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Author: S. Raidal, N. Bonne and P. Shearer  
Author Address: shraidal@csu.edu.au  
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Abstract: Psittacine beak and feather disease is a significant disease of both captive and wild psittacine birds worldwide (ref). Vaccination has proven an effective means of controlling the causative agent; beak and feather disease virus (BFDV) (Raidal et al., 1993). 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., 1993). Therefore, full length recombinant BFDV capsid protein was expressed using a baculovirus expression system. This recombinant protein has previously been proven to react with anti-BFDV sera from naturally immune psittacines 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 tenurostris) (n=18).  
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BFDV vaccination trial – an update
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Nicolai Bonne\textsuperscript{a}, Patrick Shearer\textsuperscript{a}, Margaret Sharp\textsuperscript{a}, Phillip Clark\textsuperscript{a} & Shane Raidal\textsuperscript{b}

\textsuperscript{a} School of Veterinary & Biomedical Sciences, Murdoch University, Murdoch WA, 6150

\textsuperscript{b} School of Agricultural & Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW 2678

Introduction
Psittacine beak and feather disease is a significant disease of both captive and wild psittacine birds worldwide (ref). Vaccination has proven an effective means of controlling the causative agent; beak and feather disease virus (BFDV)(Raidal et al., 1993). 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., 1993). Therefore, full length recombinant BFDV capsid protein was expressed using a baculovirus expression system. This recombinant protein has previously been proven to react with anti-BFDV sera from naturally immune psittacines 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 (\textit{Cacatua tenrostris}) (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 \textit{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 (INSERT BRAND) 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 regime:**

Birds designated vaccinates (n=13) (age range: 65-89 days) were injected with ….μg recombinant BFDV capsid protein in conjunction with freund’s incomplete adjuvant on days 0 and 53.94 μg on day 11. Controls (n=5) did not receive an injection.

**Live BFDV challenge of vaccinated and non-vaccinated corellas:**

*BFDV inoculum:* was produced as described in (Raidal et al., 1993) and 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.

**Sampling**

Feathers and blood collected by jugular venipuncture, was taken 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 were 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 as described by (Riddoch et al., 1996) 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 animals had PCR detectable BFDV DNA (data not shown).

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.

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 µl, vaccinated group mean HI titre = log$_2$ 0.23 HIU/50 µL). 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 µL). On day 40 (13 days post challenge) 10 of 13 vaccines had detectable HI (vaccinates mean HI titre = log$_2$ 1.92 HIU/50 µL). On this day, 4 of 5 control birds had a detectable HI (mean HI titre = log$_2$ 2.2 HIU/50 µL). By day 47 (20 days post challenge) all vaccines had detectable HI titre (mean HI titre = log$_2$ 3.46 HIU/50 µL) and 4 of 5 controls had detectable HI (mean = log$_2$ 4 HIU/50 µL). Day 53 had similar results to day 47 (vaccinates mean HI titre = log$_2$ 4.38 HIU/50 µL and controls mean HI titre was log$_2$ 4.2 HIU/50 µL). On day 68, all controls had seroconverted (mean HI titre = log$_2$ 4.4 HIU/50 µL) and vaccines had a mean HI titre of log$_2$ 5 HIU/50 µL. Finally, on day 124 of the experiment, vaccines mean HI titre was log$_2$ 5 HIU/50 µL and the control animal mean HI titre was 6 HIU/50 µL. Figures 2 and 3 show the HI development of vaccines and controls, respectively. Figure 4 illustrates the difference between vaccinated and control seroconversion.

Figure 2: Vaccinated corellas (n=13) mean log$_2$ HI titres at each time point.
Figure 3: Control corellas (n=5) mean log₂ HI titres at each time point.
Figure 4: Comparison of vaccinated and non-vaccinated HI development over the duration of the experiment.
Of 5 control birds, only 1 bird tested positive by HA for excretion of virus in feathers. This bird was a control animal 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 \( \log_2 12 \text{ HAU/50 } \mu\text{L} \) and > \( \log_2 12 \text{ HAU/50 } \mu\text{L} \), respectively. This animal’s HA and HI developments are illustrated in figure 5.

Discussion

The results presented here, indicate that a recombinant expressed BFDV capsid protein may protect *Cacatua tenuirostris* against BFDV infection.

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 animals remained PCR negative. At the same time point, control animals had a mean HI rise from...
log2 0 HIU/50 μL on the day of challenge to log2 2.2 HIU/50 μL (13 days post challenge). Vaccinated birds had a mean HI raise from log2 1.85 HIU/50 μL to log2 1.92 HIU/50 μL.

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

One control animal (Figure 5) showed a disease progression typical of acute PBFD; PCR positive for BFDV DNA in blood before HI positive and HA feather excretion. This animal was PCR positive throughout the challenge period and was the latest to develop detectable HI (day 68, log2 1 HIU/50 μL). The inability to produce early HI detectable antibody is probably the reason for this bird being the only individual with feather HA excretion.

At this stage of the project the vaccinate 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


