Respiratory Herpesvirus Infection in a Parakeet

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Introduction

This report documents a case of chronic proliferative parabronchitis and airsacculitis in an Indian ring-necked parakeet. Only limited information is available on this infection, both in Australia and internationally.

A female Indian ring-necked parakeet (*Psittacula krameri manillensis*) was purchased from a Sydney pet shop at 4 to 5 months of age. Two days post-purchase, it was presented with unknown clinical signs to a veterinarian who diagnosed chlamydiosis (the method of diagnosis is also unknown) and prescribed a 45 day course of doxycycline. Representation occurred shortly afterwards for dyspnoea. Approximately two months after purchase, the parakeet was presented to a specialist avian practitioner who detected severely laboured respiration at rest, increased lung sounds, sneezing and nasal exudate. A provisional diagnosis of severe pneumonia and probable airsacculitis was made. Early Psittacine Beak and Feather Disease was also suspected on the basis of abnormal feathering, including fault lines across the tail feathers, abnormally small stump feathers over the hips and presence of unopened quills over the head. Irregular ridges were also present over the upper beak.

The bird was prescribed enrofloxacin but the owner was reluctant to implement the recommended nebulisation therapy. The bird represented within 24 hours with exacerbated dyspnoea, and nebulisation with gentamicin in saline was undertaken by the veterinarian, with a slight improvement in the degree of dyspnoea noted. A guarded prognosis was nevertheless warranted and the bird died whilst thoracic radiographs were being taken.

The veterinarian necropsied the bird and described hepatosplenomegaly, generalised airsacculitis with caseonecrotic exudate over the surface and luminal frothy exudate, grey consolidation and oedema of virtually the entire lung fields and pink froth within the tracheal lumen.

Light microscopic examination revealed severe diffuse epithelial hyperplasia of the thoracic and abdominal air sacs, with the lining cells often tall columnar with cytoplasmic basophilia. Karyomegaly and nuclear vesiculation were evident in many of the hyperplastic cells. Numerous protruberant epithelial syncytia were present, often containing 20 to 30 or more nuclei and voluminous basophilic floccular cytoplasm. Both the syncytial and hyperplastic uninucleate cells contained intranuclear inclusions. These were pleomorphic, varying from sold eosinophilic bodies surrounded by a clear halo, with nuclear membranes accentuated by chromatin margination, through to amphophilic or basophilic bodies that filled the entire nucleus. The submucosa of the air sacs was thickened by fibrous connective tissue, with still active fibroplasia, and by moderate numbers of infiltrating macrophages, lymphocytes and plasma cells, with fewer heterophils. There was multifocal inflammatory lymphoid follicle formation in the submucosa.

Comparable pathology was present in the lung, with severe hyperplasia, cytoplasmic basophilia, syncytia formation and numerous intranuclear inclusion bodies particularly in the parabronchial (and to a lesser

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extent secondary bronchial) epithelium, which also showed focal squamous metaplasia. Where syncytia and necrotic epithelium had detached into the parabronchial lumina, there was mild mucin and heterophilic exudation. The secondary bronchial and parabronchial submucosa and the interstitium of adjacent atria and air capillaries were thickened by oedema fluid with reactive fibroplasia/fibrosis and mild mononuclear leukocytic infiltrates with perivascular lymphoid cuffing. Mesobronchi were inconsistently and less severely involved than the parabronchi and secondary bronchi. In both the air sacs and parabronchi, virus-infected uninucleate and syncytial epithelial cells frequently displayed retention of cilia, and necrosis, although present, was not a prominent feature.

No chlamydial elementary bodies were identifiable intracellularly in Machiavello-stained sections of air sacs and lungs.

Other lesions included mild chronic active nonsuppurative interstitial nephritis, mild splenic lymphoid depletion with haemosiderosis, mild segmental enteritis, mild nonsuppurative proventriculitis and superficial candidiasis of the crop. No viral inclusion bodies were identifiable in these sites. Samples of dystrophic powder down revealed typical lesions of circovirus infection (Psittacine Beak and Feather Disease), with random necrosis of both follicular and feather epithelium, basophilic intranuclear inclusions in the epithelial cells especially of the feather epithelium, and similar basophilic inclusions and debris within the cytoplasm of feather pulp macrophages.

The syncytia and intranuclear inclusions in the parabronchi and air sacs were strongly reminiscent of the lesions of infectious laryngotracheitis of domestic poultry. A diagnosis of respiratory herpesvirus infection was made on morphological grounds.

Respiratory herpesvirus infection in psittacines was first reported in 1980 in the USA, following the sudden death of a Bourke's parrot (*Neophema bourkii*) (the bird was termed a Bourke's parakeet in the report)[12]. The report added to the long list of pathological manifestations of herpesvirus infections in birds (Table 1). Necropsy lesions in the parrot were pneumonic consolidation of the caudal lungs and mild airsacculitis. Parabronchial epithelial segmental necrosis, cuboidal metaplasia, syncytia formation, intranuclear inclusion bodies and nonsuppurative inflammation with lymphoid aggregates were the chief microscopic lesions in the lungs. Although viral isolation attempts from lung proved negative, electron microscopy revealed 90 nm diameter intranuclear viral particles with a 45 nm electron-dense core and enveloped 150 nm viral particles in the cytoplasm of infected cells, consistent with herpesvirus[12].

The most thorough study to date on respiratory herpesvirus infection of psittacines is that of Tsai *et al* on a series of 14 parakeets (*Psittacula krameri manillensis*) introduced as pet birds to Japan from India and dying within two weeks of quarantine[29]. The consistent lesions in these birds were syncytial formation and both eosinophilic and basophilic intranuclear inclusions in particularly ciliated epithelial cells of the air sacs and of the bronchi of the lungs, particularly the secondary bronchi. Necrosis and desquamation of infected cells were observed but usually only a minimal leukocytic response. One of the birds also had inclusion bodies in the tracheal epithelium. Concurrent infections in the parakeets were common - 13/14 birds had chlamydiosis, 6/14 aspergillosis and 3/14 candidiasis[29].

Electron microscopy revealed herpesvirus-like particles in the syncytia and uninucleate bronchial epithelial cells and also occasionally within endothelial cells of respiratory blood vessels. Naked viral particles were present in both the nucleus and cytoplasm, with enveloped particles in the cytoplasm of one of six birds examined. The viral cores measured 40 to 55 nm in diameter and were described as of variably dense, partial or clear appearance. Naked and enveloped particles measured 88 to 110 nm and 125 to 164 nm in diameter respectively. Five of the six birds showed intranuclear tubular viral structures with cross striations, a central clear core, an inner diameter of 32 to 46 nm and an outer diameter of 48 to 74 nm[29].

The initial report of herpesvirus infection in a Bourke's parrot suggested that the virus had a tropism for the lung and might have been an aberrant strain of the infectious laryngotracheitis (ILT) virus[12]. Although

the most dramatic lesions of ILT infection occur in the upper respiratory tract (nasal cavity, infraorbital sinuses, larynx and trachea) and the conjunctiva, proliferative or exudative airsacculitis, bronchitis (at primary, secondary and tertiary levels) and pneumonia, with syncytial and intranuclear inclusion body formation, are also a common feature of the natural disease and of experimental infection via deep aerosol rather than nasal inoculation[22,28]. Moreover, so-called Amazon-tracheitis herpesvirus, which is capable of producing a severe necrotohaemorrhagic to diphtheritic laryngotracheitis in psittacines (to date chiefly reported in the genus *Amazona*), shows group-specific serological relation to the ILT virus and is currently regarded as a mutant of that virus[11,33].

However, the electron microscopic findings reported by Tsai *et al* indicate that the herpesvirus in the lower respiratory tract of parakeets is distinct from that of ILT[29]. The parakeet virus appears to have a strong tropism for the ciliated epithelium of the lower respiratory tract and does not provoke a severe inflammatory response. ILT infections are characterised by severe haemorrhagic to fibrinonecrotising inflammation particularly of the upper respiratory tract, with formation of opaque viral cores within intranuclear tubular structues[22,31]. In contrast, intranuclear tubular structures in infected parakeets have a clear core (Table 2)[29].

The herpesvirus responsible for Pacheco's disease of parrots can also produce intranuclear inclusions and syncytia in epithelial cells of the distal bronchi and of the air sacs[11,21,24,29]. Lung, trachea and air sacs from infected birds are also useful sites from which to obtain infective material for chick embryo and cell culture line inoculation for viral isolation and identification[19,21].

However, Pacheco's disease is a systemic infection, akin to herpesvirus infections of pigeons, owls and raptors[1,4,10,11,23,27,29]. Both the pigeon and falcon herpesviruses appear to be transmissible to psittacine species[1,2], and both viruses are capable of mimicking the lesions of Pacheco's disease in inoculated psittacines[11]. Hepatic parenchymal syncytial formation with intranuclear inclusion bodies and multifocal coagulative necrosis are characteristic and usually predominant lesions in Pacheco's disease, overshadowing any bronchial or pulmonary lesions. Hepatocellular necrosis is similarly a dominant feature of herpesvirus infections in pigeons and falcons[5,14,18,26,30], although interstitial pneumonia and airsacculitis are also reported in infected pigeons[25]. Multifocal splenic necrosis is also typical of the alphaherpesvirus infections of birds, and renal and pancreatic necrosis and a necrotising to haemorrhagic enteritis are also common lesions in Pacheco's disease[1,10,11,21].

Syncytia and inclusion bodies have been described in parathyroid chief cells, thyroid follicular epithelium, intestinal crypt epithelium, reticulum cells of bone marrow and adrenal glands, ovarian germinal epithelium, cloaca, pancreas, endothelium, bile ductules, larynx, trachea, bronchi, thymus and bursa of Fabricius in Pacheco's disease, with inclusions but no syncytia present in the spleen[29]. Hence, the truly systemic nature of these infections contrasts with the lower respiratory tract tropism of the herpesvirus reported in parakeets and *Neophema* species.

Both Pacheco's disease virus and the respiratory herpesvirus produce tubular viral structures with a clear core. However, those of Pacheco's disease have been reported only in the cytoplasm (of hepatocytes) in a small proportion of affected birds, those of the respiratory virus only in the nuclei of bronchial epithelium in most birds examined by electron microscopy (Table 2)[29]. Therefore, on morphological grounds and tissue tropism, the infections appear to be distinct. Moreover, Pacheco's disease virus inoculated into parakeets experimentally produces a typical systemic infection with prominent hepatic lesions[20]. However, thorough definition of the respiratory herpesvirus' identity still awaits virus isolation and attempts at neutralisation with specific antisera to determine if it is a truly independent virus[10,29].

The herpesvirus expands the list of viral agents that are potential respiratory pathogens in psittacines (Table 3). Of these, respiratory adenovirus infection may produce a proliferative bronchitis and interstitial pneumonia resembling herpesvirus infection.

Respiratory adenovirus infection was first diagnosed in 1990 in an adult female rose-ringed parakeet (*Psittacula krameri*) in Belgium[6]. The bird had died suddenly within 24 hours of purchase, with only excitation and panting noted prior to death. Splenomegaly, hyperaemia and consolidation of the lungs and fibrinous thickening of the air sacs were noted at necropsy and an interstitial pneumonia and proliferative bronchitis on histopathology. Parabronchi were chiefly affected, with bronchial epithelial hyperplasia, necrosis and nonsuppurative inflammation. Bronchial epithelial cells contained amphophilic intranuclear inclusions with karyomegaly, nuclear chromatin margination, cytoplasmic eosinophilia and degenerative vacuolation. Viral particles typical of adenovirus were identified in the nuclei, rarely in the cytoplasm, on electron microscopy. The particles often formed crystalline lattices, displayed icosahedral symmetry, and consisted of a hollow or electron dense core. The diameter of the capsids was approximately 80 nm and, unlike herpesviruses, there was no evidence of budding at the nuclear membranes and hence no viral envelope formation[6].

The bird also had chlamydiosis and it was surmised that the concurrent infection, possibly exacerbated by the stress of relocation after purchase, may have favoured replication of the adenovirus, possibly by activation of a latent infection[6].

The report of respiratory adenovirus infection in the parakeet did not describe epithelial syncytia formation in the lungs or virus-induced lesions in the air sacs. The airsacculitis noted at necropsy was presumably referable to chlamydiosis. Therefore, there appear to be morphological differences between adenovirus and herpesvirus infections in parakeets that could permit tentative differentiation at the light microscopic level.

There are too few reports of respiratory herpesvirus infection to define the range of psittacine species susceptible to infection. In addition to the original description in a Bourke's parrot and the series of Indian ring-necked parakeets[12,29], there are independent descriptions of proliferative tracheobronchitis with intranuclear inclusion bodies and herpesvirus particles on electron microscopy in the *Neophema* genus of small grass parrots native to Australia[8,10,15,16]. Infected birds displayed respiratory signs and some also showed such central nervous signs as stargazing, torticollis and ataxia. Unfortunately, viral culture and isolation attempts in the *Neophema* birds proved negative[15].

There is scant information on the lesions responsible for nervous system signs. However, neurological signs including convulsions, tremors, torticollis, ataxia, paresis, paralysis and head tilt have been reported in psittacines in Pacheco's disease and in herpesvirus infections in pigeons, and there is a distinct neuropathogenic herpesvirus strain in pigeons. Nonsuppurative meningoencephalomyelitis has been described in some affected birds, with cerebellar Purkinje cell loss also a feature in infected pigeons[11,14,21,25,30]. Virus can also be isolated from the brain in some cases of Pacheco's disease[19].

Intriguingly, one report of a series of 47 psittacines diagnosed in Texas with Pacheco's disease included, perhaps erroneously, a sulphur-crested cockatoo (*Cacatua galerita*) which died with respiratory signs, including mucoid nasal discharge, and a unilateral head tilt and ataxia. No reference was made to lesions responsible for the neurological signs but no hepatocyte viral inclusions were detected and herpesvirus was not isolated from the liver. Moreover, the chief lesion was airsacculitis with epithelial cell hyperplasia and intranuclear inclusion bodies[21]. Although details are limited, the cockatoo may have been infected with a herpesvirus with lower respiratory tract tropism distinct from Pacheco's disease virus.

The parakeet of this case report had clinically displayed respiratory signs for at least a two month period. The reactivity of fibroblasts and the presence of plasma cells and inflammatory lymphoid aggregates histologically in the lungs and air sacs indicated a minimum lesion duration of 72 hours. However, the maturity of hypocellular scar tissue in the respiratory tract suggested a period of several weeks and possibly longer since initial tissue injury.

In experimental aerosol infections of chickens with infectious laryngotracheitis (ILT) virus, discrete

lymphoid nodule formation and mature fibrous scar tissue development in lungs and air sacs were a prominent feature from 7 to 15 days post-infection, with both persisting at least up to 6 months post-infection. However, in ILT, faint inclusion bodies first appeared 48 hours post-inoculation, became strongly eosinophilic with a distinct halo by 72 hours but then disappeared by 4 to 5 days post-infection[22]. Although pathogenicity trials with the parakeet respiratory herpesvirus have yet to be undertaken, it would appear that the clinical course of infection is more protracted and that diagnostic inclusion bodies and syncytia persist longer than in ILT virus infections of poultry.

The epidemiological features of the respiratory herpesvirus infection require elucidation, but it is probably valid to extrapolate from epidemiological characteristics of other herpesvirus infections of birds and mammals. The 14 parakeets reported with the infection in Japan and the Indian ring-necked parakeet documented here were all potentially stressed as a result of transportation and change of environment prior to the onset of clinical signs or death, and also by concurrent infections. All herpesvirus infections are characterised by persistent infection with periodic or continuous virus shedding, and asymptomatic carriers are common. Reactivation of latent infections can be triggered by stress associated with intercurrent disease, transport, crowding and cold and, under such conditions, mortality in groups of birds could be anticipated to be high. Clinically ill birds tend to shed large quantities of virus and are probably more important amplifiers of infection within an aviary than asymptomatic carriers. Transmission of herpesviruses in general is associated with close contact of mucosal surfaces and also by aerosol droplets. The latter is probably more important in respiratory herpesvirus infections unless upper respiratory tract lesions are more common than the literature so far suggests. Viral contamination of feed or water could also be a potentially important means of transmission as it is for Pacheco's disease[1,7,10,11,21,25,30].

Electron microscopic studies are intended on formalin-fixed air sac and pulmonary tissues from this parakeet. However, definitive characterisation and identification of the causal virus will require isolation by culture in chick embryo cell lines and possibly via passage in embryonated chicken eggs, physicochemical testing of the isolate, and cross neutralisation testing using specific antisera against known avian herpesviruses[7,10,11,13,19]. Fresh or frozen specimens of air sac and lung would be the samples of choice for viral isolation.

The poor documentation of this infection in the Australian literature raises concerns as to whether the virus may be essentially exotic to this country and hence infected individuals potentially illegal importations. Only by increasing practitioner awareness of the condition, full characterisation of the virus and reporting of all diagnosed cases will it be possible to determine the prevalence of infection in Australia and its importance in wild and aviary psittacine species.

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