Application Number DOCDM	AEC443 APPLICATION DOC-7483666
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, takahē

Revised September 2023

DEPARTMENT OF CONSERVATION
APPLICATION TO MANIPULATE LIVE ANIMALS
Code of Ethical Conduct for the Care and Manipulation of Live Animals

1. APPLICANT'S DETAILS:

Name: Kate McInnes

Date: 24 October 2023

Role: DOC Veterinarian

Unit: BH&V Group, Wellington

APPLICANT'S ADDRESS:

Phone no: 9(2)(a) [redacted]

Email: kmcinnes@doc.govt.nz

2. ACCOUNTABLE MANAGER'S DETAILS:

Name: John Lyall

ACCOUNTABLE MANAGER'S ADDRESS:

As above or: DOC, Hokitika

Phone no: 9(2)(a) [redacted]
Email: jlyall@doc.govt.nz

2a. AEC443

2b. **MANIPULATION TITLE: Avian Influenza vaccination safety and efficacy trial Takahē**

2d. **Duration of the manipulation**

- *Over what timeframe are you seeking the approval?*
- *You must not commence the manipulation until you have received the approval, signed by you, your accountable manager, and the AEC Chair.*

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- *NOTE: The AEC will not generally give an approval for longer than two years at one time. Please state if this manipulation is likely to extend longer than two years from the commencement date.*

Anticipated start date:	February 2024	Anticipated finish date:	June 2025

2e. What months of the year is the manipulation most likely to be undertaken? e.g., October – March
For the duration of the dates specified but breeding birds will not be handled during September to January.

3a. Summary of the proposed manipulation for a LAYPERSON

- *Provide an abstract describing the manipulation (maximum 400 words).*

Avian influenza is a viral disease which can cause mass mortality events in birds. The current strain is decimating many populations of wild birds overseas, is predicted to reach the Southern Ocean by 2024/25 and was confirmed in South Georgia, October 2023.

We want to test the safety and efficacy of vaccination to protect critically endangered species.
The vaccine is a commercial product registered in New Zealand by Ministry for Primary Industries. It is considered very safe and highly effective. It contains inactivated (dead) virus so it cannot cause avian influenza. Vaccination reduces risk of illness or death and reduces shedding of virus, thus protecting the individual and its flock.

Takahē are a critically threatened species where it is possible to reliably administer a full course of vaccine (2 injections under the skin, one month apart) to individually identified birds. At the captive Burwood Takahē Centre, we are able to handle them repeatedly for veterinary examination and blood testing to detect any effects on health status, and measure the immune response by detection of antibodies over a 12 month period.

Captive takahē will be caught outside of the breeding season, commencing in February/March 2024. Each bird will receive a pre-vaccination health check by a veterinarian, and a blood test for health and antibody testing. Up to 2mL of blood will be collected from the leg or wing vein, as is standard for this species.

The vaccine is given under the skin. One month later the bird will receive a second vaccination and blood test. Further blood will be collected at 2-3, 6 and 12 months post vaccination to determine the level of antibody response and how long it lasts.

A cloacal and choanal (oral) swab will be collected on day 0 for PCR testing to demonstrate the birds were not incubating avian influenza at the time of vaccination.

Normal husbandry practices will be undertaken including observation of the bird's activity and food intake to monitor of any adverse reactions.

We propose to work with a total of 10 adult or juvenile takahē, divided into two cohorts. Cohort 1 will first receive the vaccination & blood tests, and a recheck at 1 month. If no safety issues are identified, then Cohort 2 will receive vaccinations & blood tests. This allows a careful start to the trial where the first 1 month is the most important to test vaccine safety. The following blood samples will determine level of immune response and duration of antibody presence and determine when further boosters would be required.

Additional approval given on 16/4/24 to collect a 10-14 week blood sample to target peak antibody levels, and to collect an opportunistic blood sample for further antibody testing, if birds are being handled for routine management purposes, with no more than twice per bird over the 12 months of the trial.

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3b. Description of the proposed manipulation (methods)

- *Provide a more detailed explanation. Describe the equipment, the location, and any environmental factors: weather, time of the year. Why have you decided to undertake the manipulation in this way? What advice have you sought? Include the species, the number of individuals, the source of animals, and the disposal/fate of animals at the conclusion of the manipulation.*
- *Be specific about the timelines for the proposed investigation and the purpose of the research, testing or teaching.*
- *Include some consideration and planning for when things might not go right.*

Please note: this is one of five trials to assess avian influenza vaccination safety and efficacy in nationally critical threatened species (takahē, kākāpō, kakī, tūturuatu, kākāriki karaka). Manipulation details which are specific to this species (takahē) have been highlighted in yellow. All other details are consistent across the five trials. By highlighting the species specific details I hope to assist the AEC with the volume of workload associated with simultaneously assessing these trials.

This trial is designed to test the safety and efficacy of a vaccine in a critically threatened species.

Selection of the species for potential vaccination is based on the risk that they could undergo an extinction event when Highly Pathogenic Avian Influenza (HPAI) reaches New Zealand. 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 risk of extinction.

The current wild adult takahē population is approximately 290 individuals, and modelling suggests that the species would be functionally extinct in 15-20 years without the intensive intervention from DOC's captive rearing programme. The captive management programme, and intensively managed insurance population across 17 mainland and island sites, produces between 40-50 birds per year. These individuals are primarily used to support existing wild populations, or establish new ones.

Based on the global evidence during this HPAI epizootic, the species most at risk of infection are those which exhibit congregation behaviours e.g. feeding, breeding or roosting in groups, those which are exposed to at risk species e.g. where seabirds overlap with another threatened species, and birds held in captive facilities where biosecurity options are limited e.g. open pens and large aviaries.

Takahē are one of 5 species identified by the DOC HPAI Technical Advisory Group as at risk where administration of a full vaccination programme is feasible in sufficient number of individuals to provide protection against species extinction. See DOC-7111177 Mitigation Options Guideline for HPAI.

Use of the accine is dependent on Ministry for Primary Industries approval, and currently requires the birds to be held in captivity or in a defined restricted area. Birds require two injections one month apart and must be individually identified with a permanent mark e.g. microchip or leg band. Takahe already have an individual leg band as part of the routine husbandry of the species.

Effective vaccination reduces susceptibility to infection. When infection does occur, it reduces clinical signs of disease and the amount of virus shed into the environment (Animal Health Australia, 2021).

There is precedent for undertaking a vaccination program in takahē. Since 2005 there has been a vaccination program in place to protect takahē against death from a bacterial disease – erysipelas. This current vaccination program means that there are staff highly skilled in this procedure on takahē and that

vaccination is a standard management tool for this species. No adverse events in takahē have been recorded as a result of the erysipelas vaccination programme.

Additionally, vaccination of California Condor was approved in the United States following an outbreak in the wild population. This was the first avian influenza vaccination programme in a wild endangered species. Advice from the veterinary and technical advisors to the condor vaccination programme has been received and is incorporated into this trial design.

We wish to undertake a limited trial to determine the safety and efficacy of the avian influenza specific vaccine in takahē as a preparedness measure for the arrival of HPAI in New Zealand.

The vaccine is produced commercially by Zoetis for use in poultry: Poulvac Flufend i AI H5N3 RG inactivated (killed) vaccine - see Appendix 1. It has been in production since 2006 and is widely used in the poultry industry. Publications on AI vaccine use in poultry and avian species in zoos have indicated a very high level of safety across a wide range of species, and efficacy has been well established. (Kandeil et al 2018, Philippa et al 2006, Philippa 2007a, Philippa 2007b, Pitman 2006). The vaccine is inactivated, so there is no live virus present, and it cannot cause avian influenza.

Advice from Zoetis (USA) indicates that this vaccine should provide good protection against the current strain of HPAI with 91% amino acid homology with the circulating strain. A newer vaccine based on the circulating strain is in production but is will not be available until the end of 2024 at the earliest.

Vaccine will be obtained from PacificVet in Christchurch and transported in a chilly bin with ice-packs by overnight courier (as per their standard transportation procedures for vaccines) to ensure cold chain is maintained. Use in the field will be managed by extraction of sterile aliquots into sterile vials or syringes. This enables sustainable use of the 1000 dose vial and maintenance of sterility of product. This process was discussed with the Zoetis Senior Research Advisor responsible for poultry products and is considered safe and appropriate.

Sterile aliquots will be obtained by using a sterile needle and syringe to extract the aliquot from the closed vaccine vial. The vial will be shaken to homogenise the contents, then the rubber stopper will be swabbed with alcohol. The sterile needle will be attached to the sterile syringe and the needle inserted via the rubber stopper. The aliquot will be drawn up into the syringe, then the needle & syringe removed from the stopper and the cap replaced on the needle. The needle will be swapped for a new sterile needle or a sterile vaccine cap. Both the aliquots and the vaccine vial will be stored refrigerated in accordance with the packaging instructions. Stored vaccine will be shaken to homogenise and drawn up immediately before use, then warmed to room temperature just prior to injection.

DOC veterinarians Kate McInnes and Lydia Uddstrom will administer the vaccination.

All birds will receive a full veterinary physical examination at the start of the trial. Only birds in good body condition exhibiting signs of good health will be included. (Any birds which show signs off poor health will be further investigated as per normal veterinary practices).

The takahē recovery team have been involved in the design of the study, and selection of study animals. The Burwood Takahē Centre holds takahē in enclosures for breeding and feeding training. Use of these birds, as opposed to those on offshore islands or in the Murchison Mountains, will enable reliable capture and monitoring. Additionally, it meets the MPI requirements for the birds to be contained during the trial.

Individuals for vaccination will be selected by the takahē recovery team at the end of the 2023.24 breeding season, based on the programme's planning for any translocations or releases.

Each individually permanently marked (leg band) captive bird will receive two doses of vaccine by subcutaneous injection into the inguinal (groin) region with a 1 month interval (no less than 3 weeks apart and a maximum of 6 weeks apart). The first vaccination will be into the left inguinal region, and the second vaccination into the right inguinal region.

Birds <1.5kg will receive 0.20ml per dose. Birds >1.5kg will receive 0.5ml per dose (as per dosages used in Vergara-Alert et al 2011).

Individual birds within the two cohorts will be determined on the day by takahē Lead rangers based on available birds' suitability and any management requirements.

At the start of the trial, each bird will receive a cloacal and oral swab to determine presence/absence of virus at day 0.
The technique will follow the draft SOP Avian swab sampling DOC-6840491 which has undergone veterinary peer review & user testing and is awaiting AEC endorsement before Director sign-off. These types of swabs are used in standard health testing on avian species and would be undertaken by the veterinarian. The test would be considered a baseline health test to demonstrate the birds were not incubating avian influenza at the time of vaccination. The swabs will undergo PCR testing at BioPacifica to look for avian influenza virus.
This is important to be able to demonstrate that any antibody response is due to the vaccination rather than the bird being infected by a wild strain of avian influenza.

First trial - Cohort 1: Five individuals (or 4-6 dependent on the aviary groupings) will be vaccinated as per the described protocol above. Blood (up to 2ml) will be collected at 0, 1 and 2-3 months to measure health parameters (white cell count & differential) and antibody response (commercial serum ELISA test to measure antibody titre). Antibody testing will be undertaken at a commercial laboratory (BioPacifica, Christchurch).

Note: 1% of body weight is considered an acceptable amount of blood to collect from a healthy bird. Adult takahē weigh ~2kg, therefore up to 20 ml would be within the safe range. We propose only up to 2ml will be collected to maintain a high margin of safety.

Second trial - Cohort 2: Based on consideration of the results of the first trial, if safety has been demonstrated, a second cohort of 5 (4-6) individuals will receive the vaccination as per the described protocol above, and blood (up to 1.5mL) will be collected at 0, 1, and 2-3 months to measure health parameters (white cell count & differential) and antibody response (commercial serum ELISA test). We will wait 1 month until we have established the vaccine is safe in cohort 1 before we start cohort 2.

Note: If antibody response at 2-3 months is noted to be muted (i.e. a low response) then the DOC vets (Kate McInnes and Lydia Uddstrom) will discuss the use of a third dose of vaccine. This was used in some species in European zoos where the initial antibody responses were considered insufficient. Consideration will be given to the level of response detected, the impacts of additional handling, and any other welfare factors noted during the preceding handling events. The benefits of testing a third dose of vaccine will be carefully considered, and this will only be undertaken if the welfare impacts are considered minimal. The justification for a third dose in this trial would be to confirm if this dose is warranted and would deliver protection for the takahē population in the event of an outbreak of HPAI in Aotearoa New Zealand.

Two additional blood samples will be collected from both cohorts at approximately 6 months and 12 months to measure duration of antibody response.

A maximum of 10 birds will be included in the trial. It is estimated that there will be 70-100 takahē on site at the Burwood Takahē Centre at the time of the trial.

These trials are modelled on the vaccination of California Condor in the USA. (FWS.gov 2023) however the 1 month interval is based on the European zoo data, and considered more appropriate to allow for recovery between handling events in birds which normally have minimal handling by humans.

Estimated timing/schedule of manipulations:

First vaccination and blood sample	Second vaccination and blood sample	2-3 month blood sample	6 month blood sample	12 month blood sample
First cohort of 5 birds (4-6)				
~ 1st March 2024	~ 1st April 2024	~1st May 2024	~30th August 2024	~15th March 2025
Second cohort of 5 birds (4-6, maximum 10 total))				
~ 1st April 2024	~1st May 2024	~1st June 2024	~30th August 2024	~15th March 2025

Second cohort of 5 birds (4-6 maximum 10 total) – same timeline but 1 month after first cohort have been vaccinated and shown no negative reaction. Blood collection at ~6 and ~12 months may be undertaken at the same time for both cohorts – these sample are about longevity of antibody presence, so the exact timing is less critical.

During a handling event, all involved staff will gather and have a pre-handling briefing by the veterinarian and the team leader to ensure all roles and responsibilities are clearly understood. Any issues can be raised at that time for clarification. The takahē team leader will be responsible for the safe capture and handling of the bird. The veterinarian will be responsible for the health examination, vaccination and blood collection.

All equipment will be prepared prior to capture to minimise handling time. Staff will know where to situate themselves and what actions are required so that an efficient process is maintained. Captive takahē receive annual vaccinations to protect them against erysipelas (a bacterial disease) so the staff at Burwood Takahē Centre are highly skilled in carrying out blood collection as this is a routine process for them.

The takahē selected for this trial will be housed in their normal enclosures (in pairs, family groups or small groups of juveniles (>3 months old)). They are either lured into a small catch pen using supplementary food or takahē team rangers will slowly and steadily walk across the enclosure corralling the birds into a small area where they can safely be caught by hand, as per normal procedures. Catching and handling of the birds will be carried out by takahē team rangers who have extensive experience in these procedures.

Takahē team rangers will be consulted to select appropriate birds based on their previous observations. It is possible to encounter birds with a "stressy" personality. Such a bird is not a good candidate for a trial which requires repeated handling events, and therefore it would be excluded from the trial. If any birds are observed to exhibit any significant distress during any of the procedures, the takahē team rangers and DOC vets will call a stop to the procedure and reassess the procedures being undertaken and the suitability of the bird(s) for inclusion in the trial. If necessary, changes will be made to reduce issues such as, but not limited to; reducing number of people present, slowing down the capture process, taking a break so birds and humans can calm down, rejecting individual birds or enclosures of birds from the trial,

changing handlers/vet to allow a rest break, abandoning the work for the day, or if serious issues are encountered, potentially stopping the trial and discussing a redesign or full abandonment. At all times bird welfare is paramount and will determine the actions of the takahē team and veterinarians.

Once caught the takahē will be weighed using a hanging scale (standard technique) and undergo a physical examination to determine its health status prior to any further manipulation. If the takahē is determined to be healthy blood collection and vaccination will proceed.

For blood collection and vaccination a trained takahē handler will restrain the bird on its side or back.

The blood collection site (usually the leg vein but may include the wing vein) will be swabbed with a sterile alcohol wipe immediately prior to collection. Blood will be collected using a 1ml or 3ml sterile syringe attached to a 25 – 22 gauge ¾ to 1 inch sterile hypodermic needle.

In the event that the temperature is cool, and on examination of the wing vein we determine that blood collection likely to be slow due to the presence of small contracted veins, the foot/leg will be warmed for 3-5 minutes using Kathmandu hand warmers wrapped in gauze, to boost circulation and enhance blood flow to enable effective blood collection.

After collection, the site of blood collection will be covered with a gauze swab and pressure applied to control any bleeding. In the very unlikely event of uncontrolled bleeding, pressure will be applied for a further 1-5 minutes. If still uncontrolled, alcohol will be rubbed onto the foot and leg to cool the limb to reduce blood flow. If required, a silver nitrate stick will be carefully used to stop the bleeding.

Blood will be transferred into a blood microtainer and spun in a centrifuge to separate the serum from the blood cells. Serum will be drawn off using a sterile pipette and transferred into an epindorf tube for storage in the freezer, prior to transfer to the commercial laboratory in batches for antibody testing.

1-2 drops of blood will be used to make blood smears which will be sent to a commercial veterinary pathology laboratory for a white cell count and differential. This provides a baseline health analysis which can detect infection or inflammation. Any abnormal results will be further investigated by the veterinarian in consultation with the takahē team staff.

Once bleeding has stopped, the bird will be vaccinated using a 1mL syringe attached to a 20 gauge ½ inch needle. The vaccination site will be swabbed with a sterile alcohol wipe immediately prior to vaccination. See Appendix 2 for details of the vaccination technique.

The bird will then be checked for any abnormalities and the veterinarian will determine if any further actions are required for health or welfare. It will be quietly released back and observed as it moves away. Regular observations during routine husbandry will continue for all birds, and any abnormalities will be reported to the veterinarian.

At subsequent handling events, the vaccination site will be examined and any discolouration, swelling, granuloma formation or unexpected abnormality will be noted and reported to the veterinarian. Photographs of each bird's injection sites will be taken to provide a clear record of the trial.

Location & timing:
The trials will be undertaken at the Burwood Takahē Centre outside of the breeding season (breeding takahē are not handled from September to January).

Safety:

Results from a meta-analysis of use of vaccine in European Zoos found very low adverse reaction rate at 0.04% local reactions and 0.015% general reactions reported (EFSA 2007). Based on this, we do not anticipate significant issues with the vaccine, however we will be prepared for immediate veterinary care if any reactions to occur.

The vaccine packaging label states: "Local or systemic post-vaccination reactions can occur due to the use of oily vaccines. Symptoms observed are generally transitory and can include oedema and granulation at the injection site, anorexia and dehydration. Such reactions can be minimised by good aseptic vaccination technique."

Anaphylaxis
A severe immediate immune hypersensitivity response could occur if the vaccine produc stimulates such a response. This is considered unlikely due to the extensive use of this vaccine and other similar vaccine products in Europe, however it is possible and needs to be considered as a potential adverse event. The vaccination team will include a veterinarian who will have access to emergency drugs and supportive care for management of anaphylaxis (including corticosteroids, adrenaline, oxygen, fluids).

Injection site reactions:
The vaccine contains an adjuvant (oil) which is present so that it stimulates a stronger immune response with greater antibody production. This can sometimes be associated with a small pea-sized lump at the site of injection. This is normal and expected, although generally not all birds will develop a lump. This will be checked at the 1 month mark, and recorded with notes and a photograph. If an excessive sized reaction is detected in an individual (>1cm), then the vaccination will be paused until it is determined that the lump does not enlarge further, or cause any impacts on the bird(s) – this is likely to be a period of 2-4 weeks. The food intake, body weight etc will be reviewed and a full physical examination undertaken.

A localised bacterial infection could result if poor sterile technique is used. Only veterinarians or specifically trained DOC staff members will be administering the vaccine, and these operators will have training in appropriate sterile techniques. If a bird experiences an infection at the site, it will receive veterinary care and follow-up to ensure the issue is managed.

Mis-injection could occur if the bird is poorly restrained and moves during vaccination. This will be managed by only using well trained experienced takahē handlers to restrain the birds. For some individual birds, they are calmer with head cover which can aid in handling. This will be determined on an individual bird basis. If a mis-injection occurs, the veterinarian will determine the appropriate next steps. This may include, re-injection if the first injection merely failed to enter the bird, appropriate first aid measures if any injury was caused, and/or exclusion from the trial and follow-up care. As noted previously, "stressy" birds will not be included in the trial which will reduce the risk of injury or mis-injection.

Injury could occur during capture and handling. This is minimised by only using trained experienced staff, careful selection of trial birds, and a "stop for safety" approach which resets the work programme and ensures time out to reassess and replan the work and procedures if necessary.

In the event of a serious reaction or injury during the vaccination trial, the bird will be taken to Dunedin Wildlife Hospital for specialist care by Dr Lisa Argilla. This has been done in the past for sick takahe. Birds can be transported to the Dunedin Wildlife Hospital within 4 hours from the Burwood Takahē Centre.

Results:

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The results of this trial will determine if this vaccine is safe to use in this species, and the level of antibody response produced by a 2-dose vaccination. In some other species, notably penguins, the antibody levels following vaccination remain low and, in some species a third vaccination was used to ensure a stronger response (ESFA 2007). The duration of antibody presence also varied between species. Therefore, this trial will help to determine the appropriate vaccination regime for takahē in the event that more widespread vaccination is required during a highly pathogenic avian influenza outbreak in New Zealand.

3c. Attach Photos of equipment, the species, the location (or a map); to help set the context.

Image: Servane Kiss

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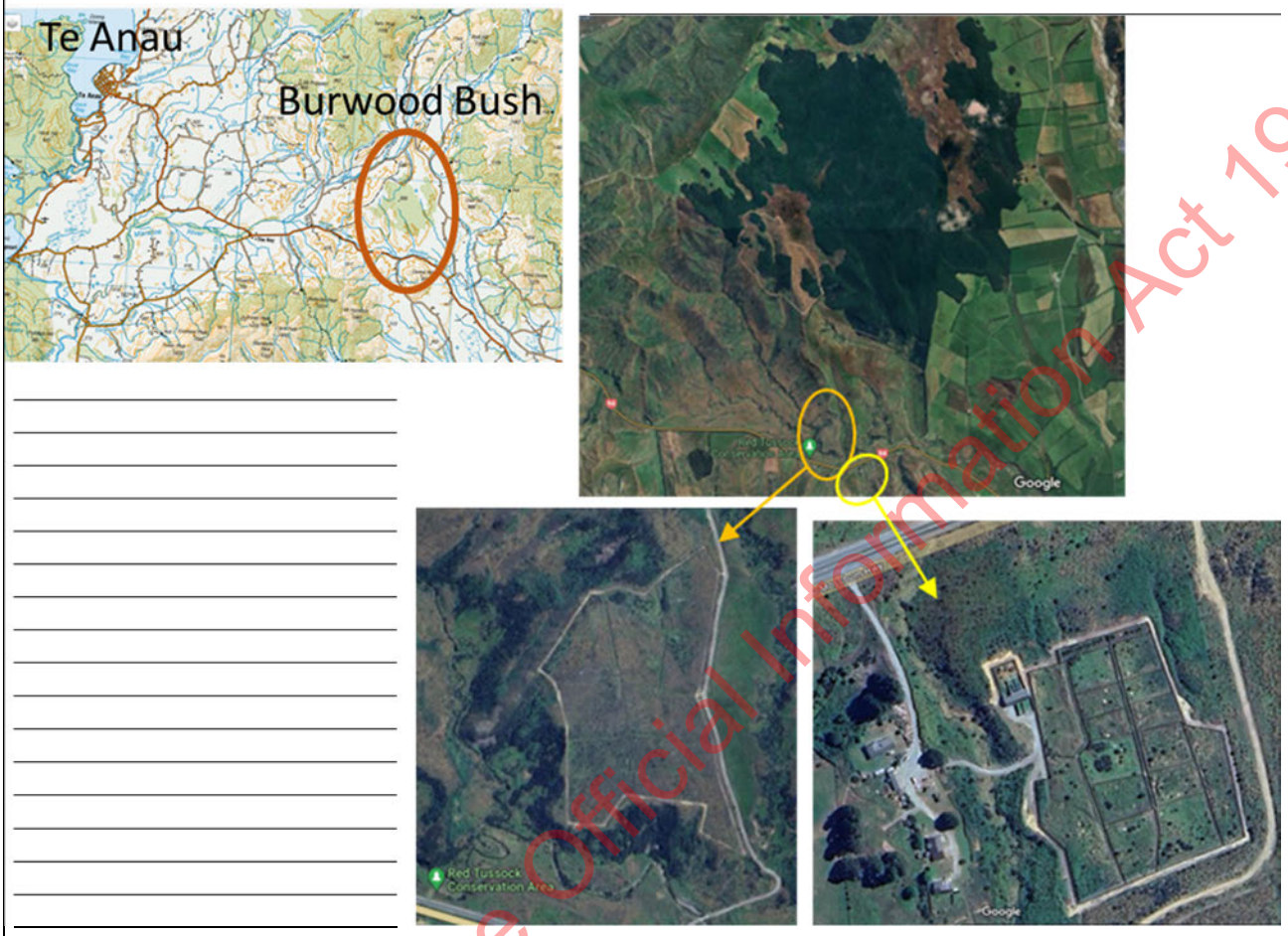


Takahē pens at Burwood Takahē Centre**, Te Anau. Breeding enclosures (left) and -quarantine pens (right)**

Takahē reciving an erysipelas vaccination at Burwood Takahē Centre

3d. References

- *List the references referred to in the application*

DOC-7111177 Mitigation Options Guideline for HPAI
https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7111177

EFSA 2007. Vaccination against avian influenza of H5 and H7 subtypes as a preventative measure carried out in Member States in birds kept in zoos under Community approved programmes. ESFA journal, 450. ESFA-Q 20006-156
https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499835

Health Australia (2021). Response strategy: Avian influenza (version 5.0). Australian Veterinary Emergency Plan (AUSVETPLAN), edition 5, Canberra, ACT. Response_Avian-influenza.pdf (animalhealthaustralia.com.au) Animal

FWS.gov 2023 Southwest California Condor Flock HPAI Information Updates - 2023 | U.S. Fish & Wildlife Service (fws.gov)

Kandeil A, Sabir SM, Abdelaal A, Mattar EH, El-Taeel AN, Sabir MJ, Khalil AA, Webby R, Kayali G, Ali MA. Efficacy of commercial vaccines against newly emerging avian influenza H5N8 in Egypt. Nature Scientific Reports, 2018. 8:9697 | DOI:10.1038/s41598-018-28057-x
https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499854

Philippa JDW, Munster VJ, van Bolhuis H, Bestebroer TM, Schaftenaar W, Beyer WEP, Fouchier RAM, Kuiken T, Osterhaus, ADME. Highly pathogenic avian influenza (H7N7): Vaccination of zoo birds and transmission to non-poultry species. Vaccine, 2005, 23:5743-5750. https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499837

Philippa JWD 2007a, in XI. Vaccination of Non-domestic Avian Species, Transmissible Disease Handbook. European Zoo Vets 5th Edition link

Philippa J, Bass C, Beyer W, Bestebroer T, Fouchier R, Smith D, Schaftenaar W, Osterhaus A. Vaccination against highly pathogenic avian influenza H5Na virus in zoos using an adjuvanted inactivated H5N2 vaccine. Vaccine, 2007b, 25: 3800-3808. https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499841

Pitman 2006. M Pittman, European Commission 12th Annual meeting of national avian influenza laboratories Veterinary and Agrochemical Research Centre (VAR) Uccle, Brussels, 16-18 October 2006 LINK: link.

Vergara-alert J, Ferhandez-Bellon H, Busquets B, Alcantara G, Delclaux M, Pizarro B, Sandchez C, Sanchez A, Majo N, Darju A. Comprehensive serological analysis of two successive heterologous vaccines against H5N1 Avian Influenza virus in exotic birds in zoos. Clinical and Vaccine Immunology, 2011. P. 697-706. https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499845

4. INVOLVEMENT OF OTHER ANIMAL ETHICS COMMITTEES:

4a. Is this Application; or a related or similar application; been or is being considered by another Animal Ethics Committee. Has this project been requested to be considered by any other AEC?

If so, please provide details.

No

4b. Does this manipulation interact with a manipulation approved by other Animal Ethics Committee? If so, detail your communications with those committee(s), and state any conditions imposed by (an)other AEC.

No

5. JUSTIFICATION FOR PROPOSED MANIPULATION:

5a. Detail any action undertaken to determine that the proposed work has not already been done.

Avian Influenza vaccine safety and efficacy has been undertaken on other avian species, however it has not been undertaken in New Zealand endemic species. Although we expect similar results, it is prudent to undertake this trial to provide more evidence of safety and efficacy in the species which we intend to vaccinate in the event of an HPAI outbreak.

5b. Have alternatives been considered to the proposed manipulation involving reduction, or replacement of live animals, or refinement of techniques?

We are looking at the species-specific response and have selected a minimum size divided into two cohorts, so other methods of reduction are not appropriate for this work.

The cohort approach allows us to cautiously approach the safety issue, and assess initial results before involving the full number of birds.

5c. To what extent has there been assessment of the suitability of using non-sentient or non-living alternatives in the project; or replacement of animals as subjects with suitable non-sentient or non-living alternatives?

N/A, see above

5d. How will the proposed work result in the extension of knowledge relevant to the health, welfare, or conservation of animals?

This work will specifically contribute to the future health of the species for conservation purposes by providing evidence of the safety and efficacy (or not) of this vaccine in this species, and inform the appropriate vaccine schedule for the species. This will determine if and how, the vaccine is employed in the future in the face of an avian influenza outbreak in New Zealand.

5e. Is the manipulation required as part of an approved training programme?

No.

5f. How will the results of this work be made available to staff within and outside DOC? (*For example internal report, journal paper, best practice guide, workshops etc*).
Internal report, journal paper, conference presentations.

6. SELECTION OF SPECIES & NUMBER OF INDIVIDUALS FOR PROPOSED MANIPULATION

6a. What will be the source of the animals to be manipulated, and how many from each source will be manipulated?
Takahē at Burwood Takahē Centre. 10 birds in total from a range of adults and juveniles (>3 months old), dependent on time of year and breeding success.

6b. Will any of the animals involved be used more than once, and if so, how many times will each animal be used?
Only once (but each animal handled/manipulated multiple times – twice for vaccinating and four more times for blood sampling, although the 6 & 12 month handling for blood collection will be planned to coincide with routine handling for health management)

6c. What factors have been taken into account in the choice of the animal species?
Takahē are one of 5 species identified by the DOC HPAI Technical Advisory Group as at risk where administration of a full vaccination programme is feasible in sufficient number of individuals to provide protection against species extinction.

6d. Could the information being sought be obtained by work on some other species?
No. The trial specifically uses takahē since the safety and efficacy needs to be tested in the target species, and due to our unique avifauna, there are no reasonable surrogate species in the world.

6e. Will the question be answered with the size of the sample?
Yes.

6f. Is the number of animals proposed to be manipulated the minimum necessary to provide a scientifically interpretable result, consistent with the level of statistical precision required? What consideration has been given to the design of the study with regard to:

- **The level of precision necessary in the results?**

The purpose of the small trial is to establish if there is a species-specific sensitivity to the vaccine and its adjuvant. For this purpose, we require only a small number of birds to extrapolate a species sensitivity. Similarly, for determining vaccine response by antibody response levels, a sample size of 10 will provide sufficient individual variation to establish an overall species response level. Additionally if a bird is removed from the trial for any reason (e.g. other health issues, injury, behavioural), starting with 10 birds allows sufficient number to still be able to make a reasonable conclusion on the vaccine efficacy for future management purposes.

A larger sample size would ensure a more nuanced examination of the species' response to vaccination, however we are examining a general level of impact/effect, rather than subtle differences.
Thus, results which showed >1 bird having a safety issue, or the majority or average antibody response to be low, would be sufficient to inform the next steps for decision making regarding takahē vaccination.

- **The possible confounding effects of animal variation?**

We expect some individual variation since the immune response is affected by individual health status and biological variation. This sample size is sufficient to ensure we have a range of individual responses to examine.

- **The needs of statistical analysis?**

There is likely to be individual variation, which, for the antibody response, requires a reasonable sample size. We determined that 10 was the maximum which was feasible to include in the trial, and also sufficient to allow for individual variation to establish some baseline parameters of antibody response.

Ultimately, in an outbreak situation, the results of a sample size of 10 will be sufficient to make reasonably informed decisions about the use of a commercially produced killed vaccine which has a good history of safety and efficacy across a wide range of species.

7. WELFARE OF ANIMALS DURING PROPOSED MANIPULATION:

7a. What measures will be taken to ensure: the general health and welfare of animals before, during and after manipulation, including the adequacy and cleanliness of housing, caging and equipment; the provision of food and water; prevention of over-crowding, and prevention and control of disease?

Birds will be sourced from the Burwood Takahē Centre where DOC staff already maintain appropriate husbandry practices and monitoring of all the birds. Each bird will be held within its normal enclosure so that there is minimum disturbance to their daily lives. Staff will continue to monitor birds throughout the trial, including food consumption and behaviour.

Each bird will receive a veterinary examination at the start of the trial. Equipment will be disinfected between individuals, or new equipment will be used. Once blood has been collected and vaccination undertaken the bird will be re-released in their home enclosure.

The vaccination trial will occur after the breeding season has finished, so will not interfere with any breeding behaviour. No female birds will be gravid at the time of manipulation, and any chicks will be over 3 months old before the trial begins (takahē chicks typically stay with their parents for 1-2 years).

7b. What movement and transportation measures will be followed for the animals to be manipulated to ensure their welfare and humane treatment?

Birds will be vaccinated on site, within their -enclosure, therefore there will be no transport should be required.

However, if a bird requires specialist veterinary care e.g. in the event of an injury or serious reaction, then it will be transferred to Dunedin Wildlife Hospital (DWH) by car, using the standard takahē transport crates, and in accordance with normal takahē transportation procedures. Briefly, birds will have non-slip flooring the crate, be transported via car to Dunedin. Transport will be managed to reduce noise and allow for temperature control. Radio will be off and driver/passenger will ensure minimal noise. Travel will be direct and the hospital will be alerted ahead of time to enable a fast hand-over a and rapid care.

Dr Lisa Argilla at DWH has treated multiple takahē during her time at Massey University Wildbase hospital and DWH and is familiar with their requirements for hospital care.

Supportive therapy would be provided prior to transport which may include pain relief and fluids, the DOC staff are trained and competent in administering medications on direction from the veterinarians.

7c. What measures are to be taken to minimise the pain or distress of any animal manipulated? *Stating there will not be any impact is not acceptable. The AEC is looking for the Applicant to (1) provide analysis about the potential for pain and/or distress to the animal(s), and (2) describe how they will manage that pain or distress. Identify how you would ascertain pain or distress animal's behaviour, environmental conditions likely to lead to pain or distress.*

Birds will be captured and handled by experienced DOC takahē staff using their routine techniques. Only experienced staff will handle the birds. Initial physical examination, vaccination and blood collection will be undertaken by a veterinarian.

Any bird detected to have abnormalities will be examined and rejected from the trial, and receive normal veterinary investigation/intervention.

Subsequent examination and blood collection may be undertaken by DOC staff trained in blood collection from takahē, provided the initial results (0,1 & 2-3 months vaccination and check) are normal across the cohorts.

The subcutaneous injection is not considered painful, and the vaccine dose will be warmed to room temperature prior to injection. Blood collection is associated with a minorly painful pin-prick when the needle is inserted. This will be minimised by careful planning and handling.

If birds are observed to have any pain response to the vaccination, the staff will report it to the veterinarian who will investigate. In the event that there is an injection site reaction (painful inflammation) then an anti-inflammatory such as Metacam may be prescribed veterinarian, as well as antibiotics if infection is also present. These can be administered by staff by hiding the drug inside some food treats.

As noted earlier, if any serious adverse reactions occur, veterinary care by the attending veterinarian will be undertaken, and transfer to Dunedin Wildlife Hospital undertaken if required for more intensive specialist care.

8. CONTINGENCY PLAN:

8a. What arrangements have been made for the abandonment of any manipulation and/or the euthanasia of animals where pain or distress cannot be held within reasonable levels?

If pain or distress is apparent, during handling or following the procedure, the veterinarian will investigate. If the veterinarian deems the level to be unreasonable, then the manipulation will be abandoned and all efforts made to ameliorate the event e.g. anti-inflammatory, pain relief medication, antibiotics

In the unlikely event that the pain is not temporary and cannot be managed, transfer to Dunedin Wildlife Hospital will allow for intensive veterinary intervention and care. This includes the ability to undertake orthopaedic intervention e.g. in the event of a broken bone, or intensive surgery e.g. in the event of a severe localised vaccine reaction.

Supportive therapy would be provided prior to transport which may include pain relief and fluids.

9. PEOPLE TO UNDERTAKE PROPOSED MANIPULATION:

9a. Who are the person(s) primarily involved in carrying out the proposed manipulation?

Drs Kate McInnes and Lydia Uddstrom are the primary persons.

9b. What is the experience and qualifica ions of the person primarily responsible (9a) for the undertaking and supervising the manipulation (including selection of animals, their care and disposal?)

Kate McInnes has been the DOC vet since 2000 and has worked across a range of avian species, and is currently the lead technical advisor for the DOC HPAI response.

Lydia Uddstrom is contracted full time to the DOC kakapo team, has undertaken postgraduate training as a zoo veterinarian and has experience with a wide range of New Zealand native species veterinary care.

Both Kate and Lydia have previously been involved in capture and handling of threatened species including takahē undertaking vaccination and blood collection for a range of threatened species, and have trained multiple DOC staff to safely and effectively undertake these procedures.

9c. Who else is in the team undertaking the manipulation? State their role in the team, and their relevant experience with the procedure(s) proposed in the application? Include DOC and non-DOC staff in the team.

Name of Manipulation Team member	*Role in the manipulation*	*Experience and qualifications relevant to the manipulation*
Nichollette Brown	Team leader for catching, handling,	**Takahē Recovery Programme Supervisor**

	monitoring & follow-up blood collection of takahē.	Trains personnel in blood collection and vaccination in takahē. Sub-cut vaccinations completed over past 5 years = 154 Blood sampling completed over 6 years on programme = ~48
Phil Marsh	Team leader for catching, handling, monitoring & follow-up blood collection of takahē	**Project Lead – Takahē Sanctuary Sites** Trains personnel in blood collection and vaccination in takahē. Sub-cut vaccinations completed over past 5 years = 128 Blood sampling completed over 10 years on programme = ~80
Glen Greaves	Team leader for catching, handling, monitoring & follow-up blood collection of takahē	**Takahē Recovery Programme Senior Ranger** Sub-cut vaccinations completed over past 5 years = 16 (plus many others over his 17 years on the programme) Blood sampling completed over 17 years on programme = ~80
Jason van de Wetering	Team leader for catching, handling, monitoring & follow-up blood collection of takahē	**Project Lead – Takahē Future Sites** Sub-cut vaccinations completed over past 5 years = 78 Blood sampling completed over 7 years on programme = ~56
James Bohan	Team leader for catching, handling, monitoring & follow-up blood collection of takahē	**Takahē team Site Lead at** Burwood Takahē Centre Sub-cut vaccinations completed over past 5 years = 243 Blood sampling completed over 5 years on programme = ~40
Lisa van Beek	Team leader for catching, handling, monitoring. Carry out follow-up blood collection with supervision.	**Takahē Ranger** based at Burwood Takahē Centre since December 2019 Sub-cut vaccinations completed over past 5 years = 112 Blood sampling completed over 4 years on programme = ~10
Tommy McKerras	Assist during handling procedures, follow	Takahē and kakapo ranger since August 2020

	veterinary and team leader directions	
Erica Hansen	Assist during handling procedures, follow veterinary and team leader directions	Takahē ranger based at Burwood Takahē Centre for the last 2 years

9d. What training will be given to the people identified in 9c to help them undertake the manipulation proposed in the application?

Team leaders have undertaken prior training in blood collection and vaccination of takahē (for the bacterial disease erysipelas) and have trained takahē rangers over repeated seasons to undertake normal husbandry practices including capture and handling.

The capture and handling will be undertaken according to the direction of the team leaders.

The handling and vaccination procedures used in this trial are all normal management procedures that are carried out under a Veterinary Operating Instruction for Eryvac vaccination (for prevention of erysipelas). Vaccination for this trial will be undertaken by the veterinarians present. Blood collection may be done by suitably skilled rangers independently of veterinarians being present.

Vaccination will be undertaken by the DOC veterinarian.

10. COMPLIANCE WITH CONDITIONS of the APPROVAL:

- Please outline any opportunities for a member, or members, of the DOC Animal Ethics Committee to observe this work.

10a. Identify ways that the manipulation(s) can be monitored by the AEC.

AEC members could attend a vaccination session at Burwood Takahē Centre or receive a video or photographs of the manipulation being undertaken.

11. Are there any other aspects which ought to be brought to the attention of the DOC Animal Ethics Committee?

No

12. Does the research, testing or teaching involve a species which is covered by a Department of Conservation Species Recovery Plan and if so, has the Recovery Group been consulted and their endorsement for the work received? Please provide a summary of communication.

Yes. The Takahē Recovery team have been fully involved in the development of this trial and it has been specifically requested by the team that this work is carried out urgently.
Lydia Uddstrom and Kate McInnes met with the takahē team members (Nicholette Brown and Glen Greaves) to discuss the inclusion of takahē in the trial and the logistics of undertaking the work on 26th September. Their advice has been incorporated into the trial design.
This application has been shared with the takahē team on 2nd November for review prior to submission to the AEC.

13. What month of year is most useful to report back to the AEC (depending on the project schedule and the animal's biology)?

September

13. Manipulation Grading

Please work through the document *'Grading of Manipulations'* (Please refer to DOCDM-870472), and determine the grading you believe best applies to the manipulation proposed in this application. Please also provide a rationale for the grading.

Grade A: No impact or virtually no impact.
Grade B: Little impact. Manipulations of minor impact and short duration.
Grade C: Moderate impact. Includes manipulations of minor impact and long duration or moderate impact and short duration.
Grade D: High impact. Includes manipulations of moderate impact and long duration or high impact and short duration.
Grade E: Very high impact. Manipulations of high impact and long duration.

Grading determined by the Applicant: B

Your rationale for the grading:
Grade B includes "**Disease/injury/functional impairment:** Studies of vaccines using killed pathogens." The animals will be kept in their normal husbandry conditions for the duration of the study. There will be capture and handling for blood collection and vaccination, and two doses of a killed vaccine administered. Handling time & stress will be minimised by using only skilled staff and it will be undertaken at site.

Note: The grading determined by the Applicant is not the grading assigned by the AEC. The Applicant will be advised of the AEC's grading and any conditions in writing.

DECLARATION by the APPLICANT

Tick boxes [☑] to indicate your agreement to conditions: *[Copy and paste this tick object ☑]*

[☑] I declare that the information in this Application is correct; and
[☑] I agree to comply with the conditions imposed by DOC's AEC for the manipulation; and
[☑] I agree to ensure all personnel involved in this manipulation will be properly trained and/or qualified to undertake the manipulation and will be aware of the contents of this AEC application; and
[☑] I declare the proposed manipulation has the necessary resources to undertake the manipulation with regard to the health and safety of the animals and staff
[☑] I agree to advise the AEC of any changes in the details of the manipulation as described in this Application.
[☑] I agree to comply with the reporting requirements stipulated by the AEC on approval of this research project.

9(2)(a) [redacted]

Application Number DOCDM	AEC443 APPLICATION DOC-7483666
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, takahē

Signed by the Applicant _____

Full Name: Catherine McInnes Date: 13/11/2023

DECLARATION by the ACCOUNTABLE MANAGER

Tick boxes [☑] to indicate your agreement to conditions: *[Copy and paste this tick object ☑]*

[☑] I agree to ensure my staff member complies with the conditions imposed by DOC's AEC for this manipulation; and

[☑] I agree to ensure all personnel involved in this manipulation will be properly trained and/or qualified to undertake the manipulation and will be made aware of the contents of this AEC application; and

[☑] I agree the proposed manipulation has the necessary resources to undertake the manipulation with regard to the health and safety of the animals and staff

[☑] I agree to oversee this Application via MORs, PDPs and other means to ensure the manipulation remains within the scope of the Application and the Approval, and all reporting required by the AEC is delivered on time;

[☑] I agree to advise the AEC of any changes in the details of the manipulation as described in this Application, and to advise the AEC if the Applicant leaves the Department, or if the work should be transferred to another staff member for operational reasons' or if the manipulations is abandoned for any reason.

Signed by the Manager: 9(2)(a)

Full Name: John Lyall

Role: Fauna Advice Manager

Date: 15/11/2023

Application Number DOCDM	AEC443 APPLICATION DOC-7483666
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, takahē

Appendix 1: Avian Influenza vaccine information

Registration number A009733: **Poulvac Flufend i AI H5N3 RG**

Registrant: Ministry for Primary Industries

Draft label information:

PRESENTATION

Bottles of 500 mL (1000 doses). Packs of 1 or 10 bottles.

DIRECTIONS FOR USE

By law the distribution and use of this product must comply with the requirements of the relevant operating plan.

General:
• Inject 0.5 mL (0.5 cc) subcutaneously, using aseptic technique, into healthy birds at 3 to 4 weeks of age or older.
• Shake well before use.
• Allow the vaccine to reach room temperature (18-29°C) before use.

Chickens:
• Administer another dose of 0.5 mL not less than 2 weeks later, if required.
• The second dose should be administered at least 4 weeks before point of lay.

Ducks:
• Ducks less than two weeks of age:
• Administer 0.2 mL of vaccine subcutaneously at the back of the neck.
• Administer another dose of 0.5 mL not less than 2 weeks later.
• Ducks two or more weeks of age:
• Administer 0.5 mL of vaccine subcutaneously at the back of the neck.
• Administer another dose of 0.5 mL not less than 2 weeks later.

ADVERSE EFFECTS, CAUTIONS AND CONTRAINDICATIONS

ADVERSE EFFECT
• Vaccinate only healthy chickens or ducks and avoid stressing the birds at the time of vaccination.
• Do not mix with any other vaccine or injectable product.
• The use of this product in laying birds has not been evaluated.
• Local or systemic post-vaccination reactions can occur due to the use of oily vaccines. Symptoms observed are generally transitory and can include oedema and granulation at the injection site, anorexia and dehydration. Such reactions can be minimised by good aseptic vaccination technique.

CAUTIONS
• Destroy any unused vaccine and containers after vaccination (including syringes and needles) by burning.
• Do not mix the vaccine with other vaccines or administer another vaccine shortly before or after vaccination with this product.
• Consult a physician immediately for an accidental self-injection and show this package insert to the physician.
• KEEP OUT OF REACH OF CHILDREN AND UNINFORMED PERSONS

CONTRAINDICATIONS
• None.

WITHHOLDING PERIODS
Meat: Nil.

STORAGE
• Store in the dark between 2 °C and 8 °C. Do not freeze.
• Protect from direct sunlight.
• Use contents of each vial within 6 hours of opening

Appendix 2: General Instructions for subcutaneous injection of vaccine

- The vaccine is supplied in a 500mL bottle and is given to the bird using a needle and syringe.
- The vaccine is injected under the skin but NOT into the muscle below.
- The vaccine should be drawn up into the syringe and then allowed to warm to room temperature (this is more comfortable for the bird).

EQUIPMENT NEEDED

1. Vaccine container
2. 1 mL syringe
3. 25 gauge 5/8th inch needle
4. alcohol swab (mediswab or cotton wool and meths)
5. dry swab (gauze or cotton wool)
6. Sharps container for needle disposal
7. Bird
8. Bird handler
9. Veterinarian

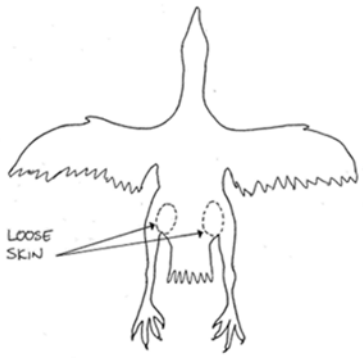
PREPARING THE VACCINATION

1. Store the vaccine in the fridge at 2-8 degrees C in the dark. Do not Freeze.
2. When ready to use, take the vaccine out of the fridge and shake well to mix.
3. Write the date on the vaccine bottle.
4. Break off the metal seal on the top of the rubber injection port.
5. Swab the injection port on the vaccine with alcohol to sterilise it with a mediswab or cotton ball soaked in methylated spirits.
6. Firmly attach the needle to the syringe – 25 gauge 5/8th inch needle to a 1mL syringe.
7. Insert the needle through the centre of the rubber stopper CAREFULLY.
8. Hold the vaccine upside down and slowly suck vaccine into the syringe until you have a little more than the prescribed dose of vaccine.
9. Flick the syringe to dislodge any air bubbles and squirt them slowly back into the vaccine bottle.
10. Keep squirting until all the bubbles are gone and you have the prescribed dose of vaccine left in the syringe.
11. Pull the needle out of the vaccine bottle and CAREFULLY recap the needle.
12. Leave the syringe and needle to warm to room temperature.
13. Repeat this procedure to draw up all the doses you need for your vaccination session.
14. Put the vaccine back in the fridge.
15. Once open, the vaccine can be used for 30 days. (Note that this expiry is based on Zoetis technical advice for limited use of the vaccine in this trial, and only applies when following the above instructions for maintaining sterility of the product and correct storage.)
16. If you are in doubt that the vaccine has been stored correctly (kept refrigerated), then discard it and get a new bottle.

GIVING THE INJECTION

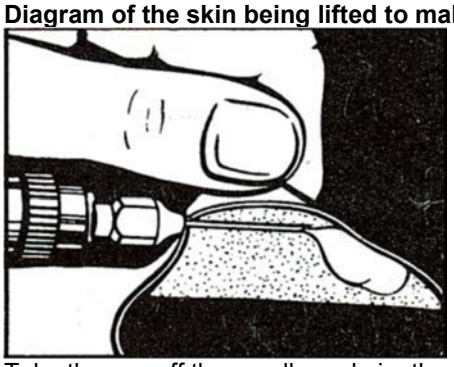
1. Have the following equipment ready for use:
    - The correct dose of vaccine drawn up in syringe with needle attached and warmed to room temperature. (the cap should be on the needle to avoid accidental stabbing or contamination of the needle)
    - One alcohol swab (mediswab or cotton wool in meths)
    - One dry swab (gauze or cotton wool)
2. Have an assistant restrain the bird on its back or side with its legs restrained to provide access to the groin (where the bird's leg joins its belly).

Diagram of ventral (belly) of a bird showing the groin region for subcutaneous vaccination sites:



3. Spread the feathers in the groin area.
4. Wet down the feathers with the swab to clear a patch of skin and swab the skin.
5. Lift the loose skin 1-2cm off the body to make a "tent".

Diagram of the skin being lifted to make a "tent" for a subcutaneous injection



6. Take the cap off the needle and aim the needle about halfway down the side of the tent. Keep the needle parallel to the body wall. When the needle goes through the skin, it should still be above the muscle of the groin i.e. you are injecting into the space inside the tent, not into the muscle.
7. Suck back on the syringe to check for blood. This is to avoid injecting into a blood vessel.
8. Inject the vaccine with a steady firm pressure.
9. Withdraw the needle and place it into the Sharps container.
10. Use the dry swab to press over the injection site if there is any bleeding.
11. Release the bird.

12. Record:	Bird ID	Date	Dose	L or R side	Vaccinator	Holder	Vaccine Batch	Expiry Date	Notes

13. Transfer this data to the vaccination record spreadsheet
14. Note any other specifics about the injection process not described above. E.g. if there was bleeding at the injection site.

Application Number DOCDM	AEC444 APPLICATION DOC-7483665
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, kākāpō

Revised September 2023

DEPARTMENT OF CONSERVATION
APPLICATION TO MANIPULATE LIVE ANIMALS
Code of Ethical Conduct for the Care and Manipulation of Live Animals

1. APPLICANT'S DETAILS:

Name: Kate McInnes

Date: 24 October 2023

Role: DOC Veterinarian

Unit: BH&V Group, Wellington

APPLICANT'S ADDRESS:

Phone no: 9(2)(a) [redacted]

Email: kmcinnes@doc.govt.nz

2. ACCOUNTABLE MANAGER'S DETAILS:

Name: John Lyall

ACCOUNTABLE MANAGER'S ADDRESS:

As above or: DOC, Hokitika

Phone no: 9(2)(a) [redacted]
Email: jlyall@doc.govt.nz

2a. AEC444

2b. **MANIPULATION TITLE: Avian Influenza vaccination safety and efficacy trial kākāpō**

2d. **Duration of the manipulation**

- *Over what timeframe are you seeking the approval?*
- *You must not commence the manipulation until you have received the approval, signed by you, your accountable manager, and the AEC Chair.*

Application Number DOCDM	AEC444 APPLICATION DOC-7483665
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, kākāpō

- *NOTE: The AEC will not generally give an approval for longer than two years at one time. Please state if this manipulation is likely to extend longer than two years from the commencement date.*

Anticipated start date:	February 2024	Anticipated finish date:	June 2025

2e. What months of the year is the manipulation most likely to be undertaken? e.g., October – March
For the duration of the dates specified

3a. Summary of the proposed manipulation for a LAYPERSON

- *Provide an abstract describing the manipulation (maximum 400 words).*

Avian influenza is a viral disease which can cause mass mortality events in birds. The current strain is decimating many populations of wild birds overseas, is predicted to reach the Southern Ocean by 2024/25 and was confirmed in South Georgia, October 2023.

We want to test the safety and efficacy of vaccination to protect critically endangered species.
The vaccine is a commercial product registered in New Zealand by Ministry for Primary Industries. It is considered very safe and highly effective. It contains inactivated (dead) virus so it cannot cause avian influenza. Vaccination reduces risk of illness or death and reduces shedding of virus, thus protecting the individual and its flock.

Kākāpō are a critically threatened species where it is possible to reliably administer a full course of vaccine (2 injections under the skin, one month apart) to individually identified birds. We are able to handle them repeatedly for a veterinary examination and blood testing to detect any effects on health status, and measure the immune response by detection of antibodies over a 12 month period.

Free-living kākāpō will be tracked using telemetry and captured on the predator free off-shore island of Whenua Hou. Each bird will receive a pre-vaccination health check by a veterinarian, and a blood test for health and antibody testing. Up to 1.5mL of blood will be collected from the leg or wing vein, as is standard for this species.

The vaccine is given under the skin. One month later the bird will receive a second vaccination and blood test. Further blood will be collected at 2-3, 6 and 12 months post vaccination to determine the level of antibody response and how long it lasts.

A cloacal and choanal (oral) swab will be collected on day 0 for PCR testing to demonstrate the birds were not incubating avian influenza at the time of vaccination.

Smart transmitters that report individual birds' daily activity levels will be used to remotely monitor for any adverse reactions.

We propose to work with a total of 10 adult or juvenile kākāpō, divided into two cohorts. Cohort 1 will first receive the vaccination & blood tests, and a recheck at 1 month. If no safety issues are identified, then Cohort 2 will receive vaccinations & blood tests. This allows a careful start to the trial where the first 1 month the most important to test vaccine safety. The following blood sample months will determine level and duration of antibody presence and determine when further boosters would be required.

Additional approval given on 16/4/24 to collect a 10-14 week blood sample to target peak antibody levels, and to collect an opportunistic blood sample for further antibody testing, if birds are being handled for routine management purposes, with no more than twice per bird over the 12 months of the trial.

Application Number DOCDM	AEC444 APPLICATION DOC-7483665
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, kākāpō

3b. Description of the proposed manipulation (methods)

- *Provide a more detailed explanation. Describe the equipment, the location, and any environmental factors: weather, time of the year. Why have you decided to undertake the manipulation in this way? What advice have you sought? Include the species, the number of individuals, the source of animals, and the disposal/fate of animals at the conclusion of the manipulation.*
- *Be specific about the timelines for the proposed investigation and the purpose of the research, testing or teaching.*
- *Include some consideration and planning for when things might not go right.*

Please note: this is one of five trials to assess avian influenza vaccination safety and efficacy in nationally critical threatened species (takahē, kākāpō, kakī, tūturuatu, kākāriki karaka). Manipulation details which are specific to this species (kākāpō) have been highlighted in yellow. All other details are consistent across the five trials. By highlighting the species specific details I hope to assist the AEC with the volume of workload associated with simultaneously assessing these trials.

This trial is designed to test the efficacy and safety of a vaccine in a critically threatened species.

Selection of the species for potential vaccination is based on the risk that they could undergo an extinction event when highly pathogenic avian influenza (HPAI) reaches New Zealand. 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 risk of extinction.

The current kākāpō population is 247. Kākāpō are very slow breeding with chicks only produced in years with rimu fruit masting. This last happened in 2022 and they will not breed again until at least 2026. This leaves the species exceptionally vulnerable to disease outbreaks as individuals who die are not quickly replaced. Kākāpō are also very slow to start breeding – females do not breed until 5 years of age and males generally are in their teens before they successfully breed. Kākāpō do not do well in a confined space (captivity) therefore all management must be done at their wild sites.

Based on the global evidence from overseas during this epizootic, the species most at risk of infection are those which exhibit congregation behaviours e.g. feeding, breeding or roosting in groups, those which are exposed to at risk species e.g. where seabirds overlap with another threatened species, and birds held in captive facilities where biosecurity options are limited e.g. open pens and large aviaries.

Kākāpō are one of 5 species identified by the DOC HPAI Technical Advisory Group as at risk where administration of a full vaccination programme is feasible in sufficient number of individuals to provide protection against species extinction. See DOC-7111177 Mitigation Options Guideline for HPAI.

There are only 247 kākāpō in existence and they have very low reproductive rates only breeding every 2-4 years. Whenua Hou has been the primary breeding island for many years and also contains large breeding petrel and shearwater populations. There has previously been evidence of disease transfer from petrels and shearwaters to kākāpō.

Use of the vaccine is dependent on Ministry for Primary Industries approval, and currently requires the birds to be held in captivity or in a defined restricted area. Birds require two injections one month apart and must be individually identified with a permanent mark e.g. microchip or leg band. All kakapo have a microchip for individual identification as part of routine husbandry.

Effective vaccination reduces susceptibility to infection. When infection does occur, it reduces clinical signs of disease and the amount of virus shed into the environment (Animal Health Australia, 2021).

There is precedent for undertaking a vaccination program in kākāpō. Since 2004 there has been a vaccination program in place to protect kākāpō against death from a bacterial disease – erysipelas. This current vaccination program means that there are staff highly skilled in this procedure on kākāpō and that vaccination is a standard management tool for this species. No adverse events in kākāpō have been recorded as a result of the erysipelas vaccination programme.

Additionally, vaccination of California Condor was approved in the United States following an outbreak in the wild population. This was the first avian influenza vaccination programme in a wild endangered species. Advice from the veterinary and technical advisors to the condor vaccination programme has been received and is incorporated into this trial design.

We wish to undertake a limited trial to determine the safety and efficacy of the avian influenza specific vaccine in kākāpō as a preparedness measure for the arrival of HPAI in New Zealand.

The vaccine is produced commercially by Zoetis for use in poultry: Poulvac Flufend i AI H5N3 RG inactivated (killed) vaccine - see Appendix 1. It has been in production since 2006 and is widely used in the poultry industry. Publications on AI vaccine use in poultry and avian species in zoos have indicated a very high level of safety across a wide range of species, and efficacy has been well established. (Kandeil et al 2018, Philippa et al 2006, Philippa 2007a, Philippa 2007b, Pitman 2006). The vaccine in inactivated, so there is no live virus present, and it cannot cause avian influenza.

Advice from Zoetis (USA) indicates that this vaccine should provide good protection against the current strain of HPAI with 91% amino acid homology with the circulating strain. A newer vaccine based on the circulating strain is in production but is will not be available until the end of 2024 at the earliest.

Vaccine will be obtained from PacificVet in Christchurch and transported in a chilly bin with ice-packs by overnight courier (as per their standard transportation procedures for vaccines) to ensure cold chain is maintained. Use in the field will be managed by extraction of sterile aliquots into sterile vials or syringes. This enables sustainable use of the 1000 dose vial and maintenance of sterility of product. This process was discussed with the Zoetis Senior Research Advisor responsible for poultry products and is considered safe and appropriate.

Sterile aliquots will be obtained by using a sterile needle and syringe to extract the aliquot from the closed vaccine vial. The vial will be shaken to homogenise the contents, then the rubber stopper will be swabbed with alcohol. The sterile needle will be attached to the sterile syringe and the needle inserted via the rubber stopper. The aliquot will be drawn up into the syringe, then the needle & syringe removed from the stopper and the cap replaced on the needle. The needle will be swapped for a new sterile needle or a sterile vaccine cap. Both the aliquots and the vaccine vial will be stored refrigerated in accordance with the packaging instructions. Vaccine doses will be drawn up immediately before use and allowed to warm to body temperature just prior to injection.

DOC veterinarians Kate McInnes and Lydia Uddstrom will administer the vaccination.

All birds will receive a full veterinary physical examination at the start of the trial. Only birds in good body condition exhibiting signs of good health will be included. (Any birds which show signs off poor health will be further investigated as per normal veterinary practices).

The kākāpō recovery team have been involved in the design of the study, and selection of study animals. Whenua Hou is the easiest location to access, track and capture the kākāpō required for this trial.

Individuals for vaccination will be selected by the kākāpō recovery team, based on the knowledge of individual home ranges and ability to be captured as well as the programme's planning for any translocations.

Each individually permanently marked (microchipped) bird will receive two doses of vaccine by subcutaneous injection into the inguinal (groin) region with a 1 month interval (no less than 3 weeks apart and a maximum of 6 weeks apart). The first vaccination will be into the left inguinal region, and the second vaccination into the right inguinal region.

Birds <1.5kg will receive 0.20ml per dose. Birds >1.5kg will receive 0.5ml per dose (as per dosages used in Vergara-Alert et al 2011).

At the start of the trial, each bird will receive a cloacal and oral swab to determine presence/absence of virus at day 0.
The technique will follow the draft SOP Avian swab sampling DOC-6840491 which has undergone veterinary peer review & user testing and is awaiting AEC endorsement before Director sign-off. These types of swabs are used in standard health testing on avian species and would be undertaken by the veterinarian. The test would be considered a baseline health test to demonstrate the birds were not incubating avian influenza at the time of vaccination. The swabs will undergo PCR testing at BioPacifica to look for avian influenza virus.
This is important to be able to demonstrate that any antibody response is due to the vaccination rather than the bird being infected by a wild strain of avian influenza.

First trial - Cohort 1: Five individuals will be vaccinated as per the described protocol above. Blood (up to 1.5ml) will be collected at 0, 1 and 2-3 months to measure health parameters (white cell count & differential) and antibody response (commercial serum ELISA test to measure antibody titre). Antibody testing will be undertaken at a commercial laboratory (BioPacifica, Christchurch).

Note: 1% of body weight is considered an acceptable amount of blood to collect from a healthy bird. Adult kākāpō weigh 1.5-2kg, therefore 15-20ml would be within the safe range. We propose only up to 1.5ml will be collected to maintain a high margin of safety.)

Second trial - Cohort 2: Based on consideration of the results of the first trial, if safety has been demonstrated, a second cohort of 5 individuals will receive the vaccination as per the described protocol above, and blood (up to 1.5mL) will be collected at 0, 1 and 2-3 months to measure health parameters (white cell count & differential) and antibody response (commercial serum ELISA test). We will wait 1 month until we have established the vaccine is safe in cohort 1 before we start cohort 2.

Amendment April 2024: where birds have received a blood sample at 8 weeks, they will receive an additional blood sample at 10-14 weeks. Otherwise birds will not be tested at 8 weeks and will be tested at 10-14 weeks instead.

Two additional blood samples will be collected from both cohorts at approximately 6 and 12 months to measure duration of antibody response.

Note: If antibody response at 2-3 months is noted to be muted (i.e. a low response) then the DOC vets (Kate McInnes and Lydia Uddstrom) will discuss the use of a third dose of vaccine. This was used in some species in European zoos where the initial responses were considered insufficient. Consideration will be

given to the level of response detected, the impacts of additional handling, and any other welfare factors noted during the preceding handling events. The benefits of testing a third dose of vaccine will be carefully considered, and this will only be undertaken if the welfare impacts are considered minimal. The justification for a third dose in this trial would be to confirm if this dose is warranted and would deliver protection for the kākāpō population in the event of an outbreak of HPAI in Aotearoa New Zealand.

A maximum of 10 birds will be included in the trial. There will be 70-80 kākapō on Whenua Hou at the time of the trial.

These trials are modelled on the vaccination of California Condor in the USA. (FWS.gov 2023) however the 1 month interval is based on the European zoo data, and considered more appropriate to allow for recovery between handling events in birds which normally have minimal handling by humans.

Estimated timing/schedule of manipulations:

First vaccination and blood sample	Second vaccination and blood sample	2-3 month blood sample	6 month blood sample	12 month blood sample
First cohort of 5 birds				
~ 1st March 2024	~ 1st April 2024	~1st May 2024	~30th August 2024	~15th March 2025
Second cohort of 5 birds (maximum 10 total)				
~ 1st April 2024	~1st May 2024	~1st June 2024	~30th August 2024	~15th March 2025

Second cohort of 5 birds – same timeline but 1 month after first cohort have been vaccinated and shown no negative reaction. Blood collection at ~6 and ~12 months may be undertaken at the same time for both cohorts – these samples are about longevity of antibody presence, so the exact timing is less critical.

During a handling event, all involved staff will gather and have a pre-handling briefing by the veterinarian and the team leader to ensure all roles and responsibilities are clearly understood. Any issues can be raised at that time for clarification. The kākāpō team leader will be responsible for the safe capture and handling of the bird. The veterinarian will be responsible for the health examination, vaccination and blood collection.

All equipment will be prepared prior to capture to minimise handling time. Staff will know where to situate themselves and what actions are required so that an efficient process is maintained. Kākāpō chicks and juveniles receive vaccinations to protect them against erysipelas (a bacterial disease). The kākāpō rangers involved in this trial will be team members with previous experience in vaccination and blood collection in kākāpō.

Kākāpō team rangers will be consulted to select appropriate birds based on their previous observations. It is possible to encounter birds with a "stressy" personality. Such a bird is not a good candidate for a trial which requires repeated handling events, and therefore it would be excluded from the trial. If any birds are observed to exhibit any significant distress during any of the procedures, the kākāpō team rangers and DOC vets will call a stop to the procedure and reassess the procedures being undertaken and the suitability of the bird(s) for inclusion in the trial. If necessary, changes will be made to reduce issues such as, but not limited to; reducing number of people present, slowing down the capture process, taking a break so birds and humans can calm down, rejecting individual birds from the trial, changing handlers/vet to allow a rest break, abandoning the work for the day, or if serious issues are encountered, potentially

stopping the trial and discussing a redesign or full abandonment. At all times bird welfare is paramount and will determine the actions of the kākāpō team and veterinarians.

Catching a kākāpō involves walking to their home range then using an aerial and receiver to track the VHF channel produced by an individual's transmitter. This allows the rangers to determine where the bird is located. Kākāpō may be anywhere from an underground roost to perching in the canopy. Once a bird is sighted the rangers assess the access to the bird and catch them by hand. Often once caught the bird is placed in a 'catch bag' (a soft cotton bag with a draw string which provides a safe restraint for temporary holding and transport of kākāpō) to allow them to be carefully and safely carried a short distance to a suitable location for examination.

Once caught the kākāpō will be weighed in the bag using a hanging scale (standard technique) and then will be carefully removed to undergo a physical examination to determine its health status prior to any further manipulation. If the kākāpō is determined to be healthy, blood collection and vaccination will proceed.

For blood collection and vaccination, a trained kākāpō handler will restrain the bird on its side or back.

The blood collection site (leg or wing vein) will be swabbed with a sterile alcohol wipe immediately prior to collection. Blood will be collected using a 1ml or 3ml sterile syringe attached to a 25 – 26 gauge ¾ - ½ inch sterile hypodermic needle.

In the event that the temperature is cool and blood collection likely to be slow due to the presence of small contracted veins, the feet will be warmed for 3-5 minutes using Kathmandu hand warmers wrapped in gauze to boost circulation and enhance blood flow to enable effective blood collection.

After collection, the site of blood collection will be covered with a gauze swab and pressure applied to control any bleeding. In the very unlikely event of uncontrolled bleeding, pressure will be applied for a further 1-5 minutes. If still uncontrolled, alcohol will be rubbed onto the foot and leg to cool the limb to reduce blood flow If required, a silver nitrate stick will be carefully used to stop the bleeding.

Blood will be transferred into a blood microtainer and spun in a centrifuge to separate the serum from the blood cells. Serum will be drawn off using a sterile pipette and transferred into an epindorf tube for storage in the freezer, prior to transfer to the commercial laboratory in batches for antibody testing.

1-2 drops of blood will be used to make blood smears which will be sent to a commercial veterinary pathology laboratory for a white cell count and differential. This provides a baseline health analysis which can detect infection or inflammation. Any abnormal results will be further investigated by the veterinarian in consultation with the kākāpō staff.

Once bleeding has stopped, the bird will be vaccinated using a 1mL syringe attached to a 20 gauge ½ inch needle. The vaccination site will be swabbed with a sterile alcohol wipe immediately prior to vaccination. See Appendix 2 for details of the vaccination technique.

The bird will then be checked for any abnormalities and the veterinarian will determine if any further actions are required for health or welfare. It will be quietly released and observed as it moves away.

The bird will be monitored via the remote monitoring system on Whenua Hou which allows tracking of birds and records their daily activity levels. This is used to monitor health and behaviour and is capable of alerting staff to changes which indicate reduced activity and possible health concerns. If any bird's data

indicates a significant reduction in activity following vaccination, the bird will be recaptured for a physical examination, and appropriate steps taken by the DOC veterinarian if ill health is detected.

At subsequent handling events, the vaccination site will be examined and any discolouration, swelling, granuloma formation or unexpected abnormality will be noted and reported to the veterinarian. Photographs will be taken to provide a clear record of the trial.

Location & timing:
The trials will be undertaken on Whenua Hou between Feb 2024 and June 2025. Whenua Hou/Codfish Island is 1396 ha and 3km off the north-west coast of Rakiura/Stewart Island. Vegetation is mostly coastal and podocarp forest, similar to that on Rakiura/Stewart Island. It is one of the islands used in the Kākāpō Recovery Programme. Whenua Hou was chosen because it holds a large portion of the kākāpō population and has well-established logistics.

Safety:

Results from a meta-analysis of use of vaccine in European Zoos found very low adverse reaction rate at 0.04% local reactions and 0.015% general reactions reported. EFSA 2007. Based on this, we do not anticipate significant issues with the vaccine, however we will be prepared for immediate veterinary care if any reactions to occur.

The vaccine packaging label states: “Local or systemic post-vaccination reactions can occur due to the use of oily vaccines. Symptoms observed are generally transitory and can include oedema and granulation at the injection site, anorexia and dehydration. Such reactions can be minimised by good aseptic vaccination technique.”

Anaphylaxis:
A severe immediate immune hypersensitivity response could occur if the vaccine product stimulates such a response. This is considered unlikely due to the extensive use of this vaccine and other similar vaccine products in Europe, however it is possible and needs to be considered as a potential adverse event. The vaccination team will include a veterinarian who will have access to emergency drugs and supportive care for management of anaphylaxis (including corticosteroids, adrenaline, oxygen, fluids).

Injection site reactions:
The vaccine contains an adjuvant (oil) which is present so that it stimulates a stronger immune response with greater antibody production. This can sometimes be associated with a small pea-sized lump at the site of injection. This is normal and expected, although generally not all birds will develop a lump. This will be checked at the 1 month mark, and records kept of any reactions detected. If an excessive size reaction is detected in an individual, then the vaccination will be paused until it is determined that the lump does not enlarge further, or cause any impacts on the birds – this is likely to be a period of 2-4 weeks. Body weight, activity levels etc will be reviewed and a full physical examination undertaken.

A localised bacterial infection could result if poor sterile technique is used. Only veterinarians or specifically trained DOC staff members will be administering the vaccine, and these operators all have training in appropriate sterile techniques. If a bird experiences an infection at the site, it will receive veterinary care and follow-up to ensure the issue is managed.

Mis-injection could occur if the bird is poorly restrained and moved during vaccination. This will be managed by only using well trained, experienced bird handlers to restrain the birds. For some individual birds, they are calmer with head cover which can aid in handling. This will be determined on an individual bird basis. If a mis-injection occurs, the veterinarian will determine the appropriate next steps. This may

include, re-injection if the first injection merely failed to enter the bird, appropriate first aid measures if any injury was caused, and/or exclusion from the trial and follow-up care. As noted previously, "stressy" birds will not be included in the trial which will reduce the risk of injury or mis-injection.

In the event of a serious reaction or injury during the vaccination trail, the bird will be taken to Dunedin Wildlife Hospital for specialist care by Dr Lisa Argilla. This is standard procedure for kākāpō requiring a high level of veterinary care.

Results:
The results of this trial will determine if this vaccine is safe to use in this species, and the level of antibody response produced by a 2 dose vaccination. In some other species, notably penguins, the antibody levels following vaccination remain low and, in some species, a third vaccination was used to ensure a stronger response (ESFA 2007). The duration of antibody presence also varied between species. Therefore, this trial will help to determine the appropriate vaccination regime for kākāpō in the event that more widespread vaccination is required during a highly pathogenic avian influenza outbreak in New Zealand.

3c. Attach Photos of equipment, the species, the location (or a map); to help set the context.

Kākāpō positioned for eysipelas vaccination

3d. References

- *List the references referred to in the application*

DOC-7111177 Mitigation Options Guideline for HPAI.

DOC-7111177 Mitigation Options Guideline for HPAI
https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7111177

EFSA 2007. Vaccination against avian influenza of H5 and H7 subtypes as a preventative measure carried out in Member States in birds kept in zoos under Community approved programmes. ESFA journal, 450. ESFA-Q-20006-156
https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499835

Health Australia (2021). Response strategy: Avian influenza (version 5.0). Australian Veterinary Emergency Plan (AUSVETPLAN), edition 5, Canberra, ACT. Response_Avian-influenza.pdf (animalhealthaustralia.com.au) Animal

FWS.gov 2023 Southwest California Condor Flock HPAI Information Updates - 2023 | U.S. Fish & Wildlife Service (fws.gov)

Kandeil A, Sabir SM, Abdelaal A, Mattar EH, El-Taeel AN, Sabir MJ, Khalil AA, Webby R, Kayali G, Ali MA. Efficacy of commercial vaccines against newly emerging avian influenza H5N8 in Egypt. Nature Scientific Reports, 2018. 8:9697 | DOI:10.1038/s41598-018-28057-x https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499854

Philippa JDW, Munster VJ, van Bolhuis H, Bestebroer TM, Schaftenaar W, Beyer WEP, Fouchier RAM, Kuiken T, Osterhaus, ADME. Highly pathogenic avian influenza (H7N7): Vaccination of zoo birds and transmission to non-poultry species. Vaccine, 2005, 23:5743-5750. https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499837

Philippa JWD 2007a, in XI. Vaccination of Non-domestic Avian Species, Transmissible Disease Handbook. European Zoo Vets 5th Edition link

Philippa J, Bass C, Beyer W, Bestebroer T, Fouchier R, Smith D, Schaftenaar W, Osterhaus, A. Vaccination against highly pathogenic avian influenza H5Na virus in zoos using an adjuvanted inactivated H5N2 vaccine. Vaccine, 2007b, 25: 3800-3808. https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499841

Pitman 2006. M Pittman, European Commission 12th Annual meeting of national avian influenza laboratories Veterinary and Agrochemical Research Centre (VAR) Uccle, Brussels, 16-18 October 2006 LINK: link.

Vergara-alert J, Ferhandez-Bellon H, Busquets B, Alcantara G Delclaux M, Pizarro B, Sandchez C, Sanchez A, Majo N, Darju A. Comprehensive serological analysis of two successive heterologous vaccines against H5N1 Avian Influenza virus in exotic birds in zoos. Clinical and Vaccine Immunology, 2011. P. 697-706. https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499845

4. INVOLVEMENT OF OTHER ANIMAL ETHICS COMMITTEES:

4a. Is this Application; or a related or similar application; been or is being considered by another Animal Ethics Committee. Has this project been requested to be considered by any other AEC?

If so, please provide details.

No

4b. Does this manipulation interact with a manipulation approved by other Animal Ethics Committee? If so, detail your communications with those committee(s), and state any conditions imposed by (an)other AEC.

No

5. JUSTIFICATION FOR PROPOSED MANIPULATION:

5a. Detail any action undertaken to determine that the proposed work has not already been done.

Avian influenza vaccine efficacy and safety has been undertaken on other avian species, however it has not been undertaken in New Zealand endemic species. Although we expect similar results, it is prudent to undertake this trial to provide more evidence of safety and efficacy in the species which we intend to vaccinate in the event of a HPAI outbreak.

5b. Have alternatives been considered to the proposed manipulation involving reduction, or replacement of live animals, or refinement of techniques?

We are looking at the species-specific response and have selected a minimum size divided into two cohorts, so other methods of reduction are not appropriate for this work.

The cohort approach allows us to cautiously approach the safety issue and assess initial results before involving the full number of birds.

Typically, kākāpō are handled once every 18 months for a transmitter change. If this timing falls within the trial period, every effort will be made to coincide the required transmitter change with the trial handling to reduce the number of times an individual is handled.

5c. To what extent has there been assessment of the suitability of using non-sentient or non-living alternatives in the project; or replacement of animals as subjects with suitable non-sentient or non-living alternatives?

N/A, see above

5d. How will the proposed work result in the extension of knowledge relevant to the health, welfare, or conservation of animals?

This work will specifically contribute to the future health of the species for conservation purposes by providing evidence of the safety and efficacy (or not) of this vaccine in this species, and inform the appropriate vaccine schedule for the species. This will determine if the vaccine is employed in the future in the face of an avian influenza outbreak in New Zealand.

5e. Is the manipulation required as part of an approved training programme?

No.

5f. How will the results of this work be made available to staff within and outside DOC? (*For example internal report, journal paper, best practice guide, workshops etc*).
Internal report, journal paper, conference presentations.

6. SELECTION OF SPECIES & NUMBER OF INDIVIDUALS FOR PROPOSED MANIPULATION

6a. What will be the source of the animals to be manipulated, and how many from each source will be manipulated?
Kākāpō free-living on Whenua Hou. 10 birds in total from a range of adults and juveniles.

6b. Will any of the animals involved be used more than once, and if so, how many times will each animal be used?
Only once (but each animal handled/manipulated multiple times – twice for vaccinating and four more times for blood sampling).

6c. What factors have been taken into account in the choice of the animal species?

Kākāpō are one of 5 species identified by the DOC HPAI Technical Advisory Group as at risk where administration of a full vaccination programme is feasible in sufficient number of individuals to provide protection against species extinction.

6d. Could the information being sought be obtained by work on some other species?
No. The trial specifically uses kākāpō since the safety and efficacy needs to be tested in the target species, and due to our unique avifauna, there are no reasonable surrogate species in the world.

6e. Will the question be answered with the size of the sample?
Yes.

6f. Is the number of animals proposed to be manipulated the minimum necessary to provide a scientifically interpretable result, consistent with the level of statistical precision required? What consideration has been given to the design of the study with regard to:

- **The level of precision necessary in the results?**

The purpose of the small trial is to establish if there is a species-specific sensitivity to the vaccine and its adjuvant. For this purpose, we require only a small number of birds to extrapolate a species sensitivity. Similarly, for determining vaccine response, a sample size of 10 will provide sufficient individual variation to establish an overall species response level.

A larger sample size would ensure a more nuanced examination of the species' response to vaccination; however we are examining a general level of impact/effect, rather than subtle differences. Thus, results which showed >1 bird having a safety issue, or the majority or average antibody response to be low, would be sufficient to inform the next steps for decision making regarding kākāpō vaccination.

- **The possible confounding effects of animal variation?**

We expect some individual variation since the immune response is affected by individual health status and biological variation. This sample size is sufficient to ensure we have a range of individual responses to examine.

- **The needs of statistical analysis?**

There is likely to be individual variation, which, for the antibody response, requires a reasonable sample size. We determined that 10 was the maximum which was feasible to include in the trial, and also sufficient to allow for individual variation to establish some baseline parameters of antibody response.

Ultimately, in an outbreak situation, the results of a sample size of 10 will be sufficient to make reasonably informed decisions about the use of a commercially produced killed vaccine which has a good history of safety and efficacy across a wide range of species.

7. WELFARE OF ANIMALS DURING PROPOSED MANIPULATION:

7a. What measures will be taken to ensure: the general health and welfare of animals before, during and after manipulation, including the adequacy and cleanliness of housing, caging and equipment; the provision of food and water; prevention of over-crowding, and prevention and control of disease?

Kākāpō will be caught and handled by experienced rangers as per usual kākāpō protocols on Whenua hou/Codfish Island.

Each bird will receive a veterinary examination at the start of the trial. Equipment will be disinfected between individuals, or new equipment will be used. Once blood has been collected and vaccination undertaken the bird will be re-released where they were found.

Ongoing monitoring will be undertaken using remote monitoring tools to minimise disturbance of the bird.

There will not be any kākāpō breeding for the coming seasons (2023/24/25), so this trial will not interfere with any breeding behaviour.

7b. What movement and transportation measures will be followed for the animals to be manipulated to ensure their welfare and humane treatment?

Birds will be vaccinated on site, where they are captured, therefore there will be no transport required.

However, if a bird requires specialist veterinary care e.g. in the event of an injury or serious reaction, then it will be transferred to Dunedin Wildlife Hospital (DWH), using the standard kākāpō transport crates, and in accordance with normal kākāpō transportation procedures. Briefly, birds will have non-slip flooring the crate, be transported via helicopter to Invercargill, then car to Dunedin. Transport will be managed to reduce noise and allow for temperature control. Radio will be off and driver/passenger will ensure minimal noise. Travel will be direct and the hospital will be alerted ahead of time to enable a fast hand-over a and rapid care.

Dr Lisa Argilla at DWH has treated multiple kākāpō during her time at Massey University Wildbase hospital and DWH and is familiar with their requirements for hospital care.

Supportive therapy would be provided prior to transport which may include pain relief and fluids. The kakapo veterinary advisor would travel with the bird if ongoing supportive care was required during transport.

7c. What measures are to be taken to minimise the pain or distress of any animal manipulated? *Stating there will not be any impact is not acceptable. The AEC is looking for the Applicant to (1) provide analysis about the potential for pain and/or distress to the animal(s), and (2) describe how they will manage that pain or distress. Identify how you would ascertain pain or distress animal's behaviour, environmental conditions likely to lead to pain or distress.*

Birds will be captured and handled by experienced DOC kākāpō staff using their routine techniques. Only experienced staff will handle the birds. Initial physical examination, vaccination and blood collection will be undertaken by a veterinarian.

Any bird detected to have abnormalities will be examined and rejected from the trial, and receive normal veterinary investigation/intervention.

Subsequent examination and blood collection may be undertaken by DOC staff trained in blood collection from kākāpō, provided the initial results (0,1 and 2-3 months vaccination and check) are normal across the cohorts.

The subcutaneous injection is not considered painful, and the vaccine dose will be warmed to room temperature prior to injection. Blood collection is associated with a minorly painful pin-prick when the needle is inserted. This will be minimised by careful planning and handling.

If birds are observed to have any pain response to the vaccination, the staff will report it to the veterinarian who will investigate. In the event that there is an injection site reaction (painful inflammation) then an anti-

inflammatory such as Metacam may be prescribed by the attending veterinarian, as well as antibiotics if infection is also present.

As noted earlier, if any serious adverse reactions occur, veterinary care by the attending veterinarian will be undertaken, and transfer to Dunedin Wildlife Hospital undertaken if required for more intensive specialist care.

8. CONTINGENCY PLAN:

8a. What arrangements have been made for the abandonment of any manipulation and/or the euthanasia of animals where pain or distress cannot be held within reasonable levels?

If pain or distress is apparent, during handling or following the procedure, the veterinarian will investigate. If the veterinarian deems the level to be unreasonable, then the manipulation will be abandoned and all efforts made to ameliorate the event e.g. anti-inflammatory, pain relief medication, antibiotics.

In the unlikely event that the pain is not temporary and cannot be managed, transfer to Dunedin Wildlife Hospital will allow for intensive veterinary intervention and care. This includes the ability to undertake orthopaedic intervention e.g. in the event of a broken bone, or intensive surgery e.g. in the event of a severe localised vaccine reaction.

Supportive therapy would be provided during any transport which may include pain relief and fluids.

9. PEOPLE TO UNDERTAKE PROPOSED MANIPULATION:

9a. Who are the person(s) primarily involved in carrying out the proposed manipulation?
Kate McInnes and Lydia Uddstrom are the primary persons.

9b. What is the experience and qualifications of the person primarily responsible (9a) for the undertaking and supervising the manipulation (including selection of animals, their care and disposal?)
Kate McInnes has been the DOC vet since 2000 and has worked across a range of avian species, and is currently the lead technical advisor for the DOC HPAI response.
Lydia Uddstrom is contracted full time to the DOC kākāpō team and has undertaken postgraduate training as a zoo veterinarian

Both Kate and Lydia have previously been involved in capture and handling of threatened species including kākāpō, undertaking vaccination and blood collection for a range of threatened species, and have trained multiple DOC staff to safely and effectively undertake these procedures.

9c. Who else is in the team undertaking the manipulation? State their role in the team, and their relevant experience with the procedure(s) proposed in the application? Include DOC and non-DOC staff in the team.

Name of Manipulation Team member	*Role in the manipulation*	*Experience and qualifications relevant to the manipulation*

Experienced kākāpō vaccinator - Deidre Vercoe - Andrew Digby - Daryl Eason - Jake Osborne - Bryony Hitchcock - Petrus Hedman - Brodie Philps - Sarah Little - Daniella Whitaker - Theo Thompson - Tommy McKerras	Team leader for tracking, catching, handling & monitoring	These are team members who have extensive kākāpō tracking, capture and handling experience. They have also undergone additional training to carry out vaccinations (as per the Veterinary Operating Instructions for Eryvac vaccination in Kākāpō).
Experienced kākāpō blood sampler - Deidre Vercoe - Andrew Digby - Daryl Eason - Jake Osborne - Bryony Hitchcock - Petrus Hedman - Brodie Philps - Sarah Little - Theo Thompson	Team leader for tracking, catching, handling, monitoring & follow-up blood collection in kākāpō.	These are team members who have extensive kākāpō tracking, capture and handling experience. They have also undergone additional training to carry out blood sampling in kākāpō.
Kākāpō field rangers - Everyone listed in the sections above, also: - Maddy Whittiker - Sarah Manktelow - Scott Latimer - Tim Raemaekers - Jenny Ricketts	Team member for tracking, catching, handling, and monitoring kākāpō	These are team members who have extensive kākāpō tracking, capture and handling experience.
Takahē field rangers - Nicholette Brown - Glen Greaves - Phil Marsh - Jason van de Wetering - James Bohan - Erica Hansen	Team member for tracking, catching, handling, and monitoring kākāpō	These are members of the takahē team who have also spent extensive time tracking, capturing and handling kākāpō

9d. What training will be given to the people identified in 9c to help them undertake the manipulation proposed in the application?

Team leaders have undertaken prior training in blood collection and vaccination of kākāpō (for the bacterial disease erysipelas). Team leaders have trained kākāpō rangers over repeated seasons to undertake normal practices including capture and handling.

The capture and handling will be undertaken according to the direction of the team leaders.

The handling and vaccination procedures used in this trial are all normal management procedures that are carried out under a Veterinary Operating Instruction for Eryvac vaccination (for prevention of erysipelas).

Vaccination for this trial will be undertaken by the veterinarians present. Blood collection may be done by suitably skilled rangers independently of veterinarians being present.

Vaccination will be undertaken by the DOC veterinarian.

10. COMPLIANCE WITH CONDITIONS of the APPROVAL:

- Please outline any opportunities for a member, or members, of the DOC Animal Ethics Committee to observe this work.

10a. Identify ways that the manipulation(s) can be monitored by the AEC.

Due to the "permit only" requirements which limit visitation to Whenua Hou, it is suggested that AEC members could attend a vaccination session at Burwood Takahe Centre to see the procedure in takahē and then receive a video or photographs of the manipulation being undertaken in kākāpō.

11. Are there any other aspects which ought to be brought to the attention of the DOC Animal Ethics Committee?

No

12. Does the research, testing or teaching involve a species which is covered by a Department of Conservation Species Recovery Plan and if so, has the Recovery Group been consulted and their endorsement for the work received? Please provide a summary of communication.

Yes. Kākāpō management is guided by a Kākāpō Recovery Plan (Draft 2021-2026). Objective 14.1 Is to analyse and develop detection and mitigation measures for emerging health issues and understand the subsequent risk to the population. The Kākāpō Recovery Group support the objectives of this trial.

This trial has been developed with the consensus of the Kākāpō Recovery Team, which has a close working relationship with Kaitiaki Rōpū o nga Murihiku Rūnanga o Ngāi Tahu who are also supportive of this work.

All involved have requested that this work is undertaken urgently to increase our understanding of the ability of the HPAI vaccine to protect kākāpō should HPAI arrive in New Zealand.

13. What month of year is most useful to report back to the AEC (depending on the project schedule and the animal's biology)?

September

13. Manipulation Grading

Please work through the document '*Grading of Manipulations*' (Please refer to DOCDM-870472), and determine the grading you believe best applies to the manipulation proposed in this application. Please also provide a rationale for the grading.

Grade A: No impact or virtually no impact.
Grade B: Little impact. Manipulations of minor impact and short duration.
Grade C: Moderate impact. Includes manipulations of minor impact and long duration or moderate impact and short duration.
Grade D: High impact. Includes manipulations of moderate impact and long duration or high impact and short duration.
Grade E: Very high impact. Manipulations of high impact and long duration.

Grading determined by the Applicant: B

Application Number DOCDM	AEC444 APPLICATION DOC-7483665
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, kākāpō

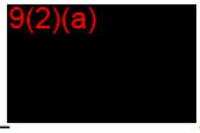
Your rationale for the grading:
Grade B includes "**Disease/injury/functional impairment:** Studies of vaccines using killed pathogens." The animals will be kept in their normal husbandry conditions for the duration of the study. There will be capture and handling for blood collection and vaccination, and two doses of a killed vaccine administered. Handling time & stress will be minimised by using only skilled staff and it will be undertaken at site.

Note: The grading determined by the Applicant is not the grading assigned by the AEC. The Applicant will be advised of the AEC's grading and any conditions in writing.

DECLARATION by the APPLICANT

Tick boxes [☑] to indicate your agreement to conditions: *[Copy and paste this tick object ☑]*

[☑] I declare that the information in this Application is correct; and
[☑] I agree to comply with the conditions imposed by DOC's AEC for the manipulation; and
[☑] I agree to ensure all personnel involved in this manipulation will be properly trained and/or qualified to undertake the manipulation and will be aware of the contents of this AEC application; and
[☑] I declare the proposed manipulation has the necessary resources to undertake the manipulation with regard to the health and safety of the animals and staff
[☑] I agree to advise the AEC of any changes in the details of the manipulation as described in this Application.
[☑] I agree to comply with the reporting requirements stipulated by the AEC on approval of this research project.

Signed by the Applicant  9(2)(a)

Full Name: Catherine McInnes Date: 13/11/2023

DECLARATION by the ACCOUNTABLE MANAGER

Tick boxes [☑] to indicate your agreement to conditions: *[Copy and paste this tick object ☑]*

[☑] I agree to ensure my staff member complies with the conditions imposed by DOC's AEC for this manipulation; and
[☑] I agree to ensure all personnel involved in this manipulation will be properly trained and/or qualified to undertake the manipulation and will be made aware of the contents of this AEC application; and

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[☑] I agree the proposed manipulation has the necessary resources to undertake the manipulation with regard to the health and safety of the animals and staff

[☑] I agree to oversee this Application via MORs, PDPs and other means to ensure the manipulation remains within the scope of the Application and the Approval, and all reporting required by the AEC is delivered on time;

[☑] I agree to advise the AEC of any changes in the details of the manipulation as described in this Application, and to advise the AEC if the Applicant leaves the Department, or if the work should be transferred to another staff member for operational reasons' or if the manipulations is abandoned for any reason.

Signed by the Manager: 9(2)(a) [redacted]

Full Name: John Lyall

Role: Fauna Advice Manager

Date: 15/11/2023

Application Number DOCDM	AEC444 APPLICATION DOC-7483665
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, kākāpō

Appendix 1: Avian Influenza vaccine information

Registration number A009733: **Poulvac Flufend i AI H5N3 RG**

Registrant: Ministry for Primary Industries

Draft label information:

PRESENTATION

Bottles of 500 mL (1000 doses). Packs of 1 or 10 bottles.

DIRECTIONS FOR USE

By law the distribution and use of this product must comply with the requirements of the relevant operating plan.

General:
• Inject 0.5 mL (0.5 cc) subcutaneously, using aseptic technique, into healthy birds at 3 to 4 weeks of age or older.
• Shake well before use.
• Allow the vaccine to reach room temperature (18-29°C) before use.

Chickens:
• Administer another dose of 0.5 mL not less than 2 weeks later, if required.
• The second dose should be administered at least 4 weeks before point of lay.

Ducks:
• Ducks less than two weeks of age:
• Administer 0.2 mL of vaccine subcutaneously at the back of the neck.
• Administer another dose of 0.5 mL not less than 2 weeks later.
• Ducks two or more weeks of age:
• Administer 0.5 mL of vaccine subcutaneously at the back of the neck.
• Administer another dose of 0.5 mL not less than 2 weeks later.

ADVERSE EFFECTS, CAUTIONS AND CONTRAINDICATIONS

ADVERSE EFFECT
• Vaccinate only healthy chickens or ducks and avoid stressing the birds at the time of vaccination.
• Do not mix with any other vaccine or injectable product.
• The use of this product in laying birds has not been evaluated.
• Local or systemic post-vaccination reactions can occur due to the use of oily vaccines. Symptoms observed are generally transitory and can include oedema and granulation at the injection site, anorexia and dehydration. Such reactions can be minimised by good aseptic vaccination technique.

CAUTIONS
• Destroy any unused vaccine and containers after vaccination (including syringes and needles) by burning.
• Do not mix the vaccine with other vaccines or administer another vaccine shortly before or after vaccination with this product.
• Consult a physician immediately for an accidental self-injection and show this package insert to the physician.
• KEEP OUT OF REACH OF CHILDREN AND UNINFORMED PERSONS

CONTRAINDICATIONS
• None.

WITHHOLDING PERIODS
Meat: Nil.

STORAGE
• Store in the dark between 2 °C and 8 °C. Do not freeze.
• Protect from direct sunlight.

• Use contents of each vial within 6 hours of opening

Appendix 2: General Instructions for subcutaneous injection of vaccine

- The vaccine is supplied in a 500mL bottle and is given to the bird using a needle and syringe.
- The vaccine is injected under the skin but NOT into the muscle below.
- The vaccine should be drawn up into the syringe and then allowed to warm to room temperature (this is more comfortable for the bird).

EQUIPMENT NEEDED

1. Vaccine container
2. 1 mL syringe
3. 25 gauge 5/8th inch needle
4. alcohol swab (mediswab or cotton wool and meths)
5. dry swab (gauze or cotton wool)
6. Sharps container for needle disposal
7. Bird
8. Bird handler
9. Veterinarian

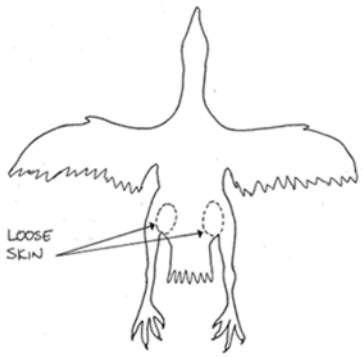
PREPARING THE VACCINATION

1. Store the vaccine in the fridge at 2-8 degrees C in the dark. Do not Freeze.
2. When ready to use, take the vaccine out of the fridge and shake well to mix.
3. Write the date on the vaccine bottle.
4. Break off the metal seal on the top of the rubber injection port.
5. Swab the injection port on the vaccine with alcohol to sterilise it with a mediswab or cotton ball soaked in methylated spirits.
6. Firmly attach the needle to the syringe – 25 gauge 5/8th inch needle to a 1mL syringe.
7. Insert the needle through the centre of the rubber stopper CAREFULLY.
8. Hold the vaccine upside down and slowly suck vaccine into the syringe until you have a little more than the prescribed dose of vaccine.
9. Flick the syringe to dislodge any air bubbles and squirt them slowly back into the vaccine bottle.
10. Keep squirting until all the bubbles are gone and you have the prescribed dose of vaccine left in the syringe.
11. Pull the needle out of the vaccine bottle and CAREFULLY recap the needle.
12. Leave the syringe and needle to warm to room temperature.
13. Repeat this procedure to draw up all the doses you need for your vaccination session.
14. Put the vaccine back in the fridge.
15. Once open, the vaccine can be used for 30 days. (Note that this expiry is based on Zoetis technical advice for limited use of the vaccine in this trial, and only applies when following the above instructions for maintaining sterility of the product and correct storage.)
16. If you are in doubt that the vaccine has been stored correctly (kept refrigerated), then discard it and get a new bottle.

GIVING THE INJECTION

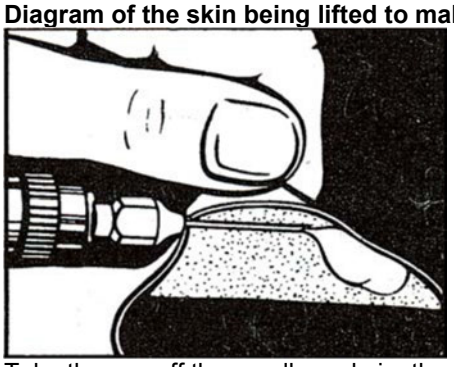
1. Have the following equipment ready for use:
    - The correct dose of vaccine drawn up in syringe with needle attached and warmed to room temperature. (the cap should be on the needle to avoid accidental stabbing or contamination of the needle)
    - One alcohol swab (mediswab or cotton wool in meths)
    - One dry swab (gauze or cotton wool)
2. Have an assistant restrain the bird on its back or side with its legs restrained to provide access to the groin (where the bird's leg joins its belly).

Diagram of ventral (belly) of a bird showing the groin region for subcutaneous vaccination sites:



3. Spread the feathers in the groin area.
4. Wet down the feathers with the swab to clear a patch of skin and swab the skin.
5. Lift the loose skin 1-2cm off the body to make a "tent".

Diagram of the skin being lifted to make a "tent" for a subcutaneous injection



6. Take the cap off the needle and aim the needle about halfway down the side of the tent. Keep the needle parallel to the body wall. When the needle goes through the skin, it should still be above the muscle of the groin i.e. you are injecting into the space inside the tent, not into the muscle.
7. Suck back on the syringe to check for blood. This is to avoid injecting into a blood vessel.
8. Inject the vaccine with a steady firm pressure.
9. Withdraw the needle and place it into the Sharps container.
10. Use the dry swab to press over the injection site if there is any bleeding.
11. Release the bird.

12. Record:	Bird ID	Date	Dose	L or R side	Vaccinator	Holder	Vaccine Batch	Expiry Date	Notes

13. Transfer this data to the vaccination record spreadsheet
14. Note any other specifics about the injection process not described above. E.g. if there was bleeding at the injection site.

Application Number DOCDM	AEC445 APPLICATION DOC-7483662
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, kakī

Revised September 2023

DEPARTMENT OF CONSERVATION
APPLICATION TO MANIPULATE LIVE ANIMALS
Code of Ethical Conduct for the Care and Manipulation of Live Animals

1. APPLICANT'S DETAILS:

Name: Kate McInnes

Date: 24 October 2023

Role: DOC Veterinarian

Unit: BH&V Group, Wellington

APPLICANT'S ADDRESS:

Phone no: 9(2)(a)

Email: kmcinnes@doc.govt.nz

2. ACCOUNTABLE MANAGER'S DETAILS:

Name: John Lyall

ACCOUNTABLE MANAGER'S ADDRESS:

As above or: DOC, Hokitika

Phone no: 9(2)(a)
Email: jlyall@doc.govt.nz

2a. AEC443

2b. **MANIPULATION TITLE: Avian Influenza vaccination safety and efficacy trial in kāki**

2d. **Duration of the manipulation**

- *Over what timeframe are you seeking the approval?*
- *You must not commence the manipulation until you have received the approval, signed by you, your accountable manager, and the AEC Chair.*

Application Number DOCDM	AEC445 APPLICATION DOC-7483662
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, kakī

- *NOTE: The AEC will not generally give an approval for longer than two years at one time. Please state if this manipulation is likely to extend longer than two years from the commencement date.*

Anticipated start date:	February 2024	Anticipated finish date:	June 2025

2e. What months of the year is the manipulation most likely to be undertaken? e.g., October – March
For the duration of the dates specified but breeding birds will not be handled during September to January

3a. Summary of the proposed manipulation for a LAYPERSON

- *Provide an abstract describing the manipulation (maximum 400 words).*

Avian influenza is a viral disease which can cause mass mortality events in birds. The current strain is decimating many populations of wild birds overseas, is predicted to reach the Southern Ocean by 2024/25 and was confirmed in South Georgia, October 2023.

We want to test the safety and efficacy of vaccination to protect critically endangered species.
The vaccine is a commercial product registered in New Zealand by Ministry for Primary Industries. It is considered very safe and highly effective. It contains inactivated (dead) virus so it cannot cause avian influenza. Vaccination reduces risk of illness or death and reduces shedding of virus, thus protecting the individual and its flock.

Kāki are a critically threatened species which is reliant on a captive breeding programme where it is possible to reliably administer a full course of vaccine (2 injections under the skin, one month apart) to individually identified birds, and where we are able to handle them again for a veterinary examination and blood testing to detect any effects on health status, and measure the immune response by detection of antibodies over a 12 month period.

Captive kakī will be captured in the aviary and receive a pre-vaccination health check by a veterinarian, and a blood test for health and antibody testing. Up to 1 mL of blood will be collected from the wing vein, as is standard for this species.

The vaccine is given under the skin. One month later the bird will receive a second vaccination and blood test. Further blood will be collected at 2-3, 6 and 12 months post vaccination to determine the level of antibody response and how long it lasts.

A cloacal and choanal (oral) swab will be collected on day 0 for PCR testing to demonstrate the birds were not incubating avian influenza at the time of vaccination.

Normal husbandry practices will be undertaken including observation of the bird's activity and food intake to monitor of any adverse reactions.

We propose to work with a total of 10 adult or juvenile kāki, divided into two cohorts. Cohort 1 will first receive the vaccination & blood tests, and a recheck at 1 month. If no safety issues are identified, then Cohort 2 will receive vaccinations & blood tests. This allows a careful start to the trial where the first month is the most important to test vaccine safety. The following blood samples at 6 – 12 months will determine level and duration of antibody presence and determine when further boosters would be required.

Additional approval given on 16/4/24 to collect a 10-14 week blood sample to target peak antibody levels, and to collect an opportunistic blood sample for further antibody testing, if birds are being handled for routine management purposes, with no more than twice per bird over the 12 months of the trial.

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3b. Description of the proposed manipulation (methods)

- *Provide a more detailed explanation. Describe the equipment, the location, and any environmental factors: weather, time of the year. Why have you decided to undertake the manipulation in this way? What advice have you sought? Include the species, the number of individuals, the source of animals, and the disposal/fate of animals at the conclusion of the manipulation.*
- *Be specific about the timelines for the proposed investigation and the purpose of the research, testing or teaching.*
- *Include some consideration and planning for when things might not go right.*

Please note: this is one of five trials to assess avian influenza vaccination safety and efficacy in nationally critical threatened species (takahē, kākāpō, kakī, tūturuatu, kākāriki karaka). Manipulation details which are specific to this species (kakī) have been highlighted in yellow. All other details are consistent across the five trials. By highlighting the species specific details I hope to assist the AEC with the volume of workload associated with simultaneously assessing these trials.

This trial is designed to test the efficacy and safety of a vaccine in a nationally critical threatened species.

Selection of the species for potential vaccination is based on the risk that they could undergo an extinction event when highly pathogenic avian influenza (HPAI) reaches New Zealand. 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 risk of extinction.

The current wild adult kakī population is approximately 150 individuals, and modelling suggests that the species would be functionally extinct in 6-8 years without the intensive intervention from DOC's captive rearing programme. The captive management programme produces between 100-200 birds per year for release back into the wild population.

Based on the evidence from overseas during this epizootic, the species most at risk of infection are those which exhibit congregation behaviours e.g. feeding, breeding or roosting in groups, those which are exposed to at risk species e.g. where seabirds overlap with another threatened species, and birds held in captive facilities where biosecurity options are limited e.g. open pens and large aviaries.

Kāki are one of 5 species identified by the DOC HPAI Technical Advisory Group as at risk where administration of a full vaccination programme is feasible in sufficient number of individuals to provide protection against species extinction. See DOC-7111177 Mitigation Options Guideline for HPAI.

Use of the vaccine is dependent on Ministry for Primary Industries approval, and currently requires the birds to be held in captivity. Birds require two injections one month apart and must be individually identified with a permanent mark e.g. microchip or leg band. Birds are currently individual marked with leg bands as part of routine husbandry.

Effective vaccination reduces susceptibility to infection. When infection does occur, it reduces clinical signs of disease and the amount of virus shed into the environment (Animal Health Australia, 2021).

Additionally, vaccination of California Condor was approved in the United States following an outbreak in the wild population. This was the first avian influenza vaccination programme in a wild endangered species. Advice from the veterinary and technical advisors to the condor vaccination programme has been received and is incorporated into this trial design.

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Key Words	avian influenza, vaccine, safety, efficacy, kakī

We wish to undertake a limited trial to determine the safety and efficacy of the avian influenza specific vaccine in kakī as a preparedness measure for the arrival of HPAI in New Zealand.

The vaccine is produced commercially by Zoetis for use in poultry: Poulvac Flufend i AI H5N3 RG inactivated (killed) vaccine - see Appendix 1. It has been in production since 2006 and is widely used in the poultry industry. Publications on AI vaccine use in poultry and avian species in zoos have indicated a very high level of safety across a wide range of species, and efficacy has been well established. (Kandeil et al 2018, Philippa et al 2006, Philippa 2007a, Philippa 2007b, Pitman 2006, Vergara-Alert 2011). The vaccine is inactivated, so there is no live virus present and it cannot cause avian influenza.

Advice from Zoetis (USA) indicates that this vaccine should provide good protection against the current strain of HPAI with 91% amino acid homology with the circulating strain. A newer vaccine based on the circulating strain is in production but is will not be available until the end of 2024 at the earliest.

Vaccine will be obtained from 9(2)(c) in Christchurch and transported in a chilly bin with ice-packs by overnight courier (as per their standard transportation procedures for vaccines) to ensure cold chain is maintained. Use in the field will be managed by extraction of sterile aliquots into sterile vials or syringes. This enables sustainable use of the 1000 dose vial and maintenance of sterility of product. This process was discussed with the Zoetis Senior Research Advisor responsible for poultry products and is considered safe and appropriate.

Sterile aliquots will be obtained by using a sterile needle and syringe to extract the aliquot from the closed vaccine vial. The vial will be shaken to homogenise the contents, then the rubber stopper will be swabbed with alcohol. The sterile needle will be attached to the sterile syringe and the needle inserted via the rubber stopper. The aliquot will be drawn up into the syringe, then the needle& syringe removed from the stopper and the cap replace on the needle. The needle will be swapped for a new sterile needle or a sterile vaccine cap. Both the aliquots and the vaccine vial will be stored refrigerated in accordance with the packaging instructions. Vaccine doses will be drawn up immediately before use and allowed to warm to room temperature just prior to injection.

DOC veterinarians Kate McInnes and Lydia Uddstrom will administer the vaccination.

All birds will receive a full veterinary physical examination at the start of the trial. Only birds in good body condition exhibiting signs of good health will be included. (Any birds which show signs of poor health will be further investigated as per normal veterinary practices).

The kāki recovery team have been involved in the design of the study, and selection of study animals. The Twizel kakī breeding centre was chosen for being one of only 2 facilities holding kakī, presence of experienced staff, and the ability to access a total of 10 birds.

Individuals for vaccination will be selected by the kāki recovery team, based on the programme's planning for any translocations.

Each individually permanently marked (leg band) bird will receive two doses of vaccine by subcutaneous injection into the inguinal (groin) region with a 1 month interval (no less than 3 weeks apart and a maximum of 6 weeks apart). The first vaccination will be into the left inguinal region, and the second vaccination into the right inguinal region.

Birds will receive 0.2ml per dose (as per dosages used in Vergara-Alert et al 2011).

Individual birds within the two cohorts will be determined on the day by Kakī Project Lead Liz Brown based on available birds' suitability and any management requirements. Cohorts will aim to hold 5 birds in each, however depending on the birds' group numbers, this may be 4, 5 or 6 birds in each cohort, up to a maximum total of 10 birds altogether. This allows for some flexibility based on outcomes of the current breeding season.

At the start of the trial, each bird will receive a cloacal and oral swab to determine presence/absence of virus at day 0.
The technique will follow the draft SOP Avian swab sampling DOC-6840491 which has undergone veterinary peer review & user testing and is awaiting AEC endorsement before Director sign-off. These types of swabs are used in standard health testing on avian species and would be undertaken by the veterinarian. The test would be considered a baseline health test to demonstrate the birds were not incubating avian influenza at the time of vaccination. The swabs will undergo PCR testing at BioPacifica to look for avian influenza virus.
This is important to be able to demonstrate that any antibody response is due to the vaccination rather than the bird being infected by a wild strain of avian influenza.

First trial - Cohort 1: Five (4-6) individuals will be vaccinated as per the described protocol above. Blood (up to 1ml) will be collected at 0, 1, and 2-3 months to measure health parameters (white cell count & differential) and antibody response (commercial serum ELISA test to measure antibody titre). Antibody testing will be undertaken at a commercial laboratory (BioPacifica, Christchurch).

Note: 1% of body weight is considered an acceptable amount of blood to collect from a healthy bird. Adult weigh ~220g, therefore up to 2.2 ml would be within the safe range for an adult. We propose to only collect up to 1ml to maintain a high margin of safety. For juveniles or subadults birds, the maximum collection would be up to 1% of body weight of the bird at the time of sampling.

Second trial - Cohort 2: Based on consideration of the results of the first trial, if safety has been demonstrated, a second cohort of 5 individuals (4-6) will receive the vaccination as per the described protocol above, and blood will be collected at 0, 1, and 2-3 months to measure health parameters (white cell count & differential) and antibody response (commercial serum ELISA test). We will wait 1 month until we have established the vaccine is safe in cohort 1 before we start cohort 2.

Note: If antibody response at 2-3 months is noted to be muted (i.e. a low response) then the DOC vets (Kate McInnes and Lydia Uddstrom) will discuss the use of a third dose of vaccine. This was used in some species in European zoos where the initial antibody responses were considered insufficient. Consideration will be given to the level of response detected, the impacts of additional handling, and any other welfare factors noted during the preceding handling events. The benefits of testing a third dose of vaccine will be carefully considered, and this will only be undertaken if the welfare impacts are considered minimal. The justification for a third dose in this trial would be to confirm if this dose is warranted and would deliver protection for the kakī population in the event of an outbreak of HPAI in Aotearoa New Zealand.

Two additional blood samples will be collected from both cohorts at approximately 6 months and 12 months to measure duration of antibody response.

A maximum of 10 birds will be included in the trial. It is estimated that there will be 80-120 kakī on site at the DOC facility at the time of the trial.

These trials are modelled on the vaccination of California Condor in the USA. (FWS.gov 2023) however the 1 month interval is based on the European zoo data, and considered more appropriate to allow for recovery between handling events in birds which normally have minimal handling by humans.

Estimated timing/schedule of manipulations:

First vaccination and blood sample	Second vaccination and blood sample	2-3 month blood sample	~6 month blood sample	~12 month blood sample
First cohort of 5 birds (4-6)				
~ 1st February 2024	~ 1st March 2024	~1st April 2024	~ 14th August 2024	~ 14th February 2025
Second cohort of 5 birds (4-6, maximum 10 total)				
~ 1st March 2024	~1st April 2024	~1st May 2024	~14th August 2024	~ 14th February 2025

Second cohort of 5 birds (4-6, with maximum total of 10) – same timeline but 1 month after first cohort have been vaccinated and shown no negative reaction. Blood collection at ~6 and ~12 months may be undertaken at the same time for both cohorts – these sample are about longevity of antibody presence, so the exact timing is less critical.

During a handling event, all involved staff will gather and have a pre-handling briefing by the veterinarian and the team leader to ensure all roles and responsibilities are clearly understood. Any issues can be raised at that time for clarification. The kāki team leader will be responsible for the safe capture and handling of the bird. The veterinarian will be responsible for the health examination, vaccination, and blood collection.

All equipment will be prepared prior to capture to minimise handling time. Staff will know where to situate themselves and what actions are required so that an efficient process is maintained. The kāki rangers involved in this trial will be team members with previous experience in blood collection in kāki.

Kakī are caught according to the following procedures as detailed in the kaki husbandry manual:

4.4.2 Adults, juveniles and sub-adults

Birds in aviaries should not be caught in wet weather or in high winds unless unavoidable. . Handling wet birds can damage their plumage, and strong wind increases the risk of injury. Various methods have been trialled for catching birds in the aviaries, including drop nets, tunnel nets and hand nets. Hand nets are mainly used in holding aviaries, as this is the quickest method. Ensure that the net is kept dry, and fabric does drag on the ground or through the pond. This method is most effective with three or more people, in the larger breeding compartments more people will be needed. In small aviaries it is possible for one or two people to catch birds. Birds should not be caught when they are in the water and should not be caught in flight. The safest method for capture is to gently hover the net over the bird and slowly lower the net to ensure you do not damage the bird. The bird can then be gently untangled from the net by placing your hands over it. Most birds will fly when approached and you will need to wait for them to land, before gently placing the net over them. Inexperienced catchers should watch experienced staff first, and not be pressured to catch birds until they feel confident. Adults can be more difficult to catch, and occasionally it may be necessary to catch them while flying. This should be done as a last resort and only if catching is essential to their wellbeing, and the catching attempt has already exceeded 25 minutes. Catching while in flight should only be attempted when the bird is slowing down to land, or just taking off, and the movement should be followed with the net to prevent damaging the bird. Birds should never be caught when flying at full speed.

Figure 1. Catching kakī with large whitebait nets, the bird in the foreground is being held through the net before it can be extracted. Photo: Ursula Paul

Birds are held by cradling them in both hands, with thumbs on top holding the wings and little fingers hooked behind the birds' legs to keep the bird secure. The hold should be light, without undue pressure on the bird.

Once caught the bird is placed in a 'catch bag' (a soft cotton bag with a draw string which provides a safe restraint for temporary holding) to be weighed in the bag using a hanging scale (standard technique) and

then carefully removed and undergo a physical examination to determine its health status prior to any further manipulation. If the kāki is determined to be healthy, blood collection and vaccination will proceed.

For blood collection and vaccination a trained kakī handler will restrain the bird on its side or back.

The blood collection site (wing vein) will be swabbed with a sterile alcohol wipe immediately prior to collection. Blood will be collected from the wing vein either using a 1ml sterile syringe attached to a 25 or 26 gauge ¾ inch sterile hypodermic needle or via the pin-prick method where the vein is pricked using a sterile 25 or 26 gauge hypodermic needle, and then the bleb of blood is collected in capillary tubes (also known as haematocrit tubes). The method will be dependent on the skills and confidence of the collector. Both methods are valid for blood collection from a small bird.

In the event that the temperature is cool, and on examination of the wing vein we determine that blood collection likely to be slow due to the presence of small contracted veins, the wing will be warmed for 3-5 minutes using Kathmandu hand warmers wrapped in gauze, to boost circulation and enhance blood flow to enable effective blood collection.

After collection, the site of blood collection will be covered with a gauze swab and pressure applied to control any bleeding. In the very unlikely event of uncontrolled bleeding pressure will be applied for a further 1-5 minutes. If still uncontrolled, an icepack wrapped in gauze swabs will be held on the wing area to cool the limb and reduce blood flow. If required, a silver nitrate stick will be carefully used to stop the bleeding.

Blood will be transferred into a blood microtainer and spun in a centrifuge to separate the serum from the blood cells. Serum will be drawn off using a sterile pipette and transferred into an epindorf tube for storage in the freezer, prior to transfer to the commercial laboratory in batches for antibody testing.

1-2 drops of blood will be used to make blood smears which will be sent to a commercial veterinary pathology laboratory for a white cell count and differential. This provides a baseline health analysis which can detect infection or inflammation. Any abnormal results will be further investigated by the veterinarian in consultation with the kakī staff.

Once bleeding has stopped, the bird will be vaccinated using a 1mL syringe attached to a 20 gauge ½ inch needle. The vaccination site will be swabbed with a sterile alcohol wipe immediately prior to vaccination. See Appendix 2 for details of the vaccination technique.

The bird will then be checked for any abnormalities and the veterinarian will determine if any further actions are required for health or welfare. Once the procedures are complete the bird will be quietly released and observed as it moves away. Regular observations during routine husbandry will continue for all birds, and any abnormalities will be reported to the veterinarian. The birds are visited and monitored at least twice daily by DOC staff when they are being fed the artificial diet.

At subsequent handling events, the vaccination site will be examined and any discolouration, swelling, granuloma formation or unexpected abnormality will be noted and reported to the veterinarian. Photographs of each bird's injection sites will be taken to provide a clear record of the trial.

Location & timing:
The trials will be undertaken at the DOC Twizel kakī breeding facility between Feb 2024 and June 2025 (breeding kakī will not be handled).

Safety:

Results from a meta-analysis of use of vaccine in European Zoos found very low adverse reaction rate at 0.04% local reactions and 0.015% general reactions reported. EFSA 2007. Based on this, we do not anticipate significant issues with the vaccine, however we will be prepared for immediate veterinary care if any reactions to occur.

The vaccine packaging label states: "Local or systemic post-vaccination reactions can occur due to the use of oily vaccines. Symptoms observed are generally transitory and can include oedema and granulation at the injection site, anorexia and dehydration. Such reactions can be minimised by good aseptic vaccination technique."

Anaphylaxis:
A severe immediate immune hypersensitivity response could occur if the vaccine produc stimulates such a response. This is considered unlikely due to the extensive use of this vaccine and other similar vaccine products in Europe, however it is possible and needs to be considered as a potential adverse event. The vaccination team will include a veterinarian who will have access to emergency drugs and supportive care for management of anaphylaxis (including corticosteroids, adrenaline, oxygen, fluids).

Injection site reactions:
The vaccine contains an adjuvant (oil) which is present so that it stimulates a stronger immune response with greater antibody production. This can sometimes be associated with a small pea-sized lump at the site of injection. This is normal and expected, although generally not all birds will develop a lump. This will be checked at the 1 month mark, and records kept of any reactions detected. If an excessive size reaction is detected in an individual (>1cm), then the vaccination will be paused until it is determined that the lump does not enlarge further, or cause any impacts on the bird(s) – this is likely to be a period of 2-4 weeks. Body weight, activity levels etc will be reviewed and a full physical examination undertaken.

A localised bacterial infection could result if poor sterile technique is used. Only veterinarians or specifically trained DOC staff members will be administering the vaccine, and these operators all have training in appropriate sterile techniques. If a bird experiences an infection at the site, it will receive veterinary care and follow-up to ensure the issue is managed.

Mis-injection could occur if the bird is poorly restrained and moved during vaccination. This will be managed by only using well trained, experienced kakī handlers to restrain the birds. For some individual birds, they are calmer with head cover which can aid in handling. This will be determined on an individual bird basis. If a mis-injection occurs, the veterinarian will determine the appropriate next steps. This may include, re-injection if the first injection merely failed to enter the bird, appropriate first aid measures if any injury was caused, and/or exclusion from the trial and follow-up care. As noted previously, "stressy" birds will not be included in the trial which will reduce the risk of injury or mis-injection.

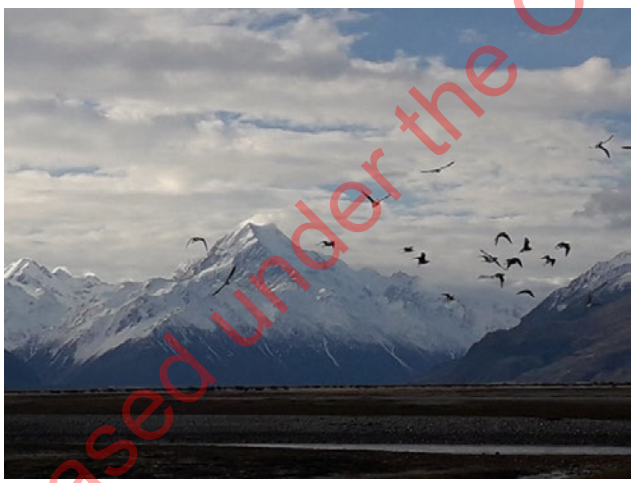
Injury could occur during capture and handling. This is minimised by only using trained experienced staff, careful selection of trial birds, and a "stop for safety" approach which resets the work programme and ensures time out to reassess and replan the work and procedures if necessary.

In the event of a serious reaction or injury during the vaccination trail, the bird will be taken to Dunedin Wildlife Hospital for specialist care by Dr Lisa Argilla. This is standard procedure for kāki requiring a high level of veterinary care. Birds can be transported to the Dunedin Wildlife Hospital within 4 hours from the DOC Twizel site.

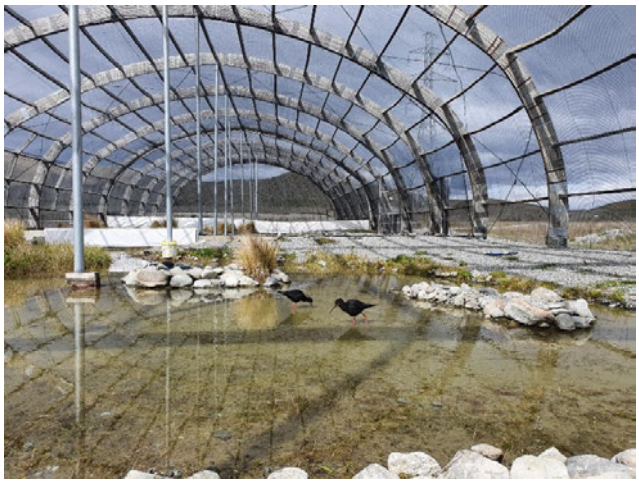
Results:

The results of this trial will determine if this vaccine is safe to use in this species, and the level of antibody response produced by a 2 dose vaccination. In some other species, notably penguins, the antibody levels following vaccination remain low and, in some species, a third vaccination was used to ensure a stronger response (ESFA 2007). The duration of antibody presence also varied between species. Therefore, this trial will help to determine the appropriate vaccination regime for kāki in the event that more widespread vaccination is required during a highly pathogenic avian influenza outbreak in New Zealand.

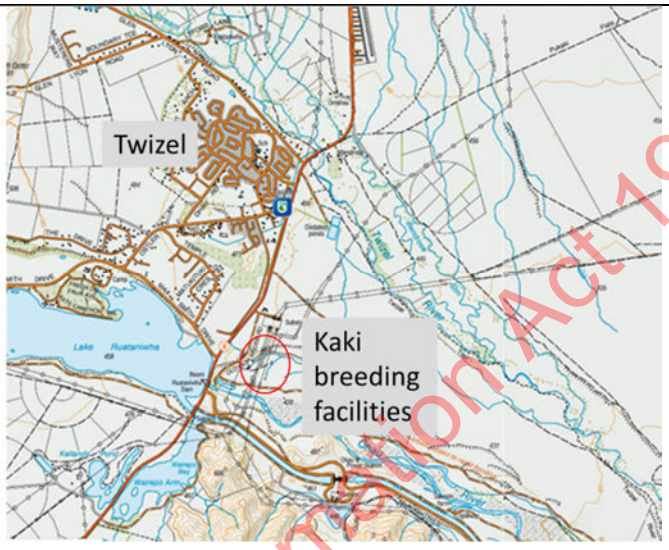
3c. Attach Photos of equipment, the species, the location (or a map); to help set the context.



Kakī release 2018



Kakī in aviary, Twizel



DOC Twizel aviaries

3d. References

- *List the references referred to in the application*

DOC-7111177 Mitigation Options Guideline for HPAI
https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7111177

EFSA 2007. Vaccination against avian influenza of H5 and H7 subtypes as a preventative measure carried out in Member States in birds kept in zoos under Community approved programmes. ESFA journal, 450. ESFA-Q-20006-156
https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499835

Health Australia (2021). Response strategy: Avian influenza (version 5.0). Australian Veterinary Emergency Plan (AUSVETPLAN), edition 5, Canberra, ACT. Response_Avian-influenza.pdf (animalhealthaustralia.com.au) Animal

FWS.gov 2023 Southwest California Condor Flock HPAI Information Updates - 2023 | U.S. Fish & Wildlife Service (fws.gov)

Kandeil A, Sabir SM, Abdelaal A, Mattar EH, El-Taeel AN, Sabir MJ, Khalil AA, Webby R, Kayali G, Ali MA. Efficacy of commercial vaccines against newly emerging avian influenza H5N8 in Egypt. Nature Scientific Reports, 2018. 8:9697 | DOI:10.1038/s41598-018-28057-x https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499854

Kakī husbandry manual (in draft). Internal DOC document https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7495734

Philippa JDW, Munster VJ, van Bolhuis H, Bestebroer TM, Schaftenaar W, Beyer WEP, Fouchier RAM, Kuiken T, Osterhaus, ADME. Highly pathogenic avian influenza (H7N7): Vaccination of zoo birds and transmission to non-poultry species. Vaccine, 2005, 23:5743-5750. https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499837

Philippa JWD 2007a, in XI. Vaccination of Non-domestic Avian Species, Transmissible Disease Handbook. European Zoo Vets 5th Edition link

Philippa J, Bass C, Beyer W, Bestebroer T, Fouchier R, Smith D, Schaftenaar W, Osterhaus, A. Vaccination against highly pathogenic avian influenza H5Na virus in zoos using an adjuvanted inactivated H5N2 vaccine. Vaccine, 2007b, 25: 3800-3808. https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499841

Pitman 2006. M Pittman, European Commission 12th Annual meeting of national avian influenza laboratories Veterinary and Agrochemical Research Centre (VAR) Uccle, Brussels, 16-18 October 2006 LINK: link.

Vergara-alert J, Ferhandez-Bellon H, Busquets B, Alcantara G, Delclaux M, Pizarro B, Sandchez C, Sanchez A, Majo N, Darju A. Comprehensive serological analysis of two successive heterologous vaccines against H5N1 Avian Influenza virus in exotic birds in zoos. Clinical and Vaccine Immunology, 2011. P. 697-706. https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499845

4. INVOLVEMENT OF OTHER ANIMAL ETHICS COMMITTEES:

4a. Is this Application; or a related or similar application; been or is being considered by another Animal Ethics Committee. Has this project been requested to be considered by any other AEC?

If so, please provide details.

No

4b. Does this manipulation interact with a manipulation approved by other Animal Ethics Committee? If so, detail your communications with those committee(s), and state any conditions imposed by (an)other AEC.

No

5. JUSTIFICATION FOR PROPOSED MANIPULATION:

5a. Detail any action undertaken to determine that the proposed work has not already been done.

Avian Influenza vaccine efficacy and safety has been undertaken on other avian species, however it has not been undertaken in New Zealand endemic species. Although we expect similar results, it is prudent to

undertake this trial to provide more evidence of safety and efficacy in the species which we intend to vaccinate in the event of a HPAI outbreak.

5b. Have alternatives been considered to the proposed manipulation involving reduction, or replacement of live animals, or refinement of techniques?

We are looking at the species-specific response and have selected a minimum size divided into two cohorts, so other methods of reduction are not appropriate for this work.

The cohort approach allows us to cautiously approach the safety issue, and assess initial results before involving the full number of birds.

All captive kakī undergo twice yearly health screens, including testing and treatment for internal parasites and a physical check requiring capture. The first vaccination will be timed to coincide with the post-breeding season health screen, and the 6 month blood test will coincide with a pre-breeding season health screen. This reduces the number of times the birds are handled specifically for this trial from six to four.

5c. To what extent has there been assessment of the suitability of using non-sentient or non-living alternatives in the project; or replacement of animals as subjects with suitable non-sentient or non-living alternatives?

N/A, see above

5d. How will the proposed work result in the extension of knowledge relevant to the health, welfare, or conservation of animals?

This work will specifically contribute to the future health of the species for conservation purposes by providing evidence of the safety and efficacy (or not) of this vaccine in this species, and inform the appropriate vaccine schedule for the species. This will determine if the vaccine is employed in the future in the face of an avian influenza outbreak in New Zealand.

5e. Is the manipulation required as part of an approved training programme?

No.

5f. How will the results of this work be made available to staff within and outside DOC? (*For example internal report, journal paper, best practice guide, workshops etc*).
Internal report, journal paper, conference presentations, shared with other captive institutions that hold kakī.

6. SELECTION OF SPECIES & NUMBER OF INDIVIDUALS FOR PROPOSED MANIPULATION

6a. What will be the source of the animals to be manipulated, and how many from each source will be manipulated?
Kakī at the DOC Twizel breeding centre. 10 birds in total from a range of adults, sub-adults and juveniles. It is estimated that there will be 80-120 kakī on site at the DOC facility at the start of the time of the trial.

6b. Will any of the animals involved be used more than once, and if so, how many times will each animal be used?

Only once (but each animal handled/manipulated multiple times – twice for vaccinating and four more times for blood sampling, although the 6 & 12 month handling for blood collection will be planned to coincide with routine handling for health management)

6c. What factors have been taken into account in the choice of the animal species?
Kakī are one of 5 species identified by the DOC HPAI Technical Advisory Group as at risk where administration of a full vaccination programme is feasible in sufficient number of individuals to provide protection against species extinction.

6d. Could the information being sought be obtained by work on some other species?
No. The trial specifically uses kakī since the safety and efficacy needs to be tested in the target species, and there are no captive populations available of a reasonable surrogate species, such as pied stilt, nor would it be good welfare to obtain wild pied stilt to hold in captivity due to the stress this would induce, and finally, there are no appropriate facilities available to hold wild pied stilt for this trial.

6e. Will the question be answered with the size of the sample?
Yes.

6f. Is the number of animals proposed to be manipulated the <u>minimum</u> necessary to provide a scientifically interpretable result, consistent with the level of statistical precision required? What consideration has been given to the design of the study with regard to:

- **The level of precision necessary in the results?**

The purpose of the small trial is to establish if there is a species-specific sensitivity to the vaccine and its adjuvant. For this purpose, we require only a small number of birds to extrapolate a species sensitivity. Similarly, for determining vaccine response by antibody response levels, a sample size of 10 will provide sufficient individual variation to establish an overall species response level. Additionally, if a bird is removed from the trial for any reason (e.g. other health issues, injury, behavioural), starting with 10 birds allows sufficient number to still be able to make a reasonable conclusion on the vaccine efficacy for future management purposes.

A larger sample size would ensure a more nuanced examination of the species' response to vaccination; however, we are examining a general level of impact/effect, rather than subtle differences. Thus, if results which showed >1 bird having a safety issue, or the majority or average antibody response to be low, that would be sufficient to inform the next steps for decision making regarding kakī vaccination.

- **The possible confounding effects of animal variation?**

We expect some individual variation since the immune response is affected by individual health status and biological variation. This sample size is sufficient to ensure we have a range of individual responses to examine.

- **The needs of statistical analysis?**

There is likely to be individual variation, which, for the antibody response, requires a reasonable sample size. We determined that 10 was the maximum which was feasible to include in the trial, and also sufficient to allow for individual variation to establish some baseline parameters of antibody response.

Ultimately, in an outbreak situation, the results of a sample size of 10 will be sufficient to make reasonably informed decisions about the use of a commercially produced killed vaccine which has a good history of safety and efficacy across a wide range of species.

7. WELFARE OF ANIMALS DURING PROPOSED MANIPULATION:

7a. What measures will be taken to ensure: the general health and welfare of animals before, during and after manipulation, including the adequacy and cleanliness of housing, caging and equipment; the provision of food and water; prevention of over-crowding, and prevention and control of disease?

Kakī will be caught and handled by experienced rangers as per usual kakī protocols at DOC Twizel breeding centre where DOC staff already maintain appropriate husbandry practices and monitoring of all the birds. Each bird will be held within its normal enclosure so that there is minimum disturbance to their daily lives. Staff will continue to monitor birds throughout the trial, including food consumption and behaviour.

Each bird will receive a veterinary examination at the start of the trial. Equipment will be disinfected between individuals, or new equipment will be used. Once blood has been collected and vaccination undertaken the bird will be re-released in their home aviary.

The vaccination trial will occur after the breeding season has finished, so wil not interfere with any breeding behaviour. No female birds will be gravid at the time of manipulation, and any breeding birds will have raised and fledged their chicks before the trial begins.

All captive kakī undergo twice yearly health screens, including testing and treatment for internal parasites and a physical check requiring capture. The first vaccination will be timed to coincide with the post-breeding season health screen, and the 6 month blood test will coincide with a pre-breeding season health screen. This reduces the number of times the birds are handled specifically for this trial from six to four.

7b. What movement and transportation measures will be followed for the animals to be manipulated to ensure their welfare and humane treatment?

Birds will be vaccinated on site, in their aviary where they are captured, therefore there will be no transport required.

However, if a bird requires special st veterinary care e.g. in the event of an injury or serious reaction, then it will be transferred to Dunedin Wildlife Hospital (DWH), using the standard kakī transport crates, and in accordance with normal kakī transportation procedures. Briefly, birds will have non-slip flooring the crate, be transported via car to Dunedin. Transport will be managed to reduce noise and allow for temperature control. Radio will be off and driver/passenger will ensure minimal noise. Travel will be direct, and the hospital will be alerted ahead of time to enable a fast hand-over and rapid care.

More than 20 kakī have been treated by the Dunedin Wildlife Hospital in the past 3 years, and they are familiar with their requirements for hospital care.

Supportive therapy would be provided prior to transport which may include pain relief and fluids, the DOC staff are trained and competent in administering medications on direction from the veterinarians.

7c. What measures are to be taken to minimise the pain or distress of any animal manipulated? *Stating there will not be any impact is not acceptable. The AEC is looking for the Applicant to (1) provide analysis about the potential for pain and/or distress to the animal(s), and (2) describe how they will manage that pain or distress. Identify how you would ascertain pain or distress animal's behaviour, environmental conditions likely to lead to pain or distress.*

Birds will be captured and handled by experienced DOC kakī staff using their routine techniques. Only experienced staff will handle the birds. Initial physical examination, vaccination and blood collection will be undertaken by a veterinarian.

Any bird detected to have abnormalities will be examined and rejected from the trial, and receive normal veterinary investigation/intervention.

Subsequent examination and blood collection may be undertaken by DOC staff trained in blood collection from kakī, provided the initial results (0,1, and 2-3 month check) are normal across the cohorts

The subcutaneous injection is not considered painful, and the vaccine dose will be warmed to room temperature prior to injection. Blood collection is associated with a minorly painful pin-prick when the needle is inserted. This will be minimised by careful planning and handling.

If birds are observed to have any pain response to the vaccination, the staff will report it to the veterinarian who will investigate. Ifthere is an injection site reaction (painful inflammation) then an anti-inflammatory such as Metacam may be prescribed by the attending veterinarian, as well as antibiotics if infection is also present.

As noted earlier, if any serious adverse reactions occur, veterinary care by the attending veterinarian will be undertaken, and transfer to Dunedin Wildlife Hospital undertaken if required for more intensive specialist care.

8. CONTINGENCY PLAN:

8a. What arrangements have been made for the abandonment of any manipulation and/or the euthanasia of animals where pain or distress cannot be held within reasonable levels?

If pain or distress is apparent, during handling or following the procedure, the veterinarian will investigate. If the veterinarian deems the level to be unreasonable, then the manipulation will be abandoned and all efforts made to ameliorate the event e.g. anti inflammatory, pain relief medication, antibiotics.

In the unlikely event that the pain is not temporary and cannot be managed, transfer to Dunedin Wildlife Hospital will allow for intensive veterinary intervention and care. This includes the ability to undertake orthopaedic intervention e.g. in the event of a broken bone, or intensive surgery e.g. in the event of a severe localised vaccine reaction.

Supportive therapy would be provided during any transport which may include pain relief and fluids.

9. PEOPLE TO UNDERTAKE PROPOSED MANIPULATION:

9a. Who are the person(s) primarily involved in carrying out the proposed manipulation?
Kate McInnes and Lydia Uddstrom are the primary persons.

9b. What is the experience and qualifications of the person primarily responsible (9a) for the undertaking and supervising the manipulation (including selection of animals, their care and disposal?)
Kate McInnes has been the DOC vet since 2000 and has worked across a range of avian species , and is currently the lead technical advisor for the DOC HPAI response.

Lydia Uddstrom is contracted full time to the DOC kākāpō team, has undertaken postgraduate training as a zoo veterinarian and has experience with a wide range of New Zealand native species veterinary care.

Both Kate and Lydia have previously been involved in capture and handling of threatened species undertaking vaccination and blood collection for a range of threatened species, and have trained multiple DOC staff to safely and effectively undertake these procedures.

9c. Who else is in the team undertaking the manipulation? State their role in the team, and their relevant experience with the procedure(s) proposed in the application? Include DOC and non-DOC staff in the team.

Name of Manipulation Team member	*Role in the manipulation*	*Experience and qualifications relevant to the manipu ation*
Liz Brown	Lead person for catching, handling, monitoring & follow-up blood collection of kakī.	Kakī Recovery Programme - project lead for the captive kakī programme. Liz has 15 years experience managing kakī in captivity, and has routinely been collecting blood samples from all kakī raised in captivity for the past 8 years.
Taleigha Tuer	Catching, handling monitoring & follow-up blood collection of kakī	Kakī Recovery Programme – captive kakī ranger. Taleigha has 5 years of experience working with kakī in captivity, including routine blood sampling.
Serena O'Brien	Catching, handling, monitoring & follow-up blood collection of kakī	Kakī Recovery Programme – captive kakī ranger. Serena has 2 year of experience working with kakī in captivity, including routine blood sampling.
Scott Bourke	catching and handling for the first vaccination session	currently employed on a seasonal contract working with the captive kakī team. Scott has also assisted with catching kakī for several releases, as a student volunteer
Claudia Mischler	May be called on to assist with helping catch and handle during vaccination sessions or follow up sampling	kakī operations team specialising in wild population management. Claudia has 6 years of experience catching and handling kakī in captivity.
Cody Thyne	May be called on to assist with helping catch and handle during vaccination sessions or follow up sampling.	Biodiversity supervisor (and previously kakī operations team). Cody has 10 years of experience catching and handling kakī in captivity

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9d. What training will be given to the people identified in 9c to help them undertake the manipulation proposed in the application?

Team leader and kakī rangers are trained in blood collection of kakī as per the DOC Avian Blood Collection SOP training requirements, and to undertake normal practices including capture & handling.

The capture and handling will be undertaken according to the direction of the team leader.

Vaccination will be undertaken by the DOC veterinarian.

10. COMPLIANCE WITH CONDITIONS of the APPROVAL:

- Please outline any opportunities for a member, or members, of the DOC Animal Ethics Committee to observe this work.

10a. Identify ways that the manipulation(s) can be monitored by the AEC

AEC members could attend a vaccination session at Twizel to see the procedure in kakī and/or receive a video or photographs of the manipulation being undertaken.

11. Are there any other aspects which ought to be brought to the attention of the DOC Animal Ethics Committee?

No

12. Does the research, testing or teaching involve a species which is covered by a Department of Conservation Species Recovery Plan and if so has the Recovery Group been consulted and their endorsement for the work received? Please provide a summary of communication.

Yes. Kakī management is guided by a Kaki advisory group. The Advisory group have been consulted and are supportive of this trial. Liz Brown Pers comm.

This application has been shared with the kakī team on 10th November for review prior to submission to the AEC.

13. What month of year is most useful to report back to the AEC (depending on the project schedule and the animal's biology)?

September

13. Manipulation Grading

Please work through the document '*Grading of Manipulations*' (Please refer to DOCDM-870472), and determine the grading you believe best applies to the manipulation proposed in this application. Please also provide a rationale for the grading.

Grade A: No impact or virtually no impact.
Grade B: Little impact. Manipulations of minor impact and short duration.
Grade C: Moderate impact. Includes manipulations of minor impact and long duration or moderate impact and short duration.
Grade D: High impact. Includes manipulations of moderate impact and long duration or high impact and short duration.
Grade E: Very high impact. Manipulations of high impact and long duration.

Grading determined by the Applicant: B

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Your rationale for the grading:
Grade B includes "**Disease/injury/functional impairment:** Studies of vaccines using killed pathogens." The animals will be kept in their normal husbandry conditions for the duration of the study. There will be capture and handling for blood collection and vaccination, and two doses of a killed vaccine administered. Handling time & stress will be minimised by using only skilled staff and it will be undertaken at site.

Note: The grading determined by the Applicant is not the grading assigned by the AEC. The Applicant will be advised of the AEC's grading and any conditions in writing.

DECLARATION by the APPLICANT

Tick boxes [☑] to indicate your agreement to conditions: *[Copy and paste this tick object ☑]*

[☑] I declare that the information in this Application is correct; and
[☑] I agree to comply with the conditions imposed by DOC's AEC for the manipulation; and
[☑] I agree to ensure all personnel involved in this manipulation will be properly trained and/or qualified to undertake the manipulation and will be aware of the contents of this AEC application; and
[☑] I declare the proposed manipulation has the necessary resources to undertake the manipulation with regard to the health and safety of the animals and staff
[☑] I agree to advise the AEC of any changes in the details of the manipulation as described in this Application.
[☑] I agree to comply with the reporting requirements stipulated by the AEC on approval of this research project.

Signed by the Applicant 9(2)(a) [redacted]

Full Name: Catherine McInnes Date: 13/11/2023

DECLARATION by the ACCOUNTABLE MANAGER

Tick boxes [☑] to indicate your agreement to conditions: *[Copy and paste this tick object ☑]*

[☑] I agree to ensure my staff member complies with the conditions imposed by DOC's AEC for this manipulation; and
[☑] I agree to ensure all personnel involved in this manipulation will be properly trained and/or qualified to undertake the manipulation and will be made aware of the contents of this AEC application; and

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[☑] I agree the proposed manipulation has the necessary resources to undertake the manipulation with regard to the health and safety of the animals and staff

[☑] I agree to oversee this Application via MORs, PDPs and other means to ensure the manipulation remains within the scope of the Application and the Approval, and all reporting required by the AEC is delivered on time;

[☑] I agree to advise the AEC of any changes in the details of the manipulation as described in this Application, and to advise the AEC if the Applicant leaves the Department, or if the work should be transferred to another staff member for operational reasons' or if the manipulations is abandoned for any reason.

Signed by the Manager: 9(2)(a) [redacted]

Full Name: John Lyall

Role: Fauna Advice Manager

Date: 14/11/2023

Application Number DOCDM	AEC445 APPLICATION DOC-7483662
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, kakī

Appendix 1: Avian Influenza vaccine information

Registration number A009733: **Poulvac Flufend i AI H5N3 RG**

Registrant: Ministry for Primary Industries

Draft label information:

PRESENTATION

Bottles of 500 mL (1000 doses). Packs of 1 or 10 bottles.

DIRECTIONS FOR USE

By law the distribution and use of this product must comply with the requirements of the relevant operating plan.

General:
• Inject 0.5 mL (0.5 cc) subcutaneously, using aseptic technique, into healthy birds at 3 to 4 weeks of age or older.
• Shake well before use.
• Allow the vaccine to reach room temperature (18-29°C) before use.

Chickens:
• Administer another dose of 0.5 mL not less than 2 weeks later, if required.
• The second dose should be administered at least 4 weeks before point of lay.

Ducks:
• Ducks less than two weeks of age:
• Administer 0.2 mL of vaccine subcutaneously at the back of the neck.
• Administer another dose of 0.5 mL not less than 2 weeks later.
• Ducks two or more weeks of age:
• Administer 0.5 mL of vaccine subcutaneously at the back of the neck.
• Administer another dose of 0.5 mL not less than 2 weeks later.

ADVERSE EFFECTS, CAUTIONS AND CONTRAINDICATIONS

ADVERSE EFFECT
• Vaccinate only healthy chickens or ducks and avoid stressing the birds at the time of vaccination.
• Do not mix with any other vaccine or injectable product.
• The use of this product in laying birds has not been evaluated.
• Local or systemic post-vaccination reactions can occur due to the use of oily vaccines. Symptoms observed are generally transitory and can include oedema and granulation at the injection site, anorexia and dehydration. Such reactions can be minimised by good aseptic vaccination technique.

CAUTIONS
• Destroy any unused vaccine and containers after vaccination (including syringes and needles) by burning.
• Do not mix the vaccine with other vaccines or administer another vaccine shortly before or after vaccination with this product.
• Consult a physician immediately for an accidental self-injection and show this package insert to the physician.
• KEEP OUT OF REACH OF CHILDREN AND UNINFORMED PERSONS

CONTRAINDICATIONS
• None.

WITHHOLDING PERIODS
Meat: Nil.

STORAGE
• Store in the dark between 2 °C and 8 °C. Do not freeze.
• Protect from direct sunlight.
• Use contents of each vial within 6 hours of opening

Appendix 2: General Instructions for subcutaneous injection of vaccine

- The vaccine is supplied in a 500mL bottle and is given to the bird using a needle and syringe.
- The vaccine is injected under the skin but NOT into the muscle below.
- The vaccine should be drawn up into the syringe and then allowed to warm to room temperature (this is more comfortable for the bird).

EQUIPMENT NEEDED

1. Vaccine container
2. 1 mL syringe
3. 25 gauge 5/8th inch needle
4. alcohol swab (mediswab or cotton wool and meths)
5. dry swab (gauze or cotton wool)
6. Sharps container for needle disposal
7. Bird
8. Bird handler
9. Veterinarian

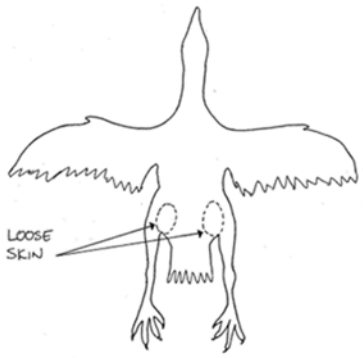
PREPARING THE VACCINATION

1. Store the vaccine in the fridge at 2-8 degrees C in the dark. Do not Freeze.
2. When ready to use, take the vaccine out of the fridge and shake well to mix.
3. Write the date on the vaccine bottle.
4. Break off the metal seal on the top of the rubber injection port.
5. Swab the injection port on the vaccine with alcohol to sterilise it with a mediswab or cotton ball soaked in methylated spirits.
6. Firmly attach the needle to the syringe – 25 gauge 5/8th inch needle to a 1mL syringe.
7. Insert the needle through the centre of the rubber stopper CAREFULLY.
8. Hold the vaccine upside down and slowly suck vaccine into the syringe until you have a little more than the prescribed dose of vaccine.
9. Flick the syringe to dislodge any air bubbles and squirt them slowly back into the vaccine bottle.
10. Keep squirting until all the bubbles are gone and you have the prescribed dose of vaccine left in the syringe.
11. Pull the needle out of the vaccine bottle and CAREFULLY recap the needle.
12. Leave the syringe and needle to warm to room temperature.
13. Repeat this procedure to draw up all the doses you need for your vaccination session.
14. Put the vaccine back in the fridge.
15. Once open, the vaccine can be used for 30 days. (Note that this expiry is based on Zoetis technical advice for limited use of the vaccine in this trial, and only applies when following the above instructions for maintaining sterility of the product and correct storage.)
16. If you are in doubt that the vaccine has been stored correctly (kept refrigerated), then discard it and get a new bottle.

GIVING THE INJECTION

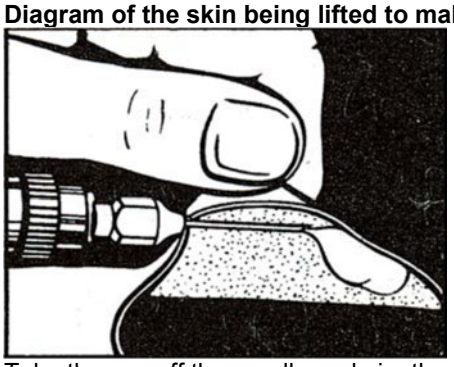
1. Have the following equipment ready for use:
    - The correct dose of vaccine drawn up in syringe with needle attached and warmed to room temperature. (the cap should be on the needle to avoid accidental stabbing or contamination of the needle)
    - One alcohol swab (mediswab or cotton wool in meths)
    - One dry swab (gauze or cotton wool)
2. Have an assistant restrain the bird on its back or side with its legs restrained to provide access to the groin (where the bird's leg joins its belly).

Diagram of ventral (belly) of a bird showing the groin region for subcutaneous vaccination sites:



3. Spread the feathers in the groin area.
4. Wet down the feathers with the swab to clear a patch of skin and swab the skin.
5. Lift the loose skin 1-2cm off the body to make a "tent".

Diagram of the skin being lifted to make a "tent" for a subcutaneous injection



6. Take the cap off the needle and aim the needle about halfway down the side of the tent. Keep the needle parallel to the body wall. When the needle goes through the skin, it should still be above the muscle of the groin i.e. you are injecting into the space inside the tent, not into the muscle.
7. Suck back on the syringe to check for blood. This is to avoid injecting into a blood vessel.
8. Inject the vaccine with a steady firm pressure.
9. Withdraw the needle and place it into the Sharps container.
10. Use the dry swab to press over the injection site if there is any bleeding.
11. Release the bird.

12. Record:	Bird ID	Date	Dose	L or R side	Vaccinator	Holder	Vaccine Batch	Expiry Date	Notes

13. Transfer this data to the vaccination record spreadsheet
14. Note any other specifics about the injection process not described above. E.g. if there was bleeding at the injection site.

Application Number DOCDM	AEC446 APPLICATION DOC-7483658
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, tūturuatu

Revised September 2023

DEPARTMENT OF CONSERVATION
APPLICATION TO MANIPULATE LIVE ANIMALS
Code of Ethical Conduct for the Care and Manipulation of Live Animals

1. APPLICANT'S DETAILS:

Name: Kate McInnes

Date: 15 November 2023

Role: DOC Veterinarian

Unit: BH&V Group, Wellington

APPLICANT'S ADDRESS:

Phone no: 9(2)(a) [redacted]

Email: kmcinnes@doc.govt.nz

2. ACCOUNTABLE MANAGER'S DETAILS:

Name: John Lyall

ACCOUNTABLE MANAGER'S ADDRESS:

As above or: DOC, Hokitika

Phone no: 9(2)(a) [redacted]
Email: jlyall@doc.govt.nz

2a. AEC446

2b. **MANIPULATION TITLE: Avian Influenza vaccination safety and efficacy trial tūturuatu**

2d. **Duration of the manipulation**

- *Over what timeframe are you seeking the approval?*
- *You must not commence the manipulation until you have received the approval, signed by you, your accountable manager, and the AEC Chair.*

Application Number DOCDM	AEC446 APPLICATION DOC-7483658
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, tūturuatu

- *NOTE: The AEC will not generally give an approval for longer than two years at one time. Please state if this manipulation is likely to extend longer than two years from the commencement date.*

Anticipated start date:	February 2024	Anticipated finish date:	June 2025

2e. What months of the year is the manipulation most likely to be undertaken? e.g., October – March

For the duration of the dates specified but breeding birds will not be handled during September to January

3a. Summary of the proposed manipulation for a LAYPERSON

- *Provide an abstract describing the manipulation (maximum 400 words).*

Avian influenza is a viral disease which can cause mass mortality events in birds. The current strain is decimating many populations of wild birds overseas, is predicted to reach the Southern Ocean by 2024/25 and was confirmed in South Georgia, October 2023.

We want to test the safety and efficacy of vaccination to protect critically endangered species.
The vaccine is a commercial product registered in New Zealand by Ministry for Primary Industries. It is considered very safe and highly effective. It contains inactivated (dead) virus so it cannot cause avian influenza. Vaccination reduces risk of illness or death and reduces shedding of virus, thus protecting the individual and its flock.

Tūturuatu are a critically threatened species which is reliant on a captive breeding programme where it is possible to reliably administer a full course of vaccine (2 injections under the skin, one month apart) to individually identified birds, and where we are able to handle them again for a veterinary examination and blood testing to detect any effects on health status, and measure the immune response by detection of antibodies over a 12 month period.

Captive tūturuatu will be captured in the aviary and receive a pre-vaccination health check by a veterinarian, and a blood test for health and antibody testing. Up to 0.4 mL of blood will be collected from the wing vein, as is standard for this species.

The vaccine is given under the skin. One month later the bird will receive a second vaccination and blood test. Further blood will be collected at 2-3, 6 and 12 months post vaccination to determine the level of antibody response and how long it lasts.

A cloacal and choanal (oral) swab will be collected on day 0 for PCR testing to demonstrate the birds were not incubating avian influenza at the time of vaccination.

Normal husbandry practices will be undertaken including observation of the bird's activity and food intake to monitor of any adverse reactions.

We propose to work with a total of 10 adult or juvenile tūturuatu, divided into two cohorts. Cohort 1 will first receive the vaccination & blood tests, and a recheck at 1 month. If no safety issues are identified, then Cohort 2 will receive vaccinations & blood tests. This allows a careful start to the trial where the month is the most important to test vaccine safety. The following blood samples (at 2-3, 6 & 12 months) will determine level and duration of antibody presence and determine when further boosters would be required.

Additional approval given on 16/4/24 to collect a 10-14 week blood sample to target peak antibody levels, and to collect an opportunistic blood sample for further antibody testing, if birds are being handled for routine management purposes, with no more than twice per bird over the 12 months of the trial.

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3b. Description of the proposed manipulation (methods)

- *Provide a more detailed explanation. Describe the equipment, the location, and any environmental factors: weather, time of the year. Why have you decided to undertake the manipulation in this way? What advice have you sought? Include the species, the number of individuals, the source of animals, and the disposal/fate of animals at the conclusion of the manipulation.*
- *Be specific about the timelines for the proposed investigation and the purpose of the research, testing or teaching.*
- *Include some consideration and planning for when things might not go right.*

Please note: this is one of five trials to assess avian influenza vaccination safety and efficacy in nationally critical threatened species (takahē, kākāpō, kakī, tūturuatu, kākāriki karaka). Manipulation details which are specific to this species (tūturuatu) have been highlighted in yellow. All other detai s are consistent across the five trials. By highlighting the species specific details I hope to assist the AEC with the volume of workload associated with simultaneously assessing these trials.

This trial is designed to test the efficacy and safety of a vaccine in a critically threatened species.

Selection of the species for potential vaccination is based on the risk that they could undergo an extinction event when highly pathogenic avian influenza (HPAI) reaches New Zealand. 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 risk of extinction.

The current wild adult tūturuatu population is approximately 130-150 adult birds on Rangatira, Chatham Islands. The captive management programme produces up to ~50 birds per year for release into mainland sites to establish/maintain additional populations.

Based on the evidence from overseas during this epizootic, the species most at risk of infection are those which exhibit congregation behaviours e.g. feeding, breeding or roosting in groups, those which are exposed to at risk species e.g. where seabirds overlap with another threatened species, and birds held in captive facilities where biosecurity options are limited e.g. open pens and large aviaries.

Tūturuatu are one of 5 species identified by the DOC HPAI Technical Advisory Group as at risk where administration of a full vaccination programme is feasible in sufficient number of individuals to provide protection against species extinction. See DOC-7111177 Mitigation Options Guideline for HPAI.

Use of the vaccine is dependent on Ministry for Primary Industries approval, and currently requires the birds to be held in captivity. Birds require two injections one month apart and must be individually identified with a permanent mark e.g. microchip or leg band. Birds in this trial will already have these marks as part of routine husbandry/management procedures.

Effective vaccination reduces susceptibility to infection. When infection does occur, it reduces clinical signs of disease and the amount of virus shed into the environment (Animal Health Australia, 2021).

Additionally, vaccination of California Condor was approved in the United States following an outbreak in the wild population. This was the first avian influenza vaccination programme in a wild endangered species. Advice from the veterinary and technical advisors to the condor vaccination programme has been received and is incorporated into this trial design.

Application Number DOCDM	AEC446 APPLICATION DOC-7483658
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, tūturuatu

We wish to undertake a limited trial to determine the safety and efficacy of the avian influenza specific vaccine in tūturuatu as a preparedness measure for the arrival of HPAI in New Zealand.

The vaccine is produced commercially by Zoetis for use in poultry: Poulvac Flufend i AI H5N3 RG inactivated (killed) vaccine - see Appendix 1. It has been in production since 2006 and is widely used in the poultry industry. Publications on AI vaccine use in poultry and avian species in zoos have indicated a very high level of safety across a wide range of species, and efficacy has been well established. (Kandeil et al 2018, Philippa et al 2006, Philippa 2007a, Philippa 2007b, Pitman 2006, Vergara-Alert 2011). The vaccine is inactivated, so there is no live virus present and it cannot cause avian influenza.

Advice from Zoetis (USA) indicates that this vaccine should provide good protection against the current strain of HPAI with 91% amino acid homology with the circulating strain. A newer vaccine based on the circulating strain is in production but is will not be available until the end of 2024 at the earliest.

Vaccine will be obtained from PacificVet in Christchurch and transported in a chilly bin with ice-packs by overnight courier (as per their standard transportation procedures for vaccines) to ensure cold chain is maintained. Use in the field will be managed by extraction of sterile aliquots into sterile vials or syringes. This enables sustainable use of the 1000 dose vial and maintenance of sterility of product. This process was discussed with the Zoetis Senior Research Advisor responsible for poultry products and is considered safe and appropriate.

Sterile aliquots will be obtained by using a sterile needle and syringe to extract the aliquot from the closed vaccine vial. The vial will be shaken to homogenise the contents, then the rubber stopper will be swabbed with alcohol. The sterile needle will be attached to the sterile syringe and the needle inserted via the rubber stopper. The aliquot will be drawn up into the syr nge, then the needle& syringe removed from the stopper and the cap replace on the needle. The needle will be swapped for a new sterile needle or a sterile vaccine cap.. Both the aliquots and the vaccine vial will be stored refrigerated in accordance with the packaging instructions. Vaccine doses will be drawn up immediately before use and allowed to warm to room temperature just prior to injection.

DOC veterinarians Kate McInnes or Lydia Uddstrom will administer the vaccination.

All birds will receive a full veterinary physical examination at the start of the trial. Only birds in good body condition exhibiting signs of good health will be included. (Any birds which show signs off poor health will be further investigated as per normal veterinary practices).

The tūturuatu recovery team have been involved in the design of the study, and selection of study animals. The Christchurch tūturuatu breeding centre (Isaacs Conservation & Wildlife Trust) was chosen for being one of only 3 facilities holding tūturuatu, the presence of experienced staff, and the ability to access a total of 10 birds at one site.

Individuals for vaccination will be selected by the tūturuatu recovery team, based on the programme's planning for any translocations.

Each individually permanently marked (leg band) bird will receive two doses of vaccine by subcutaneous injection into the inguinal (groin) region with a 1 month interval (no less than 3 weeks apart and a maximum of 6 weeks apart). The first vaccination will be into the left inguinal region, and the second vaccination into the right inguinal region.

Birds will receive 0.2ml per dose (as per dosages used in Vergara-Alert et al 2011).

Individual birds within the two cohorts will be determined on the day by tūturuatu staff based on available birds' suitability and any management requirements.

At the start of the trial, each bird will receive a cloacal and oral swab to determine presence/absence of virus at day 0.
The technique will follow the draft SOP Avian swab sampling DOC-6840491 which has undergone veterinary peer review & user testing and is awaiting AEC endorsement before Director sign-off. These types of swabs are used in standard health testing on avian species and would be undertaken by the veterinarian. The test would be considered a baseline health test to demonstrate the birds were not incubating avian influenza at the time of vaccination. The swabs will undergo PCR testing at BioPacifica to look for avian influenza virus.
This is important to be able to demonstrate that any antibody response is due to the vaccination rather than the bird being infected by a wild strain of avian influenza

First trial - Cohort 1: Four individuals will be vaccinated as per the described protocol above. Blood (up to 0.4ml) will be collected at 0, 1, and 2-3 months to measure health parameters (white cell count & differential) and antibody response (commercial serum ELISA test to measure antibody titre). Antibody testing will be undertaken at a commercial laboratory (BioPacifica, Christchurch).

Note: 1% of body weight is considered an acceptable amount of blood to collect from a healthy bird. Adult weigh ~60g, therefore up to 0.6 ml would be within the safe range for an adult. We propose to only collect up to 0.4ml to maintain a high margin of safety.

Second trial - Cohort 2: Based on consideration of the results of the first trial, if safety has been demonstrated, a second cohort of six individuals will receive the vaccination as per the described protocol above, and blood will be collected at 0, 1, and 2-3 months to measure health parameters (white cell count & differential) and antibody response (commercial serum ELISA test). We will wait 1 month until we have established the vaccine is safe in cohort 1 before we start cohort 2.

Note: If antibody response at 2-3 months is noted to be muted (i.e. a low response) then the DOC vets (Kate McInnes and Lydia Uddstrom) will discuss the use of a third dose of vaccine. This was used in some species in European zoos where the initial antibody responses were considered insufficient. Consideration will be given to the level of response detected, the impacts of additional handling, and any other welfare factors noted during the preceding handling events. The benefits of testing a third dose of vaccine will be carefully considered, and this will only be undertaken if the welfare impacts are considered minimal. The justification for a third dose in this trial would be to confirm if this dose is warranted and would deliver protection for the tūturuatu population in the event of an outbreak of HPAI in Aotearoa New Zealand.

Two additional blood samples will be collected from both cohorts at approximately 6 months and 12 months to measure duration of antibody response.

A maximum of 10 birds will be included in the trial. It is estimated that there will be ~12 breeding tūturuatu on site at the DOC facility at the time of the trial.

These trials are modelled on the vaccination of California Condor in the USA. (FWS.gov 2023) however the 1 month interval is based on the European zoo data, and considered more appropriate to allow for recovery between handling events in birds which normally have minimal handling by humans.

Estimated timing/schedule of manipulations:

First vaccination and blood sample	Second vaccination and blood sample	2-3 month blood sample	~6 month blood sample	~12 month blood sample
First cohort of 4 birds				
~ 1st February 2024	~ 1st March 2024	~1st April 2024	~ 1st August 2024	~ 1st February 2025
Second cohort of 6 birds (maximum 10 total)				
~ 1st March 2024	~1st April 2024	~1st May 2024	~1st August 2024	~ 1st February 2025

Second cohort of 6 birds – same timeline but 1 month after first cohort have been vaccinated and shown no negative reaction. Blood collection at ~6 and ~12 months may be undertaken at the same time for both cohorts – these sample are about longevity of antibody presence, so the exact timing is less critical.

During a handling event, all involved staff will gather and have a pre-handling briefing by the veterinarian and the team leader to ensure all roles and responsibilities are clearly understood. Any issues can be raised at that time for clarification. The tūturuatu team leader will be responsible for the safe capture and handling of the bird. The veterinarian will be responsible for the health examination, vaccination and blood collection.

All equipment will be prepared prior to capture to minimise handling time. Staff will know where to situate themselves and what actions are required so that an efficient process is maintained. The tūturuatu staff involved in this trial will be team members with previous experience in blood collection in tūturuatu.

Tūturuatu are caught according to the following procedures as detailed in the tūturuatu husbandry manual:

4.1.1 Noose mat

Shore plover are usually captured using a noose mat (Fig. 2). Noose mats consist of fishing line loops ('nooses'), attached to a strip of plastic mesh approximately 900 x 100 mm in size. When birds walk across the mat, their feet are caught in the nooses.

Noose mats work well with shore plover, as they prefer to walk rather than fly when displaced. They can be easily herded across a noose mat if they do not recognise what it is.

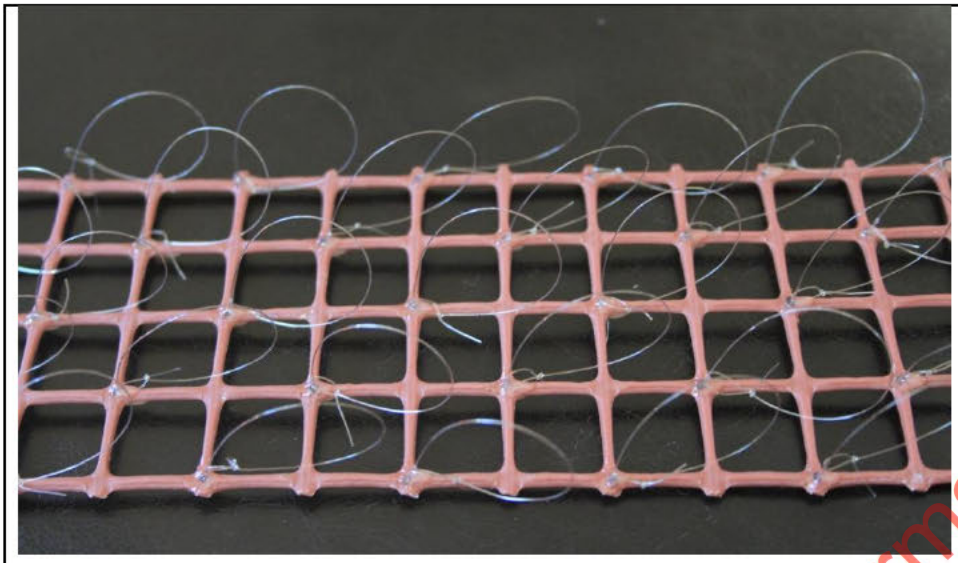


Figure 1. Noose mat used to capture shore plover

Tips for safe and successful noose mat use:

- When setting up the noose mat it is critical that the nooses are all standing upright and in the same direction, i.e. the noose sits parallel with the length of the mat, and there are no gaps between adjacent nooses that birds could walk through. Nooses will need to be replaced from time to time if they crinkle or twist in the wrong direction (refer to appendix 14.7 Making or fixing a noose mat).
- Place the noose mat across a logical pathway, or create one by placing obstacles either end of the mat so the birds are funnelled towards and across it.
- The noose mat must be pegged or weighed down so that when a bird is caught the mat does not lift or flip over and birds cannot fly away with it attached. This is easily done by tying the end tie cords securely to a heavy stone.
- Ensure the noose mat lies completely flat on the substrate. If possible, disguise it by sprinkling shingle over it so the plastic is less visible.
- Do not try to catch birds in windy conditions as movement of the nooses may alert birds and put them off walking across the noose mat. Otherwise place the noose mat in an area sheltered from the wind.
- It is often easier for just one person to do the 'herding' part of capture. Shore plover tend to get suspicious and nervous when there are two or more people involved.
- Never walk far away from a noose mat or leave it unattended. If a bird is snared, you need to be able to get to the mat very quickly to extract it (i.e. within 5-10 seconds).
- Once a bird is snared, walk quickly up to the noose mat to free it, but be careful not to stumble as the bird will be flapping around and there is the potential to stand or kneel on it.
- Be careful to only target birds that really need to be caught for a specific purpose. Unnecessary captures should be avoided whenever possible, because they will make birds wary and potentially jeopardise capture

attempts in future, when it may be particularly important to catch a bird (e.g. to replace a band). Aim to minimise the number of captures in any bird's lifetime (Dowding 1998).

Shore plover become more wary of a noose mat after being caught in one a few times, or witnessing other birds being captured. If it becomes difficult to catch particular individuals with a noose mat the following options can be tried:

- Place mealworms next to the noose mat as a lure or distraction.
- Play recordings of chick or juvenile alarms calls from the other side of the noose mat (these can be recorded when handling chicks in brooders). Most birds will be curious and come over to investigate. If using this method on a group of shore plover, ensure there are helpers standing by to release the birds if a number are captured at once.

Once caught the bird is placed in a 'catch bag' (a soft cotton bag with a draw string which provides a safe restraint for temporary holding) to be weighed in the bag using a hanging scale (standard technique) and then carefully removed undergo a physical examination to determine its health status prior to any further manipulation. If the tūturuatu is determined to be healthy blood collection and vaccination will proceed.

For blood collection and vaccination, a trained tūturuatu handler will restrain the bird on its side or back.

The blood collection site (brachial/wing vein) will be swabbed with a sterile alcohol wipe immediately prior to collection. Blood will be collected from the wing vein via the pin-prick method where the vein is pricked using a sterile 25 or 26 gauge hypodermic needle, and then the bleb of blood is collected in capillary tubes (also known as haematocrit tubes which can contain 75microlitres of blood).

In the event that the temperature is cool, and on examination of the wing vein we determine that blood collection likely to be slow due to the presence of small contracted veins, the wing will be warmed for 3-5 minutes using Kathmandu hand warmers wrapped in gauze, to boost circulation and enhance blood flow to enable effective blood collection.

After collection, the site of blood collection will be covered with a gauze swab and pressure applied to control any bleeding. In the very unlikely event of uncontrolled bleeding, pressure will be applied for a further 1-5 minutes. If still uncontrolled, an icepack wrapped in gauze swabs will be held on the wing area to cool the limb and reduce blood flow. If required, a silver nitrate stick will be carefully used to stop the bleeding.

Blood will be spun in a centrifuge to separate the serum from the blood cells. The tubes will be kept chilled and transferred ASAP to the commercial laboratory in batches for antibody testing.

1-2 drops of blood will be used to make blood smears which will be sent to a commercial veterinary pathology laboratory for a white cell count and differential. This provides a baseline health analysis which can detect infection or inflammation. Any abnormal results will be further investigated by the veterinarian in consultation with the tūturuatu staff.

Once bleeding has stopped, the bird will be vaccinated using a 1mL syringe attached to a 20 gauge ½ inch needle. The vaccination site will be swabbed with a sterile alcohol wipe immediately prior to vaccination. See Appendix 2 for details of the vaccination technique.

The bird will then be checked for any abnormalities and the veterinarian will determine if any further actions are required for health or welfare. Once the procedures are complete the bird will be quietly released and observed as it moves away. Regular observations during routine husbandry will continue for

all birds, and any abnormalities will be reported to the veterinarian. The birds are visited and monitored at least twice daily by staff when they are being fed the artificial diet.

At subsequent handling events, the vaccination site will be examined and any discolouration, swelling, granuloma formation or unexpected abnormality will be noted and reported to the veterinarian. Photographs of each bird's injection sites will be taken to provide a clear record of the trial.

Location & timing:
The trials will be undertaken at the Isaacs Conservation and Wildlife Trust tūturuatu breeding facility in Christchurch between Feb 2024 and June 2025 (breeding tūturuatu will not be handled which is from approximately September to January, dependent on the season and breeding success).

Safety:

Results from a meta-analysis of use of vaccine in European Zoos found very low adverse reaction rate at 0.04% local reactions and 0.015% general reactions reported. EFSA 2007. Based on this, we do not anticipate significant issues with the vaccine, however we will be prepared for immediate veterinary care if any reactions to occur.

The vaccine packaging label states: "Local or systemic post-vaccination reactions can occur due to the use of oily vaccines. Symptoms observed are generally transitory and can include oedema and granulation at the injection site, anorexia and dehydration. Such reactions can be minimised by good aseptic vaccination technique."

Anaphylaxis:
A severe immediate immune hypersensitivity response could occur if the vaccine product stimulates such a response. This is considered unlikely due to the extensive use of this vaccine and other similar vaccine products in Europe, however it is possible and needs to be considered as a potential adverse event. The vaccination team will include a veterinarian who will have access to emergency drugs and supportive care for management of anaphylaxis (including corticosteroids, adrenaline, oxygen, fluids).

Injection site reactions:
The vaccine contains an adjuvant (oil) which is present so that it stimulates a stronger immune response with greater antibody production. This can sometimes be associated with a small pea-sized lump at the site of injection. This is normal and expected, although generally not all birds will develop a lump. This will be checked at the 1 month mark, and records kept of any reactions detected. If an excessive size reaction is detected in an individual (>1cm), then the vaccination will be paused until it is determined that the lump does not enlarge further, or cause any impacts on the bird(s) – this is likely to be a period of 2-4 weeks. Body weight, act vity levels etc will be reviewed and a full physical examination undertaken.

A localised bacterial infection could result if poor sterile technique is used. Only registered veterinarians will be administering the vaccine, and these operators all have training in appropriate sterile techniques. If a bird experiences an infection at the site, it will receive veterinary care and follow-up to ensure the issue is managed.

Mis-injection could occur if the bird is poorly restrained and moved during vaccination. This will be managed by only using well trained, experienced tūturuatu handlers to restrain the birds. For some individual birds, they are calmer with head cover which can aid in handling. This will be determined on an individual bird basis. If a mis-injection occurs, the veterinarian will determine the appropriate next steps. This may include, re-injection if the first injection merely failed to enter the bird, appropriate first aid

measures if any injury was caused, and/or exclusion from the trial and follow-up care. As noted previously, "stressy" birds will not be included in the trial which will reduce the risk of injury or mis-injection.

Injury could occur during capture and handling. This is minimised by only using trained experienced staff, careful selection of trial birds, and a "stop for safety" approach which resets the work programme and ensures time out to reassess and replan the work and procedures if necessary.

In the event of a serious reaction or injury during the vaccination trial, the bird will be taken to Wildbase Hospital Massey University for specialist care by Drs Megan Jolly and Brett Gartrell. This is standard procedure for tūturuatu requiring a high level of veterinary care. Birds have been transported this way via Air New Zealand on many occasions.

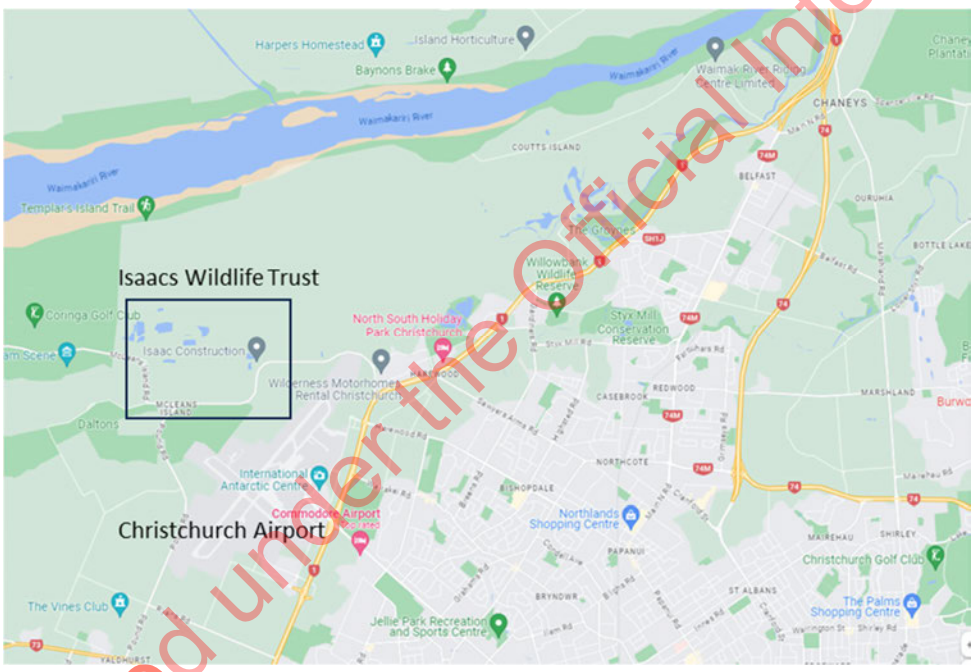
Results:
The results of this trial will determine if this vaccine is safe to use in this species, and the level of antibody response produced by a 2 dose vaccination. In some other species, notably penguins, the antibody levels following vaccination remain low and, in some species, a third vaccination was used to ensure a stronger response (ESFA 2007). The duration of antibody presence also varied between species. Therefore, this trial will help to determine the appropriate vaccination regime for tūturuatu in the event that more widespread vaccination is required during a highly pathogenic avian influenza outbreak in New Zealand.

3c. Attach Photos of equipment, the species, the location (or a map); to help set the context.

Application Number DOCDM	AEC446 APPLICATION DOC-7483658
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, tūturuatu

Figure 2.Standard hold for shore plover

Figure 1. ICWT shore plover aviary block with service corridor.



3d. References

- *List the references referred to in the application*

DOC-7111177 Mitigation Options Guideline for HPAI
https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7111177

EFSA 2007. Vaccination against avian influenza of H5 and H7 subtypes as a preventative measure carried out in Member States in birds kept in zoos under Community approved programmes. ESFA journal, 450. ESFA-Q-20006-156
https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499835

Health Australia (2021). Response strategy: Avian influenza (version 5.0). Australian Veterinary Emergency Plan (AUSVETPLAN), edition 5, Canberra, ACT. Response_Avian-influenza.pdf (animalhealthaustralia.com.au) Animal

FWS.gov 2023 Southwest California Condor Flock HPAI Information Updates - 2023 | U.S. Fish & Wildlife Service (fws.gov)

Kandeil A, Sabir SM, Abdelaal A, Mattar EH, El-Taeel AN, Sabir MJ, Khalil AA, Webby R, Kayali G, Ali MA. Efficacy of commercial vaccines against newly emerging avian influenza H5N8 in Egypt. Nature Scientific Reports, 2018. 8:9697 | DOI:10.1038/s41598-018-28057-x
https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499854

Tūturuatu/shoreplover husbandry manual. Internal DOC document
https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOCDM-1203057

Philippa JDW, Munster VJ, van Bolhuis H, Bestebroer TM, Schaftenaar W, Beyer WEP, Fouchier RAM, Kuiken T, Osterhaus, ADME. Highly pathogenic avian influenza (H7N7): Vaccination of zoo birds and transmission to non-poultry species. Vaccine, 2005, 23:5743-5750.
https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499837

Philippa JWD 2007a, in XI. Vaccination of Non-domestic Avian Species, Transmissible Disease Handbook. European Zoo Vets 5th Edition link

Philippa J, Bass C, Beyer W, Bestebroer T, Fouchier R, Smith D, Schaftenaar W, Osterhaus, A. Vaccination against highly pathogenic avian influenza H5Na virus in zoos using an adjuvanted inactivated H5N2 vaccine. Vaccine, 2007b, 25: 3800-3808. https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499841

Pitman 2006. M Pittman, European Commission 12th Annual meeting of national avian influenza laboratories Veterinary and Agrochemical Research Centre (VAR) Uccle, Brussels, 16-18 October 2006 LINK: link.

Vergara-alert J, Ferhandez-Bellon H, Busquets B, Alcantara G, Delclaux M, Pizarro B, Sandchez C, Sanchez A, Majo N, Darju A. Comprehensive serological analysis of two successive heterologous vaccines against H5N1 Avian Influenza virus in exotic birds in zoos. Clinical and Vaccine Immunology, 2011. P. 697-706. https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499845

4. INVOLVEMENT OF OTHER ANIMAL ETHICS COMMITTEES:

4a. Is this Application; or a related or similar application; been or is being considered by another Animal Ethics Committee. Has this project been requested to be considered by any other AEC?

If so, please provide details.

No

4b. Does this manipulation interact with a manipulation approved by other Animal Ethics Committee? If so, detail your communications with those committee(s), and state any conditions imposed by (an)other AEC.

No

5. JUSTIFICATION FOR PROPOSED MANIPULATION:

5a. Detail any action undertaken to determine that the proposed work has not already been done.

Avian Influenza vaccine efficacy and safety has been undertaken on other avian species, however it has not been undertaken in New Zealand endemic species. Although we expect similar results, it is prudent to undertake this trial to provide more evidence of safety and efficacy in the species which we intend to vaccinate in the event of a HPAI outbreak.

5b. Have alternatives been considered to the proposed manipulation involving reduction, or replacement of live animals, or refinement of techniques?

We are looking at the species-specific response and have selected a minimum size divided into two cohorts, so other methods of reduction are not appropriate for this work.

The cohort approach allows us to cautiously approach the safety issue, and assess initial results before involving the full number of birds.

5c. To what extent has there been assessment of the suitability of using non-sentient or non-living alternatives in the project; or replacement of animals as subjects with suitable non-sentient or non-living alternatives?

N/A, see above

5d. How will the proposed work result in the extension of knowledge relevant to the health, welfare, or conservation of animals?

This work will specifically contribute to the future health of the species for conservation purposes by providing evidence of the safety and efficacy (or not) of this vaccine in this species, and inform the appropriate vaccine schedule for the species.. This will determine if the vaccine is employed in the future in the face of an avian influenza outbreak in New Zealand.

5e. Is the manipulation required as part of an approved training programme?

No.

5f. How will the results of this work be made available to staff within and outside DOC? (*For example internal report, journal paper, best practice guide, workshops etc*).
Internal report, journal paper, conference presentations, shared with other captive institutions that hold tūturuatu.

6. SELECTION OF SPECIES & NUMBER OF INDIVIDUALS FOR PROPOSED MANIPULATION

6a. What will be the source of the animals to be manipulated, and how many from each source will be manipulated?
Tūturuatu at the Isaacs Conservation & Wildlife Trust Christchurch breeding centre. 10 birds in total. It is estimated that there will be 12 breeding tūturuatu on site at the facility at the start of the time of the trial.

6b. Will any of the animals involved be used more than once, and if so, how many times will each animal be used?
Only once (but each animal handled/manipulated multiple times – twice for vaccinating and four more times for blood sampling, although the 6 & 12 month handling for blood collection will be planned to coincide with routine handling for health management)

6c. What factors have been taken into account in the choice of the animal species?
Tūturuatu are one of 5 species identified by the DOC HPAI Technical Advisory Group as at risk where administration of a full vaccination programme is feasible in sufficient number of individuals to provide protection against species extinction.

6d. Could the information being sought be obtained by work on some other species?
No. The trial specifically uses tūturuatu since the safety and efficacy needs to be tested in the target species.

6e. Will the question be answered with the size of the sample?
Yes.

6f. Is the number of animals proposed to be manipulated the <u>minimum</u> necessary to provide a scientifically interpretable result, consistent with the level of statistical precision required? What consideration has been given to the design of the study with regard to:

- **The level of precision necessary in the results?**

The purpose of the small trial is to establish if there is a species-specific sensitivity to the vaccine and its adjuvant. For this purpose, we require only a small number of birds to extrapolate a species sensitivity. Similarly, for determining vaccine response by antibody response levels, a sample size of 10 will provide sufficient individual variation to establish an overall species response level. Additionally if a bird is removed from the trial for any reason (e.g. other health issues, injury, behavioural), starting with 10 birds allows sufficient number to still be able to make a reasonable conclusion on the vaccine efficacy for future management purposes.

A larger sample size would ensure a more nuanced examination of the species' response to vaccination; however we are examining a general level of impact/effect, rather than subtle differences. Thus, if results which showed >1 bird having a safety issue, or the majority or average antibody response to be low, that would be sufficient to inform the next steps for decision making regarding tūturuatu vaccination.

- **The possible confounding effects of animal variation?**

We expect some individual variation since the immune response is affected by individual health status and biological variation. This sample size is sufficient to ensure we have a range of individual responses to examine.

- **The needs of statistical analysis?**

There is likely to be individual variation, which, for the antibody response, requires a reasonable sample size. We determined that 10 was the maximum which was feasible to include in the trial, and also sufficient to allow for individual variation to establish some baseline parameters of antibody response.

Ultimately, in an outbreak situation, the results of a sample size of 10 will be sufficient to make reasonably informed decisions about the use of a commercially produced killed vaccine which has a good history of safety and efficacy across a wide range of species.

7. WELFARE OF ANIMALS DURING PROPOSED MANIPULATION:

7a. What measures will be taken to ensure: the general health and welfare of animals before, during and after manipulation, including the adequacy and cleanliness of housing, caging and equipment; the provision of food and water; prevention of over-crowding, and prevention and control of disease?

Tūturuatu will be caught and handled by experienced staff as per usual tūturuatu protocols at Isaacs Conservation & Wildlife Trust Christchurch breeding centre where DOC staff already maintain appropriate husbandry practices and monitoring of all the birds. Each bird will be held within its normal enclosure so that there is minimum disturbance to their daily lives. Staff will continue to monitor birds throughout the trial, including food consumption and behaviour

Each bird will receive a veterinary examination at the start of the trial. Equipment will be disinfected between individuals, or new equipment will be used. Once blood has been collected and vaccination undertaken the bird will be re-released in their home aviary.

The vaccination trial will occur after the breeding season has finished, so will not interfere with any breeding behaviour. No female birds will be gravid at the time of manipulation, and any breeding birds will have raised and fledged their chicks before the trial begins.

7b. What movement and transportation measures will be followed for the animals to be manipulated to ensure their welfare and humane treatment?

Birds will be vaccinated on site, in their aviary where they are captured, therefore there will be no transport required.

However, if a bird requires specialist veterinary care e.g. in the event of an injury or serious reaction, then the bird will be taken to Wildbase Hospital Massey University for specialist care by Drs Megan Jolly and Brett Gartrell. This is standard procedure for tūturuatu requiring a high level of veterinary care. Birds have been transported this way via Air New Zealand on many occasions.

Brids will be transported using the standard tūturuatu transport crates, and in accordance with normal tūturuatu transportation procedures. Briefly, birds will have non-slip flooring the crate, be transported via car to Christchurch Airport and flown direct to Palmerston North. Transport will be managed to reduce noise and allow for temperature control. Radio will be off and driver/passenger will ensure minimal noise. Travel will be direct and the hospital will be alerted ahead of time to enable a fast hand-over a and rapid care.

Supportive therapy would be provided prior to transport which may include pain relief and fluids, the staff are trained and competent in administering medications on direction from the veterinarians.

7c. What measures are to be taken to minimise the pain or distress of any animal manipulated? *Stating there will not be any impact is not acceptable. The AEC is looking for the Applicant to (1) provide analysis about the potential for pain and/or distress to the animal(s), and (2) describe how they will manage that pain or distress. Identify how you would ascertain pain or distress animal's behaviour, environmental conditions likely to lead to pain or distress.*

Birds will be captured and handled by experienced DOC tūturuatu staff using their routine techniques. Only experienced staff will handle the birds. Initial physical examination, vaccination and blood collection will be undertaken by a veterinarian.

Any bird detected to have abnormalities will be examined and rejected from the trial, and receive normal veterinary investigation/intervention.

Subsequent examination and blood collection may be undertaken by DOC staff trained in blood collection from tūturuatu, provided the initial results (0,1, and 2-3 month check) are normal across the cohorts.

The subcutaneous injection is not considered painful, and the vaccine dose will be warmed to room temperature prior to injection. Blood collection is associated with a minorly painful pin-prick when the needle is inserted. This will be minimised by careful planning and handling.

If birds are observed to have any pain response to the vaccination, the staff will report it to the veterinarian who will investigate. In the event that there is an injection site reaction (painful inflammation) then an anti-inflammatory such as Metacam may be prescribed by the attending veterinarian, as well as antibiotics if infection is also present.

As noted earlier, if any serious adverse reactions occur, veterinary care by the attending veterinarian will be undertaken, and transfer to Wildbase Hospital undertaken if required for more intensive specialist care.

8. CONTINGENCY PLAN:

8a. What arrangements have been made for the abandonment of any manipulation and/or the euthanasia of animals where pain or distress cannot be held within reasonable levels?

If pain or distress is apparent, during handling or following the procedure, the veterinarian will investigate. If the veterinarian deems the level to be unreasonable, then the manipulation will be abandoned and all efforts made to ameliorate the event e.g. anti-inflammatory, pain relief medication, antibiotics.

In the unlikely event that the pain is not temporary and cannot be managed, transfer to Dunedin Wildlife Hospital will allow for intensive veterinary intervention and care. This includes the ability to undertake orthopaedic intervention e.g. in the event of a broken bone, or intensive surgery e.g. in the event of a severe localised vaccine reaction.

Supportive therapy would be provided during any transport which may include pain relief and fluids.

9. PEOPLE TO UNDERTAKE PROPOSED MANIPULATION:

9a. Who are the person(s) primarily involved in carrying out the proposed manipulation?
Kate McInnes and Lydia Uddstrom are the primary persons.

9b. What is the experience and qualifications of the person primarily responsible (9a) for the undertaking and supervising the manipulation (including selection of animals, their care and disposal?)
Kate McInnes has been the DOC vet since 2000 and has worked across a range of avian species , and is currently the lead technical advisor for the DOC HPAI response.

Lydia Uddstrom is contracted full time to the DOC kākāpō team, has undertaken postgraduate training as a zoo veterinarian and has experience with a wide range of New Zealand native species veterinary care.

Both Kate and Lydia have previously been involved in capture and handling of threatened species undertaking vaccination and blood collection for a range of threatened species, and have trained multiple DOC staff to safely and effectively undertake these procedures.

9c. Who else is in the team undertaking the manipulation? State their role in the team, and their relevant experience with the procedure(s) proposed in the application? Include DOC and non-DOC staff in the team.

Name of Manipulation Team member	*Role in the manipulation*	*Experience and qualifications relevant to the manipulation*
Anne Richardson	Lead person for catching, handling & monitoring.	Anne has over 20 years experience managing tūturuatu in captivity, and has routinely been collecting blood samples from birds for >15 years.
Leigh Percasky	Catching, handling, monitoring & follow-up blood collection of tūturuatu	Leigh has 6 years of experience working with tūrutuatu in captivity, including routine blood sampling of birds.

9d. What training will be given to the people identified in 9c to help them undertake the manipulation proposed in the application?

Team leader and tūturuatu staff are trained in blood collection of tūturuatu as per the DOC Avian Blood Collection SOP training requirements, and to undertake normal practices including capture & handling.

The capture and handling will be undertaken according to the direction of the team leader.

Vaccination will be undertaken by the DOC veterinarian.

10. COMPLIANCE WITH CONDITIONS of the APPROVAL:

- Please outline any opportunities for a member, or members, of the DOC Animal Ethics Committee to observe this work.

10a. Identify ways that the manipulation(s) can be monitored by the AEC

AEC members could attend a vaccination session at Christchurch to see the procedure in tūturuatu and/or receive a video or photographs of the manipulation being undertaken

11. Are there any other aspects which ought to be brought to the attention of the DOC Animal Ethics Committee?

No

12. Does the research, testing or teaching involve a species which is covered by a Department of Conservation Species Recovery Plan and if so has the Recovery Group been consulted and their endorsement for the work received? Please provide a summary of communication.

Yes. Tūturuatu management is guided by the tūturuatu Recovery group which has been consulted and are supportive of this trial.

This application has been shared with the tūturuatu team on 10th November for review prior to submission to the AEC, and the contents were discussed and agreed with the team on 15th November 2023.

13. What month of year is most useful to report back to the AEC (depending on the project schedule and the animal's biology)?

September

13. Manipulation Grading

Please work through the document '*Grading of Manipulations*' (Please refer to DOCDM-870472), and determine the grading you believe best applies to the manipulation proposed in this application. Please also provide a rationale for the grading.

Grade A: No impact or virtually no impact.
Grade B: Little impact. Manipulations of minor impact and short duration.
Grade C: Moderate impact. Includes manipulations of minor impact and long duration or moderate impact and short duration.
Grade D: High impact. Includes manipulations of moderate impact and long duration or high impact and short duration.
Grade E: Very high impact. Manipulations of high impact and long duration.

Grading determined by the Applicant: B

Application Number DOCDM	AEC446 APPLICATION DOC-7483658
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, tūturuatu

Your rationale for the grading:
Grade B includes "**Disease/injury/functional impairment:** Studies of vaccines using killed pathogens." The animals will be kept in their normal husbandry conditions for the duration of the study. There will be capture and handling for blood collection and vaccination, and two doses of a killed vaccine administered. Handling time & stress will be minimised by using only skilled staff and it will be undertaken at site.

Note: The grading determined by the Applicant is not the grading assigned by the AEC. The Applicant will be advised of the AEC's grading and any conditions in writing.

DECLARATION by the APPLICANT

Tick boxes [☑] to indicate your agreement to conditions: *[Copy and paste this tick object ☑]*

[☑] I declare that the information in this Application is correct; and
[☑] I agree to comply with the conditions imposed by DOC's AEC for the manipulation; and
[☑] I agree to ensure all personnel involved in this manipulation will be properly trained and/or qualified to undertake the manipulation and will be aware of the contents of this AEC application; and
[☑] I declare the proposed manipulation has the necessary resources to undertake the manipulation with regard to the health and safety of the animals and staff
[☑] I agree to advise the AEC of any changes in the details of the manipulation as described in this Application.
[☑] I agree to comply with the reporting requirements stipulated by the AEC on approval of this research project.

Signed by the Applicant 9(2)(a) [redacted]

Full Name: Catherine McInnes Date: 13/11/2023

DECLARATION by the ACCOUNTABLE MANAGER

Tick boxes [☑] to indicate your agreement to conditions: *[Copy and paste this tick object ☑]*

[☑] I agree to ensure my staff member complies with the conditions imposed by DOC's AEC for this manipulation; and
[☑] I agree to ensure all personnel involved in this manipulation will be properly trained and/or qualified to undertake the manipulation and will be made aware of the contents of this AEC application; and

Application Number DOCDM	AEC446 APPLICATION DOC-7483658
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Key Words	avian influenza, vaccine, safety, efficacy, tūturuatu

[☑] I agree the proposed manipulation has the necessary resources to undertake the manipulation with regard to the health and safety of the animals and staff

[☑] I agree to oversee this Application via MORs, PDPs and other means to ensure the manipulation remains within the scope of the Application and the Approval, and all reporting required by the AEC is delivered on time;

[☑] I agree to advise the AEC of any changes in the details of the manipulation as described in this Application, and to advise the AEC if the Applicant leaves the Department, or if the work should be transferred to another staff member for operational reasons' or if the manipulations is abandoned for any reason.

Signed by the Manager: 9(2)(a) [redacted]

Full Name: John Lyall

Role: Fauna Advice Manager

Date: 16/11 / 2023

Application Number DOCDM	AEC446 APPLICATION DOC-7483658
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, tūturuatu

Appendix 1: Avian Influenza vaccine information

Registration number A009733: **Poulvac Flufend i AI H5N3 RG**

Registrant: Ministry for Primary Industries

Draft label information:

PRESENTATION

Bottles of 500 mL (1000 doses). Packs of 1 or 10 bottles.

DIRECTIONS FOR USE

By law the distribution and use of this product must comply with the requirements of the relevant operating plan.

General:
• Inject 0.5 mL (0.5 cc) subcutaneously, using aseptic technique, into healthy birds at 3 to 4 weeks of age or older.
• Shake well before use.
• Allow the vaccine to reach room temperature (18-29°C) before use.

Chickens:
• Administer another dose of 0.5 mL not less than 2 weeks later, if required.
• The second dose should be administered at least 4 weeks before point of lay.

Ducks:
• Ducks less than two weeks of age:
• Administer 0.2 mL of vaccine subcutaneously at the back of the neck.
• Administer another dose of 0.5 mL not less than 2 weeks later.
• Ducks two or more weeks of age:
• Administer 0.5 mL of vaccine subcutaneously at the back of the neck.
• Administer another dose of 0.5 mL not less than 2 weeks later.

ADVERSE EFFECTS, CAUTIONS AND CONTRAINDICATIONS

ADVERSE EFFECT
• Vaccinate only healthy chickens or ducks and avoid stressing the birds at the time of vaccination.
• Do not mix with any other vaccine or injectable product.
• The use of this product in laying birds has not been evaluated.
• Local or systemic post-vaccination reactions can occur due to the use of oily vaccines. Symptoms observed are generally transitory and can include oedema and granulation at the injection site, anorexia and dehydration. Such reactions can be minimised by good aseptic vaccination technique.

CAUTIONS
• Destroy any unused vaccine and containers after vaccination (including syringes and needles) by burning.
• Do not mix the vaccine with other vaccines or administer another vaccine shortly before or after vaccination with this product.
• Consult a physician immediately for an accidental self-injection and show this package insert to the physician.
• KEEP OUT OF REACH OF CHILDREN AND UNINFORMED PERSONS

CONTRAINDICATIONS
• None.

WITHHOLDING PERIODS
Meat: Nil.

STORAGE
• Store in the dark between 2 °C and 8 °C. Do not freeze.
• Protect from direct sunlight.
• Use contents of each vial within 6 hours of opening

Appendix 2: General Instructions for subcutaneous injection of vaccine

- The vaccine is supplied in a 500mL bottle and is given to the bird using a needle and syringe.
- The vaccine is injected under the skin but NOT into the muscle below.
- The vaccine should be drawn up into the syringe and then allowed to warm to room temperature (this is more comfortable for the bird).

EQUIPMENT NEEDED

1. Vaccine container
2. 1 mL syringe
3. 25 gauge 5/8th inch needle
4. alcohol swab (mediswab or cotton wool and meths)
5. dry swab (gauze or cotton wool)
6. Sharps container for needle disposal
7. Bird
8. Bird handler
9. Veterinarian

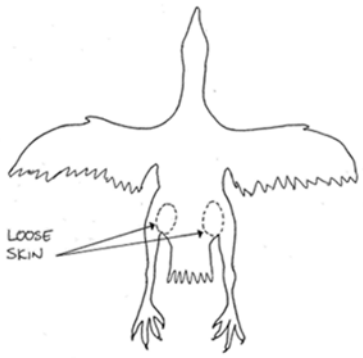
PREPARING THE VACCINATION

1. Store the vaccine in the fridge at 2-8 degrees C in the dark. Do not Freeze.
2. When ready to use, take the vaccine out of the fridge and shake well to mix.
3. Write the date on the vaccine bottle.
4. Break off the metal seal on the top of the rubber injection port.
5. Swab the injection port on the vaccine with alcohol to sterilise it with a mediswab or cotton ball soaked in methylated spirits.
6. Firmly attach the needle to the syringe – 25 gauge 5/8th inch needle to a 1mL syringe.
7. Insert the needle through the centre of the rubber stopper CAREFULLY.
8. Hold the vaccine upside down and slowly suck vaccine into the syringe until you have a little more than the prescribed dose of vaccine.
9. Flick the syringe to dislodge any air bubbles and squirt them slowly back into the vaccine bottle.
10. Keep squirting until all the bubbles are gone and you have the prescribed dose of vaccine left in the syringe.
11. Pull the needle out of the vaccine bottle and CAREFULLY recap the needle.
12. Leave the syringe and needle to warm to room temperature.
13. Repeat this procedure to draw up all the doses you need for your vaccination session.
14. Put the vaccine back in the fridge.
15. Once open, the vaccine can be used for 30 days. (Note that this expiry is based on Zoetis technical advice for limited use of the vaccine in this trial, and only applies when following the above instructions for maintaining sterility of the product and correct storage.)
16. If you are in doubt that the vaccine has been stored correctly (kept refrigerated), then discard it and get a new bottle.

GIVING THE INJECTION

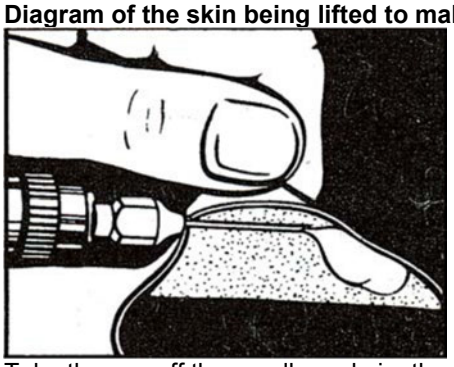
1. Have the following equipment ready for use:
    - The correct dose of vaccine drawn up in syringe with needle attached and warmed to room temperature. (the cap should be on the needle to avoid accidental stabbing or contamination of the needle)
    - One alcohol swab (mediswab or cotton wool in meths)
    - One dry swab (gauze or cotton wool)
2. Have an assistant restrain the bird on its back or side with its legs restrained to provide access to the groin (where the bird's leg joins its belly).

Diagram of ventral (belly) of a bird showing the groin region for subcutaneous vaccination sites:



3. Spread the feathers in the groin area.
4. Wet down the feathers with the swab to clear a patch of skin and swab the skin.
5. Lift the loose skin 1-2cm off the body to make a "tent".

Diagram of the skin being lifted to make a "tent" for a subcutaneous injection



6. Take the cap off the needle and aim the needle about halfway down the side of the tent. Keep the needle parallel to the body wall. When the needle goes through the skin, it should still be above the muscle of the groin i.e. you are injecting into the space inside the tent, not into the muscle.
7. Suck back on the syringe to check for blood. This is to avoid injecting into a blood vessel.
8. Inject the vaccine with a steady firm pressure.
9. Withdraw the needle and place it into the Sharps container.
10. Use the dry swab to press over the injection site if there is any bleeding.
11. Release the bird.

12. Record:	Bird ID	Date	Dose	L or R side	Vaccinator	Holder	Vaccine Batch	Expiry Date	Notes

13. Transfer this data to the vaccination record spreadsheet
14. Note any other specifics about the injection process not described above. E.g. if there was bleeding at the injection site.

Application Number DOCDM	AEC447 APPLICATION DOC-7483661
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, kākāriki

Revised September 2023

DEPARTMENT OF CONSERVATION
APPLICATION TO MANIPULATE LIVE ANIMALS
Code of Ethical Conduct for the Care and Manipulation of Live Animals

1. APPLICANT'S DETAILS:

Name: Kate McInnes

Date: 17 November 2023

Role: DOC Veterinarian

Unit: BH&V Group, Wellington

APPLICANT'S ADDRESS:

Phone no: 9(2)(a)

Email: kmcinnes@doc.govt.nz

2. ACCOUNTABLE MANAGER'S DETAILS:

Name: John Lyall

ACCOUNTABLE MANAGER'S ADDRESS:

As above or: DOC, Hokitika

Phone no: 9(2)(a)
Email: jlyall@doc.govt.nz

2a. AEC443

2b. **MANIPULATION TITLE: Avian Influenza vaccination safety and efficacy trial kākāriki**

2d. **Duration of the manipulation**

- *Over what timeframe are you seeking the approval?*
- *You must not commence the manipulation until you have received the approval, signed by you, your accountable manager, and the AEC Chair.*

Application Number DOCDM	AEC447 APPLICATION DOC-7483661
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, kākāriki

- *NOTE: The AEC will not generally give an approval for longer than two years at one time. Please state if this manipulation is likely to extend longer than two years from the commencement date.*

Anticipated start date:	February 2024	Anticipated finish date:	June 2025

2e. What months of the year is the manipulation most likely to be undertaken? e.g., October – March
For the duration of the dates specified

3a. Summary of the proposed manipulation for a LAYPERSON

- *Provide an abstract describing the manipulation (maximum 400 words).*

Avian influenza is a viral disease which can cause mass mortality events in birds. The current strain is decimating many populations of wild birds overseas, is predicted to reach the Southern Ocean by 2024/25 and was confirmed in South Georgia, October 2023.

We want to test the safety and efficacy of vaccination to protect critically endangered species.
The vaccine is a commercial product registered in New Zealand by Ministry for Primary Industries. It is considered very safe and highly effective. It contains inactivated (dead) virus so it cannot cause avian influenza. Vaccination reduces risk of illness or death and reduces shedding of virus, thus protecting the individual and its flock.

Kākāriki karaka are a critically threatened species which is reliant on a captive breeding programme where it is possible to reliably administer a full course of vaccine (2 injections under the skin, one month apart) to individually identified birds, and where we are able to handle them again for a veterinary examination and blood testing to detect any effects on health status, and measure the immune response by detection of antibodies over a 12 month period.

Captive Red-crowned kākāriki (as a surrogate species) will be captured in their aviary and receive a pre-vaccination health check by a veterinarian, and a blood test for health and antibody testing. Up to 0.4 mL of blood will be collected from the wing vein, as is standard for this species.

The vaccine is given under the skin. Once month later the bird will receive a second vaccination and blood test. Further blood will be collected at 2-3, 6 and 12 months post vaccination to determine the level of antibody response and how long it lasts.

A cloacal and choanal (oral) swab will be collected on day 0 for PCR testing to demonstrate the birds were not incubating avian influenza at the time of vaccination.

Normal husbandry practices will be undertaken including observation of the bird's activity and food intake to monitor of any adverse reactions.

We propose to work with a total of 10 adult or juvenile kākāriki, divided into two cohorts. Cohort 1 will first receive the vaccination & blood tests, and a recheck at 1 month. If no safety issues are identified, then Cohort 2 will receive vaccinations & blood tests. This allows a careful start to the trial where the month is the most important to test vaccine safety. The following blood samples (at 2-3, 6 and 12 months) will determine level and duration of antibody presence and determine when further boosters would be required.

Additional approval given on 16/4/24 to collect a 10-14 week blood sample to target peak antibody levels, and to collect an opportunistic blood sample for further antibody testing, if birds are being handled for routine management purposes, with no more than twice per bird over the 12 months of the trial.

Application Number DOCDM	AEC447 APPLICATION DOC-7483661
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, kākāriki

3b. Description of the proposed manipulation (methods)

- *Provide a more detailed explanation. Describe the equipment, the location, and any environmental factors: weather, time of the year. Why have you decided to undertake the manipulation in this way? What advice have you sought? Include the species, the number of individuals, the source of animals, and the disposal/fate of animals at the conclusion of the manipulation.*
- *Be specific about the timelines for the proposed investigation and the purpose of the research, testing or teaching.*
- *Include some consideration and planning for when things might not go right.*

Please note: this is one of five trials to assess avian influenza vaccination safety and efficacy in nationally critical threatened species (takahē, kākāpō, kakī, tūturuatu, kākāriki karaka). Manipulation details which are specific to this species (kākāriki) have been highlighted in yellow. All other details are consistent across the five trials. By highlighting the species specific details I hope to assist the AEC with the volume of workload associated with simultaneously assessing these trials.

This trial is designed to test the efficacy and safety of a vaccine in a critically threatened species, however for this species we plan to use a closely related surrogate.

Selection of the species for potential vaccination is based on the risk that they could undergo an extinction event when highly pathogenic avian influenza (HPAI) reaches New Zealand. 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 risk of extinction.

The wild adult kākāriki karaka numbers in the Hawdon, Andrews, and Poulter valleys are extremely low. The south branch of the Hurunui currently has the largest population on the mainland, being comprised almost entirely of birds that have been released from captivity. Even so, there are probably fewer than 100 mature parakeets on the mainland, and perhaps 200-300 on islands following translocations.

The surrogate species in this trial, red-crowned kakariki, are widely distributed throughout the New Zealand region, and very common on some islands, they are almost entirely absent from the two main islands. They are held in captivity for advocacy (display) and many institutions around New Zealand, and by private holders under permit form DOC. There are thriving populations on off-shore islands. Their conservation status in "endemic" and they are not considered threatened.

Based on the evidence from overseas during this epizootic, the species most at risk of infection are those which exhibit congregation behaviours e.g. feeding, breeding or roosting in groups, those which are exposed to at risk species e.g. where seabirds overlap with another threatened species, and birds held in captive facilities where biosecurity options are limited e.g. open pens and large aviaries.

Kākāriki karaka are one of 5 species identified by the DOC HPAI Technical Advisory Group as at risk where administration of a full vaccination programme is feasible in sufficient number of individuals to provide protection against species extinction. See DOC-7111177 Mitigation Options Guideline for HPAI.

Use of the vaccine is dependent on Ministry for Primary Industries approval, and currently requires the birds to be held in captivity. Birds require two injections one month apart and must be individually identified with a permanent mark e.g. microchip or leg band. Kākāriki will already have a leg band as part of their normal husbandry.

Effective vaccination reduces susceptibility to infection. When infection does occur, it reduces clinical signs of disease and the amount of virus shed into the environment (Animal Health Australia, 2021).

Additionally, vaccination of California Condor was approved in the United States following an outbreak in the wild population. This was the first avian influenza vaccination programme in a wild endangered species. Advice from the veterinary and technical advisors to the condor vaccination programme has been received and is incorporated into this trial design.

We wish to undertake a limited trial to determine the safety and efficacy of the avian influenza specific vaccine in kākāriki (using red crowned kakariki as a surrogate) as a preparedness measure for the arrival of HPAI in New Zealand.

The vaccine is produced commercially by Zoetis for use in poultry: Poulvac Flufend i AI H5N3 RG inactivated (killed) vaccine - see Appendix 1. It has been in production since 2006 and is widely used in the poultry industry. Publications on AI vaccine use in poultry and avian species in zoos have indicated a very high level of safety across a wide range of species, and efficacy has been well established. (Kandeil et al 2018, Philippa et al 2006, Philippa 2007a, Philippa 2007b, Pitman 2006, Vergara-Alert 2011). The vaccine is inactivated, so there is no live virus present and it cannot cause avian influenza.

Advice from Zoetis (USA) indicates that this vaccine should provide good protection against the current strain of HPAI with 91% amino acid homology with the circulating strain. A newer vaccine based on the circulating strain is in production but is will not be available until the end of 2024 at the earliest.

Vaccine will be obtained from PacificVet in Christchurch and transported in a chilly bin with ice-packs by overnight courier (as per their standard transportation procedures for vaccines) to ensure cold chain is maintained. Use in the field will be managed by extraction of sterile aliquots into sterile vials or syringes. This enables sustainable use of the 1000 dose vial and maintenance of sterility of product. This process was discussed with the Zoetis Senior Research Advisor responsible for poultry products and is considered safe and appropriate.

Sterile aliquots will be obtained by using a sterile needle and syringe to extract the aliquot from the closed vaccine vial. The vial will be shaken to homogenise the contents, then the rubber stopper will be swabbed with alcohol. The sterile needle will be attached to the sterile syringe and the needle inserted via the rubber stopper. The aliquot will be drawn up into the syringe, then the needle& syringe removed from the stopper and the cap replace on the needle. The needle will be swapped for a new sterile needle or a sterile vaccine cap.. Both the aliquots and the vaccine vial will be stored refrigerated in accordance with the packaging instructions. Vaccine doses will be drawn up immediately before use and allowed to warm to room temperature just prior to injection.

DOC veterinarians Kate McInnes and Lydia Uddstrom will administer the vaccination. DOC veterinarian Rachel Stayner who is contracted to assist with HPAI preparedness work may also participate in this trial.

All birds will receive a full veterinary physical examination at the start of the trial. Only birds in good body condition exhibiting signs of good health will be included. (Any birds which show signs off poor health will be further investigated as per normal veterinary practices).

The kākāriki karaka recovery team have been involved in discussions about this study, and are in agreement with the value of the vaccination to protect the breeding programme. The actual study design and selection of study animals has been determined by Kate McInnes, due to the accessibility of a suitable

surrogate species which can be held more widely in captive institutions and by private breeders. Red-crowned kakariki are approximately twice the size of kākāriki karaka (70-80g vs 30-50g) and are considered by aviculturalists to be a mor robust bird to tolerance of handling stress, making them a better candidate for this trial.

Natureland Wildlife Trust was selected because they are a Zoo Aquarium Association accredited professional wildlife conservation facility willing to participate to support the conservation outcomes, they have experienced staff, and they hold sufficient red-crowned kakariki for the completion of the trial.

Individual birds for vaccination will be selected by the staff at Natureland, based on the captive programme requirements.

Each individually permanently marked (leg band) bird will receive two doses of vaccine by subcutaneous injection into the inguinal (groin) region with a 1 month interval (no less than 3 weeks apart and a maximum of 6 weeks apart). The first vaccination will be into the left inguinal region, and the second vaccination into the right inguinal region.

Birds will receive 0.2ml per dose (as per dosages used in Vergara-Alert et al 2011).

Individual birds within the two cohorts will be determined on the day by kākāriki staff based on available birds' suitability and any management requirements.

Male and female kākāriki are held in separate aviaries. Currently there are 3 males and 6 females, however in the next 3 months, a few additional birds may be added to the collection. The cohort sizes below will be adjusted based on birds in the aviary at the commencement of the trial, up to a maximum of 10 birds in total.

At the start of the trial, each bird will receive a cloacal and oral swab to determine presence/absence of virus at day 0.
The technique will follow the draft SOP Avian swab sampling DOC-6840491 which has undergone veterinary peer review & user testing and is awaiting AEC endorsement before Director sign-off. These types of swabs are used in standard health testing on avian species and would be undertaken by the veterinarian. The test would be considered a baseline health test to demonstrate the birds were not incubating avian influenza at the time of vaccination. The swabs will undergo PCR testing at BioPacifica to look for avian influenza virus.
This is important to be able to demonstrate that any antibody response is due to the vaccination rather than the bird being infected by a wild strain of avian influenza.

First trial - Cohort 1: three to six individuals form the male group aviary will be vaccinated as per the described protocol above. Blood (up to 0.4ml) will be collected at 0, 1, and 2-3 months to measure health parameters (white cell count & differential) and antibody response (commercial serum ELISA test to measure antibody titre). Antibody testing will be undertaken at a commercial laboratory (BioPacifica, Christchurch).

Note: 1% of body weight is considered an acceptable amount of blood to collect from a healthy bird. Adult red-crowned kākāriki weigh ~70-80g, therefore up to 0.7 ml would be within the safe range for an adult. We propose to only collect up to 0.4ml to maintain a high margin of safety.

Second trial - Cohort 2: Based on consideration of the results of the first trial, if safety has been demonstrated, a second cohort of four to seven individuals from the female group aviary (maximum of 10

in total) will receive the vaccination as per the described protocol above, and blood will be collected at 0, 1, and 2-3 months to measure health parameters (white cell count & differential) and antibody response (commercial serum ELISA test). We will wait 1 month until we have established the vaccine is safe in cohort 1 before we start cohort 2.

Note: If antibody response at 2-3 months is noted to be muted (i.e. a low response) then the DOC vets (Kate McInnes and Lydia Uddstrom) will discuss the use of a third dose of vaccine, and consult with Natureland if additional vaccination is recommended. This was used in some species in European zoos where the initial antibody responses were considered insufficient. Consideration will be given to the level of response detected, the impacts of additional handling, and any other welfare factors noted during the preceding handling events. The benefits of testing a third dose of vaccine will be carefully considered and this will only be undertaken if the welfare impacts are considered minimal. The justification for a third dose in this trial would be to confirm if this dose is warranted and would likely be needed to deliver protection for the kākāriki karaka population in the event of an outbreak of HPAI in Aotearoa New Zealand.

Two additional blood samples will be collected from both cohorts at approximately 6 months and 12 months to measure duration of antibody response.

A maximum of 10 birds will be included in the trial. It is estimated that there will be a minimum of 9, but possibly more, display kākāriki on site at the DOC facility at the time of the trial. Thus it is possibly that only 9 birds will be enrolled in the trial but likely to be 10.

These trials are modelled on the vaccination of California Condor in the USA. (FWS.gov 2023) however the 1 month interval is based on the European zoo data, and considered more appropriate to allow for recovery between handling events in birds which normally have minimal handling by humans.

Estimated timing/schedule of manipulations:

First vaccination and blood sample	Second vaccination and blood sample	2-3 month blood sample	~6 month blood sample	~12 month blood sample
First cohort (3-6 birds)				
~ 1st February 2024	~1st March 2024	~1st April 2024	~ 1st August 2024	~ 1st February 2025
Second cohort (4-7 birds, to make up maximum of 10 birds)				
~ 1st March 2024	~1st April 2024	~1st May 2024	~1st August 2024	~ 1st February 2025

Second cohort of birds – similar timeline but commences 1 month after first cohort have been vaccinated and shown no negative reaction. Blood collection at 6 and 12 months may be undertaken at the same time for both cohorts – these sample are about longevity of antibody presence, so the exact timing is less critical.

During a handling event, all involved staff will gather and have a pre-handling briefing by the veterinarian and the team leader to ensure all roles and responsibilities are clearly understood. Any issues can be raised at that time for clarification. The Natureland kākāriki team leader will be responsible for the safe capture and handling of the bird. The veterinarian will be responsible for the health examination, vaccination and blood collection.

All equipment will be prepared prior to capture to minimise handling time. Staff will know where to situate themselves and what actions are required so that an efficient process is maintained. The Natureland kākāriki staff involved in this trial will be team members with previous experience in capture and handling of kākāriki.

Kākāriki are held in two flock aviaries, so they will be caught according to the following procedures:

Kākāriki will be captured using cage traps by experienced bird handlers. Briefly, the cage traps are installed permanently inside the aviary with the kakariki feeders inside so that birds are familiar with their presence . When capture is required, the birds are allowed to enter for feeding, and then the cage is triggered to close the mesh wall via a string control, capturing the bird inside. The birds is then quickly restrained and removed from the cage. By an experienced handler using the small side sliding door (see photographs below).

A second handler assists in the removal of the bird from the net directly into a catch bag (a soft cotton bag with a draw string which provides a safe restraint for temporary holding) to be weighed in the bag using a hanging scale (standard technique) and then carefully removed and undergo a physical examination to determine its health status prior to any further manipulation. If the kākāriki is determined to be healthy blood collection and vaccination will proceed.

This is the standard method of capture for kakariki used at Natureland Wildlife Trust.

For blood collection and vaccination, a trained kākāriki handler will restrain the bird on its back.

The blood collection site (wing vein) will be swabbed with a sterile alcohol wipe immediately prior to collection. Blood will be collected from the wing vein via the pin-prick method where the vein is pricked using a sterile 25 or 26 gauge hypodermic needle, and then the bleb of blood is collected in capillary tubes (also known as haematocrit tubes which can contain 75microlitres of blood).

In the event that the temperature is cool, and on examination of the wing vein we determine that blood collection likely to be slow due to the presence of small contracted veins, the wing will be warmed for 3-5 minutes using Kathmandu hand warmers wrapped in gauze, to boost circulation and enhance blood flow to enable effective blood collection.

After collection, the site of blood collection will be covered with a gauze swab and pressure applied to control any bleeding. In the very unlikely event of uncontrolled bleeding, pressure will be applied for a further 1-5 minutes. If still uncontrolled, an icepack wrapped in gauze swabs will be held on the wing area to cool the limb and reduce blood flow. If required, a silver nitrate stick will be carefully used to stop the bleeding.

Blood will be spun in a centrifuge to separate the serum from the blood cells. The tubes will be kept chilled and transferred ASAP to the commercial laboratory in batches for antibody testing.

1-2 drops of blood will be used to make blood smears which will be sent to a commercial veterinary pathology laboratory for a white cell count and differential. This provides a baseline health analysis which can detect infection or inflammation. Any abnormal results will be further investigated by the veterinarian in consultation with the kākāriki staff.

Once bleeding has stopped, the bird will be vaccinated using a 1mL syringe attached to a 20 gauge ½ inch needle. The vaccination site will be swabbed with a sterile alcohol wipe immediately prior to vaccination. See Appendix 2 for details of the vaccination technique.

The bird will then be checked for any abnormalities and the veterinarian will determine if any further actions are required for health or welfare. Once the procedures are complete the bird will be quietly released and observed as it moves away. Regular observations during routine husbandry will continue for

all birds, and any abnormalities will be reported to the veterinarian. The birds are visited and monitored at least twice daily by staff during routine husbandry.

At subsequent handling events, the vaccination site will be examined and any discolouration, swelling, granuloma formation or unexpected abnormality will be noted and reported to the veterinarian. Photographs of each bird's injection sites will be taken to provide a clear record of the trial.

Location & timing:
The trials will be undertaken using display birds only at the Natureland Wildlife Trust between February 2024 and June 2025

Safety:

Results from a meta-analysis of use of vaccine in European Zoos found very low adverse reaction rate at 0.04% local reactions and 0.015% general reactions reported. EFSA 2007. Based on this, we do not anticipate significant issues with the vaccine, however we will be prepared for immediate veterinary care if any reactions to occur.

The vaccine packaging label states: "Local or systemic post-vaccination reactions can occur due to the use of oily vaccines. Symptoms observed are generally transitory and can include oedema and granulation at the injection site, anorexia and dehydration. Such reactions can be minimised by good aseptic vaccination technique."

Anaphylaxis:
A severe immediate immune hypersensitivity response could occur if the vaccine product stimulates such a response. This is considered unlikely due to the extensive use of this vaccine and other similar vaccine products in Europe, however it is possible and needs to be considered as a potential adverse event. The vaccination team will include a veterinarian who will have access to emergency drugs and supportive care for management of anaphylaxis (including corticosteroids, adrenaline, oxygen, fluids).

Injection site reactions:
The vaccine contains an adjuvant (oil) which is present so that it stimulates a stronger immune response with greater antibody production. This can sometimes be associated with a small pea-sized lump at the site of injection. This is normal and expected, although generally not all birds will develop a lump. This will be checked at the 1 month mark, and records kept of any reactions detected. If an excessive size reaction is detected in an individual (>1cm), then the vaccination will be paused until it is determined that the lump does not enlarge further, or cause any impacts on the bird(s) – this is likely to be a period of 2-4 weeks. Body weight, activity levels etc will be reviewed and a full physical examination undertaken.

A localised bacterial infection could result if poor sterile technique is used. Only registered veterinarians will be administering the vaccine, and these operators all have training in appropriate sterile techniques. If a bird experiences an infection at the site, it will receive veterinary care and follow-up to ensure the issue is managed.

Mis-injection could occur if the bird is poorly restrained and moved during vaccination. This will be managed by only using well trained, experienced kākāriki handlers to restrain the birds. For some individual birds, they are calmer with head cover which can aid in handling. This will be determined on an individual bird basis. If a mis-injection occurs, the veterinarian will determine the appropriate next steps. This may include, re-injection if the first injection merely failed to enter the bird, appropriate first aid measures if any injury was caused, and/or exclusion from the trial and follow-up care. As noted previously, "stressy" birds will not be included in the trial which will reduce the risk of injury or mis-injection.

Injury could occur during capture and handling. This is minimised by only using trained experienced staff, careful selection of trial birds, and a "stop for safety" approach which resets the work programme and ensures time out to reassess and replan the work and procedures if necessary.

In the event of a serious reaction or injury during the vaccination trial, the bird will be taken to the local veterinary clinic of Rachel Stayner, the veterinarian who provides care for the Natureland animal collection.

Results:
The results of this trial will determine if this vaccine is safe to use in this species, and the level of antibody response produced by a 2 dose vaccination. In some other species, notably penguins, the antibody levels following vaccination remain low and, in some species, a third vaccination was used to ensure a stronger response (ESFA 2007). The duration of antibody presence also varied between species. Therefore, this trial will help to determine the appropriate vaccination regime for kākāriki in the event that more widespread vaccination is required during a highly pathogenic avian influenza outbreak in New Zealand.

3c. Attach Photos of equipment, the species, the location (or a map); to help set the context.

Vaccine image

kākāriki karaka (orange-fronted)

red-crowned kākāriki

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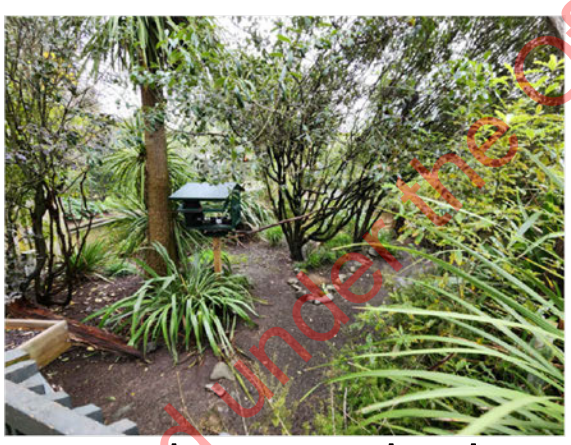
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, kākāriki

Aviary 1 – males **Aviary 2 - females**



Cage trap – The cage traps are located permanently within the kakariki aviary and birds are fed in the cages daily. The captured bird is accessed via the small sliding side door.

When capture is required, the side of the cage can be closed using the string control

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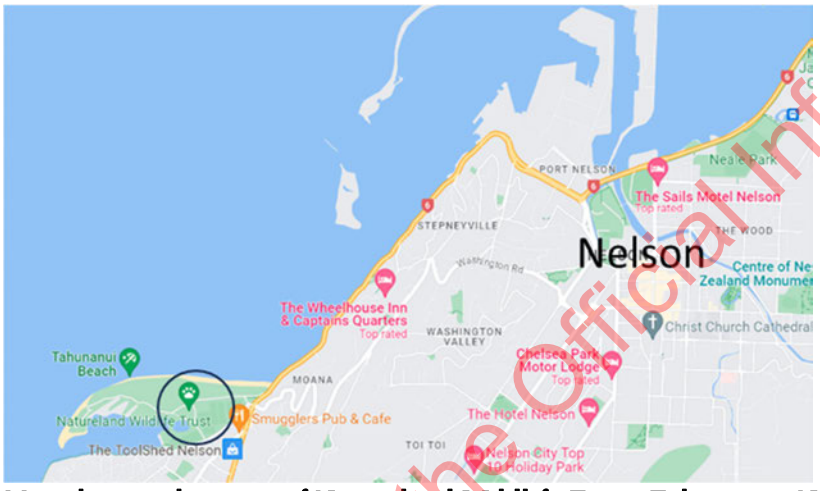
Cage trap open, string control of sliding door.

Cage trap - door closing.



Map showing location of Natureland Wildlife Trust, Tahunanui, Nelson

Aerial view of Natureland Wildlife Trust, Tahunanui, Nelson

3d. References

- *List the references referred to in the application*

DOC-7111177 Mitigation Options Guideline for HPAI
https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7111177

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Pitman 2006. M Pittman, European Commission 12th Annual meeting of national avian influenza laboratories Veterinary and Agrochemical Research Centre (VAR) Uccle, Brussels, 16-18 October 2006 LINK: link.

Vergara-alert J, Ferhandez-Bellon H, Busquets B, Alcantara G, Delclaux M, Pizarro B, Sandchez C, Sanchez A, Majo N, Darju A. Comprehensive serological analysis of two successive heterologous vaccines against H5N1 Avian Influenza virus in exotic birds in zoos. Clinical and Vaccine Immunology, 2011. P. 697-706. https://doccm.doc.govt.nz/cwxv4/wcc/faces/wccdoc?dDocName=DOC-7499845

4. INVOLVEMENT OF OTHER ANIMAL ETHICS COMMITTEES:

4a. Is this Application; or a related or similar application; been or is being considered by another Animal Ethics Committee. Has this project been requested to be considered by any other AEC?

If so, please provide details.

No

4b. Does this manipulation interact with a manipulation approved by other Animal Ethics Committee? If so, detail your communications with those committee(s), and state any conditions imposed by (an)other AEC.

No

5. JUSTIFICATION FOR PROPOSED MANIPULATION:

5a. Detail any action undertaken to determine that the proposed work has not already been done.

Avian Influenza vaccine efficacy and safety has been undertaken on other avian species, however it has not been undertaken in New Zealand endemic species. Although we expect s milar results, it is prudent to undertake this trial to provide more evidence of safety and efficacy in the species which we intend to vaccinate in the event of a HPAI outbreak.

5b. Have alternatives been considered to the proposed manipulation involving reduction, or replacement of live animals, or refinement of techniques?

We are looking at the species-specific response and have selected a minimum size divided into two cohorts, so other methods of reduction are not appropriate for this work.

The cohort approach allows us to cautiously approach the safety issue, and assess initial results before involving the full number of birds.

5c. To what extent has there been assessment of the suitability of using non-sentient or non-living alternatives in the project; or replacement of animals as subjects with suitable non-sentient or non-living alternatives?

N/A, see above

5d. How will the proposed work result in the extension of knowledge relevant to the health, welfare, or conservation of animals?

This work will specifically contribute to the future health of the species for conservation purposes by providing evidence of the safety and efficacy (or not) of this vaccine in this species, and inform the appropriate vaccine schedule for the species. This will determine if the vaccine is employed in the future in the face of an avian influenza outbreak in New Zealand.

5e. Is the manipulation required as part of an approved training programme?

No.

5f. How will the results of this work be made available to staff within and outside DOC? (*For example internal report, journal paper, best practice guide, workshops etc*).
Internal report, journal paper, conference presentations, shared with other captive institutions that hold kākāriki.

6. SELECTION OF SPECIES & NUMBER OF INDIVIDUALS FOR PROPOSED MANIPULATION

6a. What will be the source of the animals to be manipulated, and how many from each source will be manipulated?

Natureland Wildlife Trust was selected for the trial because it is a readily accessible facility willing to participate in the trial to support the work, with experienced staff, and the ability to provide access a total of 10 red-crowned kākāriki at one site.

6b. Will any of the animals involved be used more than once, and if so, how many times will each animal be used?

Only once (but each animal handled/manipulated multiple times – twice for vaccinating and three more times for blood sampling)

6c. What factors have been taken into account in the choice of the animal species?

Kākāriki karaka are one of 5 species identified by the DOC HPAI Technical Advisory Group as at risk where administration of a full vaccination programme is feasible in sufficient number of individuals to provide protection against species extinction. We have selected a suitable surrogate species, red-crowned kakariki, which are approximately twice the size of kākāriki karaka (70-80g vs 30-50g) and are considered by aviculturalists to be a more robust bird to high tolerance of handling stress, making them a better candidate for this trial.

6d. Could the information being sought be obtained by work on some other species?

No. This trial is using the more common surrogate species instead of the nationally critical kakariki karaka.

6e. Will the question be answered with the size of the sample?

Yes.

6f. Is the number of animals proposed to be manipulated the <u>minimum</u> necessary to provide a scientifically interpretable result, consistent with the level of statistical precision required? What consideration has been given to the design of the study with regard to:

- **The level of precision necessary in the results?**

The purpose of the small trial is to establish if there is a species-specific sensitivity to the vaccine and its adjuvant. For this purpose, we require only a small number of birds to extrapolate a species sensitivity. Similarly, for determining vaccine response by antibody response levels, a sample size of 10 will provide sufficient individual variation to establish an overall species response level. Additionally if a bird is removed from the trial for any reason (e.g. other health issues, injury, behavioural), starting with 10 birds allows sufficient number to still be able to make a reasonable conclusion on the vaccine efficacy for future management purposes.

A larger sample size would ensure a more nuanced examination of the species' response to vaccination; however we are examining a general level of impact/effect, rather than subtle differences. Thus, if results which showed >1 bird having a safety issue, or the majority or average antibody response to be low, that would be sufficient to inform the next steps for decision making regarding kākāriki vaccination.

- **The possible confounding effects of animal variation?**

We expect some individual variation since the immune response is affected by individual health status and biological variation. This sample size is sufficient to ensure we have a range of individual responses to examine.

- **The needs of statistical analysis?**

There is likely to be individual variation, which, for the antibody response, requires a reasonable sample size. We determined that 10 was the maximum which was feasible to include in the trial, and also sufficient to allow for individual variation to establish some baseline parameters of antibody response.

Ultimately, in an outbreak situation, the results of a sample size of 10 will be sufficient to make reasonably informed decisions about the use of a commercially produced killed vaccine which has a good history of safety and efficacy across a wide range of species.

7. WELFARE OF ANIMALS DURING PROPOSED MANIPULATION:

7a. What measures will be taken to ensure: the general health and welfare of animals before, during and after manipulation, including the adequacy and cleanliness of housing, caging and equipment; the provision of food and water; prevention of over-crowding, and prevention and control of disease?

Kākāriki will be caught and handled by experienced staff as per usual kākāriki protocols at Natureland Wildlife Trust where staff already maintain appropriate husbandry pract ces and monitoring of all the birds. Each bird will be held within its normal enclosure so that there is minimum disturbance to their daily lives. Staff will continue to monitor birds throughout the trial, including food consumption and behaviour.

Each bird will receive a veterinary examination at the start of the trial. Equipment will be disinfected between individuals, or new equipment will be used. Once blood has been collected and vaccination undertaken the bird will be re-released in their home aviary.

7b. What movement and transportation measures will be followed for the animals to be manipulated to ensure their welfare and humane treatment?

Birds will be vaccinated on site, in their aviary where they are captured, therefore there will be no transport required.

However, if a bird requires specialist veterinary care e.g. in the event of an injury or serious reaction, then the bird will be taken to the local veterinary clinic of Rachel Stayner, the veterinarian who provides care for the Natureland animal collection.

Birds will be transported using the standard transport boxes, and in accordance with normal transportation procedures. Briefly, birds will have non-slip flooring the box, be transported via car to the veterinary clinic Transport will be managed to reduce noise and allow for temperature control. Radio will be off and driver/passenger will ensure minimal noise. Travel will be direct and the clinic will be alerted ahead of time to enable a fast hand-over a and rapid care.

Supportive therapy may be provided prior to transport which may include pain relief and fluids by the veter narian if on site, otherwise the bird will be rapidly transferred to the clinic for immediate vet care.

7c. What measures are to be taken to minimise the pain or distress of any animal manipulated? *Stating there will not be any impact is not acceptable. The AEC is looking for the Applicant to (1) provide analysis about the potential for pain and/or distress to the animal(s), and (2) describe how they will manage that pain or distress. Identify how you would ascertain pain or distress animal's behaviour, environmental conditions likely to lead to pain or distress.*

Birds will be captured and handled by experienced staff using their routine techniques. Only experienced staff will handle the birds. Initial physical examination, vaccination and blood collection will be undertaken by a veterinarian.

Any bird detected to have abnormalities will be examined and rejected from the trial, and receive normal veterinary investigation/intervention.

Subsequent examination and blood collection may be undertaken by staff trained in blood collection from kākāriki, provided the initial results (0,1, and 2-3 month check) are normal across the cohorts

The subcutaneous injection is not considered painful, and the vaccine dose will be warmed to room temperature prior to injection. Blood collection is associated with a minorly painful pin-prick when the needle is inserted. This will be minimised by careful planning and handling.

If birds are observed to have any pain response to the vaccination, the staff will report it to the veterinarian who will investigate. In the event that there is an injection site reaction (painful inflammation) then an anti-inflammatory such as Metacam may be prescribed by the attending veterinarian, as well as antibiotics if infection is also present.

As noted earlier, if any serious adverse reactions occur, veterinary care by the attending veterinarian will be undertaken, and transfer to local veterinary clinic of Rachel Stanyer undertaken if required for more intensive specialist care.

8. CONTINGENCY PLAN:

8a. What arrangements have been made for the abandonment of any manipulation and/or the euthanasia of animals where pain or distress cannot be held within reasonable levels?

If pain or distress is apparent, during handling or following the procedure, the veterinarian will investigate. If the veterinarian deems the level to be unreasonable, then the manipulation will be abandoned and all efforts made to ameliorate the event e.g. anti inflammatory, pain relief medication, antibiotics.

In the unlikely event that the pain is not temporary and cannot be managed, transfer to the local veterinary clinic of Rachel Stayner will allow for intensive veterinary intervention and care.

Supportive therapy would be provided during any transport which may include pain relief and fluids.

9. PEOPLE TO UNDERTAKE PROPOSED MANIPULATION:

9a. Who are the person(s) primarily involved in carrying out the proposed manipulation?
Veterinarians Kate McInnes, Lydia Uddstrom and Rachel Stayner are the primary persons.

9b. What is the experience and qualifications of the person primarily responsible (9a) for the undertaking and supervising the manipulation (including selection of animals, their care and disposal?)

Kate McInnes has been the DOC vet since 2000 and has worked across a range of avian species , and is currently the lead technical advisor for the DOC HPAI response.

Lydia Uddstrom is contracted full time to the DOC kākāpō team, has undertaken postgraduate training as a zoo veterinarian and has experience with a wide range of New Zealand native species veterinary care.

Both Kate and Lydia have previously been involved in capture and handling of threatened species undertaking vaccination and blood collection for a range of threatened species, and have trained multiple DOC staff to safely and effectively undertake these procedures.

Rachel Stayner is a local veterinarian in Nelson with post-graduate training in avian medicine, and currently provides veterinary care to the animal collection at Natureland Wildlife Trust. She is also contracted to DOC to assist with the DOC Avian Influenza preparedness and response work.

9c. Who else is in the team undertaking the manipulation? State their role in the team, and their relevant experience with the procedure(s) proposed in the application? Include DOC and non-DOC staff in the team.

Name of Manipulation Team member	*Role in the manipulation*	*Experience and qualifications relevant to the manipulation*
Leah Foster	Team leader for capture, handling and monitoring of birds	Manager and Keeper at Natureland Wildlife Trust. 11 Years at Australia Zoo. 2 years as Wildlife carer for Fauna Rescue North QLD. 6 years as Veterinary Nurse at Belmont Veterinary Hospital. Experience in handling, medicating, and rehabilitation of native Australian Birds.
Alix Rimmer	Assist in capture handling and monitoring of birds	Certificate in Captive Wild Animal Management. Care Team Leader at Natureland Wildlife Trust. 7+ years at Auckland Zoo and Ti Point Reptile Park. Breed for release: Yellow crowned kākāriki, Kiwi, Kākā, Kōkako, Pāteke, Tuatara and rehab. In situ breeding monitoring of wild native birds. Split band banding for 3 years. Safe capture and handling of native and introduced/exotic birds. Mentoring and training Tertiary students for Unitec, Vet Nursing, Animal Care, college students, new staff. Coordinating volunteers.
Claire Daniel	Assist in capture handling and monitoring of birds	Certificate in Captive Wild Animal Management, BSc and Msc in Biological Science. Keeper at Natureland Wildlife Trust. Five years at Birdcare Aotearoa husbandry and medical treatment of wild native, introduced and captive exotic birds. Two years with WIRES (Sydney) rescue, husbandry

		and medical treatment of Australian native birds. Thesis fieldwork including capture, blood sampling, banding and measurements of 100+ Australasian gannets.
Hani Fern	Assist in capture handling and monitoring of birds	Certificate in Captive Wild Animal Management Keeper at Natureland Wildlife Trust. I have experience with safe capture, restraint and handling techniques for native bird species such as Kaka and Red crowned Kakariki for medical treatment, banding and transportat on.
Natalie Kerr	Assist in capture handling and monitoring of birds	Diploma in animal Management. Keeper at Natureland Wildlife Trust. 6 years experience in training, handling and restraining birds of prey in England. Trained in administering oral medication and topical treatment.

9d. What training will be given to the people identified in 9c to help them undertake the manipulation proposed in the application?

The capture and handling will be undertaken according to the direction of the team leader.

Vaccination will be undertaken by the DOC veterinarian.

10. COMPLIANCE WITH CONDITIONS of the APPROVAL:

- Please outline any opportunities for a member, or members, of the DOC Animal Ethics Committee to observe this work.

10a. Identify ways that the manipulation(s) can be monitored by the AEC.

AEC members could attend a vaccination session at Nelson to see the procedure in kākāriki and/or receive a video or photographs of the manipulation being undertaken.

11. Are there any other aspects which ought to be brought to the attention of the DOC Animal Ethics Committee?

No

12. Does the research, testing or teaching involve a species which is covered by a Department of Conservation Species Recovery Plan and if so, has the Recovery Group been consulted and their endorsement for the work received? Please provide a summary of communication.

Yes. Kākāriki karaka management is guided by the kākāriki karaka Recovery Group which has been consulted and are supportive of this trial. The use of a surrogate species is acknowledged as a desirable alternative to the additional capture and handling which would be required for the captive kākāriki karaka population.

This application has been discussed with the kākāriki karaka RG at a captive management meeting in August. 2023, and again with the kākāriki karaka captive management coordinator on 15th November 2023.

13. What month of year is most useful to report back to the AEC (depending on the project schedule and the animal's biology)?

September

13. Manipulation Grading

Please work through the document '*Grading of Manipulations*' (Please refer to DOCDM-870472), and determine the grading you believe best applies to the manipulation proposed in this application. Please also provide a rationale for the grading.

Grade A: No impact or virtually no impact.
Grade B: Little impact. Manipulations of minor impact and short duration.
Grade C: Moderate impact. Includes manipulations of minor impact and long duration or moderate impact and short duration.
Grade D: High impact. Includes manipulations of moderate impact and long duration or high impact and short duration.
Grade E: Very high impact. Manipulations of high impact and long duration.

Grading determined by the Applicant: B

Your rationale for the grading:
Grade B includes "**Disease/injury/functional impairment:** Studies of vaccines using killed pathogens." The animals will be kept in their normal husbandry conditions for the duration of the study. There will be capture and handling for blood collection and vaccination, and two doses of a killed vaccine administered. Handling time & stress will be minimised by using only skilled staff and it will be undertaken at site.

Note: The grading determined by the Applicant is not the grading assigned by the AEC. The Applicant will be advised of the AEC's grading and any conditions in writing.

DECLARATION by the APPLICANT

Tick boxes [☑] to indicate your agreement to conditions: *[Copy and paste this tick object ☑]*

[☑] I declare that the information in this Application is correct; and
[☑] I agree to comply with the conditions imposed by DOC's AEC for the manipulation; and
[☑] I agree to ensure all personnel involved in this manipulation will be properly trained and/or qualified to undertake the manipulation and will be aware of the contents of this AEC application; and
[☑] I declare the proposed manipulation has the necessary resources to undertake the manipulation with regard to the health and safety of the animals and staff
[☑] I agree to advise the AEC of any changes in the details of the manipulation as described in this Application.
[☑] I agree to comply with the reporting requirements stipulated by the AEC on approval of this research project.

9(2)(a) [redacted]

Application Number DOCDM	AEC447 APPLICATION DOC-7483661
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, kākāriki

Signed by the Applicant _____

Full Name: Catherine McInnes Date: 13/11/2023

DECLARATION by the ACCOUNTABLE MANAGER

Tick boxes [☑] to indicate your agreement to conditions: *[Copy and paste this tick object ☑]*

[☑] I agree to ensure my staff member complies with the conditions imposed by DOC's AEC for this manipulation; and

[☑] I agree to ensure all personnel involved in this manipulation will be properly trained and/or qualified to undertake the manipulation and will be made aware of the contents of this AEC application; and

[☑] I agree the proposed manipulation has the necessary resources to undertake the manipulation with regard to the health and safety of the animals and staff

[☑] I agree to oversee this Application via MORs, PDPs and other means to ensure the manipulation remains within the scope of the Application and the Approval, and all reporting required by the AEC is delivered on time;

[☑] I agree to advise the AEC of any changes in the details of the manipulation as described in this Application, and to advise the AEC if the Applicant leaves the Department, or if the work should be transferred to another staff member for operational reasons' or if the manipulations is abandoned for any reason.

Signed by the Manager: 9(2)(a)

Full Name: John Lyall

Role: Fauna Advice Manager

Date: 17/11 / 2023

Application Number DOCDM	AEC447 APPLICATION DOC-7483661
Applicant	Kate McInnes
Key Words	avian influenza, vaccine, safety, efficacy, kākāriki

Appendix 1: Avian Influenza vaccine information

Registration number A009733: **Poulvac Flufend i AI H5N3 RG**

Registrant: Ministry for Primary Industries

Draft label information:

PRESENTATION

Bottles of 500 mL (1000 doses). Packs of 1 or 10 bottles.

DIRECTIONS FOR USE

By law the distribution and use of this product must comply with the requirements of the relevant operating plan.

General:
• Inject 0.5 mL (0.5 cc) subcutaneously, using aseptic technique, into healthy birds at 3 to 4 weeks of age or older.
• Shake well before use.
• Allow the vaccine to reach room temperature (18-29°C) before use.

Chickens:
• Administer another dose of 0.5 mL not less than 2 weeks later, if required.
• The second dose should be administered at least 4 weeks before point of lay.

Ducks:
• Ducks less than two weeks of age:
• Administer 0.2 mL of vaccine subcutaneously at the back of the neck.
• Administer another dose of 0.5 mL not less than 2 weeks later.
• Ducks two or more weeks of age:
• Administer 0.5 mL of vaccine subcutaneously at the back of the neck.
• Administer another dose of 0.5 mL not less than 2 weeks later.

ADVERSE EFFECTS, CAUTIONS AND CONTRAINDICATIONS

ADVERSE EFFECT
• Vaccinate only healthy chickens or ducks and avoid stressing the birds at the time of vaccination.
• Do not mix with any other vaccine or injectable product.
• The use of this product in laying birds has not been evaluated.
• Local or systemic post-vaccination reactions can occur due to the use of oily vaccines. Symptoms observed are generally transitory and can include oedema and granulation at the injection site, anorexia and dehydration. Such reactions can be minimised by good aseptic vaccination technique.

CAUTIONS
• Destroy any unused vaccine and containers after vaccination (including syringes and needles) by burning.
• Do not mix the vaccine with other vaccines or administer another vaccine shortly before or after vaccination with this product.
• Consult a physician immediately for an accidental self-injection and show this package insert to the physician.
• KEEP OUT OF REACH OF CHILDREN AND UNINFORMED PERSONS

CONTRAINDICATIONS
• None.

WITHHOLDING PERIODS
Meat: Nil.

STORAGE
• Store in the dark between 2 °C and 8 °C. Do not freeze.
• Protect from direct sunlight.
• Use contents of each vial within 6 hours of opening

Appendix 2: General Instructions for subcutaneous injection of vaccine

- The vaccine is supplied in a 500mL bottle and is given to the bird using a needle and syringe.
- The vaccine is injected under the skin but NOT into the muscle below.
- The vaccine should be drawn up into the syringe and then allowed to warm to room temperature (this is more comfortable for the bird).

EQUIPMENT NEEDED

1. Vaccine container
2. 1 mL syringe
3. 25 gauge 5/8th inch needle
4. alcohol swab (mediswab or cotton wool and meths)
5. dry swab (gauze or cotton wool)
6. Sharps container for needle disposal
7. Bird
8. Bird handler
9. Veterinarian

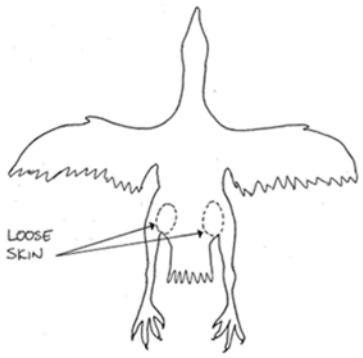
PREPARING THE VACCINATION

1. Store the vaccine in the fridge at 2-8 degrees C in the dark. Do not Freeze.
2. When ready to use, take the vaccine out of the fridge and shake well to mix.
3. Write the date on the vaccine bottle.
4. Break off the metal seal on the top of the rubber injection port.
5. Swab the injection port on the vaccine with alcohol to sterilise it with a mediswab or cotton ball soaked in methylated spirits.
6. Firmly attach the needle to the syringe – 25 gauge 5/8th inch needle to a 1mL syringe.
7. Insert the needle through the centre of the rubber stopper CAREFULLY.
8. Hold the vaccine upside down and slowly suck vaccine into the syringe until you have a little more than the prescribed dose of vaccine.
9. Flick the syringe to dislodge any air bubbles and squirt them slowly back into the vaccine bottle.
10. Keep squirting until all the bubbles are gone and you have the prescribed dose of vaccine left in the syringe.
11. Pull the needle out of the vaccine bottle and CAREFULLY recap the needle.
12. Leave the syringe and needle to warm to room temperature.
13. Repeat this procedure to draw up all the doses you need for your vaccination session.
14. Put the vaccine back in the fridge.
15. Once open, the vaccine can be used for 30 days. (Note that this expiry is based on Zoetis technical advice for limited use of the vaccine in this trial, and only applies when following the above instructions for maintaining sterility of the product and correct storage.)
16. If you are in doubt that the vaccine has been stored correctly (kept refrigerated), then discard it and get a new bottle.

GIVING THE INJECTION

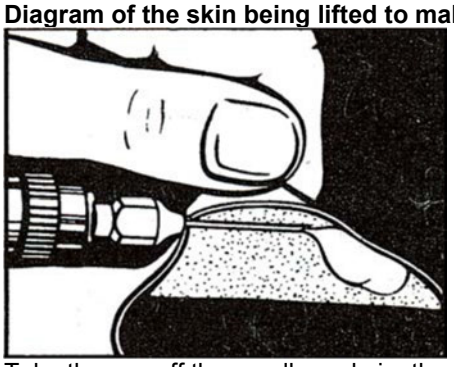
1. Have the following equipment ready for use:
    - The correct dose of vaccine drawn up in syringe with needle attached and warmed to room temperature. (the cap should be on the needle to avoid accidental stabbing or contamination of the needle)
    - One alcohol swab (mediswab or cotton wool in meths)
    - One dry swab (gauze or cotton wool)
2. Have an assistant restrain the bird on its back or side with its legs restrained to provide access to the groin (where the bird's leg joins its belly).

Diagram of ventral (belly) of a bird showing the groin region for subcutaneous vaccination sites:



3. Spread the feathers in the groin area.
4. Wet down the feathers with the swab to clear a patch of skin and swab the skin.
5. Lift the loose skin 1-2cm off the body to make a "tent".

Diagram of the skin being lifted to make a "tent" for a subcutaneous injection



6. Take the cap off the needle and aim the needle about halfway down the side of the tent. Keep the needle parallel to the body wall. When the needle goes through the skin, it should still be above the muscle of the groin i.e. you are injecting into the space inside the tent, not into the muscle.
7. Suck back on the syringe to check for blood. This is to avoid injecting into a blood vessel.
8. Inject the vaccine with a steady firm pressure.
9. Withdraw the needle and place it into the Sharps container.
10. Use the dry swab to press over the injection site if there is any bleeding.
11. Release the bird.

12. Record:	Bird ID	Date	Dose	L or R side	Vaccinator	Holder	Vaccine Batch	Expiry Date	Notes

13. Transfer this data to the vaccination record spreadsheet
14. Note any other specifics about the injection process not described above. E.g. if there was bleeding at the injection site.

Approvals Group
New Zealand Food Safety
Ministry for Primary Industries
Charles Fergusson Building, 34-38 Bowen St, Pipitea
PO Box 2526, Wellington, New Zealand 6140
Tel: 04 894 2550
Email: approvals@mpi.govt.nz

New Zealand Food Safety
Haumaru Kai Aotearoa

Research Approval Product Data Sheet
(or Variation of Existing Research Approval) of Agricultural Chemical or Veterinary Medicine or Vertebrate Toxic Agent
ACVM 5 (July 2021)

- **This form is to be completed by the Applicant or their nominated Agent/Consultant.**
- A research approval, which is an "Approval in special circumstances" with the Director-General, MPI, is required under section 8(C) of the Agricultural Compounds and Veterinary Medicines (ACVM) Act 1997.
- If you wish to make an application for research approval of an agricultural compound (i.e. agricultural chemical, veterinary medicine or vertebrate toxic agent), under section 10 of the ACVM Act you must fill out this form.
- If the agricultural compound is being imported and it contains an ingredient originating from an organism (such as from a plant, animal, fungus, bacteria, virus), you must also submit the Biosecurity Assessment of ACVMs application form, which is attached as Appendix 1.
- Send the completed application form electronically together with any fee and other required documentation (see section D1 of this form) to the Ministry for Primary Industries at the above email address.
- If there are any changes to the details provided in this application form subsequent to approval, you must inform MPI in writing at the above address.
- Refer to the Privacy Act 2020 and Official Information Act 1982 notices at the end of this form regarding collection of information by MPI.

Processing time is up to 40 working days from the time we determine that your application is complete.

Part A: General Information

Refer to ACVM Information Requirements for Research Approval in New Zealand and the Research Standard

Depending on your product type, use the veterinary medicine, agricultural chemical or vertebrate toxic agent product data sheet guideline (on our website) to help you complete this form.

A1 Trade Name or Company Code of the Product	
Trade Name	**Approval number** (if assigned)

A2 Applicant Information		
Full Legal Name Registered company name or partnership names (including the trading name) or individual name.		
Applicant's New Zealand Business Number (NZBN)		
Overseas applicants, provide Companies Act reference number		
Street/Physical Address (for service)	**Postal Address** (for communication)	
Contact Name	**Tel**	
	Mobile	
	Email	

A3 New Zealand Agent

Complete only if you have appointed an agent in New Zealand. (This is compulsory for overseas companies.) Any official MPI documents (such as certificates of registration, suspension of registration, prohibition notices, recall notices) will be sent to this person/organisation. Note that a Letter of Authorisation is required.

If you are in New Zealand, you may also nominate an agent to accept service of documents on your behalf.

This agent is only a contact person and is not legally responsible for the product. The responsibility remains with the registrant.

Name of Organisation/Company		
Agent's New Zealand Business Number (NZBN)		
Street/Physical Address (for service)	**Postal Address** (for communication)	
Nominated Contact's Name	**Tel**	
	Mobile	
Is the person named above the primary contact for this product? (delete one) YES NO	**Email**	

A4 Consultant

Complete only if a consultant is managing the application process for you and is the point of contact during the process. Note that a Letter of Authorisation is required.

Name of Organisation/Company		
Consultant's New Zealand Business Number (NZBN) (if applicable)		
Street/Physical Address (for service)	**Postal Address** (for communication)	
Contact Name	**Tel**	
	Mobile	
	Email	

A5 Study

Detailed reason for carrying out this study.

To assess the safety and efficacy of an inactivated avian influenza vaccine on 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).

Avian influenza is a viral disease which can cause mass mortality events in birds. The current strain of HPAI has had severe impacts on wild bird populations overseas. It is predicted to reach the Southern Ocean by 2024/25 and was confirmed in South Georgia in the southern Atlantic subantarctic region in October 2023.

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 in a confined area (off-shore island). Protection of captive/confined breeding birds from HPAI morbidity and mortality, and reduction of viral shedding is essential 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 titer peak levels and duration). See EFSA 2007 & Vergara-Alert et al 2011.

We aim to determine the safety and efficacy of the vaccine in these five species. We will establish if there are any adverse events associated with vaccination which can be mitigated or which indicate the vaccine is not suitable for use in these species.

We will measure the antibody response (elevation and duration) to a standard vaccine regime. This will determine the effectiveness of the dosage and the duration of expected protection (using antibody titer as a proxy for protection).

The results will inform the species recovery programmes of any safety issues relating to the vaccine which might affect its use in these species, the duration of protective antibody titers which may determine if vaccination is feasible and effective as a protection against HPAI morbidity and mortality, and any the requirements for subsequent booster vaccination to extend the level of protection.

Overall, the results will inform any future use of vaccination to provide a small insurance population against the risk of extinction from HPAI, and the potential for use in birds bred for release into the wild which might be considered at risk in the face of a major mortality event.

References:

EFSA 2007. Vaccination against avian influenza of H5 and H7 subtypes as a preventative measure carried out in Member States in birds kept in zoos under Community approved programmes. ESFA journal, 450. https://doi.org/10.2903/j.efsa.2007.450

Vergara-Alert J, Ferhandez-Bellon H, Busquets B, Alcantara G, Delclaux M, Pizarro B, Sandchez C, Sanchez A, Majo N, Darju A. Comprehensive serological analysis of two successive heterologous vaccines against H5N1 Avian Influenza virus in exotic birds in zoos. Clinical and Vaccine Immunology, 2011. P. 697-706 https://europepmc.org/article/PMC/PMC3122527

Anticipated start date (Month and year)	February 2024
Anticipated duration of study (Length)	13 months

A6 Identification of Investigational Product See relevant guideline (as mentioned before question A1).	
Product type	Vaccine
Formulation type	Inactivated virus, oil emulsion
Application/Administration method	Subcutaneous injection
Target species/host For veterinary medicines or VTAs, state animal species. For agricultural chemicals, state target host species.	kakī/black stilt (*Himantopus novaezelandiae)* tūturuatū/shore plover (*Thinornis novaeseelandiae*) kākāpō (*Strigops habroptilus*) takahē (*Porphyrio hochstetteri*) red-crowned parakeet *(Cyanoramphus novaezelandiae novaezelandiae*) (as a surrogate species for kākāriki karaka/orange-fronted parakeet (*Cyanoramphus malherbi))*
Trial design Provide a description of the trial design. State clearly the plot size/number of experimental animals, and justification of dose/ application rate and treatment frequency.	The dose volume and frequency of the vaccine is based on dosages used in Vergara-Alert et al 2011 for a range of avian species, whereby birds <1.5kg will receive 0.2ml per dose and birds >1.5kg will receive 0.5ml per dose of vaccine subcutaneously one month apart to stimulate humoral immunity and antibody production. For each species, ten individuals held at the captive facility/offshore island will be selected for the trial by the lead husbandry expert. Birds will be chosen based on normal behaviour and appearance, and suitability for capture and handling. Bird will be permanently banded with individual identifying leg bands or microchips. The birds will be divided into two cohorts of roughly 5 birds each (this may be cohorts of 4 and 6, 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 commences the trial and if all results/observations are favourable, then one month later cohort 2 commences the trial. This allows initial results from cohort 1 to be received and reviewed, prior to starting cohort 2. Time point: 0 months Each animal receives a physical examination by an experienced wildlife veterinarian. A dose of vaccine of 0.2ml for kākāriki, tūturuatu & kakī, or 0.2-0.5ml for takahē and kākāpō (depending on bodyweight) is delivered by subcutaneous injection in the left groin region. Blood samples are taken for a blood smear for white cell count and differential, and serum antibody titer. Birds are monitored daily as per normal husbandry practices, and any abnormalities are reported to the veterinarian and thoroughly investigated. Time point: 1 month One month later, the birds are re-examined for any abnormalities. If none are detected which indicate a safety issue from the vaccine, the second dose of vaccination is administered at 0.2ml for kākāriki, tūturuatu & kakī, or 0.2-0.5ml for takahē and kākāpō (depending on bodyweight), in the right groin region. If any abnormalities are detected, these are documented and thoroughly investigated. The bird may be removed from the trial if its welfare is compromised. Blood samples are taken for a blood smear for white cell count and differential,

	and serum antibody titer. Time point: 2-3 months One to 2 months later, the birds are re-examined for any abnormalities. Blood samples are taken for a blood smear for white cell count and differential, and serum antibody titer. Antibody titer results are reviewed. If a species has a low antibody response at the 2-3 month blood sample, the veterinarian may decide to administer a third dose. The birds will receive the same dose as previously given to that species according to the size of the individual bird (either 0.2 or 0.5ml). Time points: 6 months & 12 months If any abnormalities are detected, these are documented and thoroughly investigated. The bird may be removed from the trial if its welfare is compromised. Blood samples are taken for serum antibody titer.
Amount of product State clearly the total amount and unit (i.e. the total to be used in all trial work).	The product is produced in 500ml bottles. Each species will be dosed from a separate bottle. 5 species x 5 bottles = 2500ml total product.
Justification for amount of product Explanation should include full amount needed, taking into consideration trial numbers, design, and application rates, as well as practical considerations (such as overage or container sizes).	The product is supplied in 5 x 500ml bottles = 2500ml total. There will be 30 birds in the trial which will receive two doses each of 0.2ml per dose: 60 doses x 0.2ml = 12 ml. There will be 20 birds in the trial which will receive two doses each of up to 0.5ml per dose (dependent on body weight): 40 doses x 0.5ml = 20 ml. There may be a third dose given to any of the species, based on antibody response results. This additional volume could be up to 16 ml if all 5 species require the third dose: (30 x 0.2ml) + (20 x 0.5ml) = 16 ml Sterile practices will be used to draw up aliquots of 2 mL of vaccine from the main bottle and stored in sterile glass 5 mL vials to enable the product to be broken down into smaller doses. 50 aliquots will be made, with up to 3 birds vaccinated from each vial. The total used as a result of the trial is 2500ml because the five full bottles will be partly used and the remainer is discarded as medical waste at the end of the trial.

A7 Formulation Details

See relevant guideline.

Ingredient Name (Common or Chemical)	CAS Number	Quantity (g/kg or g/L)	Function
See registration data for this product			

Specific gravity	

Other information about formulation (for example, overage, isomers)

Part B: Other Approvals

B1 Biosecurity Approval (only applies to imported products)	
Does the product contain any ingredient of biological (animal, plant, or micro-organism) origin?	☐ ~~No~~ ☐ Yes If yes, you will need a Biosecurity approval. Submit a completed **Biosecurity Assessment of ACVMs** application (appended to this form) with the information you have available. If necessary we will contact you or the manufacturer for further information. Note this incurs a biosecurity processing fee in addition to the fee for processing this research approval application. Both fees will be invoiced together. If you have questions, contact animal.imports@mpi.govt.nz

B2 Importation Approval	
Do you require an Approval to Import for goods being imported under your research approval?	☒ ~~No~~ ☐ Yes
If yes, physical address to which goods must be released from the border	
kākāpō and takahē - 2x 500ml bottles Department of Conservation, Kakapo Recovery Team, Level 7, 33 Don Street, Invercargill, 9810. ATTN Dr Lydia Uddstrom **kakī: 1 x 500ml bottle** Department of Conservation, 15 Wairepo Road, Twizel 7901. ATTN Liz Brown **tūturuatu: 1 x 500ml bottle** Isaac Conservation and Wildlife Trust, Isaac Construction, McArthurs Road, Harewood, Christchurch, 8051 ATTN Anne Richardson/Leigh Percasky **kākāriki: 1 x 500ml bottle** Dr Rachel Stanyer, Nelson Vets - Saxton, 2 Findlay Place, Stoke. 7011	

B3 HSNO Approval HSNO status must be obtained before MPI approval will be issued. (See note at end of form, before appendices.) **Tick one box below.**	
Status of a Substance issued by the EPA or section 26 declaration under the Hazardous Substances and New Organisms Act 1996 **Provide a copy of the approval with your application.**	☐ SOS # ☐ section 26 declaration ☐ EPA Approval Code: HSR
OR Self-determination that the product fits an existing HSNO or group standard approval	☐ EPA Approval Code: ☐ EPA Group Standard: HSR100757
OR Self-determination that the trade name product is non-hazardous	☐
OR EPA generic containment **Provide written confirmation from EPA that your trials**	☐ EPA Approval Code: HSC

have been notified for use under this approval.	

B4 Animal Ethics Committee Approval Animal Ethics Committee approval is required for all animal-based trial work and must be obtained before that trial can commence. A copy of that approval must be provided to the ACVM team. The Animal Ethics Committee approval must remain current for all trials conducted under this approval for the duration of the approval period.		
Has Animal Ethics Committee Approval been obtained? (Put an X next to correct answer and add other information as indicated.)		
✓	Yes	A letter from the chairperson of the AEC or other proof of AEC approval has been provided. AEC Approval Numbers: AEC443 – DOC7522196 AEC444 – DOC7522200 AEC445 – DOC7522201 AEC446 – DOC7522198 AEC447 – DOC7522199.
	No	Attach information on the status of the approval (e.g. a letter from the AEC chairperson that the application is pending or other information on the status of the application).
	Not applicable	

Part C: Security of Product, Place and People

Refer to ACVM Information Requirements for Research Approval in New Zealand

If appropriate, provide references from your research documentation that will enable the following information to be found during an audit.

C1 Personnel	
Name of Study Director	Grant Matthews
Names of personnel involved in the study and their responsibilities	Catherine (Kate) McInnes BVSc DOC Veterinarian, HPAI response work Vet lead Responsible for design and oversight of the trials, Animal Ethics Committee application, delivery of veterinary services including physical examination, vaccination and blood collection and records pertaining to this, adverse event investigation, collating and reporting data. Lydia Uddstrom, BVSc DOC veterinarian (kakapo team), assisting with HPAI vaccination trial Assist with design and oversight of the trials, Animal Ethics Committee application, delivery of veterinary services including physical examination, vaccination and blood collection and records pertaining to this, adverse event investigation, collating and reporting data. The site lead will be responsible for the product while stored at the site, direct staff for catching, handling, monitoring & follow-up blood collection at 2-3, 6 & 12 months post vaccination. Site leads: Kakī: DOC Twizel breeding centre, Liz Brown Tūturuatu: Isaacs Conservation and Wildlife Trust, Christchurch. Anne Richardson Kākāriki: Natureland Wildlife Trust, Leah Foster, Manager (on site activities), Dr Rachel Stanyer (veterinarian responsible for product storage) Takahē: Burwood Takahē Centre: James Bohan Kākāpō: Whenua Hou/Codfish Island: Petrus Hedman

C2 Site
Identify the study location/site, the method used to select it, and the means by which access to it is limited.

Kaki: Department of Conservation Twizel breeding centre. This is a purpose-built site for captive breeding of kakī, operated by the Department of Conservation at 6199 Tekapo-Twizel Road, Pukakai, 7999. It was chosen because it holds a sufficient number of birds to complete the trial and has experienced staff on site. The site is surrounded by an electric fence, and security swipe pass is needed to enter the grounds. Only persons authorised by DOC are permitted to enter the site.

Tūturuatu: Isaacs Conservation and Wildlife Trust, Christchurch. It was chosen because it holds a sufficient number of birds to complete the trial and has experienced staff on site. The site is surrounded by a security fence and security swipe pass is needed to enter the grounds. Only persons authorised by Isaacs Construction are allowed to enter the site, via security sign in at the front office.

Kākāriki: Natureland Wildlife Trust, Nelson. It was chosen because it holds a sufficient number of birds to complete the trial and has experienced staff on site. It is a Zoo and Aquarium Association (ZAA) accredited facility. The site is surrounded by a security fence and meets MPI zoo containment requirements.

Takahē: Burwood Takahē Centre: holds takahē in enclosures for breeding. It was chosen because it holds a sufficient number of birds to complete the trial and has experienced staff on site. It is located at 3860 Te Anau-Mossburn Highway, Centre Hill. Only persons authorised by DOC are permitted to enter the site. The site is not marked and not widely known. There are DOC rangers living on-site to monitor people accessing the location.

Kākāpō: Whenua Hou/Codfish Island: is a 1396 ha island located 3km off the north-west coast of Rakiura/Stewart Island. It was chosen because it holds a sufficient number of birds to complete the trial and has experienced staff available to work on site. Whenua Hou is a nature reserve under the Reserves Act 1977 – access is by permit only. There is no public access to this site.

C3 Security	
Give assurance that persons who have access to the product are suitably qualified or trained to use it and that they have had any conditions specified on the research approval given to them in writing	The product will only be directly handled and administered by a registered veterinarian. This will be either Dr Catherine (Kate) McInnes BVSc or Dr Lydia Uddstrom BVSc or Dr Rachel Stanyer who are all employed by the Department of Conservation. Access to the product by other staff only relates to the storage in the refrigerator at the captive facility. See below for more details.

Measures implemented to limit access to the investigational product	This process is outlined in the document: "SOP for receiving, storing and access to Poulvac Flufend vaccine DOC-7521640" which will be provided to the veterinarians and site leads for all species/sites. The product will be delivered via courier from the supplier to the facility, or it will be directly collected from the supplier by the veterinarian. If delivered by courier, the local DOC ranger/ facility staff member will receive the package and store it in the captive facility refrigerator in a closed container which is clearly labelled "Veterinary Access Only – keep refrigerated" in accordance with the SOP. If collected form the facility, the Veterinarian will maintain the cold chain until it is delivered to the captive facility where it will be stored. The refrigerator at the facility has access limited to authorised staff only. All staff will be made aware of the restricted access to the vaccine.
Measures implemented to limit off-target exposure to the investigational product by animals or plants	The vaccine will be administered by the veterinarian directly to the test subject only. Care will be taken in all handling of the product to ensure no spillage, breakage or contamination of the environment occurs. Used needles and syringes will be disposed of directly into Sharps containers for disposal via medical waste. If a bird dies, it will be detected by staff and collected immediately. It will be double bagged and chilled, then sent for necropsy examination at Massey University (takahē, kakariki, kakī & tūturuatu) or Auckland Zoo (kākāpō). Its remains will be disposed of via medical waste or held frozen for genetic, cultural or conservation purposes. Bodies will not enter the food chain.

Describe the method of disposal of any unused investigational product	Any unused product will be disposed of by placing in a medical waste container for disposal by a commercial medical waste company. Disposal will be recorded by the veterinarian in a shared document record in the DOC Document Content system, which will also be used to record delivery of the vaccine, and all use of the vaccine including vet ID, animal ID, date, dose given, injection site, and any relevant notes. "HPAI vaccination safety and efficacy trial VACCINE USE DOCCM-7521354" A stock audit (similar to the controlled drug register audit) will be undertaken each time the vaccine is accessed – that is monthly for 3 months. Once the final audit is complete, the disposal will occur.

C4 Residues

Describe how residues in treated crops/animals will be managed, including animal transfer if crops are to be used as animal feed.

The animals receiving the vaccine will not enter the food chain.

Site residues

Describe measures implemented to ensure that the study site(s) are free from residues that might compromise further use of the site(s), including the period of time for which the site will remain secure to avoid residues affecting its further use.

In addition, for agricultural chemicals only, discuss management of potential residues in soil and subsequent crops.

There will be no site residues. The vaccine is administered directly into the bird and is not excreted via urine/urates or faeces.

C5 Approval for Sale of Treated Produce or Declaration

If you do not seek an approval for sale* of treated produce (animal or plant matter), you must indicate YES in the declaration below and confirm by your signature (section D2).

*Definition of 'sale' under the ACVM Act:

Sale includes barter, and also includes offering, exposing, or attempting to sell, or having in possession for sale, or sending or delivering for sale, or causing or allowing to be sold, offered, or exposed for sale; and also includes—

(a) delivering or disposing of by way of gift, loan, or otherwise; and

(b) giving or distributing, in the course of business, as a sample or otherwise, without charge

Will the treated crop/animals be destroyed? (Put an X next to correct answer.)	Yes	
	No	X
I confirm that the treated plants or animals or their produce will NOT be sold or used for human/ animal consumption or enter the food chain at any time. (Put an X next to correct answer.)	Yes	X
	No	

If NO, provide information to support sale

Part D: Documentation, Applicant Statement and Payment

D1 Additional Documentation Requirements
Provide electronic copies of the following documents with this application: ▪ Letter of authorisation for agent/consultant (if applicable) ▪ Letter of consent (Confidential Supporting Information) (if applicable) ▪ Biosecurity assessment application if a product being imported contains an ingredient of biological origin ▪ Status of substance (SOS) advice or non-hazardous declaration from EPA (if applicable) ▪ Animal Ethics Committee approval documentation (if applicable) ▪ Request for import approval (if applicable) ▪ Existing import approval if application is for a variation

D2 Applicant Statement			
I confirm that: ▪ I am authorised to make this application as the applicant OR a person with legal authority to act on behalf of the applicant noted in section A2; and ▪ the information supplied in and with this application is truthful and accurate to the best of my knowledge; and ▪ I understand that any change to the information provided in this application must go through MPI's 'variation' process or I will be in breach of the research approval conditions.			
Name		**Tel**	
		Email	
Signature		**Date**	

D3 MPI Service Charge

ON PAYMENT THIS BECOMES A TAX INVOICE GST No: 64-558-838

APPLICATION FEE: Refer to schedule of fees on website.

PAYMENT OPTIONS:

Payments comprising multiple fees must be supported by a remittance advice. Please attach your payment confirmation to this application or send it separately to: **approvals@mpi.govt.nz**

MPI does not accept cash. Payment must be made using **credit/debit card or direct credit**. (Please mark your choice with an X and fill in the appropriate section.)

APPROVED CREDITOR ☐

CREDIT /DEBIT CARD (preferred option): ☐

Go to https://www.mpi.govt.nz/food-safety/payments and follow the instructions.

☐ I have attached my credit card payment receipt

DIRECT CREDIT ☐

1. Pay into Bank Account no. **03 0049 0001709 002**
2. In the 'Reference' details, put the code: **RESAPP**
3. Enter the date of deposit and the payer name on this form below:

Date of Deposit		**Payer Name**	

Collection of Information

Collection of Personal Information

Pursuant to Principle 3 of the Privacy Act 2020, we advise that:

1. This information is being collected for the purpose of approval in special circumstances (research) under the Agricultural Compounds and Veterinary Medicines (ACVM) Act 1997; and
2. The recipient of this information, which is the agency that will collect and hold the information, is the Ministry for Primary Industries, PO Box 2526, Wellington 6140; and
3. The collection of information is authorised under section 10 of the ACVM Act; and
4. The provision of this information is necessary in order to process this application; and
5. The supply of this information is voluntary; and
6. Failure to provide the requested information is likely to result in a return of the application form to the applicant, and in accordance with the ACVM Act, may ultimately result in a refusal of this application; and
7. Under Principles 6 and 7 of the Privacy Act 2020, you have the right of access to, and correction of, any personal information which you have provided.

Collection of Official Information

All information provided to the Ministry for Primary Industries is official information and may be subject to a request made under the Official Information Act 1982.

If a request is made under that Act for information you have provided in this application, the Ministry for Primary Industries will consider any such request, taking into account its obligations under the Official Information Act 1982 and any other applicable legislation.

Note on HSNO Approval

Section 21(5) of the ACVM Act states: "Where a trade name product contains an agricultural compound that is also a hazardous substance or new organism, the Director-General must not register that product under this section, unless an approval for that substance or organism has been issued under the Hazardous Substances and New Organisms (HSNO) Act 1996".

Hazardous substances or new organisms

If your product contains a hazardous substance or a new organism, or if you are unsure whether it does, contact the Environmental Protection Authority (http://www.epa.govt.nz/). EPA NZ will provide informal advice, based on information provided, on whether or not a substance is hazardous and/or whether it is covered by an existing approval. If you choose this option, provide a status of substance (SOS) number, or section 26 declaration, and the EPA approval code on the PDS, and attach a copy of the SOS letter.

If you have self-determined that the product matches an existing substance, you must provide the HSNO number and ensure that you have filled out Section B2. Your signature on this form serves as confirmation.

Alternatively, you may make the determination that a product may be assigned to a group standard approval. If you have self-determined that a product fits a group standard, MPI will require evidence of the determination process to justify the use of the group standard. If you choose this option, provide the reference number of the group standard.

Non-hazardous substances

MPI will accept a declaration if you have self-determined that the agricultural compound you wish to register is not a hazardous substance*. We may require you to provide a technical argument why the ingredients in the product are non-hazardous to support your declaration. If MPI is not certain that the determination is correct, we will advise you to obtain a determination by EPA NZ.

* For a product to be considered non-hazardous, it must either contain no hazardous substances as defined under the HSNO Act OR contain a hazardous substance at a low enough level that the product as a whole is considered non-hazardous.

Appendix 1

Biosecurity Assessment of Agricultural Compounds or Veterinary Medicines (ACVMs) Application Form for Research Approval

- If the agricultural compound contains an ingredient of a biological (animal, plant, or micro-organism) origin, this information is required by the Biosecurity Act 1993 to undertake a risk assessment for biosecurity approval. Provide as much information requested in the form as you have. We will contact you if we need further information.
- Time for assessment: If all the information requirements are met, then the assessment will be processed within the timeframe of the ACVM process.
- Cost of assessment: NZ$117.61 (inc GST) per hour. The fee will be invoiced in conjunction with the ACVM charges.
- If you have questions about this biosecurity assessment, contact animal.imports@mpi.govt.nz

1. Trade Name or Company Code of the Product	
Trade name	**Poulvac Flufend i AI H5N3 RG**
Approval number (if assigned)	Registration number A009733
List countries where product is registered	New Zealand,

2. Manufacturer(s) of the Formulated Product

Complete for all manufacturers

Company name	Site address

3. Is your product a vaccine?

☐ No
☒ Yes

If yes, skip section 4 below and complete the information requirements outlined in Appendix 2. Ensure you take note of section 5 and complete the Applicant Statement (section 6).

4. If your product is not a vaccine but contains ingredients of bacterial, fungal, viral, plant or animal origin

For products containing live organisms provide:

- systematic name and strain of the bacteria, protozoa, fungi, rickettsia, nematode or virus and the taxonomic description of the agent, serotype, strain or mutant;
- common name or alternative and superseded names;
- composition of the unformulated material, microbiological purity, nature and identity of any culture media, impurities and content of extraneous organisms.

For processed products provide:

Origin of the ingredient(s) of plant and animal origin

Complete for each ingredient (raw material) and for each manufacturer if more than one manufacturer:

Identify the raw materials used, the species and country of origin. Include health certification referring to disease country freedom and herd or flock of origin disease testing.

Describe the manufacturing processes for preparing the product.

Briefly outline the processes designed to render the product(s) sterile (e.g. heat treatment, filtration, acid or alkali treatment, irradiation, long term maturation etc). Include relevant parameters (e.g. temperature, pH level, radiation dose) and the time the product is maintained at these levels.

Each major step in the production process should be shown in a flow-chart diagram.
Each step on the flowchart should be cross-referenced to the application, which should contain details of the materials used and results of any tests conducted.

Describe the operational environment, quality systems and controls used for manufacturing. The manufacturer's GMP may include SOPs and/or specifications of the approved source, sterilisation procedure (if applicable) and pathogen testing applied to each product.

Expert opinion

If available, the applicant shall provide an opinion on the likelihood of the product containing associated organisms from an independent expert authority who is familiar with the manufacturing process. Include the following information:
Name:
Postal address:
Street address (if different from above):
Tel:
E-mail:

5. Confidential Information

If information is confidential, please ensure that you have contacted the manufacturer/supplier to arrange for information to be supplied to us directly.

6. Applicant Statement

I confirm that the information supplied in and with this application for biosecurity assessment is truthful and accurate to the best of my knowledge.

Name		**Tel**	
Job Title		**Email**	
Signature		**Date**	

Appendix 2
Additional Information Required for a Biosecurity Assessment of a Veterinary Vaccine

- Information identified below is additional to that required in the Application for Biosecurity Assessment of Agricultural Compounds or Veterinary Medicines (Appendix 1).
- Provide information in a format consistent with these requirements or as a summary document cross-referenced to registration dossiers and/or drug master files which should also be submitted.
- Provide the information you have available. If necessary we will contact you or the manufacturer for further information.
- If you have questions about this biosecurity assessment, contact animal.imports@mpi.govt.nz.

Additional information required for products that are veterinary vaccines
Materials of biological origin Provide detailed information on all components of biological origin used directly or indirectly in production of the vaccine. Such components include viral/bacterial seeds, cell lines, trypsin, nutritive factors (e.g. serum), fermentation broths/culture media and excipients. List every ingredient of animal origin contained in or used in the production of the product, the country and species of origin, approximate date of collection if available, processing/treatment and testing specified.
Testing standards MPI will normally accept procedures to test for pathogens that are specified in the Code of Federal Regulations (9CFR 113) or other standards. Submit details of all testing protocols with the application.
Certification and audit trails Provide information to show that an audit trail can track the country, species and date of origin of each product of animal origin used in production of the vaccine. Such audits should be able to correlate batches of finished product with all raw ingredients.
Other pathogens held and vaccines produced at the facility List of all pathogens held and vaccines produced within the vaccine manufacturing facility. List of other activities on the same site (e.g. vaccine research involving challenge trials, veterinary pathology and diagnostic services) and on neighbouring sites (e.g. intensive livestock production, abattoirs, animal research facilities).
Sterilisation of components of animal origin Sterilisation procedures must be validated. Submit a copy of the appropriate SOP with the application.
Master seeds (virus, bacteria and cells) A well-documented history of the master seed must be made available. Provide the origin, date of isolation, passage history, reversion to virulence, purity and identity confirmation studies. Provide details of cell lines and nutritive media used for the transport, storage and propagation of the master seed. For master seeds created many years ago, detailed information on the initial nutritive factors used may not be available. In this situation, it may be possible in some circumstances to establish the safety of the master seed by additional testing and a history of safe use over many years in live vaccines. Frequent use and extensive pathogen testing over many years in research laboratories and inactivated vaccine manufacture may also provide an additional level of biosecurity confidence. Provide details of the testing methods used to establish freedom from contamination by bacteria, fungi, mycoplasma, viruses and pathogens.
Working and production seeds (virus, bacteria and cells) Describe the tests used to identify potential pathogens in working and production seeds.

Nutritive factors Nutritive factors include serum, foetal serum, serum albumin and other serum products. Detail the country and species of origin, processing and/or any pathogen testing.
Trypsin and other enzymes of animal origin Provide details on the country of origin, species of origin, processing and any pathogen testing.
Fermentation broths and culture media List all ingredients used in the fermentation broth/production culture media in the import application. Specify country and species of origin of each ingredient of biological origin along with details of any processing, treatments or testing of either the ingredients or the final culture media/fermentation broth.
Final product testing – live vaccines Describe the testing used on live vaccines.