CLINICAL RECOVERY AND EXCRETION KINETICS OF CIRCOVIRUS IN LORIKEETS INFECTED WITH PSITTACINE BEAK AND FEATHER DISEASE

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INTRODUCTION

Psittacine beak and feather disease (PBFD), caused by beak and feather disease virus (PBFDV) is the most significant infectious disease in psittacine birds. The virus infects and kills the cells of the feather and beak as well as cells of the immune system, causing irreversible feather dystrophy and loss, beak abnormalities and variable degrees of immunosuppression which may result in lethal secondary infection. Spontaneous recovery does occur although most chronically affected birds do not recover. Clinically the disease can present as acute or chronic, affecting both young and mature birds. Acute infections are usually accompanied by signs of illness with severe mortality and feather abnormalities in young birds, whereas chronic infections can be insidious in progression, making diagnosis difficult. In these cases, infection with PBFD must be differentiated from avian polyomavirus and, to a lesser extent, herpesvirus, which appears to be a recent introduction to Australia, with very little as yet known about its incidence and distribution.

Diagnosis of the disease in live birds relies mostly on the detection of virus particles or viral DNA, as attempts at virus isolation have not yet been successful. This can be achieved by haemagglutination assays (HA, Raidal *et al*., 1993) or by molecular detection methods such as polymerase chain reaction (PCR) (Ypelaar *et al*., 1999) and real-time PCR (Raue *et al*., 2004, Shearer *et al*., 2009). HA procedures require significant expertise, suitable avian erythrocytes and standardised procedures to ensure

reliable and valid results. A positive PCR test on its own is not a demonstration of active viral infection, as non-replicating DNA may take up to 3 months to clear from blood. Haemagglutination inhibition (HI) tests allow for quantification of antibody titres and predictions of immune status and when used in conjunction with HA and PCR, are likely to provide a powerful diagnostic and prognostic tool (Khalesi *et al*., 2005). The existence of different strains of virus (Bassami *et al*, 2001; Ritchie *et al*, 2003; Raue *et al*, 2004; de Kloet and de Kloet, 2004) raises the question of the ability of PCR-based diagnostic tests to detect all strains of virus (Bassami *et al*., 2001). An advantage of utilizing a two-stage testing regime is to capture isolates that may be genetically unique, and therefore PCR-negative, but still capable of causing haemagglutination, although such isolates have not yet been demonstrated (Khalesi *et al*., 2005).

As PCR is becoming the first choice for diagnosis of PBFD in live birds, more data are needed about the excretion of virus and the most reliable samples for PCR diagnosis (Hess *et al*., 2004). Replicating virus can be detected in blood, feather dander and faeces. A study of virus excretion during a PBFD outbreak in budgerigars demonstrated that virus could be most reliably detected by PCR using feather samples rather than blood samples (Hess *et al*., 2004). In this study, virus detection in blood usually correlated with the presence of clinical signs. This contrasts with the observations of Khalesi *et al*., (2005) where blood samples from lovebirds were more sensitive in PCR tests than feather samples, possibly reflecting species differences.

Studies have demonstrated that birds (especially older ones) can intermittently excrete virus in the absence of clinical signs (Richie, 1995; Hess *et al*., 2004) but it is not clear if, or for how long, clinically recovered birds act as reservoirs of infection. This information is especially important to guide decisions about the re-introduction of rehabilitated clinically recovered birds into their wild or captive populations.

The aim of this study was to use current serological diagnostic tools and a qPCR detection assay to investigate virus excretion in different sample types collected from PBFD-infected lorikeets over a period of 18 months. Only lorikeets were studied in order to minimise the impact of possible virus genotypes affecting detection of the virus.

MATERIALS AND METHODS

Three veterinary clinics specialising in avian medicine participated in the study. Fifty-eight wild caught birds were selected based on presentation with clinical signs of beak and feather disease, and the presence of circovirus was confirmed by PCR following previously published protocols (Ypelaar *et al*., 1999). Birds were quarantined for a period of 19 months and provided with treatments and supportive therapy to assist recovery. A detailed grading system was developed to ensure uniformity of clinical assessment which was performed on a monthly basis. Blood spotted and dried onto Whatmann filter paper, feather and cloacal swab samples were collected on a monthly basis and used in real-time PCR for quantitation of viral load. DNA from blood and cloacal samples were also tested for avian polyomavirus (APV) using a PCR-based test. Feather and blood spot samples from every fourth collection were also submitted for HA and HI testing.

RESULTS AND DISCUSSION

Of the 58 birds in the trial, there were 48 rainbow lorikeets and 13 scaley-breasted lorikeets. Half of the birds lived longer than 12 months after capture (58% of the rainbow lorikeets and 23% of the scaley-breasted lorikeets). Based on clinical assessment, 48% of birds recovered physically (55% of

the rainbow lorikeets and 38% of the scaley-breasted lorikeets). Of those that survived for 19 months 21% continued to display feathering abnormalities symptomatic of BFDV.

All birds were negative on PCR for avian polyomavirus. Real time PCR data provided an indication of the amounts of circovirus excreted over time. Large amounts of virus were found in all three sample types (blood, feather and cloaca) from birds that died during the trial, presumably from the disease. Virus shedding in these birds consistently increased in the collection prior to death. Blood samples from birds that survived for at least 12 months consistently demonstrated a rapid clearance of virus from blood (within a maximum of four months) and fluctuating low levels of virus thereafter. The sensitive PCR assay could detect virus in blood spots from all recovered birds, but levels were insignificant compared to starting levels.

The amount of virus detected was consistently and significantly less in blood samples as compared to feather and cloacal samples. Feather samples in particular from some birds contained very high levels of virus. Virus shedding through the skin and faeces was intermittent and unpredictable during the 19 months of sampling, although generally reduced over time to negligible levels by 19 months. A peak in virus shedding through feathers was usually observed around 3-4 months after the peak viraemia, but shedding of virus through the faeces was more sporadic.

These results indicate that virus is more likely to be detected through feather and cloacal samples because of higher levels of virus being shed through skin and faeces but blood samples will provide a more reliable prognostic indicator of the disease. It is likely that beak and feather virus is endemic in Australian lorikeets and that the amount of virus present in blood is more informative than a simple positive viral PCR test indicating the presence or absence of viral DNA.

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