

BFDV Vaccination Trial - an Update

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Introduction

Psittacine beak and feather disease is a significant disease of both captive and wild psittacine birds worldwide (Raidal, 1994; Raidal *et al.*, 1993b). Vaccination has proven an effective means of controlling the causative agent; beak and feather disease virus (BFDV) (Raidal *et al.*, 1993a). There have been attempts at culturing BFDV in numerous cell culture systems, however there have been no reports of successful virus amplification using cell cultures (Pass and Perry, 1985) and past vaccination trials have utilized virus eluted from feathers of infected birds (Raidal *et al.*, 1993a). However, the production of antigen for vaccine production by the latter method is time consuming, expensive and ethically questionable. Therefore, full length recombinant BFDV capsid protein was expressed using a baculovirus expression system to produce vaccine. This recombinant protein has previously been proven to react with anti-BFDV sera from naturally immune psittacine birds and from chickens experimentally inoculated with native BFDV in both western immuno-blots and haemagglutination inhibition (HI) assay (Stewart *et al.*, 2007). However, the use of baculovirus expressed recombinant protein for vaccination against BFDV has not been documented. Thus, we tested the protective properties of this recombinant protein using a flock of BFDV-free long-billed corellas (*Cacatua tenirostris*) (n=18).

Materials and Methods

Recombinant BFDV Capsid Protein

Construction of recombinant baculovirus, full length recombinant BFDV capsid protein expression and protein purification was conducted as described by Stewart *et al.* (2007).

BFDV-free Corella Flock

Nestling corellas (n=18) collected by Department of Environment and Conservation (DEC) officers from a wild nesting site near Perth, were supplied and housed in Brinsea TLC-4

incubators and hand-raised using a commercial hand rearing mix (Roudybush formula 3) until they reached weaning age. As the birds reached this point, they were moved to an indoor aviary where they were kept for the duration of the experiment. During their growth the birds were tested weekly by PCR (blood) to identify individuals that might have been infected in the nest and tested by haemagglutination inhibition for anti-BFDV-antibody.

During the vaccination trial, all birds were housed in an air-conditioned, temperature controlled animal house room that was sealed to the exterior.

Vaccination

Birds designated vaccinates (n=13) (age range: 65-89 days) were injected with 40 µg recombinant BFDV capsid protein in conjunction with Freund's incomplete adjuvant on days 0 and 54 µg on day 11. Controls (n=5) did not receive an injection.

Live BFDV Challenge of Vaccinated and Non-vaccinated Corellas

BFDV inoculum: The live BFDV inoculum, produced as described by Raidal *et al.* (1993a), had a HA titre of \log_2 12 HAU/50 µL.

BFDV challenge: Vaccinates and controls were challenged 16 days after the boost injection (27 days post primary injection) with 0.5 mL BFDV, 0.4 mL administered intramuscularly in the pectoral musculature and 0.1 mL administered orally. The combination of these two routs was used as they were determined effective in causing BFDV infection by Raidal *et al.* (1993a)

Sampling

Feathers and blood (latter collected by jugular venipuncture), were collected from each vaccinate and control on days 0, 11, 27, 40, 47, 53, 68 and 124 post primary vaccination. Blood was spotted onto Whatman filter paper No. 3, allowed to dry for at least 1 hr at room temperature and all samples stored at 4°C until use.

Dried blood spots were excised from the filter paper using a stationary hole puncher (OfficeWorks, Australia), deposited into 1.5mL Eppendorf tubes and DNA extracted using the QIAamp DNA Blood Mini kit (QIAGEN, Australia). PCR for detection of BFDV DNA was performed according to Ypelaar *et al* (1999). Anti-BFDV HI antibody detection was performed on dried blood spots and HA testing was performed on feather extracts as described by Riddoch *et al* (1996).

Results

Polymerase chain reaction

PCR screening of all corellas before the start of the vaccination / challenge study were all negative (data not shown). Thirteen days post challenge (Figure 1) with live BFDV, all 5 controls (lanes 8, 9, 13, 15, 18) had become PCR positive for BFDV DNA and none of the vaccinated birds (lanes 1-7, 10-12, 14, 16-17 and 19-21) had detectable BFDV DNA. Twenty days post challenge, all controls were still positive, and still no vaccinated birds had PCR detectable BFDV DNA (data not shown).

HI

Prior to starting the vaccination and challenge studies, no birds had anti-BFDV-antibody detectable by HI. Eleven days after primary vaccination only one vaccinated bird had detectable HI ($\log_2 3$ HIU/50 μ L, vaccinated group mean HI titre = $\log_2 0.23$ HIU/50 μ L). On day 27 (16 days post boost vaccination), 9 of 13 vaccinated birds had a detectable HI (mean HI titre = $\log_2 1.85$ HIU/50 μ L). On day 40 (13 days post challenge) 10 of 13 vaccinates had detectable HI (vaccinates mean HI titre = $\log_2 1.92$ HIU/50 μ L). On this day, 4 of 5 control birds had a detectable HI (mean HI titre = $\log_2 2.2$ HIU/50 μ L). By day 47 (20 days post challenge) all vaccinates had detectable HI titre (mean HI titre = $\log_2 3.46$ HIU/50 μ L) and 4 of 5 controls had detectable HI (mean = $\log_2 4$ HIU/50 μ L). Day 53 had similar results to day 47 (vaccinates mean HI titre = $\log_2 4.38$ HIU/50 μ L and controls mean HI titre was $\log_2 4.2$ HIU/50 μ L). On day 68, all controls had seroconverted (mean HI titre = $\log_2 4.4$ HIU/50 μ L) and vaccinates had a mean HI titre of $\log_2 5$ HIU/50 μ L. Finally, on day 124 of the experiment, vaccinates mean HI titre was $\log_2 5$ HIU/50 μ L and the control bird mean HI titre was $\log_2 6$ HIU/50 μ L. Figure 2 illustrates and compares the mean HI development of vaccinated and control birds.

HA

Of 5 control birds, only 1 bird tested positive by HA for excretion of virus in feathers. This bird was a control and HA negative on days 0, 11, 27, 40, 47 and 124. However, on day 53 (26 days post challenge) and day 68 (41 days post challenge) this bird had a HA titre of $\log_2 12$ HAU/50 μ L and $> \log_2 12$ HAU/50 μ L, respectively. The development of HA and HI for this bird is illustrated in Figure 3.

Clinical observations

No birds showed any signs of hepatic infection. Control birds had dystrophy of powder-down and growing feathers after challenge.

Discussion

As early as 11 days post vaccination, HI detectable antibody may be present in vaccinated birds. As expected, control birds (having not received any injection) did not seroconvert until after challenge. Thirteen days post challenge, all control birds were PCR positive in blood, whereas vaccinated birds remained PCR negative. At the same time point, control animals had a mean HI rise from $\log_2 0$ HIU/50 μ L on the day of challenge to $\log_2 2.2$ HIU/50 μ L (13 days post challenge). Vaccinated birds had a mean HI raise from $\log_2 1.85$ HIU/50 μ L to $\log_2 1.92$ HIU/50 μ L.

Some vaccinated birds became PCR negative on separate occasions later than 13 days post challenge; however none of these remained PCR positive at subsequent time points. Control birds on the other hand were all PCR positive in blood 13 and 20 days post challenge.

One control bird (Figure 3) had test results typical of acute PBFD; PCR positive for BFDV DNA in blood before HI positive and HA feather excretion. This bird was PCR positive throughout the challenge period and was the latest to develop detectable HI (day 68, $\log_2 1$ HIU/50 μ L). The inability to produce early HI detectable antibody is probably the reason for this bird being the only individual with feather HA excretion.

The recombinant BFDV capsid protein is clearly immunogenic as vaccinated birds developed HI antibody and controls did not. Furthermore, vaccinated birds did not develop consistent PCR detectable viraemia after challenge, whereas control birds were all positive within 13 days of challenge and remained positive throughout the experimental period. The results presented here prove that a recombinant expressed BFDV capsid protein is immunogenic and protect *Cacatua tenuirostris*, at least in the short term against BFDV infection.

At this stage of the project the vaccinated and control birds are being monitored for signs of ill health and feather disease. The long term protection of the vaccine needs to be determined, and we intend to keep our birds until they molt to determine whether there may be development of chronic disease triggered by the next molting process.

References

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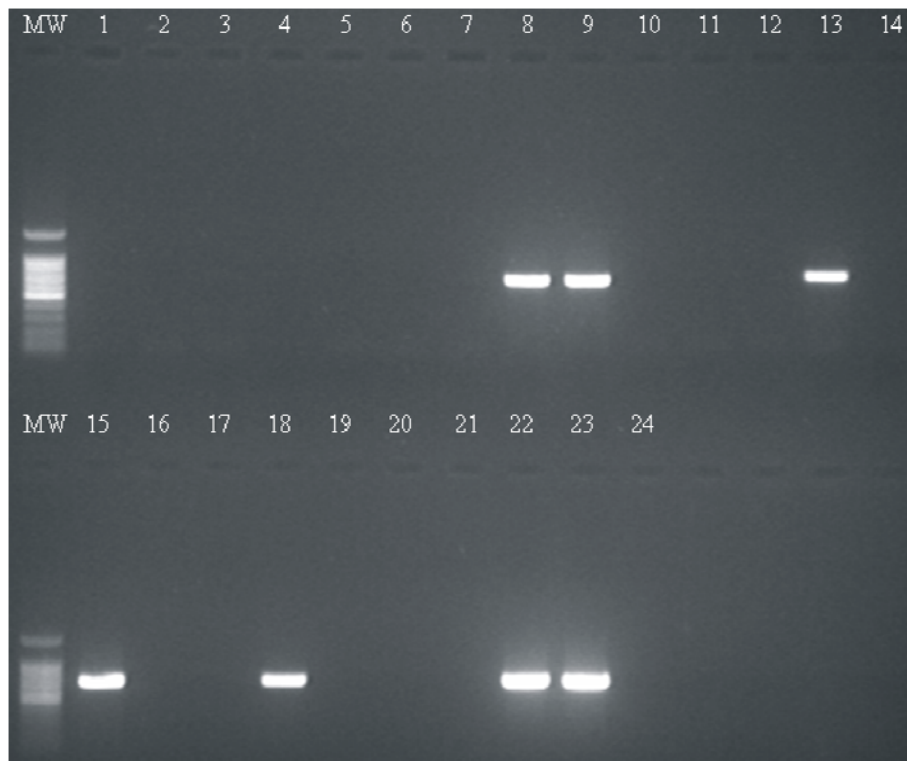


Figure 1: PCR of samples taken from vaccinated corellas and non-vaccinated control corellas 13 days post live virus challenge. Lane MW: Promega 100bp molecular weight marker. Lanes 1-7, 10-12, 14, 16, 17 and 19-21: Vaccinated corellas. Lanes 8, 9, 13, 15, 18: non-vaccinated corellas (negative controls). Lane 22: DNA extraction positive control. Lane 23: PCR positive control. Lane 24: PCR negative control. PCR products were separated by electrophoresis in a 1% agarose gel at 90V for 35 min, and viewed under UV transillumination.

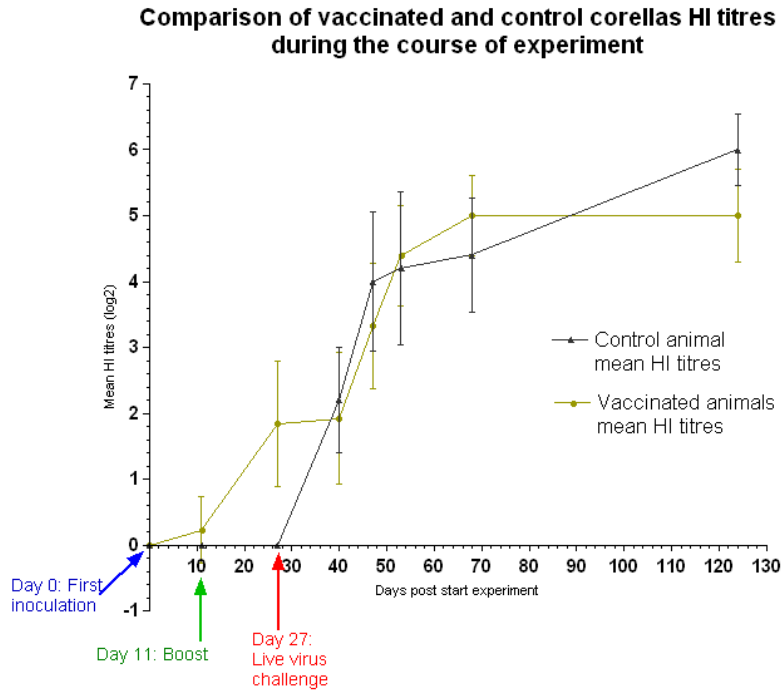


Figure 2: Comparison of vaccinated and non-vaccinated HI development over the duration of the experiment. Graph showing mean \pm SEM serum log₂ HI titres for each group.

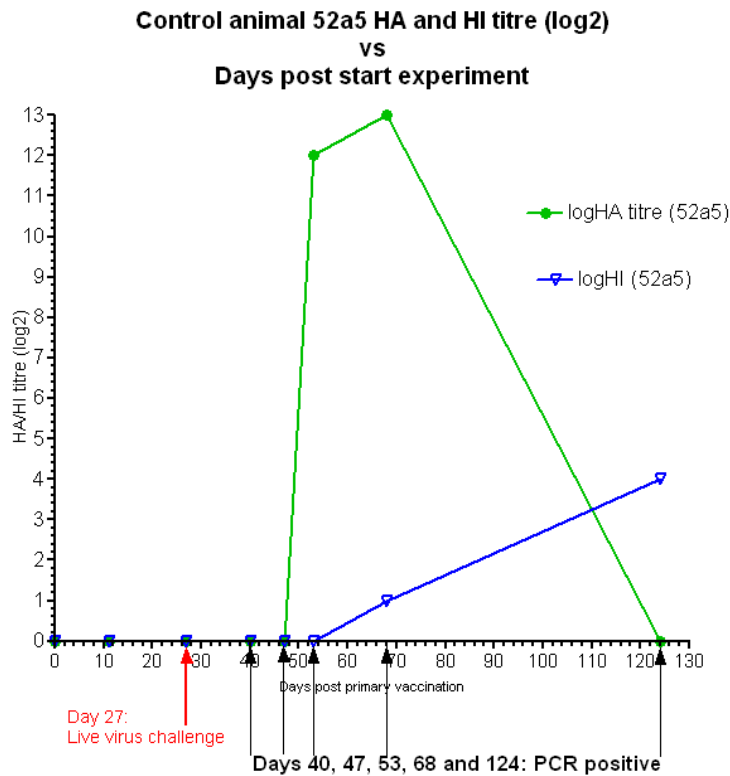


Figure 3: One control bird's HA and HI development over the duration of the experiment. Time points where this bird was PCR positive are indicated.