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AUSTRALIAN ANTARCTIC PROGRAM

Photo: Leslie Frost

Unusual Animal Mortality Response Plan 2019–22

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1.0 INTRODUCTION

Unusual animal mortalities, although rare, have been observed amongst Antarctic wildlife and may occur again. An international workshop on diseases of Antarctic wildlife was held in Hobart in 1998 resulting in a number of recommendations to reduce the risk of disease introduction into Antarctica along with provision of guidelines for response to suspected disease occurrence. The workshop report[1] also contains a useful summary of all major disease agents previously documented in penguins and seals.

1.1 Purpose and objectives

The purpose of this response plan is to provide guidance on what to do if sick or dead animals are discovered in unusually high numbers or with signs that suggest disease.

The objectives are to obtain information on the species involved, the extent of affected animals and the cause. A further objective is to reduce the likelihood of people spreading the infectious agent, or acquiring infection themselves, if a zoonotic agent is involved.

1.2 Target audience

This plan provides information for people in the field who discover unusual numbers of sick or dead animals; the Station Leader (SL), Field Leader (FL) or Voyage Leader (VL) responsible for the people in the field, and people at the Australian Antarctic Division (AAD) headquarters at Kingston responsible for coordinating a response.

1.3 Geographic scope

The plan covers the Australian Antarctic Territory (AAT), Macquarie Island and the Southern Ocean. Macquarie Island is a Tasmanian reserve and managed by the Tasmanian Parks and Wildlife Service (TASPAWS). It is included in this plan because AAD personnel will be involved in any unusual mortality response that may be required.

1.4 Health and welfare of personnel

Protection of the health and welfare of personnel must be a priority in any response to the discovery of sick or dead animals. Biohazard and Infectious risk must be assessed to prevent any risk to human health.

2.0 PREPARATION AND PLANNING

2.1 Awareness and training

SLs, FLs or VLs and Antarctic Medical Practitioners are to be briefed on the response plan and response kit as part of their pre-departure briefing by the Environmental Manager. During their briefing to new arrivals on station or ship, the SL/VL should instruct people to report the discovery of unusual numbers of sick or dead animals to them immediately.

2.2 Equipment

A response kit has been provided to each station and ship. It contains the equipment necessary to record an event, undertake post-mortem examinations and prepare samples for transport and subsequent analysis, together with instructions on procedures and safety precautions. Further information about the kit is included in **Appendix 1.**

Station Leaders hold the key to the kits on station. On occasion, stocktakes will be requested by the Polar Medical Unit (PMU) to ensure that the kit is complete and the equipment is in good order. The PMU is responsible for ordering replacement stock.

[1] Kerry K, Riddle M, Clarke J (1999) Diseases of Antarctic Wildlife. Report for SCAR and COMNAP 1999.104pp

The equipment is stored in a clearly labelled, sealed yellow box in the following locations on stations:

Station	Storage Location
Casey	Green Store – racking in the warm store section near the medical fire stock.
Davis	Flammable Liquids Store (Chemicals section)
Macquarie Island	Obsolete darkroom in the Science Building (now being used for storage)
Mawson	Green Shed among the field equipment items

On ships, the kits are to be stowed in the Deputy Voyage Leader container. All kits must have appropriate dangerous goods labelling on the outside. Any goods inside that could spill or leak should be packed properly, e.g., in a sealed plastic bag with absorbent material.

2.3 Permits

In general, permits are required to collect specimens of animals in the Antarctic. A standing permit for collection of biological samples has been issued to the Chief Scientist for emergency purposes.

A permit to import samples to Australia is required under the *Quarantine Act 1908*.

2.4 Funding

Expenditure on equipment, analysis or consultants must be approved in advance by the AAD, and funds will be provided from the Director's contingency budget.

3.0 IMMEDIATE RESPONSE

3.1 On discovery

Upon discovery of unusually high numbers of sick or dead animals:

- withdraw from immediate area;
- be aware of biohazard risks to both human and wildlife health;
- restrict access to the site to reduce the risk of transferring pathogens to uninfected populations;
- do not visit other colonies or sites with aggregations of animals without taking precautions to prevent transfer of pathogens on boots, clothes and equipment;
- immediately notify the SL, FL or VL that unusual numbers of dead or dying animals have been discovered. The Leader will refer to this response plan and provide instructions to the field party;
- on Macquarie Island, immediately notify the TASPAWS Ranger on site; and
- notify the Antarctic Medical Practitioner and/or any veterinarian/s on station or on board ship.

3.2 Criteria for determining an unusual mortality event

The decision on what constitutes unusual numbers of sick or dead animals can be made only on the basis of experience of what is normal for the species and location. If you discover dead or dying animals in numbers, or in a condition which, in your experience, is unusual, then report it.

For animals and birds, unusual behaviour that may indicate presence of disease includes:

- staggering, falling, paralysis, inability to rise or disinclination to move, lameness or trauma when approached;
- coughing, sneezing, excessive nasal discharge or respiratory distress (note that panting is a normal response of penguins in warm weather);
- ocular discharge, apparent blindness;
- diarrhoea or bloody and fetid (fowl smelling) faeces; and/or
- Skin / feather / fur lesions or abnormalities (note normal patterns of moult), bald patches that could indicate feather loss disease.

3.3 Initial data collection

Sufficient data should be collected initially to provide animal experts with information to determine whether something unusual has been discovered.

Do not walk among the sick or dead animals. Stand back, view widely and record the following information from the periphery of the group of animals:

- location including coordinates (use a GPS if available);
- area affected;
- species involved and whether adults or chicks/pups and sex ratio if identifiable;
- indication of the number of animals involved - if possible count the dead and dying and estimate the percentage of each among the colony;
- clinical signs;
- contact details of all people who were at the site;
- weather conditions now and, if known, over the previous week; and
- take lots of photographs and video.

Call the SL, FL or VL by radio again and report the information gathered during the initial data collection.

3.4 Hygiene

Boots and any other clothing or gear which may have had contact with the potentially contaminated substrate must be cleaned using sea water or snow before leaving the general area of the dead or dying animals. Repeated washing in sea-water will remove most pathogens.

The SL, FL or VL should arrange for boots, clothing, equipment and vehicles to be sanitised on return of the field party. Boots and equipment should be scrubbed in a 2% w/v solution (20g/litre) of Virkon, F10 or similar agent included in the response kit. Household bleach in a 10% solution is also effective.

3.5 Notification

The field party must inform the SL or VL as soon as they suspect they have discovered unusual numbers of sick or dying animals, and again when the initial data collection has been completed.

The SL, FL or VL will:

- advise the Antarctic Medical Practitioner at station or on board ship;
- advise personnel that access to the area is restricted to those authorised to investigate the event;

- inform the Support and Coordination Manager at the AAD headquarters at Kingston, and send the information from the initial data collection and the names of people on station who have relevant skills.; and
- lodge an incident report on the Incident Reporting System on the intranet as soon as practicable.

The incident report will automatically go to the Environmental Manager who will ensure that the following people are notified if they are not already aware of the incident:

- Director, AAD
- General Manager, Support and Operations, AAD
- Chief Medical Officer, AAD
- Chief Scientist, AAD
- AAD representative to the Australian Wildlife Health Network, who will report the incident to the **Emergency Animal Diseases Hotline on 1800 675 888.**

3.6 Preparation for further investigation

On notification of the incident, the Station Leader and/or their delegate should open the response kit in anticipation that further investigation may be required. The Station Leader is to determine on station medical/veterinary skills available to assist in any required sample collection.

Before proceeding with further investigation, advice will be sought from the AAD representative on the AWHN, or the Animal Health Centre in Geelong, or, in the instance of an event on Macquarie Island, the CVO Tasmania as to whether the event is 'significant' and requires further investigation.

4.0 ADMINISTRATION (INCIDENT CONTROL STRUCTURE)

4.1 Response team

The Director shall form a response team to determine whether the mortality is in fact unusual or may involve disease, and if so, will direct any further action that might be required.

The response team should include the following expertise and responsibilities:

- Convener – Operational responsibility for management of the response
- Advisor – Station or ship operations (as appropriate)
- Advisor – Environmental management
- Advisor – Human health / disease implications
- Advisor – Permits
- Advisor – Quarantine implications
- Advisor – Occupational health and safety aspects
- Advisor – Animal ecology
- Advisor – Veterinary aspects and pathology.
- Advisor – Treaty and Government, including Ministerial briefs and information to ATCM and other Parties

Expertise across several of these areas may reside in a single person, in which case the overall size of the response team will be reduced. The Australian Wildlife Health Network will be contacted to determine scientific/epidemiological/technical expertise available to assist in the response. In the instance of an event on Macquarie Island, the CVO Tasmania will be contacted.

4.2 Assessment for further investigation

The convenor of the response team will determine whether further investigation is required on the basis of the information provided by the station/ship and any other information available. The response team may decide that:

- the number or nature of sick or dead animals is not unusual and that no further action is required;
- it is uncertain whether the event is unusual and that the population should be observed, without sampling, to determine whether more animals are affected, or whether the symptoms worsen;
- the event is unusual and that further investigation, including sampling, is required.

Responsibility for further investigation of the incident in Antarctica will be assigned to the person on site with the most appropriate expertise, such as veterinary science, pathology, microbiology.

The priorities for further investigation are to determine the range of species affected, geographic extent of the event and to determine the cause.

4.3 Coordination of further investigation

The Director will assume overall responsibility for ensuring the event is managed correctly. This responsibility may be delegated to the Convener of the response team.

The SL, FL or VL will assume responsibility for ensuring the incident is managed correctly in Antarctica and should coordinate the response in the field. The Leader should liaise with the Medical Officer and other people with appropriate skills on station/ship. On Macquarie Island, the TASPAWS ranger in charge will assume responsibility for coordinating the response in the field.

4.4 Health and Safety

The Advisor – Human Health / Disease Implications will determine risks and mitigation techniques to prevent transmission of disease to humans.

The Antarctic Medical Practitioner on station or on board ship should ensure that all personnel who may come in contact with affected animals are aware of the need for appropriate PPE / hygiene practices, and should provide instruction on the contents and use of the response kit.

4.5 Communication

The SL, FL or VL is responsible for informing their personnel of the possibility of a disease event. The Convener will liaise with all advisors to determine required statutory notifications that need to be undertaken.

Do not contact the media. Refer all media queries to the AAD Media Liaison Officer. Dissemination of information to the public about the event is to be in accordance with the AAD Media Policy. Any external communication by expeditioners will require authorisation by the SL or VL.

The Director, AAD, will determine whether other national operators or other organisations need be informed, and will arrange for information to be passed on if required.

An Animal Unusual Mass Mortality Incident Report Form template is provided at **Appendix 2**.

4.6 Station/voyage and field personnel

Station, voyage and field personnel should be warned about potential human health risks. People in the field may be called on to determine the geographic extent of the event, but must be cautioned about the risk of transferring disease causing agents on boots and clothing when moving between locations. All personnel should be advised when appropriate PPE and hygiene precautions such as boot washing are required and of the procedures for doing this.

4.7 Legislation and permits

Before authorising sampling, the convenor of the response team is responsible for ensuring that all permits required under various acts of legislation (Section 2.3) are in place and up to date. All investigative activities, such as sampling, must be consistent with the conditions of permits. The AAD holds a permit under ATEP which allows for opportunistic collection and sampling of dead fauna. The amended EPBC Act recognises permits issued under ATEP for the purposes of EPBC listed threatened, migratory and marine species, except cetaceans.

5.0 CONTAINMENT

5.1 Site access

Access to the site should be restricted to reduce the risk of transferring pathogens to uninfected populations. Do not take vehicles close to a colony or aggregation that includes sick or dead animals. Park vehicles at least 250m from the aggregation to reduce the chance of faeces, blood or other organic matter being trapped in vehicle treads and spread to other locations. Access routes may be specified to minimise disease spread.

5.2 Removal of site access restrictions

Restrictions on site access will remain in force until the SL, FL or VL is advised that normal access may be resumed by the Director.

5.3 Disposable overshoes and protective clothing

Disposable overshoes and personal protective equipment (PPE) are provided in the response kit and should be worn when visiting colonies or aggregations during the event. On leaving the colony, overshoes should be removed at least 250m from the edge of the aggregation and then should be sealed in plastic bin liners for disposal in the station/ship incinerator. Overalls, masks etc. should be disposed of in a similar manner.

Movement between discrete aggregations of wildlife must be strictly controlled/curtailed. Protective clothing, including overshoes, must be changed before moving between discrete aggregations of wildlife, i.e. between colonies on separate islands or between colonies separated by at least 1000m of unoccupied ground.

5.4 Precautions during authorised sampling

All possible precautions are to be taken by people handling affected animals to avoid the geographical spread of disease to other colonies. If dead animals are seen on the sea-ice while travelling to a colony, they should be collected on the return trip to minimise the risk of introducing or spreading disease within the colony. As far as practicable, the shortest route from the edge of an aggregation of animals is to be taken when collecting dead specimens. Only authorised people are permitted to approach and handle affected animals.

6.0 NECROPSY AND TISSUE COLLECTION

Protocols for examination and dissection, tissue collection, sample preservation and storage are contained in **Appendix 1.**

7.0 POST-EVENT ACTIVITIES

7.1 Post-event debrief

Post event debriefs should be held at the station/ship and at AAD headquarters at Kingston.

The station/ship debrief should include:

- A full account of the discovery.
- Collation of an event timetable with details of response actions implemented.
- Report on contents of response kit, including items to be restocked, items that could be improved and new items that would improve the kit.
- Suggestions for improvements to the response plan.
- Collation of information on samples, photographs etc. including number, location, storage requirements etc.
- A record of the people responsible for ensuring all samples, photographs and records are transferred to the AAD.

AAD debrief at Kingston should include:

- Review of response to the event.
- The effectiveness of the incident reporting process.
- Collation of information on samples, photographs and records.
- Review of the response plan.
- Review of response kit.
- Initiate restocking of response kit.
- Outline of communication strategy.
- Summary of information provided to the Minister, Antarctic Treaty Consultative Meetings, other Treaty Parties, national operators and other organisations (e.g. International Association of Antarctic Tour Operators (IAATO)).
- Review of relevant parts of training programs.

This could be facilitated by a professional organisation experienced in disease outbreak investigation and management.

A final written report should be produced that is endorsed by the Division. It should be appropriately filed at the Division such that it can be rapidly accessed in future. Over time this will build a useful picture of disease incidents in the AAT, and act as a valuable resource to improve decision making and management. A summary of the disease event should also be provided to the National Wildlife Health Information System at a level of accessibility determined by the Director of the Division.

7.2 Reporting

SL, FL or VL

The following reports will be transmitted by the SL, FL or VL to the Support and Coordination Manager at AAD Headquarters, Kingston:

- Report by the people discovering the unusual mortality event to provide details of circumstances in the field including the initial data collection.
- Report by the person responsible for further investigation to provide details of further investigation, and samples including location and storage requirements.
- Report by the Station/Voyage/Antarctic Medical Practitioner to provide details of Occupational Health and Safety aspects and response kit restocking requirements.
- Report by the SL/VL to provide details of station/ship response and collation of all the above reports from station/ship personnel.

The reports will be passed to the convenor of the response team.

Convener of the Response Team

The convenor of the response team will prepare a report for the AAD Director to include:

- Collated report on the incident and response from the SL/VL.
- Review of the event and possible causes.
- Review of response to the event.
- Collated information on samples, photographs and records including location and storage requirements
- Post-activity reports where required by the ATEP or EPBC acts.
- Review of the response plan.
- Review of response kit.
- Schedule for restocking of response kit.
- Publication strategy.
- Summary of information provided to Minister, ATCM, other Treaty Parties, national operators and other organisations (e.g. IAATO).

The Convener will table the report of the response team at the next AAD Executive meeting and provide a copy to the Permits Officer in accordance with any permit requirements.

The Convener will also arrange preparation and distribution of reports as required by any external organisations.

7.3 Follow-up activities and stand-down

Restrictions on site access will remain in force until the SL/VL is directed to resume normal access by the Director. The convenor of the response team will advise the Director on the appropriate time to resume normal site access.

The response team shall direct follow-up activities until told to stand-down by the Director.

7.4 Access to tissue samples

The response team will determine the policy on access to tissue samples for each event. If an infectious disease is suspected, samples may be required to be lodged in a secure facility such as the Australian Animal Health Laboratory in Geelong. They will provide instructions on packaging samples for transport.

7.5 Post-event monitoring

The response team will determine whether post-event monitoring is required, and if so, will identify who is responsible for monitoring design and implementation.

8.0 Review of this plan

This plan will be reviewed every four years by the AAD Environmental Manager, the Chief Medical Officer, the Program Leader of the Terrestrial and Nearshore Environment Program and if possible, a representative of the Australia Wildlife Health Network.

Appendix 1
to: Unusual Animal Mortality Response Plan 2019–22

Unusual Mortality Investigation Kits and Necropsy Tissue Collection Techniques

A copy of the **Wildlife Health Investigation Manual (Rose, K. 2007)** provided by the Australian Registry of Wildlife Health, published by the Zoological Parks Board of NSW, has been provided to the Antarctic Medical Practioners at each of the four stations to be kept in the Surgery Library.

Unusual Animal Mortality Investigation Kits have been deployed to each of the AAD Stations, and for major field expeditions at locations where they are likely to encounter significant wildlife populations. These kits are to be checked in June every two years. The PMU of the AAD will request a stocktake at that time and any deficiencies or necessary reordering of equipment will be undertaken in time for the coming season's resupply of stations.

The kits contain equipment and protocols for the collection of information and biological samples required for the investigation of a suspected disease outbreak in Antarctic birds. All protocols are based on the CEMP[2] Standard Methods, Section 6: Protocols for collection of samples for pathological analysis in the event of disease being expected among monitored species of birds.

Equipment is provided for obtaining biological samples from up to 100 affected live birds, and/or up to 20 carcasses.

Note: The instructions below focus on the examination of birds / penguins. If the disease outbreak concerns other animals, then advice should be sought for the Response Team about appropriate techniques from the **Emergency Animal Diseases Hotline on 1800 675 888**.

Note: While birds may die of non-infectious causes, adult birds are rarely found dead in a colony. The presence of an infectious agent should always be assumed when an unexplained mass death is observed**.**

HUMAN HEALTH AND THE PREVENTION OF SPREAD OF DISEASE

ENSURE NO DANGER – BE ALERT TO BIOZHAZARDS- ASSESS HAZARDS AND RISKS BEFORE PROCEEDING

Before the investigation of any suspected disease outbreak begins, it is most important that measures are taken to mitigate any risks to human health and to prevent any spread of disease.

Human Health

A number of avian diseases, some of which have been recorded in Antarctic birds, are contagious to humans and some can cause serious disease (e.g. *Chlamydia* spp. (Psittacosis), *Salmonella* spp., and avian influenza). On the other hand, a number of pathogenic and non-pathogenic organisms carried by humans can cause disease in birds. Diseased or environmentally-stressed birds would be more susceptible to such pathogens. Therefore, all care should be taken to prevent the transfer and spread of disease between humans and birds. The precautions listed below should be taken when visiting sites of suspected disease outbreak and/or obtaining samples from affected animals:

- wear disposable rubber gloves or disinfect outer gloves after handling ill or dead birds;
- wear disposable protective clothing or change and wash outer clothing in disinfectant after handling ill or dead birds;
- wear a N95 PPE surgical mask if handling birds in a poorly-ventilated room or if the person in close contact with birds has a respiratory infection;

[2] CCAMLR Ecosystem Monitoring Program

- cuts and scratches on personnel should be treated with disinfectant as soon as possible;
- observe sensible hygiene measures at all times, e.g. frequent hand-washing (or use hand-wipes when in the field);
- all waste from sample collection should be bagged and disposed of by high temperature incineration; and
- advice regarding carcase disposal will be provided by AAD Head Office, Kingston.

Protective equipment provided:
• disposable overalls — Large and X-large, 10 pairs (5 of each size) • disposable gloves — Large so can be worn over other gloves for warmth • disposable N95 PPE surgical masks • 1 box disinfectant hand-wipes

Prevention of the Spread of Disease

The potential to spread disease from colony to colony by human activity is ever present, and the consequences of this may be devastating. Bacteria and viruses can survive at low temperatures and some are extremely resistant to adverse conditions. Pathogens can be spread mechanically by adhering to clothing, equipment and vehicles. While it is important to establish how widespread the suspected disease outbreak is (see Epidemiological Information section below), it is critical not to spread pathogens.

- Visits to other colonies for sampling or recording activities should not occur unless measures to reduce microbial contamination, such as cleaning and disinfection, of equipment and clothing have been carried out. Cleaning of boots is especially important, and disposable overshoes should be worn when in infected areas.
- Vehicles should be kept well away from potentially infected areas.
- Restrictions on movement of non-essential personnel in the area of the outbreak or around other concentrations of animals should be considered.

Equipment provided:
• disposable overshoes • bleach - for washing of clothes between locations. Include instructions for use • Virkon/F10 - for sterilizing boots and equipment between locations. Include instructions for use

THE DISEASE INVESTIGATION

The complete investigation of a suspected disease outbreak requires the collection of both epidemiological information and biological or pathological samples. It is not expected that the cause of the outbreak will be established at the time, as follow-up microbiological, virological and pathological analysis will be required on return of the samples to Australia.

EPIDEMIOLOGICAL INFORMATION

In any suspected disease outbreak detailed information on the species involved, signs and symptoms observed and the extent of spread is vital both for determining the cause, and in understanding the implications of the outbreak on the animal population. Information on environmental conditions is also important. While a parasitic or microbial pathogen actually causes disease, other factors such as stress, starvation, excessive predation, disturbance by humans, inclement weather, etc. can contribute to the

outbreak of disease or the death of a bird. Such data needs to be recorded in addition to the collection of pathological samples and carcasses.

It is important therefore to record such factors as:

- number of ill and dead birds at each affected location, the position of affected birds in the colony, proportion of birds which recover or clinically unaffected.
- description of any observed symptoms of the disease: clinical symptoms may or may not be apparent. If apparent, these could include: discharge from the eyes or mouth, coughing, sneezing, laboured breathing, nervous signs, tremors, convulsions and diarrhoea, skin lesions;
- demographic factors of affected birds: species, sex, age (adult/chick), reproductive status, stage of breeding cycle, colony size;
- extent of spread of the suspected outbreak: are other colonies in the general area affected (note: measures to prevent spread of disease discussed above must be undertaken before visiting each new location);
- environmental factors: location, weather, time, date, geography of the colony, human access and intervention, presence and activity of predators.

Detailed records supported by colour photographs or video will greatly assist the investigation. Where available, a GPS can be helpful for supplying accurate location data.

Recording equipment provided:
• notebooks (1 field, 1 laboratory) • pencils, marker pens • counters (for population counting) • Stock spray - to be used sparingly only if necessary to help keep track of numbers to prevent multiple counting. This should not be undertaken if it will cause more disturbance to already stressed animals. Photography and estimation are a better options.

COLLECTION OF BIOLOGICAL SAMPLES

Biological samples that should be collected from birds in affected colonies include:

- serum for antibody analysis
- swabs for bacterial and viral isolation
- ectoparasites (if observed) and blood smears for haematozoan identification
- eggs (where death is occurring among adult birds at laying time, abandoned eggs, or in the event of large numbers of hatching failures.)
- carcasses for post-mortem (in serious outbreaks when many birds are sick and dying.)

SAMPLING PROTOCOLS

The following protocols describe collection and storage of samples. All sampling equipment needed is supplied in the kit, though freezers (-18°C to -80°C) and/or liquid nitrogen may be required for the storage of samples. At all stages of the investigation, detailed labelling and recording of specimens collected is of the utmost importance to ensure all information is available to assist eventual diagnosis.

Collection of Blood for Serology

An antibody titre indicates that a bird has been in contact with a specific disease at some stage. The level of the antibody titre can indicate whether there has been recent contact or active infection. Recently infected birds have the highest antibody titres. Genomics analysis of blood samples can assist in the detection of novel viruses and bacterial pathogens from blood, but need to be stored appropriately. Birds displaying a range of symptoms as well as birds showing no evidence of disease should be sampled.

- Using aseptic techniques collect up to 2ml (small birds) or 5ml (penguins) of blood from the brachial, tibial or jugular vein of each bird into a sterile blood collection tube. and DNA/RNA blood collection tube for genomics analysis. (Avoid clotting of the blood during collection by obtaining a good blood flow. Avoid freezing of the blood during collection by performing venipuncture protected from the wind).
- Keep the blood collection tubes warm (e.g. on the inside of your jacket or in an Esky with a hot-water bottle), and stand overnight in warm conditions to encourage clotting. Avoid freezing as this will lyse the red blood cells and discolour the serum.
- Pipette off the serum with sterile plastic transfer pipettes. Avoid contamination with cell fraction. Blood clots can be spun down by centrifugation to obtain more serum (2000rpm for 10 minutes).
- Samples stored in DNA/RNA shield need to be stored at -80°C within 5 days of collection.
- Store serum in labelled cryotubes. Label tube with identification number of the bird and date of collection with a water-proof marker pen.
- Freeze serum at -18°C to -80°C.
- Prepare a full inventory of the specimens collected in laboratory notebook. Along with identification number of the bird, include place and date of collection, species, chick or adult, sex if known and who collected the specimen.

Equipment provided for serum collection from 100 birds:
• 1 box (100) 5ml syringes • 1 box (100) 23g needles - for obtaining blood samples from small birds • 1 box (100) 21g needles - for obtaining blood samples from adult penguins • 100 blood collection tubes • 100 DNA/RNA Shield blood collection tubes • 100 sterile transfer pipettes • 100 cryotubes (Nunc tubes) • 2 needle disposal containers • 1 rack for blood tubes (foam) • 1 rack for Nunc tubes (box)

Collection of Swabs for Bacterial and Viral Isolation / Genomics Analysis

Relevant wildlife experts will be consulted on the appropriate technique for collecting swabs from particular species.

Swabs of faecal material from the cloaca should be taken routinely. Where respiratory symptoms are observed, swabs should be taken from the trachea or palatine fissure. Swabs should also be taken from any scabby lesions observed on unfeathered regions or in the beak. Swabs can be taken while birds are restrained for blood collection.

- Collect faecal (or other) material on 3 sterile swabs.
- Place each swab in a sterile cryotube containing approx. 1ml of either bacterial transport media, viral transport media or DNA/RNA shield (or similarly approved product) (plastic stems of swabs can be easily broken to fit in tubes). Label tube with identification number of the bird and date of collection and sample type (i.e. viral or bacterial) with a water- proof marker pen.
- After collection keep swabs chilled and freeze as soon as possible at -80°C or in liquid nitrogen.
- Prepare a full inventory of the specimens collected in laboratory notebook. Along with identification number of the bird include place and date of collection, species, chick or adult, sex if known and who collected the specimen.

Viral transport media: Brain Heart Infusion Broth (BHIB) with penicillin and streptomycin. BHIB can be stored at room temperature, but once antibiotic solution is added should be stored at 4°C.

Bacterial transport media: (can be used for *Mycoplasma* spp and *Chlamydia spp* isolation). Brain Heart Infusion Broth (BHIB). Can be stored at room temperature.

To make BHIB: Dissolve contents of 1 BHIB sachet in 1L of sterile water. Dispense into 2 500ml plastic autoclave bottle and sterilize by autoclaving. Use one bottle as bacterial transport media. To the other bottle, when cooled, add 2.5ml of antibiotic solution. Once antibiotic solution is added, the bottle should be stored at 4°C. Dispense 1ml into cryotybes with sterile plastic transfer pipettes.

DNA/RNA preservation media: DNA/RNA Shield purchased from Integrated Sciences. Samples should be stored in <10% v/v ratio.
To make antibiotic solution: Dissolve 1 vial (600mg) penicillin and 1 vial (1g) streptomycin in 10ml sterile water. Store frozen until added to media.

Equipment provided (for collection of swabs from 100 birds):
300 sterile swabs
300 cryotubes
5 sterile plastic pipettes for transfer of 1ml transport medium to cryotubes
1 sachet BHIB powder (to make 1litre media)
1 litre DNA/RNA shield media (R100-1L)
1 litre sterile water
10ml sterile water in tube for antibiotics
1 vial (600mg) penicillin
1 vial (1g) streptomycin
2 500ml autoclave bottles
1 box for transporting cryotubes in field

Collection and Preservation of Ectoparasites

Relevant wildlife experts will be consulted on the appropriate technique for removing ectoparasites and collecting skin scrapings.

Lice and ticks are usually found where they cannot be removed by preening, e.g. under the beak, in the ear canals, on top of the head and along the back. The brood patch also may provide a possible site for ectoparasites. If any ectoparasites are observed:

- Take skin scrapings of scaly areas, in the centre and on the edges of the lesion.
- Preserve specimens in a 70% ethanol/5% glycerol solution. A few ml in a cryotube is enough.
- Label each container with animal number and date.
- Prepare a full inventory of the specimens collected including information on place of collection, date, species, approximate age of the bird and sex if known and who collected the sample.

Equipment provided:
• 1 pair of forceps • 20 sample collection tubes - cryotubes • 70% ethanol • 5ml glycerol

Identification of Haemoparasites

Haemoparasites are transmitted by ectoparasites and can be identified in blood smears of heparinized blood. When ectoparasites are found widespread on birds throughout a colony, infection with haemoparasites should be suspected and blood smears made. The smears can be stored indefinitely and examined at a later date.

- Add one drop heparin to small amount of fresh unclotted blood in a separate tube. Mix.
- Make a thin blood smear on a microscope slide. This is done by placing one drop of blood on one end of the slide and "dragging" it along the length of the slide using the edge of a second slide. This may take some practice.
- Label slide in pencil on frosted end with animal identification number
- Allow to dry in air. Avoid blowing heated air on the slide.
- Fix by immersing slide in 100% methanol for 5 minutes.
- Allow to air day and store in a dry, dark place.
- Prepare a full inventory in laboratory notebook of the specimens collected with details of the collection site, time, species, and identification number of the bird, age, sex if known and who collected the specimen.

Equipment provided:
• 1 box glass slides • Heparin • 100% methanol

Egg Collection

Several bacterial and viral diseases can affect the viability of the chick in the egg. Eggs should be collected when deaths occur among adult birds during egg incubation, resulting in abandoned eggs, or in the event of unusually large numbers of hatching failures.

- Collect fresh or abandoned eggs.
- Label each egg on the shell in pencil with sample number and collection information (where collected, when and by whom).
- Store frozen at -80°C, if possible, or -18°C.
- Prepare a full inventory of the specimens collected.

POST-MORTEM EXAMINATION

A post-mortem involves the systematic examination of the entire body, including internal organs, and the collection of samples. Avoid microbial contamination of organs and ensure that all organs are examined.

Specimens collected for post-mortem should represent a range of ages, sexes (if known) and have exhibited a range of symptoms of disease before they die or are euthanised. Carcasses should be as fresh as possible, as post-mortem changes reduce the quality of histological and microbiological analysis. While carcasses which have not been frozen are preferred, post-mortem can be conducted on thawed specimens. Carcasses which are found frozen should be maintained that way and only allowed to thaw immediately prior to the post-mortem examination.

At this stage it is important to note that performing post-mortems is not an immediate priority in a suspected disease investigation. While not ideal, post-mortems can be conducted on frozen carcasses on return to Australia. Detailed descriptions for both the storage of carcasses and post-mortem examinations (dissection procedures and sample collection) are given below. Post-mortems should only be undertaken if personnel are confident with the techniques required.

Collection and Preservation of Carcasses for Later Post-Mortem

- Collect a minimum of five birds.
- Each carcass should be labelled with a paper tag attached by string to the leg. Record, in pencil, sample number and all collection information.
- Wrap each carcass separately in a plastic garbage bag.

- Freeze (-18 to -80°C) as soon as possible.
- Prepare a full inventory of the specimens collected including sample number of each carcass, its sex if known, age, from where it was collected, (including position in a specific colony), when, by whom and any symptoms observed prior to death.

Equipment:
• plastic garbage bags • tags (20) • string (20)

Post-Mortem Examination

If authorised post-mortem examinations are undertaken in Antarctica, they are best conducted in a clean, comfortable environment as a complete, detailed pathological assessment of a bird can take several hours.

Detailed records of all observations made and samples taken throughout a post-mortem examination are vital. Progressively taken colour photographs, particularly before the tissues are collected, will also greatly assist the investigation. Description on audio tape and video may also be of value.

Health Precautions

Before beginning a post-mortem examination, it is very important that all precautions are taken to ensure the health of all personnel participating:

- wear disposable rubber gloves;
- wear a disposable N95 surgical mask as minimum;
- wear full disposable protective clothing;
- open the bird in a well-ventilated room or area, but not in windy conditions;
- cuts and scratches on personnel should be treated with disinfectants as soon as possible;
- be aware of the occupational and safety measures applying to the use of formalin;
- on completion of the examination, all equipment used should be sterilized, bagged, and disposed of by incineration.

Sample Collection and Preservation

During a post-mortem examination, tissue samples should be routinely collected from all major organs of each dead bird. This includes intestine, pancreas, liver, kidneys, spleen, lung, heart, brain, bone and the thymus, and bursa in chicks. Sections taken should include any observed macroscopic lesions (e.g. white spots on the liver). Samples from each site should be preserved both for histopathology and microbiology /toxicology by the storage methods described below. Tissue samples should also be taken from any abnormal lesions observed in other organs.

Histopathology samples

- Use a scalpel blade to remove tissue samples.
- Cut pieces of tissue no greater than 1 cm^3.
- Place in 10% buffered formalin. Organs for histology from a single carcass can be placed together in one jar as long as the volume of 10% formalin is 10x the total tissue volume. Each jar should be labelled with the type of tissue, the number/identity of the bird, the date and name of the person who collected it in water-resistant pen. A paper label with all information recorded in pencil should also be included in each jar.
- After 2–3 days, fixed tissue can be transferred to another jar containing only a small amount of formalin to keep the sample moist. The tissue samples must not be allowed to dry out. Label as for above.
- Do not let fixed tissue freeze.

- Prepare a full inventory of the specimens collected.

Microbiology /toxicology samples

- Use a scalpel blade to remove tissue samples.
- Place samples in Sterile Whirl-Pak bags. Label the outside of bag with the type of tissue, the number/identity of the bird, the date and the name of the person who collected it in water-resistant pen.
- Seal bags, freeze and store at -80°C.
- Prepare a full inventory of the specimens collected.

Post-Mortem Procedure

Before beginning the examination:

- Wet bird in warm running water. Use detergent if bird is soiled.
- If the carcass is frozen, let it defrost first.
- Place bird on its back on a well-lit dissection board or easily accessible, flat surface covered with a disposable layer, such as paper or plastic.

The examination should then begin with an external examination of the carcass. Commence with palpation to feel for broken bones or any other abnormalities. Rigor mortis may hinder detection of this. Injury and other lesions (e.g. tumours, lumps, areas of feather loss and discharges) should be described. The description should include colour, consistency and size. A systematic examination of the bird should be followed as suggested below:

- measure body weight;
- morphometric assessment – beak length and depth, wing and mid-toe lengths;
- integument – condition of the skin and plumage, signs of trauma, scabby lesions of the skin, look for external parasites;
- head – eyes, nares, beak, oral cavity and ears, look for discharges, note colour and consistency, colour of mucous membrane – lesions in the mouth, swellings;
- neck – swellings and any injuries;
- body condition – fat, normal, emaciated, dehydrated;
- abdomen – distension indicates that the bird has fed recently; flat indicates that the bird has not fed recently;
- brood patch – presence of scabby lesions; red and vascular as seen when the bird is brooding;
- vent – cloaca: soiled or caked-up; diarrhoea, blood, colour of excreta;
- preen gland (above the base of the tail);
- •wings – injuries, deformities; and
- legs – injuries, deformities.

All observations should be recorded. Swabs should be taken from any lesions seen and stored for bacterial and viral isolation as described in previous sections. Any ectoparasites found should be preserved as previously described.

Dissection technique:

- Place bird on its back. Support may be needed either side of the bird to keep it upright. Dislocate hips in all birds except penguins, if necessary.
- Part the feathers and make a skin incision over the sternum or keel. Extend incision to the midline of the beak and to the vent, taking care not to cut through the abdominal wall. Peel skin back with fingers until the neck, all the chest (pectoral) and abdominal muscles are exposed, and extend down the thighs and legs where possible. This is necessary to avoid contamination of the abdominal and thoracic cavities with feathers. Extreme care is needed in small birds and birds which have been dead for some time as pressure can rupture the abdominal musculature.

Make a subjective assessment of the bulk and the colour of the pectoral muscle mass. A score can be assigned using the following guide*:*

1 -profoundly atrophic breast muscle, paper thin to near transparency, prominent keel (cachexia).

2-mildly atrophic, keel prominent

3 -muscling adequate, keel can be felt protruding above thickness of breast muscle, but not markedly so

4 - breast muscle full, the same thickness as the keel

5 -breast muscle very full, raised convex surface, indentation where it joins the keel

Make a subjective assessment of the amount of subcutaneous and intra-abdominal fat present. A measurement of the thickness of the subcutaneous fat layer in the same position on each carcass may help.

Note and describe any haemorrhage in the muscle and under the skin. Haemorrhages can appear as red spots, splashes or bruises.

- Cut the pectoral muscles with a scalpel blade along either side of the sternum, across the surface of the ribs. Use bone cutters or sturdy scissors, depending on the size and maturity of the bird, to cut through the sternal ribs and lever the sternum up to expose the thoracic and anterior abdominal contents. This exposes the air sacs. Cut through the clavicles to remove the sternum. Alternatively, to maximise exposure of the thoracic cavity, most of the ribcage can be removed by cutting across the ribs as dorsal as possible. This will disturb the air sacs but examination of them is still possible.

  Air sacs are transparent membrane sacs located in the thorax and abdomen and should contain no fluid. Note the presence of any fluid, its colour and consistency. Take swabs of fluid or a sample for freezing. Abnormalities in the membrane thickness and transparency of the air sac wall should also be recorded.

  Record the colour and consistency of fluid and other unusual material in the thoracic cavity. Take swab or a sample. Store frozen.

- Open the abdominal cavity with scissors, cutting along the midline and the posterior border of the thoracic cavity while holding up the abdominal wall with rat-tooth forceps. Care must be taken not to pierce the gall bladder, liver or intestinal tract. Fold back the abdominal muscles so that the abdominal contents are exposed.

  Note the colour of liver, size of the gall bladder. If any fluid is present in the abdominal cavity note quantity, colour and consistency. Take sample of fluid and store frozen. Note the presence or absence of food in the stomach and intestine.

- Open the trachea and syrinx.

  Note the presence of any fluid, froth, its consistency and colour in the lumen of the trachea. Take sample or swab and store frozen.

- Cut through the right mandible and hyoid apparatus and open the oral cavity. Open the oesophagus. Examine the tongue, palatine fissure, oropharynx, glottis, larynx and thymus (in young birds).

  Look for evidence of swelling, discharges, discolouration, lesions, etc. Take samples and swabs where applicable.

- Examine the pericardial sac – the membranous sac containing the heart. The sac is normally translucent and has a shiny surface. Remove the heart and the liver.

  Record any thickening of the pericardial membrane, the presence of any fluid and or material in the pericardial sac. Take samples for freezing.

  Examine heart and liver and take samples.

- To remove the gastrointestinal tract, transect the oesophagus between two ties which occlude the lumen, low in the thoracic cavity and the large intestine, close to the cloaca. The intestine can

be lifted out while gently breaking the mesentery and suspensory ligaments. Care should be taken not to rupture the gall bladder as the bile will discolour tissues.

After removal from the abdominal cavity samples can be taken from the stomach, intestines (3 or 4 sections from different parts along the full length), pancreas and spleen (which will come away attached to the upper intestinal tract). In the case of small birds leave the intestines coiled, make sections across the coil and fix; this avoids handling the delicate tissue.

The intestinal tract is then opened from the stomach to the cloaca to investigate for parasites. Stomach contents should be collected and fixed in 70% alcohol. Any endoparasites (round and tape worms, and flukes) found should be collected and preserved. Gently remove parasites and wash off excess intestinal contents. Fix in 10% formal saline in a small tube for later examination.

Note - care should be taken to avoid contaminating other organs with intestinal contents.

- The kidneys and gonads can be observed along the dorsal side of abdominal cavity after the removal of the gastrointestinal tract. Remove and examine.

  Describe any lesions. Take samples of kidneys and gonads if required.

- Remove the lungs from the dorsal thoracic cavity. Inspect the ribs for fractures.
  Describe any tumours or other lesions in the lungs and take samples.

- In young birds remove the bursa of Fabricius - a blind, pale sac located near the vent.
  Examine and take samples.

- Skin the head before removing the brain. The head can be removed from the neck at this stage. Cut through the skull using scissors or bone cutters. The brain should be removed with minimal handling. Drop the brain out under gravity, tipping in an anterior to posterior direction.

  Examine and take samples. Take cross section of entire brain for histology.

- Open wing and leg joints.

  The joint fluid is normally clear and the cartilage white and smooth. Take swabs of fluid or material in any joint that is not clear. Bone marrow can be obtained from the medullary cavities of the femur and tibia or a rib. Collect sample and store frozen.

Equipment provided:

- forceps - 2 plain
- 2 rap-toothed bone cutters
- 1 pair bone saw or small hacksaw
- 2 x surgical scissors
- disposable scalpels with plastic handles
- sharps container
- ruler
- garbage bags
- tape measure
- jars of various sizes
- Whirlpak bags
- 10% buffered formal saline swabs

If in doubt at any time, contact the CVO Tasmania for events on Macquarie Island, and the **Emergency Animal Diseases Hotline on 1800 675 888** for events at sea or in the AAT.

Appendix 2
to: Unusual Animal Mortality Response Plan 2019–22

Australian Government
Department of the Environment and Energy
Australian Antarctic Division

AUSTRALIAN ANTARCTIC PROGRAM

Animal Unusual Mass Mortality Incident Report Form TEMPLATE

Reporters Details:
Name:
Organisation/Affiliation:
Contact Details:
Other Members on Site, plus affiliation and contact details:

Incident Details
Date incident first reported:
Date of investigation:
Location of Incident including GPS coordinates:
Description of incident

Species Affected	Total number of affected animals	Number of dead individuals/species	Number of dying individuals/species	Age Class	Sex Ratio (if possible)

Clinical Signs of Disease:
Weather Conditions on day of incident:
Weather Conditions/events for the past week:

Sample Collection Sheet

Names and Affiliations of Sample Collection Team:
Date of Sample Collection:
Location of Sampling:
Number of Collection Sheets:

Species	Sample ID	Sample Type	Collection Method	Preservation media

Sample Collection Sheet

Species	Sample ID	Sample Type	Collection Method	Preservation media