Beak and feather disease virus

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Abstract

The Beak and feather disease virus (BFDV) causes psittacine beak and feather disease, an often chronic and fatal disease in psittacine birds. The virus most commonly infects psittacine birds, but is also capable of infecting non-psittacine bird species in Australasia. The virus induces an immunosuppressive condition with chronic symmetrical irreversible loss of feather, as well as beak and claw deformities eventually leading to death. Currently, there is no specific treatment commercially available for infected birds; however, a combination of quarantine and hygiene control, diagnostic testing and enhancing flock adaptive immunity is recommended to provide the most effective and sustainable control. Recent structural determination of BFDV capsid protein provides insight into the different assemblies that can be formed from one of the smallest known DNA viruses.

History

Psittacine beak and feather disease (PBFD) was first described in the early 1980s and has become recognised as the dominant viral pathogen of psittacine birds worldwide. In wild red-rumped grass parakeets (Psephotus haematonotus), a feather loss syndrome that was highly suggestive of PBFD was first recorded case in South Australia in 1907 (Ashby, 1907). The virus causes PBFD was initially designated as psittacine circovirus but has since been renamed Beak and feather disease virus (BFDV). However, except for the recent structural determination of BFDV capsid protein (Sarker et al., 2016b), there was a lack of information on the structure of BFDV.

Structure of beak and feather disease virus

Beak and feather disease virus (BFDV) is currently a member of the family Circoviridae. Like other circoviruses, BFDV possesses small circular single-stranded DNA (ssDNA) genome (approximately 2.0 kb in length) that is encapsidated into a non-enveloped, spherical icosahedral virion (Sarker et al., 2016b). In order to replicate its genome, BFDV needs to invade the nucleus to access the transcriptional machinery of the host cell. The replication of BFDV known to occur in numerous tissues, including skin, liver, gastrointestinal tract, bursa
of Fabricus (Raidal and Cross, 1995; Wylie and Pass, 1987) while capsid antigen of BFDV found in the spleen, thymus, thyroid, parathyroid and bone marrow (Latimer et al., 1990). However, the distinction between viral entry and replication in a host cell is unclear in absence of confirmation in suitable cell culture. Viral attachment and entry into host cells may not necessarily lead to viral replication, and therefore, all cells containing viral particles may not contribute to the disease progression. However, it is thought that the BFDV encodes proteins that actively transport the viral genome into the nucleus as well as factors that direct the precursor DNA exit to the cytoplasm, where it causes large globular intracytoplasmic paracrystalline arrays (Figure 1) (Sarker et al., 2016b).

The BFDV genome is bi-directionally transcribed and encodes at least two major proteins; a replication initiation protein (rep) expressed from the virion strand and a capsid protein (cap) expressed from the complementary strand. In order to understand the functionality of cap and its interaction with a range of host and viral proteins, recently a study conducted by Sarker et al. confirmed that the cap protein forms virus-like particles (VLPs) of ~17 nm (mature form), and a smaller assembly of ~10 nm (immature form) (Figure 2), using a combination of X-ray crystallography, cryo-electron microscopy and atomic force microscopy (Sarker et al., 2016b). Furthermore, this study demonstrated that assembly of these two VLPs is regulated by single-stranded DNA (ssDNA), and provide a structural basis of capsid assembly around single-stranded DNA (Sarker et al., 2016b).

Figure 1. Transmission electron micrograph of BFDV infected cell on the right demonstrating how the nucleus (N) is relatively spared, with large crystalline arrays of mature virus particles preferentially forming intracytoplasmic inclusions (V) shown at higher magnification on the left (Sarker et al., 2016b).
Figure 2. Structural characterisation of two BFDV capsid virion. X-ray crystal structures allow modelling of the two particles to 1.9 Å (10 nm-immature virions, left), and 2.5 Å (60 nm-mature virions, right). The smaller particle is comprised of 10 capsid molecules arranged as two interlocking discs, with each disc containing five capsid molecules. The larger VLP is comprised of 12 pentamers arranged with T=1 icosahedral symmetry (Sarker et al., 2016b).

Host range and transmission

The BFDV infection was thought to be restricted to within Psittaciformes, but evidence of host switch in distantly-related Australian avian species was demonstrated recently in the rainbow bee-eater (Merops ornatus) (Sarker et al., 2015b), powerful owl (Ninox strenua) (Sarker et al., 2016a) and finches (Circella et al., 2014). A large number of other non-psittacine birds are likely susceptible to sporadic spill-over infection (Amery-Gale et al., 2017) and we have unpublished evidence of BFDV-associated feather disease in the Laughing Kookaburra (Daceolo novaeguineae), columbids, corvids and raptors including the Wedge-tailed Eagle (Aquila audax), White-breasted Sea Eagle (Haliaeetus leucogaster), Peregrine Falcon (Falco peregrinus) and Whistling Kite (Haliastur sphenurus) (Raidal and Peters, 2018).

Beak and feather disease virus is the dominant viral pathogen of Psittaciformes in Australasia, where it has been present for at least 10 million years (Raidal and Peters, 2018) and Australia has been identified as the most likely origin of the virus (Harkins et al., 2014). The richness of psittacine avifauna in this region has produced a mixture of potential hosts for the pathogens resulting in competing forces of virus co-evolution, spill-over infection and virus host-switches within parrots, cockatoos and lorikeets. Recent evidence has shown that all threatened and endangered Australian psittacine bird species can be infected by BFDV genotypes from any other closely- or distantly related host reservoir species (Raidal et al., 2015; Sarker et al., 2015a). Currently, more than 78 psittacine bird species globally have been reported to be infected by BFDV, including at least 38 of the 50 Australian native parrot species both in captivity and the wild, and over 25 non-psittacine bird species (Amery-Gale et al., 2017; Das et al., 2016b; Department of the Environment and Heritage, 2005; Eastwood et al., 2014; Fogell et al., 2016; Raidal and Peters, 2018; Sarker et al., 2014c; Sarker et al., 2016a; Sarker et al., 2015b; Sarker et al., 2014e).
Transmission is thought to include both horizontal and vertical modalities. In wild bird populations transmission of infection most likely occurs within nest hollows by oral or intraclaoacal ingestion of the virus possibly sourced from feather dust, crop secretions, or faeces (Ritchie et al., 1991; Wylie and Pass, 1987). Although there has been debate in the literature concerning the role of vertical transmission of avian circovirus, BFDV is suspected to be transmitted vertically because viral DNA can be found in embryos from infected hens (Rahaus et al., 2008). However, this could simply be the result of non-replicative transfer of viral DNA into the yolk of embryonated eggs, which requires to be investigated further.

Disease

*Beak and feather disease virus* is the cause of psittacine beak and feather disease (PBFD) which is recognised as an infectious threat for endangered Australian psittacine birds and is a well characterised threat to a wide variety of psittacine and non-psittacine bird species globally (Amery-Gale et al., 2017; Das et al., 2016b; Eastwood et al., 2014; Fogell et al., 2016; Raidal and Peters, 2018; Sarker et al., 2014a; Sarker et al., 2014b; Sarker et al., 2015a; Sarker et al., 2014c; Sarker et al., 2016a; Sarker et al., 2015b; Varsani et al., 2011). The disease presents as an immunosuppressive condition with chronic symmetrical irreversible loss of feather, as well as beak and claw deformities eventually leading to death (Figure 3) (Latimer et al., 1990; Pass and Perry, 1984; Raidal et al., 1993a; Ritchie et al., 1990; Ritchie et al., 1989) or it can be expressed peracutely, ranging from sudden death, particularly in neonates (Ritchie, 1995) or as an acute form in nestling and fledglings, characterised by feather dystrophy, diarrhoea, weakness and depression ultimately leading to death within 1-2 weeks (Ritchie, 1995).

Secondary viral, fungal, bacterial, or parasitic infections often occur as a result of diminished immunity caused by a PBFD viral infection. Additional clinical symptoms not mentioned above, including elevated white blood cell counts, are generally due to secondary infections and may not be directly related to PBFD virus infections. Furthermore, not all infected birds develop feather lesions. Some respond with an appropriate immune response and are cured. There is also considerable evidence, at least in lovebirds and orange-bellied parrots, of persistent infections in otherwise normally appearing individuals. It is likely that these subclinically infected birds, in addition to ones with feather dysplasia, are responsible for shedding into the environment and infection in susceptible birds.
The disease, psittacine beak and feather disease (PBFD) caused by BFDV have the potential to become a significant threat to all species of wild parrots and modern aviculture, due to international legal and illegal bird trade (Fogell et al., 2016). Currently, there are a large number of psittacine and non-psittacine bird species globally, both in captivity and the wild are affected with the deadly BFDV, which has the potential to disrupt vital ecosystem processes and services. (Amery-Gale et al., 2017; Department of the Environment and Heritage, 2005; Fogell et al., 2016; Sarker et al., 2016a; Sarker et al., 2015b). The PBFD was one of the first to be recognised as a threatening disease under the Endangered Species Protection Act 1992 (ESP Act) (Raidal et al., 2015). The Environment Protection and Biodiversity Conservation Act 1999 developed a threat abatement plan (TAP) which had two broad goals; ensure that PBFD does not escalate the threatened species status of affected birds; and minimise the likelihood of PBFD becoming a key threatening process (KTP) for other psittacine species (Department of the Environment and Heritage, 2005). In June 2015, a Ministerial review concluded that the goals of the TAP had not been met due to considerable deficits in knowledge concerning PBFD (Environment Media, 2015).

**Diagnosis**

Various approaches have been developed and used for the diagnosis of BFDV including histology, electron microscopy, haemagglutination (Khalesi et al., 2005; Raidal and Cross, 1994), immunohistochemistry (Shearer et al., 2008), in situ hybridisation (Ramis et al., 1994), polymerase chain reaction (PCR) (Ypelaar et al., 1999), duplex shuttle PCR (Ogawa et al., 2005), real-time PCR (Shearer et al., 2009), and PCR followed by high-resolution melting
curve analysis (Das et al., 2016a; Sarker et al., 2014d) and a swarm primer-applied loop-mediated isothermal amplification (sLAMP) (Chae et al., 2020). The serological detection of anti-BFDV antibodies has been conducted by haemagglutination inhibition (Khalesi et al., 2005; Raidal et al., 1993b) and Enzyme-Linked Immunosorbent Assay (ELISA) (Shearer et al., 2008). So far, the standard PCR-based assay has been used most frequently (>49%) to screen BFDV between 1984 and July 2015 (Fogell et al., 2016). Furthermore, a recently developed sLAMP assay may serve as a rapid, sensitive, and specific diagnostic field test for the detection of BFDV in clinical samples.

Treatment and control

Currently, there is no specific treatment commercially viable for infected birds affected with chronic PBFD. Epidemiological studies have shown a high seroprevalence in wild and captive flocks which means that infection does not always lead to the development of feather lesions. Testing regimes currently rely on a combination of viral DNA testing using PCR methods, excreted antigen detection in feather dander using haemagglutination assay (HA) alongside serology using haemagglutination inhibition (HI). The results can identify subclinical birds that are infected but not excreting virus as well as monitoring for an antibody response in those birds which have been exposed to infection. Depending on the stage of infection, infected birds can wax and wane as PCR positive or negative while developing HI antibody. In some species, a positive HI antibody result is strong evidence of freedom from infection and disease. Culling of infected birds is normally performed in infected captive or commercial flocks. There is an ongoing need to develop a vaccine to combat BFDV infection. It has been recommended that a combination of quarantine and hygiene control, diagnostic testing and enhancing flock adaptive immunity should be practised to provide the most effective and sustainable control (Raidal and Peters, 2018).

Acknowledgements

This research is supported by the Australian Research Council Discovery Early Career Researcher Award to S.S. (grant number DE200100367).

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