Control of Psittacine Beak and Feather Disease (PBFD) by vaccination

SR Raidal[**] and GM Cross[*]

Summary

Adult and nestling cockatoos were vaccinated with an experimental inactivated PBFD vaccine. PBFDV antibody responses of adult and nestling cockatoos were comparable to those induced by primary-oil emulsion vaccination regimen using Freund's adjuvants. Both vaccines protected galah and sulphur-crested cockatoo nestlings but accelerated the development of natural disease in 5 birds. Un-vaccinated control chicks developed severe acute PBFD within 4 weeks of challenge, probably from PBFDV-induced hepatitis since high concentrations of PBFDV were detected in their livers.

Introduction

Psittacine beak and feather disease (PBFD) is endemic in wild and captive psittacine bird populations in Australia (Perry 1981; McOrist *et al* 1984; Raidal *et al* 1993a). The PBFD virus (PBFDV) is epitheliotropic (Pass and Perry 1984; Latimer *et al* 1991) and high concentrations of virus are excreted in the faeces of diseased birds (Wylie 1991; Ritchie *et al* 1991a; Raidal *et al* 1993). The disease has been induced after oral, intra-cloacal or parenteral administration and the severity of disease is dependent on the dose and route of infection as well as age and species (Wylie and Pass 1987; Wylie 1991).

Failure to propagate PBFDV *in vitro* has hindered the development of quantitative infectivity assays and vaccines. Inactivated virus administered to dams has protected nestling psittacine birds (Ritchie *et al* 1992; Raidal *et al* 1993c). A safe and effective vaccine would reduce the serious losses caused by PBFD in Australian aviculture. The purpose of this report is to communicate our present research findings into an inactivated PBFD vaccine (Raidal *et al* 1993c; Raidal and Cross 1994).

Materials and Methods

Birds

Some of the birds used in the experiments were obtained from a commercial trapper (with permission from NSW National Parks and Wildlife Service). Adult birds were acclimatised in sheltered aviaries for 3 weeks before being housed in individual suspended wire cages for 2 weeks. During acclimatisation, the birds were subjected to thorough clinical examinations. Blood samples for serology and faeces and feathers for PBFDV detection were collected as described (Raidal *et al* 1993a). Only birds that remained clinically normal, seronegative and PBFDV-negative during acclimatisation were used.

Samples were taken from nestling cockatoos for PBFD diagnosis immediately after accession. The nestlings were then given the necessary treatments and raised in sibling groups. They were housed in a thermally-controlled environment and fed a complete diet.[‡]

---

[*] Department of Animal Health, The University of Sydney, Private Mailbag 3, Camden, New South Wales 2570

[**] Current address: School of Veterinary Studies, Veterinary Biology, Murdoch University, Perth, WA, 6150

[‡] **Parrot handrearing food. Vetafarm, Wagga Wagga, NSW 2650**

Purification of virus for challenge

PBFDV was purified from the feathers of PBFD-affected SCC as previously described (Raidal *et al* 1993c). Challenge virus concentration measured by HA was $\log_2$ 14 HAU/50 µL.

Preparation of vaccines

Purified PBFDV for vaccine production (Raidal *et al* 1993c) was treated with ß-propiolactone (Wylie 1991) and freshly-made aqueous paraformaldehyde added to a final concentration of 0.1% (w/v). The concentration of inactivated virus (stock antigen) was measured by haemagglutination assay (HA). Stock antigen was stored at -20°C until emulsified with adjuvant.

The purpose of experiments 1a and 2a was to compare an inactivated PBFDV vaccination regime as described by Ritchie *et al* (1992) with a double-oil emulsion adjuvant system (DOE-vacc). The vaccination regimen of Ritchie *et al* (1992) used both Freund's complete and incomplete adjuvants as primary water-in-oil emulsions (FRND-vacc). Five mL batch volumes of FRND-vacc were made fresh when required and used within 24 hours. A control FRND-vacc which did not contain antigen, was also made when required.

For the DOE-vacc, stock antigen was first made into a stable primary emulsion with light mineral oil before being re-emulsified in buffer. The DOE-vacc contained the equivalent amount of antigen as the FRND-vacc. A control DOE-vacc which did not contain antigen was also made. During the experiments, DOE-vacc was stored at 4°C for 4 months and was examined visually for signs of separation into aqueous and oil phases.

The purpose of experiment 2b was to determine the minimum dose of DOE-vacc required to induce protection. A fresh batch of DOE-vacc and two additional DOE-vacc batches (containing 1:10 and 1:100 dilutions of antigen respectively) were prepared for this experiment.

PBFDV Diagnosis

An HA test was used to detect PBFDV in faeces and feather material and a haemagglutination inhibition test (HI) was used to detect PBFDV antibody as described by Raidal *et al* (1993b).

Experiment 1: Antibody responses of adult and nestling birds to vaccination

>   *Experiment 1a.* Fourteen adult galahs were individually identified using wing tags and were randomly divided into two equal groups. Five birds in group I received two 0.5 mL IM injections of FRND-vacc 14 days apart, two birds in this group received 0.5 mL of control FRND-vacc. In group II, 5 galahs each received 0.5 mL of DOE-vacc IM, and 2 galahs each received 0.5 mL of control DOE-vacc on two occasions 14 days apart.

>   Four gang gang cockatoos (group III) received two 0.5 mL doses of DOE-vacc IM 14 days apart. Serology was performed on serum obtained two weeks before vaccination, and at the times of the primary and secondary vaccinations and 2 and 12 weeks later (Figure 1).

>   *Experiment 1b.* Group IV consited of 11 aviary-bred, nestling cockatoos [one galah, four short-billed corellas (*Cacatua sanguinea*), two SCC, two Major Mitchell's (*Cacatua leadbeateri*) and two gang gang cockatoos] of about 2 - 3 weeks age. Because of their smaller size and muscle mass, the birds were given 0.3 mL of DOE-vacc by subcutaneous injection. A second dose of 0.4 mL of DOE-vacc was given IM (thigh or pectoral muscle) 14 days after the first injection. These birds were not challenged.

Experiment 2: Vaccination and challenge of nestling birds

Experiment 2a. Seven nestling galahs and 15 nestling SCC were assigned to either one of two vaccine groups or a control group. Four galah and 4 SCC chicks were assigned to receive DOE-vacc (group V) and 5 SCC chicks were assigned to receive FRND-vacc (group VI). Three galah and 6 SCC chicks were assigned to an un-vaccinated control group (VII). One or two feather follicles plucked from the chicks were fixed in formalin for histopathology. Feather follicles and faeces collected every 7 days from the chicks for HA testing were stored at -20°C. Blood was collected by jugular venepuncture every 2 weeks into heparinised tubes and the plasma stored at -20°C.

The vaccine groups (V and VI) received their first 0.4 mL dose of vaccine subcutaneously between 2 and 3 weeks of age and a 0.4 mL dose IM 14 days later. The three groups were then challenged with 1 mL of PBFDV (0.5 mL orally and 0.5 mL IM), 10 days after the second vaccination or when the birds were about 6 to 8 weeks of age.

Experiment 2b. Nine SCC nestlings (5-7 weeks of age) were vaccinated IM and then challenged. The birds were randomly divided into 3 groups (group VIII, group IX and group X see Table 1). Three batches of DOE-vacc were prepared so that they contained neat, 1:10 and 1:100 dilutions of antigen. Group VIII received a single 0.2 mL dose of neat DOE-vacc. Group IX received a single 0.2 mL dose of 1:10 vaccine. Group X received a single 0.2 mL dose of 1:100 vaccine. The three groups were then administered 1 mL of PBFDV (0.5 mL orally and 0.5 mL IM), 10 days after vaccination.

Results

A summary of the experimental groups and their treatments is presented in Table 1.

Experiment 1

The DOE-vacc was stable at 4°C throughout the four-month experimental period. However, the primary emulsion FRND-vacc was unstable beyond 24 hours. The antibody responses of the adult galahs given DOE-vacc were not significantly different from those of the FRND-vacc birds ($p > 0.05$) (Figure 1). The mean serum HI antibody titres present in the vaccinates 6 months after vaccination were $\log_2 4.0 \pm 1.2$ (FRND-vacc) and $\log_2 4.3 \pm 1.7$ (DOE-vacc). The four galahs vaccinated with control vaccines failed to develop a rise in serum HI titre. One gang gang cockatoo had a high antibody titre before vaccination. Four month old DOE-vacc caused antibody responses in the gang gang cockatoos similar ($p > 0.05$) to those of the galahs (Figure 1).

None of the 11 aviary-bred cockatoo chicks (group IV) had detectable antibody before vaccination. In some chicks there was a transient hyperaemia around the vaccination site for 24 hours. However, chronic inflammatory reactions were not detected. The average PBFDV HI antibody titre after DOE-vacc for these chicks was $\log_2 4 \pm 0.5$ (Figure 2), significantly lower ($P < 0.05$) than that of adult galahs (group II). These chicks have remained clinically normal for 6 months after vaccination.

Experiment 2a

No clinical or histopathological evidence of PBFD was present in the wild-caught cockatoo chicks. The chicks were seronegative and had no PBFDV detectable by HA in faeces or feather pulp before vaccination.

Three of the 9 vaccinated SCC chicks developed PBFD after their second dose of vaccine and were euthanased. These 3 birds were siblings, 2 having received DOE-vacc and the other, FRND-vacc. The other vaccinates (groups V and VI) remained healthy after the virus challenge and developed serum HI antibody titres significantly higher than chicks that received DOE-vacc alone (Figure 2).

Three weeks after virus challenge, HA activity (titre $\log_2$ 3 - 6) was detected in feather pulp obtained from grossly normal contour feather follicles of two of the vaccinated galah chicks and two of the SCC chicks. However, no HA activity was detected in subsequent feather samples.

The unvaccinated (control) galah and SCC chicks (group VII) developed acute PBFD between 3 and 4 weeks after administration of PBFDV. Non-specific signs of illness, such as crop stasis, inappetence and lethargy, were seen in the SCC but not in the galah chicks. The appetites and demeanour of the control galahs did not vary during the acute disease. The dystrophic plumage of the galah chicks failed to improve and they were euthanased 8 weeks after challenge. All the control SCC chicks died within 4 weeks of challenge. Three of these appeared jaundiced and their droppings contained green urates. One SCC chick developed clinical cerebellar disease. Common necropsy findings in the SCC chicks included dehydration, hepatomegaly, shrunken kidneys and atrophy of the thymus and cloacal bursa. High PBFDV HA titres were present in the feather pulp and homogenised livers of some control birds (Table 2). High concentrations of PBFDV were detected in bile collected from 2 SCC chicks. In the week after challenge, neither SCC nor galah chicks shed significant concentrations of PBFDV in their faeces but high HA titres were detected in the contents of the gastrointestinal tracts of some birds at necropsy (Table 2).

Experiment 2b

Two sibling nestlings were removed from the trial because one had a low plasma HI titre (20 HIU/50 µL) and they subsequently developed evidence of natural PBFD (see Table 1). Two of the three birds in group VIII seroconverted (plasma HI titres of 40 and 80 HIU/50 µl) following vaccination. One bird in each of group IX and X developed plasma HI titres of 40 HIU/50 µl, following vaccination. All birds developed plasma HI antibody 21 days after PBFDV challenge (groups VIII and IX > 10,240 HIU/50µL). One bird in group X had high concentrations of PBFDV in feather pulp 21 days after PBFDV challenge. This bird later developed severe acute PBFD 30 days after challenge and was euthanased. Feather HA was not detected in the 5 remaining clinically normal birds (2 birds in group VIII, 2 birds in group IX and 1 bird in group X) 21 and 40 days after challenge. The bird in group VIII which failed to seroconvert following vaccination developed clinical PBFD 6 months after virus challenge.

Discussion

The report of Ritchie *et al* (1992) and the results of experiments 1 and 2 indicate that inactivated PBFDV vaccines can be effective. We found the DOE-vacc at least as effective and protective as FRND-vacc. The antibody responses of the chicks (Figure 2) were lower than those of adult galahs (Figure 1), possibly due to immaturity of the immune system. However, an antibody titre of $\log_2$ 3 was protective. We regard a HI titre of this magnitude as low (Raidal *et al* 1993b).

Both FRND-vacc and DOE-vacc protected chicks from experimental challenge.   Three weeks after challenge, some of the vaccinated chicks developed transient HA titres in feather pulp of grossly normal contour feather follicles. This indicates that vaccination did not prevent PBFDV infection and replication

and it may not prevent persistent infection. However, the birds were protected from clinical disease at the time of challenge by a dose of PBFDV lethal to 6-week old SCC chicks and nestlings which received two vaccinations have remained clinically normal with high serum antibody titres. The results of experiment 2b indicate that a single vaccine dose alone may not induce sufficient immunity in all chicks.

The control galah and SCC chicks developed acute PBFD three to four weeks after PBFDV experimental infection. All developed detectable PBFDV antibody. This is in contrast to the negative serological status of chronic PBFD-affected birds (Raidal *et al* 1993b). The low PBFDV HI titres detected in each chick after primary experimental infection was probably a consequence of immunosuppresion caused by the virus. Young birds may recover from acute PBFD (J Gill and R Perry, personal communication), presumably after an effective immune response. In other young birds, an ineffectual immune response may simply prolong the incubation period.

In the week after challenge, high HA titres were not detected in the faeces of vaccinated chicks. The results of experiment 2 indicate that enteritis is not a necessary component in the pathogenesis of PBFD. Until they develop strong leg muscles and a sense of balance, nestling psittacine birds sit tripod-like on their legs and abdomen. When they defaecate, the chicks slide their cloaca over nesting material. Therefore, PBFDV may gain access to the bursa of Fabricius by direct cloacal infection, a theory first put forward by Wylie (1991). After necropsy, medium to high HA titres were detected in duodenal and cloacal contents of control SCC chicks (Table 2). This was probably due to excretion of PBFDV into bile. Perry (1981) suspected that acute PBFD in nestling birds could present as fatal liver disease. The control SCC chicks in our study probably died secondarily to acute hepatitis since they showed clinical signs of hepatic disease and their livers contained high HA titres (Table 2). Similarly, we have detected high HA titres in the livers and bile of birds chronically affected by PBFD but not in PBFD-free birds (SR Raidal and GM Cross unpublished). From the present work we conclude that the gastrointestinal tract is not the primary site but may be a target organ for PBFDV replication and excretion and that PBFDV replication occurs in the liver early in the disease process and probably continues during the chronic disease.

The severity of PBFD is probably dose-related (Wylie 1991). Severe clinical illness was not a feature of the disease induced in the unvaccinated galah chicks, even though they received a relatively higher challenge dose, because of their smaller size, than the SCC chicks. This provides evidence for species variability in PBFDV susceptibility. Sudden death of chicks after PBFDV challenge has been observed by others (S Wylie personal communication). Cerebellar disease has been infrequently associated with acute PBFD in SCC (J Gill and R Perry personal communication) and could have been secondary to acute liver disease in the one SCC chick in our present study. Further pathogenesis studies are required to determine the infective dose of PBFDV and to define the role of the liver in the pathogenesis of the disease.

The lack of an infectivity assay prevented us from detecting residual infectivity in the vaccines. Residual infectivity might have caused PBFD in three of the SCC chicks. However, the method of inactivation described has been used to inactivate PBFDV (Wylie and Pass 1987; Ritchie *et al* 1992) and would be expected to inactivate other viruses with similar physicochemical properties. Also, none of the aviary-bred chicks developed the disease after vaccination alone.

The three chicks in experiment 2a and 2 chicks in experiment 2b that developed natural PBFD were two clutches of siblings taken from the wild. The results indicate that these chicks were incubating PBFDV at the time they were vaccinated. Consequently, PBFD-testing based on HA will not detect incubating or latent PBFDV infection. The results also indicate that vaccination does not halt but probably aggravates natural disease.

Histopathology is used to confirm the presence of PBFDV-induced inclusions in feather follicles but the absence of inclusions or other evidence may not indicate freedom from infection (Marshall and Crowley 1992). Consequently, histopathology alone is insufficient for the detection of birds incubating PBFDV infection. However, histopathology, HA and HI assays when used collectively increase the reliability of detecting infected birds but the results of all three procedures need to be interpreted in conjunction with clinical examination.

Adverse reactions are a major consideration with any vaccine containing mineral oil (Curtis-Velascoe 1990; Fudge 1990, 1991; Schmidt 1991; Gaskin 1991). Adjuvants that cause undesirable tissue reactions are unacceptable, especially when the reactions occur in the pectoral musculature. Because of their low tissue reactivity, double-oil emulsions might be ideal vaccine vehicles for small birds. Apart from being very stable, our double-emulsion vaccine has a low viscosity and can pass through a 30-gauge needle, which is desirable when vaccinating small psittacine birds. Such an adjuvant has been developed for poultry (GA Firth, unpublished), because it induces satisfactory immunity without unacceptable tissue reactions (Blackall *et al* 1992). The DOE-vacc was well tolerated by the chicks and caused little if any inflammation. In contrast, the FRND-vacc caused undesirable tissue reactions similar to those described by Ritchie (1992).

Despite the high prevalence of PBFD and other infectious diseases, many nestling cockatoos are sold in the Sydney region alone each summer (Marshall and Crowley 1992) and effective measures are required to control disease for this industry. Definitive PCR or antigen assays of high sensitivity and specificity would improve the diagnosis of PBFD. However, safe and effective vaccination, in the longer term, will greatly improve the control of the disease.

Acknowledgments

References

Blackall PJ, Eaves LE, Rogers DG and Firth GA (1992) *Avian Dis* **36**:632

Curtis-Velasco M (1990) *J Assoc Avian Vet* **4**:206

Fudge AM (1990) *J Assoc Avian Vet* **4**:222

Fudge AM (1991) *J Assoc Avian Vet* **5**:10

Gaskin JM (1991) *J Assoc Avian Vet* **5**:11

Latimer KS, Rakich PM, Steffens III WL, Kircher IM, Ritchie BW *et al* (1991) *Vet Pathol* **28**:300

Marshall R and Crowley A (1992) In *Proc Assoc Avian Vet,* Association of Avian Veterinarians, New Orleans, LA, p 37

McOrist S, Black DG, Pass DA, Scott PC and Marshall J (1984) *J Wildl Dis* **20**:120

Perry RA (1981) In *Aviary and Caged Birds*, University of Sydney Post-graduate Committee in Veterinary Science, Proceedings No 55, p 81

Pass DA and Perry RA (1984) *Aust Vet J* **61**:69

Raidal SR, McElnea CL and Cross GM (1993a) *Aust Vet J* **70**:137

Raidal SR, Sabine M and Cross GM (1993b) *Aust Vet J* **70**:133

Raidal SR, Firth GA and Cross GM (1993c) *Aust Vet J* **70**:437

Raidal SR and Cross GM (1994) *Aust Vet Pract* (Submitted for publication)

Ritchie BW, Niagro FD, Latimer KS, Steffens WL, Pesti D *et al* (1991a) *Am J Vet Res* **52**:1804

Ritchie BW, Niagro FD, Latimer KS, Lukert PD, Steffens WL *et al* (1991b) *Am J Vet Res* **52**:1810

Ritchie BW, Niagro FD, Latimer KS, Steffens WL, Pesti D *et al* (1992) *Am J Vet Res* **53**:1512

Schmidt RE (1991) *J Assoc Avian Vet* **5**:10

Wylie SL (1991) *Studies on Psittacine Beak and Feather Disease*, PhD thesis, Murdoch University, Perth
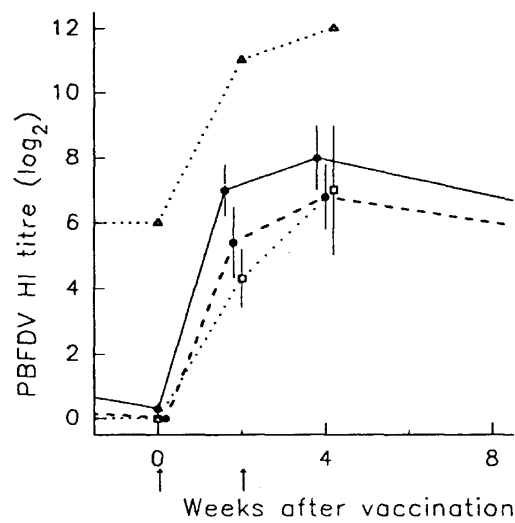
Wylie SL and Pass DA (1987) *Avian Pathol* **16**:269

Figure 1.   Mean ± SEM serum PBFDV haemagglutination inhibition (HI) antibody titres of clinically normal galahs and gang gang cockatoos after vaccination (↑) with FRND-vacc or DOE-vacc. Adult galahs that received control vaccines did not seroconvert (not shown). Galahs in groups I (M)) M) and II (M- - M) received FRND-vacc and DOE-vacc, respectively.   Three gang gang cockatoos in group III (Q - -Q) and gang gang No. 4 (△· · ·△) received DOE-vacc.
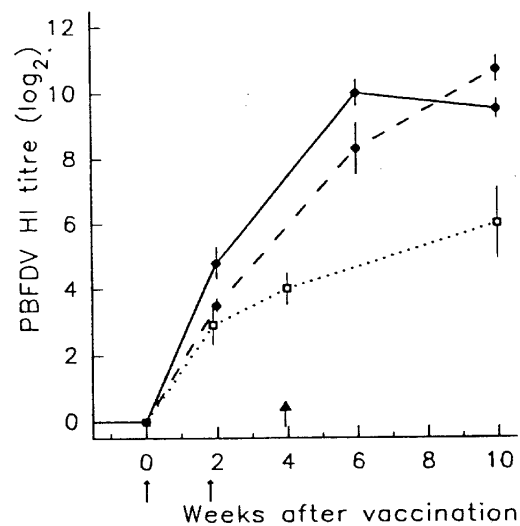

Figure 2.   Mean ± SEM serum PBFDV haemagglutination inhibition (HI) antibody titres of cockatoo chicks after vaccination (↑) alone (group IV) or vaccination and challenge (↑) with PBFDV (log₂ 14 HAU/50 µL), (groups V and VI). Group IV (Q · · Q) received DOE-vacc alone. Groups V (M)) M) and VI (M- - M) received FRND-vacc and DOE-vacc, respectively, and were challenged.

TABLE 1

SUMMARY OF EXPERIMENTAL GROUPS

EXPERIMENT	GROUPS	BIRDS	TREATMENTS	
Experiment 1a	group I	5 galahs 2 galahs	FRND-vacc ($\times$ 2)[*] control FRND-vacc ($\times$ 2)	
	group II	5 galahs 2 galahs	DOE-vacc($\times$ 2) control DOE-vacc($\times$ 2)	
	group III	4 gang gang cockatoos	DOE-vacc ($\times$ 2)	
Experiment 1b	group IV	11 nestling cockatoos	DOE-vacc ($\times$ 2)	
Experiment 2a	group V	4 nestling galahs 5 nestling SCC	DOE-vacc ($\times$ 2) DOE-vacc ($\times$ 2)	**Challenged**[†] **Challenged**
	group VI	5 nestling SCC	FRND-vacc ($\times$ 2)	**Challenged**
	group VII	3 nestling galahs 6 nestling SCC	Not vaccinated Not vaccinated	**Challenged** **Challenged**
Experiment 2b	group VIII	3 nestling SCC	DOE-vacc ($\times$ 1)	**Challenged**
	group IX	3 nestling SCC	1:10 DOE-vacc ($\times$ 1)	**Challenged**
	group X	3 nestling SCC	1:100 DOE-vacc ($\times$ 1)	**Challenged**

[*] Number of treatments

[†] The birds were challenged with 1 ml of PBFD virus (concentration $\log_2$ 14 HAU/50 µL) 10 to 16 days after the last vaccination

TABLE 2

Detection of PBFD virus (HA assay) and HI antibody in fledgling cockatoos with experimentally induced PBFD (group VII)

Fledgling number	Duodenal content HA	Cloacal content HA	Liver HA	Feather HA	Serum[‡] HI
Sulphur-crested cockatoo fledglings					
SCC0	6[†]	6	11	> 12	4
SCC1	0	2	10	> 12	3
SCC2	6	10	> 12	> 12	5
SCC3	4	3	7	> 12	0
SCC4	0	0	> 12	> 12	3
SCC5	12	12	12	> 12	3
SCC11[*]	10	2	> 12	> 12	3
SCC12[*]	0	0	11	> 12	4
SCC13[*]	10	8	> 12	> 12	3
mean	6.0 ± 1.6	5.7 ± 0.3	11.6 ± 0.3	> 12	3.6 ± 0.3
Galah fledglings					
G3	0	0	3	> 12	6
G6	0	0	0	> 12	4
G7	0	0	11	> 12	3

[*] Sibling chicks, vaccinated but not challenged (group V & VI), which developed natural PBFD.
[†] HA titre ($\log_2 \pm$ standard error)
[‡] HI titre ($\log_2 \pm$ standard error)

TABLE 3

Detection of PBFDV HI antibody in fledgling sulphur-crested cockatoos following vaccination (single dose) and PBFDV challenge (experiment 2b)

GROUP	SCC	Plasma HI titres $\log_2$ HIU/50μL (days after vaccination)		
		-1	16[†]	37
group VIII	SCC A	0	2	> 12
(DOE-vacc)	SCC B	0	8	> 12
	SCC C[‡]	0	0	> 12
group IX	SCC D	0	2	11
(1:10 DOE-vacc)	SCC E	0	0	10
	SCC F[*]	0	0	5
group X	SCC G	0	1	> 12
(1:100 DOE-vacc)	SCC H[**]	0	0	4
	SCC I[*]	2	0	7

[*]   Siblings with natural PBFD
[**] This bird developed severe acute PBFD 29 days after PBFDV challenge.
[†]   The birds were challenged with 1 ml of PBFD virus (concentration $\log_2$ 14 HAU/50 μL) 16 days after vaccination.
[‡]   This bird developed clinical signs of PBFD 6 months after vaccination.