacine birds have been identified on the basis of microscopic studies but not by clinical diagnosis. Infections with the adenovirus [10] or adenovirus-like particles [21, 57, 62, 72] have been described in a variety of psittacine birds including budgerigars (Melopsittacus undulatus), macaws (Ara spp.), Amazon parrots (Amazona spp.) and cockatoos (Cacatua spp.). However, little is known about the genomic organization of adenoviruses infecting psittacine birds. Recently, an adenovirus was detected by PCR in Senegal parrots (Poicephalus senegalus) showing clinical and pathological signs of adenovirus infection. The adenovirus was subsequently identified by its hexon gene sequence as a new avian adenovirus belonging to Aviadenovirus [81]. Designation of this virus as psittacine adenovirus (PsAdV) was approved by the ICTV [81].

Very recently, we identified a new adenovirus belonging to Siadenovirus in budgerigars showing ruffled feathers, named as Budgerigar Adenovirus type 1 (BuAdV-1) [46]. However, the prevalence and pathogenicity of BuAdV-1 is, as yet, unclear.

Psittacine poxvirus infection: Avian poxvirus infections have been observed in more than 230 of the known 9,000 species of birds, spanning 23 orders [38]. Avian poxvirus infections in psittacine birds (psittacine poxvirus (PsPoV) infections) have been reported in several psittacine species, especially Amazon and pionus parrots [8, 58], although this disease has never been reported in Japan. Avian poxviruses are all classified in the genus Avipoxvirus of the family Poxviridae with the subfamily Chordopoxvirinae. In common with other poxviruses, they contain a double stranded DNA genome, ranging from 230 to >300 kbp. Based on phylogenetic studies of the fpv167 locus, which encodes orthologues of vaccinia virus core protein P4b, PsPoV located in a distinct cluster from the avian poxvirus derived from other avian species [38].

The earliest clinical changes in affected birds are characterized by a serous ocular discharge, rhinitis, and conjunctivitis, followed by the appearance of ulcerations on the eyelid margins [8, 58]. Dry crusty lesions are noted on the lid margins and the lateral and medial canthi of the eyes.
PsPoV has been shown to vary in virulence in different hosts. Amazon parrots appear to be most susceptible to PsPoV infections. Infected Amazon parrots may also develop a severe upper respiratory tract disease. Mortality rates are highest when diphtheritic lesions cause defects in the mucosal barrier of the alimentary and respiratory tract, allowing secondary bacterial, fungal, or chlamydial organisms unrestricted access to the affected birds [8, 27, 58]. Histopathological lesions include necrosis of the heart and liver, as well as air sacculitis, pneumonia, peritonitis, and accumulation of necrotic debris on the surface of the alimentary tract. Characteristic intracytoplasmic inclusion bodies (Bollinger bodies) may be noted in lesions in the mucosa of the sinuses, trachea, crop, esophagus or throat [24].

The clinical changes associated with the cutaneous form of PsPoV are often suggestive. However, trauma and fugal, bacterial, and papillomavirus infection can cause similar lesions. Thus, recently, PsPoV infection was confirmed by PCR [38]. In other bird species, virus neutralization, ELISA, and haemagglutination-inhibition test are also used to diagnose of poxvirus infection [12, 63].

Psittacine papillomavirus infection: Internal papillomatosis of parrots (IPP) is a tumor disease characterized by progressive development of papillomas in the oral and cloacal mucosa [98]. Papillomatosis of the cloaca has been described as the appearance of large, raised, distinct masses or small, coalescing bumps that cover much of the cloacal mucosa. These lesions may cause a local inflammatory response and secondary infections of bacteria or fungi. In addition, papillomatosis of the oral cavity or esophagus may mechanically obstruct the movement of food, causing anorexia, chronic weight loss or vomiting. Birds with papillomatosis tend to develop neoplasias of the pancreas or liver [13]. Papillomatosis lesions are characterized by proliferation of epithelial cells on thin fibrovascular stalks [98]. Histopathological and microbiological studies suggest that papillomaviruses are one of the etiologic agents of IPP [68, 98]. Papillomaviruses are a large group of pathogens that cause epithelial proliferations in a wide spectrum of vertebrate species. Several genetic analyses of avian papillomaviruses have been reported [61, 99, 107]. The papillomavirus isolated from a cutaneous lesion of an African grey parrot (Psittacus erithacus) was suggested to be phylogenetically related to another papillomavirus derived from the chaffinch (Fringilla coelebs) [99]. A herpesvirus also has been suggested to be involved in the etiology of IPP [39].

Papillomatosis is diagnosed by histological examination of biopsy samples. The etiological agents of papillomatosis have been identified by PCR and in situ hybridization [39, 53].

Problems and proposals: A common problem of these DNA virus infections is that there are few etiological treatments and no efficient vaccines available at present. Thus, the main care for these diseases is symptomatic treatment and preventive therapy for bacterial secondary infection. Although only acyclovir has been shown to reduce the sickness and death of PD-affected birds, it is also associated with kidney damage in some species and it can not prevent acute death [67]. Chicken interferon-γ has been reported to alleviate manifestations of PBFD-affected African grey parrots [93].

The baculovirus-expressed recombinant BFDV capsid protein was recently reported to be immunogenic and might be a suitable candidate vaccine to prevent PBFD in psittacine birds [7]. However, an optimal vaccination regime needs to be determined to protect against PBFD. Poxvirus vaccines are available for use in several avian species, including chickens, pigeons, turkeys, quail, canaries, and psittacine birds. However, poxvirus that infects psittacine birds is serologically unrelated to other poxviruses [108]. Thus, effective prevention against PsPoV requires species-specific vaccines. Commercial vaccines for APV and PsHV-1 infection are available in U.S.A., but not in Japan [20, 85].

The goal of maintaining any bird in captivity is to keep the birds in best possible condition. In veterinary medicine, vaccination plays a major role in preventing viral infections. Therefore, effective and safe vaccines for these virus infections are urgently needed.

ACKNOWLEDGMENTS. We thank Dr. Toshiaki Masegi, Dr. Tokuma Yanai, and Dr. Hiroki Sakai, Laboratory of Veterinary pathology, Gifu University, for providing the histopathological photographs.

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