Avian Influenza vaccine safety and efficacy trial in threatened species

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Executive Summary

This trial supports the use of commercial Avian influenza vaccine in the protection of threatened species from decline or extinction due to highly pathogenic avian influenza.

The trial demonstrates safety and efficacy* of Poulvac flufend AI vaccine (Zoetis) in five native New Zealand species of endangered manu; kākāpō, takahē, kākāriki, kakī & tūturuatu. No adverse effects of the vaccine were detected. Adverse events due to capture and handling were observed in some species (kākāriki & tūturuatu). Recapture of both captive and free-living was challenging for some individual animals. These potential limitations need to be taken into account with any future use of vaccination.

Antibody testing shows that in four species a strong immune response was still present at 6 months post vaccination but absent for most individuals at 12 months. In one species (kakī), the antibody level dropped significantly at 3 months and further refinement of the protocol for that species is required (increased dose rate or frequency).

It is not appropriate to undertake virus challenge trials with these threatened species, so overall efficacy will not be known until natural challenge occurs following the arrival of the virus in Aotearoa New Zealand. Population monitoring after arrival of HPAI will further inform the level of protection provided by vaccination.

At this stage, a booster vaccination at 9-12 months is recommended where feasible for all species, and for kakī a booster at 3 months or a higher initial dose rate is recommended.

*efficacy as demonstrated by detection of antibodies in response to vaccination

Table1: percentage of birds tested showing elevated antibodies via ELISA testing at different timepoints.

Species	2 months	3 months	6 months	12 months
Takahē	100%	100%	100%	0%
Kākāpō	100%	100%	78%	25%
Kakī	100%	33%	22%	0%
Kākāriki	100%	100%	75%	0%
Tūturuatu	100%	100%	100%	25%

- The trial commenced January 2024, and ended June 2025.
- The trial demonstrated safety and efficacy of a commercial poultry vaccine for avian influenza in five critically endangered species: kākāpō, takahē, kākāriki, kakī & tuturuatu.
- No safety concerns were found with the vaccine itself.
- Adverse events detected are potential for stress or injury from capture, and individual birds may evade recapture for boosters or blood sampling.
- Duration of antibody elevation in 4 species was at least 6 months with a few individuals with antibodies present at 12 months. However duration of detectable antibodies was only around 3 months in kakī.
- Duration of antibodies is only an approximate measure of duration of protection, which makes a definitive vaccine protocol difficult to determine.
- Challenge studies to test protection are not appropriate in endangered species.
- We are still analysing the data to determine recommended booster timing. At this stage we think a booster at 9-12 months is required for most species, and for kakī give either a booster at 3 months or use a higher initial dose rate.
- Next steps are dependent on MPI approval of the technical aspects of our draft species vaccine plan. Once we have clarity on MPI requirements (e.g. blood testing, swabs, surveillance etc) we will go back to recovery groups/species leads to undertake review/consultation/engagement to develop final the plans for emergency use of vaccination for HPAI outbreaks

Purpose

To assess the safety and efficacy of an inactivated avian influenza vaccine in five nationally critically endangered species; kakī/black stilt (*Himantopus novaezelandiae),* kakariki karaka/orange-fronted parakeet (*Cyanoramphus malherbi*), tūturuatu/shore plover (*Thinornis novaeseelandiae*), kākāpō (*Strigops habroptilus*) and takahē (*Porphyrio hochstetteri*) for use in the protection against extinction from highly pathogenic avian influenza (HPAI).

Background

Avian influenza is a viral disease which can cause mass mortality events in birds. The current strain of H5N1 clade 2.3.4.4b Highly Pathogenic Avian Influenza (HPAI) has had severe impacts on wild bird and pinniped populations overseas. It was confirmed in South Georgia in the southern Atlantic subantarctic region in October 2023 and reached the southern Indian Ocean subantarctic islands in September to December 2024.

Population size is a key factor which can mitigate against extinction due to disease, however where the population is already low, has low genetic diversity or recovery is slow, a disease outbreak could have a significant impact, including loss of genetic diversity, and a high mortality rate from HPAI in an endangered species could result in extinction.

Population persistence for the five species in this trial is either dependent on captive rearing, or they are flightless birds undergoing intensive management of individual birds in a confined area (off-shore island). Use of vaccination to produce protection of captive/confined breeding birds from HPAI morbidity and mortality, and reduction of viral shedding is an important tool to mitigate against risk of extinction of these species.

Data from commercial vaccine use in other avian species in zoos indicates a high level of safety for use of avian influenza vaccines across a range of avian species, however different species have shown a variable immune response to the vaccine (as measured by antibody titre peak levels and duration). See EFSA 2007 & Vergara-Alert et al 2011).

Methods

Trial species

- kakī/black stilt (*Himantopus novaezelandiae)*
- tūturuatū/shore plover (*Thinornis novaeseelandiae*)
- kākāpō (*Strigops habroptilus*)
- takahē (*Porphyrio hochstetteri*)
- red-crowned parakeet *(Cyanoramphus novaezelandiae novaezelandiae*) (used as a surrogate species for kākāriki karaka/orange-fronted parakeet (*Cyanoramphus malherbi)* because it is less endangered and held in captive flocks for display*)*

Vaccine

Poulvac Flufend i AI H5N3 RG, Registration number A009733 held by MPI in New Zealand

Inactivated virus, oil emulsion, delivered by subcutaneous injection.

The dose volume and frequency of the vaccine was based on dosages used in Vergara-Alert et al 2011 for a range of avian species, whereby birds <1.5kg received 0.2ml per dose and birds >1.5kg received 0.5ml per dose of vaccine subcutaneously one month apart to stimulate humoral immunity and antibody production.

Animal selection

For each species, up to ten individuals were held at the captive facility/offshore island were selected for the trial by the lead husbandry expert. Birds were chosen based on normal behaviour and appearance, and suitability for capture and handling. Birds were permanently banded with individual identifying leg bands or microchips.

The birds were divided into two cohorts, depending on the birds available and their aviary distribution e.g. birds in a pair would be kept as a pair during the trial.

Cohort 1 commenced the trial and if all results/observations were favourable, then one month later cohort 2 commenced the trial. This allowed initial results from cohort 1 to be received and reviewed, prior to starting cohort 2.

Interventions

Time point: 0 months

Each animal received a physical examination by an experienced wildlife veterinarian; and choanal & cloacal swabs for an Avian Influenza PCR test.

A dose of vaccine of 0.2ml for kākāriki, tūturuatu & kakī, or 0.2-0.5ml for takahē and kākāpō (depending on bodyweight) was delivered by subcutaneous injection in the left flank region.

Blood samples were taken for a packed cell volume (PCV), total plasma protein (TP), white cell count and differential, and serum antibody titre. In small birds, blood was collected by wing stab/capillary tube method. In larger birds a needle and syringe was used to collect from the brachial vein in takahe and either the tarsal or brachial vein in kakapo.

Birds were monitored daily as per normal husbandry practices, and any abnormalities were reported to the veterinarian and thoroughly investigated.

Time point: 1 month

One month later the birds were re-examined by the veterinarian for any abnormalities and the second dose of vaccine was given.

Blood samples were taken for a PCV, TP, white cell count and differential, and serum antibody titre.

Time point: 2-3 months

One to 2 months later, the birds were re-examined for any abnormalities. (6-10 weeks after the booster is the expected maximum antibody response as measured in chickens).

In some species, opportunistic blood sampling during handling for other husbandry enabled additional antibody testing time points.

Blood samples were taken for a PCV, TP, white cell count and differential, and serum antibody titre.

Time points: 6 months & 12 months

If any abnormalities were detected, these were documented and thoroughly investigated. The bird would be removed from the trial if its welfare is compromised.

Blood samples were taken for serum antibody titre.

Avian Influenza PCR:

A nylon swab was inserted into the cloaca or choana and gently rotated to collect material from the site. The end was immersed in an Epindorf tube containing RNAshield, and the shaft cut off with scissors which wad been cleaned suing a medical alcohol wipe. The tube was sealed, labelled and stored chilled until delivery to an accredited poultry analytical laboratory within 10 days.

Samples were tested using a commercial Avian Influenza PCR test.

PCV & TP:

A heparinised capillary tube was filled and centrifuged on site at 6000RPM for 5 minutes. The PCV was calculated using a visual guide to determine percentage of volume of the tube which contained red blood cells. The TP was determined using a veterinary refractometer.

White cell count and differential:

A blood smear was made at the time of collection, stored at room temperature and submitted to a veterinary pathology laboratory for examination.

Serum antibody testing:

Blood samples were collected into serum clot tubes or non-heparinised capillary tubes and centrifuged on site on the day of collection. Serum was transferred into Epindorf tubes and delivered chilled to the lab on the same day, or frozen at -20C and delivered within 5 days. Serum was stored at -80C and then thawed on the day of testing.

ELISA testing

A commercial Avian Influenza ELISA test kit was used for antibody testing due to the small volume of blood available from some of the species. Samples were processed in batches.

Hemagglutination Inhibition testing

Haemagglutination inhibition testing for both H5N3 (vaccine type) and H5N1 (panzootic type) was undertaken at the MPI Animal Health Laboratory at Wallaceville using serum samples from the largest bird (takahē) which had been stored at -80C over the period of the trial and then delivered as a batch to the MPI lab.

This test involves serial dilution of the serum until a limit of detection of antibodies is reached. Results are reported in a log2 scale. Dilution started at 1:8 as results <1:8 are considered negative. In chickens, 1:16 is considered to offer protection from mortality, and 1:32 is considered to offer protection from morbidity (sickness) (Katzner et al, 2025).

Results

Adverse reactions

There were no adverse reactions to the vaccine, as measured by behavioural and husbandry observations, and by re-examination of the vaccine site at each subsequent handling.

Adverse events

There was one mortality due to traumatic injury during the capture process of a kākāriki.

There were two mortalities of tuturuatu from infectious disease (aspergillosis & bacterial enteritis). Both conditions had caused death in other tuturuatu not in the trial at that facility within the past 12 months and 2 years respectively. And a third tuturuatu died two days after blood sampling, with necropsy results suggesting the cause was a sudden excitation event causing heart damage, possibly a predator scare. Stress caused by capture and handling for the vaccine trial cannot be ruled out as a contributory factor.

Two events of difficulty controlling blood clotting after blood collection occurred, in kākāriki and in kakī. Stress related to capture and handling was considered a significant contributing factor and modifications of technique were employed to manage this risk in future sampling events.

Two kākāpō received only one dose of vaccine because they were unable to be recaptured for their second dose.

Recapture of kākāriki for blood sampling was difficult and unreliable due to their learned avoidance of the capture box.

Avian Influenza PCR:

No positive results occurred.

PCV & TP:

No abnormalities were detected and all results were within normal ranges for the species (where baseline normal have been determined) or within normal ranges for avian species.

White Cell count and differential:

No abnormalities were detected and all results were within normal ranges for the species (where baseline normal have been determined) or within normal ranges for avian species.

Serum antibody testing results

1. Hemagglutination Inhibition testing

Haemagglutination inhibition testing for H5N1 (panzootic type) was undertaken at the MPI Animal Health Laboratory at Wallaceville.

This test involves serial dilution of the serum until a limit of detection is reached. Results are reported in a log2 scale. Dilution started at 1:8 as results <1:8 are considered negative. In chickens, 1:16 is considered to offer protection from mortality, and 1:32 is considered to offer protection from morbidity (sickness) (Katzner et al, 2025).

In summary, the HI testing showed that takahē produced a good immune response to vaccination, and most could be expected to have protection up to six months, but not at 12 months.

Table: percentage of takahe with a minimum antibody titre or 1:16 or 1:32, indicating the expected level of protection against mortality or morbidity from HPAI.

	H5N1		
Sampling month	2	6	12
1:16 (mortality)	100%	60%	0%
1:32 (morbidity)	90%	40%	0%

2. ELISA testing

ELISA serum antibody testing shows all 5 species responded to the vaccine, with 4 out of 5 having at least 6 months positive results and 1 (kakī) having only 3 months of positive results.

The kākāpō ELISA results show a difference between a single (2 birds) or a double (8 birds) dose of vaccine. Birds with a single dose had lower antibody levels than fully vaccinated birds from 2 to 6 months. The duration of positive antibody results was possibly shorter however this was not demonstrable with the sampling interval used in this trial.

Discussion

This trial established that there were no adverse events associated with the vaccine. However, there were adverse events associated with injury and potentially stress due to capture and handling; and in both captive and free-living species, there was difficulty in recapture of some of the study animals.

We measured the antibody response (elevation and duration) to a standard vaccine regime. This determined the effectiveness of the dosage and the duration of expected protection (using antibody titre as a proxy for protection).

We showed the dose rate used in commercial poultry, and used in the studies in Europe (EFSA 2007, Vergara-Alert et al 2011) was effective in stimulating an immune response at 10-14 weeks in all of the species. Duration of antibody persistence varied across species with kakī having a very short duration of elevated antibodies.

In one species (kākāpō) two individuals only received a single dose of vaccine. The two birds had a lower peak antibody response and it may be of shorter duration. However it demonstrates the possible use of a single dose in an emergency situation to provide protection.

In one species where sufficient blood could be safely collected (takahē), haemagglutination inhibition testing was also undertaken. The results show a decline in antibodies at 6 months, however 60% of birds still had antibody levels which in poultry species would be considered protective (Katzner et al, 2025). The precautionary approach would be to administer a booster at 6 months.

These results have been used to inform the planning for emergency use of vaccine for threatened species protection from HPAI.

We recommend the vaccine be used to protect endangered species by vaccinating captive breeding birds, and a cohort of free-living birds to represent a small insurance population against the risk of extinction from HPAI. It should also be used in birds bred for release into the wild which might be considered at risk in the face of a major mortality event. It is also useful for the protection of highly managed individuals.

References:

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