



## RESEARCH COMMUNICATION

# Beak and feather disease virus haemagglutinating activity using erythrocytes from African Grey parrots and Brown-headed parrots

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### ABSTRACT

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Psittacine beak and feather disease (Pbfd) is a common viral disease of wild and captive psittacine birds characterized by symmetric feather loss and beak deformities. The causative agent, beak and feather disease virus (BFDV), is a small, circular single-stranded DNA virus that belongs to the genus *Circovirus*. BFDV can be detected by PCR or the use of haemagglutination (HA) and haemagglutination inhibition (HI) assays that detect antigen and antibodies respectively. Erythrocytes from a limited number of psittacine species of Australian origin can be used in these tests. In South Africa, the high cost of these birds makes them difficult to obtain for experimental purposes. Investigation into the use of erythrocytes from African Grey parrots and Brown-headed parrots yielded positive results showing the haemagglutinating activity of their erythrocytes with purified BFDV obtained from confirmed clinical cases of the disease. The HA activity was further confirmed by the demonstration of HI using BFDV antiserum from three different African Grey parrots previously exposed to the virus and not showing clinical signs of the disease.

**Keywords:** Beak and feather disease virus, BFDV, haemagglutination, parrots

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Psittacine beak and feather disease (Pbfd) was first described in the mid-1970s and is readily recognizable in wild and captive psittacine species in Australia (Raidal, Sabine & Cross 1993a; Raidal, Firth & Cross 1993b). The causative agent, beak and feather disease virus (BFDV), belongs to the genus *Circovirus* in the family *Circoviridae* and is closely related to porcine circovirus in the same genus and chicken anaemia virus which belongs to

the genus *Gyrovirus* within the same family. BFDV is between 14 and 17 nm in diameter, is of icosahedral or spherical symmetry and possesses a circular single-stranded DNA genome between 1992 and 2018 nucleotides (Ypelaar, Bassami, Wilcox & Raidal 1999; Bassami, Ypelaar, Berryman, Wilcox & Raidal 2001; Ritchie, Anderson & Lambert 2003).

The disease is characterized by progressive symmetric feather dystrophy and loss and the development of beak deformities (Ritchie, Niagro, Latimer, Lukert, Steffens, Rakich & Pritchard 1990; Raidal *et al.* 1993a; Sanada & Sanada 2000). Birds eventually die from secondary infections resulting from immunosuppression.

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Diagnosis of Pbfd relies on the detection of nucleic acid using the polymerase chain reaction (PCR) or the detection of antigen by haemagglutination (HA) and antibodies by the haemagglutination inhibition (HI) assay. In South Africa, only the PCR test is currently available for the diagnosis of Pbfd.

Studies have shown the ability of BFDV to haemagglutinate erythrocytes from Goffin's cockatoos (*Cacatua goffini*), some guinea pigs and galahs (*Eolophus roseicapillus*) (Raidal *et al.* 1993a). Other erythrocytes also reported to have BFDV haemagglutinating activity including those from the domestic goose (*Anser anser*) (Sexton, Penhale, Plant & Pass 1994), eastern Slender-billed corella (*Cacatua tenuirostris*), some sulphur-crested cockatoos (*Cacatua galerita*), Gang Gang cockatoo (*Callocephalon fimbriatum*), Major Mitchell's cockatoo (*Cacatua leadbeateri*) (Soares, Guimaraes & Durigon 1998), salmon-crested cockatoo (*Cacatua moluccensis*), umbrella cockatoo (*Cacatua alba*) and cockatiel (*Leptolophus hollandicus*) (Sanada & Sanada 2000). However, it has been suggested that erythrocyte suitability varies amongst species and also amongst individuals (Sanada & Sanada 2000).

Most of these birds are difficult to obtain in South Africa for experimental purposes because of their high cost, therefore an alternative source of erythrocytes was sought out for use in the HA and HI assays. Blood samples for the purpose of this study were obtained from two different African psittacine species, African grey parrots (*Psittacus erithacus erithacus*) and brown-headed parrots (*Poicephalus cryptoxanthus*).

Blood from three African grey parrots that had continuously tested negative for Pbfd by PCR over a two-year period and from five brown-headed parrots that had also tested negative for Pbfd by PCR was obtained by puncturing a wing vein and stored at 4 °C in an equal amount of Alsever's solution. Blood from the African grey parrots was pooled into a single fraction and that of the brown-headed parrots into a second fraction. The fractions were washed three times in phosphate buffered saline (PBS) and stored as a 25% erythrocyte stock solution at 4 °C. A working solution of 0.75% erythrocytes was freshly made before the start of each HA assay. Blood was stored for a maximum of 7 days unless haemolysis was observed.

Purification of BFDV was performed by adapting the method used by Raidal *et al.* (1993b). A 10% suspension of organs obtained from an African grey parrot, in PBS with added sodium dodecyl sul-

phate (0.5%) was homogenized at 4 °C until the organs were well blended (Braun hand blender). The homogenate was filtered and centrifuged at 6 500 rpm for 10 min at 4 °C (Beckman® Model J2-21 centrifuge, JA 20 rotor). Sodium chloride and solid polyethylene glycol (PEG) were added to the supernatant to a final concentration of 1 M NaCl and 10% PEG. The mixture was stirred at 4 °C for an hour and centrifuged at 9 500 rpm for 20 min at 4 °C. The supernatant was discarded and the pellet resuspended in a minimal volume of PBS. Cesium chloride (CsCl) was added to a concentration of 0.46 g/ml and centrifuged (Beckman L8-55 ultracentrifuge, SW 28 rotor; 25 000 rpms, at 4 °C for 24 h) in CsCl gradients to recover the purified virus. Presence of the virus in the pellet was confirmed by PCR according to the methods described by Albertyn, Tajbhai & Bragg (2004) without any modifications. BFDV DNA, which was previously amplified from birds with confirmed cases of Pbfd, was used as a positive control in the PCR test.

The HA tests were performed in 96 well plates as described by Ritchie, Niagro, Latimer, Steffens, Pesti & Lukert (1991) with a slight modification. Serial two-fold dilutions of purified BFDV were made in 50 µl of PBS from an original undiluted solution. An equal volume of 0.75% erythrocytes from each test species was added to the wells and the plate incubated at 25 °C for 45 min. Results were visualized and haemagglutination found up to a 1:2 dilution for each test species.

To confirm these results, HI assays were performed simultaneously with the HA assays. African grey parrot anti-BFDV serum (original undiluted solution) from three individuals, which had survived infection and had been in constant contact with infected birds for more than 12 months, was serially diluted in 50 µl of PBS. Fifty microlitres of undiluted purified virus was added to each well and the plate incubated at 25 °C for 30 min. Fifty microlitres of 0.75% erythrocytes from each test species were added and the plate incubated at 25 °C for a further 45 min. Negative controls were also performed and consisted of wells with PBS and erythrocytes only. Haemagglutination inhibition was observed by the formation of buttons at the bottom of the wells.

Haemagglutination was observed in the first two wells, representing a maximum titre of 4 HA units, for each test species in the HA assay and upon the addition of anti-BFDV serum, haemagglutination inhibition was demonstrated. The low concentration of the virus attributed to haemagglutination being

observed only up to a one in two dilution but this activity was confirmed by the HI assay using three different anti-BFDV sera.

Thus, BFDV haemagglutinating activity was found using the erythrocytes of both African grey parrots and Brown-headed parrots. In addition, erythrocytes obtained from a single African grey parrot (results not shown) showed similar results for both the HA and HI assays. The establishment that erythrocytes from more readily available species of birds can be used to perform HA and HI assays will greatly assist in the implementation of an alternative diagnostic test to PCR for the detection of PBFV in captive psittacine birds in South Africa.

It is interesting to note that BFDV is capable of agglutinating erythrocytes collected from Australian birds (Raidal *et al.* 1993a; Sanada & Sanada 2000) and African birds (this study). However, a study by Soares *et al.* (1998) found no haemagglutinating activity of BFDV using erythrocytes from red-shouldered macaws (*Ara nobilis*), orange-winged Amazon parrots (*Amazona amazonica*), blue and gold macaws (*Ara ararauna*) and blue-fronted Amazon parrots (*Amazona aestiva*) which are all South American birds. African and Australian birds are considered to be highly susceptible to BFDV while South American birds are generally regarded as being more resistant to infection by this virus. It is interesting to speculate if the ability of BFDV to agglutinate erythrocytes is related to the pathogenicity of the virus in a particular species of bird.

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