Histopathological study on dual infections of adenovirus and papovavirus in budgerigars (*Melopsittacus undulatus*)

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SUMMARY

Of a total of 293 budgerigars (*Melopsittacus undulatus*) examined histologically, 45 birds (15.4%) had dual infections with adenovirus and papovavirus. Both viruses induced intranuclear inclusion bodies in the kidney and rarely in other organs. The renal tubular epithelium was the target site for both viruses. These inclusion bodies were different in size and stainability. The adenoviral inclusions were very large and deeply basophilic or eosinophilic, whereas the papovaviral inclusions were large and clear or slightly basophilic. Ultrastructural examination of very large basophilic inclusions in the kidneys revealed viral particles typical of adenovirus. The very large eosinophilic inclusions consisted of only fine granular and filamentous material. Papovavirus particles were frequently found in the slightly basophilic intranuclear inclusions, but none was demonstrated in the clear inclusions. The dual infections in this study were regarded as latent infections because there was little or no tissue damage in the affected tissues.

INTRODUCTION

Adenoviruses are recognized as important pathogens of poultry and have also been reported in Common murre (Lowenstine & Fry, 1985), cockatiels (Scott et al., 1986), lovebirds (Pass, 1987), pigeons (Goryo et al., 1988), merlins (Schelling et al., 1989), budgerigars, rosellas and other psittacine birds (Mori et al., 1989). Infection involves a variety of organs including the liver, kidney, small intestine and pancreas.

A generalized inclusion body disease with a high mortality caused by papovavirus has been described in fledgling budgerigars (Bernier et al., 1981; Davis et al., 1981), finches (Johnston & Riddell, 1986; Forshaw et al., 1988), and lovebirds (Pass, 1985). Infection of renal tubular epithelial cells with papovavirus...
without involvement of other organs has been described in splendid parakeets (Pass, 1987).

Concurrent infections with adenovirus and papovavirus have never been reported in birds and mammals. This article describes the histopathological and electron microscopic findings of infection with both viruses in budgerigars.

MATERIALS AND METHODS

Histological examination was performed on 293 young adult and adult budgerigars (*Melopsittacus undulatus*) collected from pet shops. These birds were discarded because of illthrift. Of them, 118 birds were diagnosed as having adenovirus infection, and 91 as having papovavirus infection based on the presence of typical intranuclear inclusion bodies (IIB) in the affected organs. Among these cases, 45 birds (15.4%) with dual infections were used for this study.

For histopathological examination, tissue samples composed of the brain, heart, trachea, lung, air sac, digestive tract, liver, kidney, spleen, thymus, bursa of Fabricius, parathyroid, thyroid, adrenal, bone marrow, genital organs, eye, muscle and skin were fixed in 10% formalin and processed routinely for paraffin embedding, and sections were stained with haematoxylin and eosin (HE).

For electron microscopic examination, selected formalin-fixed kidneys were post-fixed in 1% osmium tetroxide and embedded in Quetol 812. Ultra-thin sections were stained with uranyl acetate and lead citrate, and examined in a JEM-100SX electron microscope.

RESULTS

All the birds examined had both IIB characteristic of adenovirus and papovavirus infections in the same kidney. Although some cases were abundant in adenoviral IIB (Figure 1), others had predominantly papovaviral inclusions (Figure 2). The adenoviral IIB were large and surrounded by marginated chromatin. The inclusions were either basophilic or eosinophilic. Most were basophilic and appeared as a diffusely homogeneous mass or stippled basophilic granules. There was no halo between the inclusion body and nuclear membrane. The size of the IIB varied from 2 to 4 times (large type) to 5 to 10 times (very large) that of the normal nuclei of the original cells.

The adenoviral IIB occurred mostly in the epithelial cells of the collecting ductules (39 cases; 87.7%) and distal convoluted tubules (15 cases; 33.3%), and less frequently in the proximal convoluted tubules (four cases; 8.9%). There were no IIB in the other cells of the kidney, but one bird had similar IIB in the crypt epithelium of the small intestine.

The papovavirus IIB were large and clear or slightly basophilic and were surrounded by marginted chromatin. The inclusions enlarged nuclei to 2 to 4 times that of normal nuclei. The IIB were not surrounded by a clear halo. These IIB were detected in the collecting ductules (28 cases; 62.2%), proximal tubules...
Figure 1. Many adenoviral (arrowheads) and one papovaviral IIB (arrow) are simultaneously found in the kidney. The adenoviral type is very large and basophilic, while the papovaviral type is smaller and clear. HE stain. Bar = 30 μm.

(17 cases; 37.8%) and distal convoluted tubules (11 cases; 24.4%). Occasional IIB were found in the interstitial cells (two cases) and the endothelial cells of blood vessels (one case) in the kidney. Focal infiltrates of lymphocytes and plasma cells were present in the renal interstitium (12 cases) and spleen (40 cases).

Figure 2. An adenoviral (arrow) and many papovaviral IIB are present in the same kidney. The papovaviral IIB consist of clear (C) and slightly basophilic (B) types. HE stain. Bar = 30 μm.
Papovaviral IIB occurred occasionally in other organs such as bone marrow (three cases), skin (two cases), eye (two cases) and adrenal gland (one case), and were unaccompanied by tissue damage and cellular reaction.

Electron-microscopically, the very large basophilic IIB in the kidney were composed of numerous adenovirus-like particles embedded in a fine granular material. These particles were round or hexagonal in shape, ranging from 57 to 88 nm in diameter, and sometimes aggregated in crystalline arrays (Figure 3). The very large and large eosinophilic IIB consisted of fine granular and filamentous material. Virus particles were not seen in these inclusions.

In the case of the slightly basophilic IIB in the kidney, many papovavirus-like particles were present in the nucleus and occasionally in the cytoplasm (Figure 4). These virus particles were spherical in shape and ranged from 33 to 55 nm in diameter. However, the clear IIB were composed of a fine granular matrix surrounded by margined nuclear chromatin.

Complicating infections with other pathogens were uncommon in this study. These included giardiasis in the small intestine (15 cases; 33.3%), megabacterial proventriculitis (eight cases; 17.8%), chlamydiosis in liver (seven cases; 15.6%), and candidiasis in crop (five cases).

**DISCUSSION**

The present study indicated that dual infections with adenovirus and papovavirus were highly prevalent in the budgerigars examined here (15.4%). Although all the birds had both viral IIB in the same kidney, the inclusions were quite different in...
size and stainability. Adenovirus induced larger eosinophilic and deeply basophilic IIB, while papovaviral IIB were smaller and clear or slightly basophilic. Electron-microscopically, the very large basophilic IIB contained adenovirus particles, and the large slightly basophilic IIB contained papovavirus particles. There were no viral particles in the eosinophilic and clear IIB.

Adenoviral inclusion bodies are often found in healthy birds and appear to be less significant as a cause of illness or death (Hunter et al., 1979; Lowenstine & Fry, 1985; Mori et al., 1989) than papovaviruses which often produce systemic infection and a high mortality (Bernier et al., 1981; Davis et al., 1981; Hirai et al., 1984; Randall et al., 1987; Tsai, 1987). The papovaviral IIB and the related lesions in the young adult and adult budgerigars described here were quite different from those described previously in naturally infected nestlings. However, a serological survey has indicated that papovaviruses appear to infect a wide range of captive adult psittacines without causing obvious signs (Wainright et al., 1987). The difference between our observations and the previous findings of high mortality in nestling budgerigars may be attributed to age-related susceptibility to the virus, the immunological status of the birds or different serotypes of the virus.

It is unknown why the IIB of the both viruses were common in the renal tissues and scarce in the other organs of the birds reported here. The birds appear to have latent infections with these viruses as illness and tissue damage could not be attributed to them. Disease may be stimulated by environmental stress, concurrent disease or immunosuppression.
The role that renal excretion plays in the spread of infection is not known, but one would expect it to be a major means of spread with these two viruses.

REFERENCES


RESUME

Etude histopathologique d'une infection double à adénovirus et papovavirus chez des perruches (Melopsittacus undulatus)

Sur un total de 293 perruches (Melopsittacus undulatus) examinées histologiquement, 45 oiseaux (15,4%) présentaient une infection double à adénovirus et papovavirus. Les deux virus induisaient des corps d'inclusion intranucléaire dans le rein et rarement dans les autres organes. L'épithélium tubulaire rénal était la cible des deux virus. Ces corps d'inclusion étaient différents en taille et coloration. Les inclussions adénovirales étaient très larges et très basophiles ou éosinophiles alors que les inclusions papovavirales étaient larges et claires ou légèrement basophiles. L'examen ultrastructural des très larges inclusions basophiles dans le rein a révélé la présence typique de particules virales d'adénovirus. Les très larges inclusions éosinophiles consistaient seulement en un matériel granulaire et filamentueux fin. Les particules de papovavirus étaient souvent mises en évidence dans les inclusions intra nucléaires légèrement basophiles mais aucune n'était présente dans les inclusions claires. La double infection dans
cette étude est considérée comme latente en raison du peu ou de l’absence de lésions dans les tissus affectés.

**ZUSAMMENFASSUNG**

*Histopathologische Untersuchung über Doppelinfektionen mit Adenovirus und Papovavirus bei Wellensittichen (Melopsittacus undulatus)*


**RESUMEN**

*Estudio histopatológico de infecciones dobles con adenovirus y papovavirus en periquitos*

De un total de 293 periquitos (*Melopsittacus undulatus*) examinados histológicamente, 45 aves (15.4%) presentaron una infección doble por adenovirus y papovavirus. Ambos virus produjeron cuerpos de inclusión intranucleares en el riñón y en raras ocasiones en otros órganos. El epitelio tubular renal fue la célula blanco para ambos virus. Los cuerpos de inclusión presentaron diferencias en cuanto a su tamaño y carácter tintorial. Las inclusiones de adenovirus eran muy grandes e intensamente basófilas o eosinófilas mientras que las inclusiones de papovavirus eran grandes y pálidas o ligeramente basófilas. El examen ultraestructural de inclusiones basofilicas muy grandes en los riñones mostró partículas virales típicas de adenovirus. Las inclusiones muy grandes eosinófilas consistían únicamente en material finamente granular y filamentoso. Partículas de papovavirus fueron frecuentes en inclusiones intranucleares ligeramente basófilas pero no se observaron en las inclusiones pálidas. Se consideró que estas infecciones dobles eran latentes debido a que se observó poco daño tisular o éste fue inexistente.