Sick & Dead Bird Health Surveillance

SAMPLE COLLECTION PROTOCOL

The Australian Registry of Wildlife Health

Taronga Zoo | Western Plains Zoo
Zoological Parks Board of New South Wales

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Prepared by Karrie Rose DVM, DVSc
Australian Registry of Wildlife Health, Taronga Zoo

Introduction

The purpose of this document is to provide guidelines for the collection of samples that will lead to a diagnosis being reached in sick and dead wild birds collected in Australia, and will allow testing to rule out the presence of animal diseases of concern to Australia (such as highly pathogenic avian influenza, West Nile Virus and Newcastle Disease).

Information in this document is set out in the following sequence:

- Clinical signs of infectious disease: triggers for disease investigation
- Live bird handling
- Dead bird collection
- Sample collection
- Swabbing technique
- Sample handling and transport
- Sample collection list
- Hygiene protocols for handling wildlife with signs of infectious disease
- Sick/Dead Bird Event Form - appendix 1
- Australian Wildlife Health Network State and Territory Coordinators - appendix 2
- Avian Necropsy Protocol - appendix 3

Highly pathogenic (H5N1) avian influenza virus and West Nile Virus have not been detected in Australia, thus, there is currently no need to increase occupational health and safety measures beyond the general hygiene recommendations included in this document.

If highly pathogenic avian viruses are found in birds in Australia, specific occupational health and safety guidelines will likely be issued by the Communicable Diseases Network Australia (CDNA) and distributed on the website of the Commonwealth Government Department of Health and Aging. The CDNA has issued Interim Health Advice for Poultry and Associated Industries, which is available on http://www.health.gov.au/internet/wcms/publishing.nsf/content/health-avian_influenza-index.htm.

In the event of a diagnosis in Australia of a highly pathogenic avian virus, this health surveillance and sample collection protocol will be revised and made available on the website of the Australian Registry of Wildlife Health (http://www.arwh.org/ARWH/OtherPublications).

The following document is based on the assertion that wildlife exhibiting signs of infectious disease should not be permitted to enter wildlife rehabilitation. Instead, such birds should be subject to pathological investigations where there is an increased level of hygiene and human health protection.

Clinical Signs of Infectious Disease: Triggers for Disease Investigation

- Sudden death
- Mass mortality or clusters of wild bird mortality (mortality unexpected considering the natural history of the species)
- Behavioural abnormalities - falling over, head tilt, head and neck twisting, circling, paralysis, seizures
- Locomotion abnormalities - unable to stand or flap properly in the absence of evidence of traumatic injury
- Regurgitation
- Diarrhoea
- Sneezing
- Unexplained emaciation
- Open sores
- Discharge (clear or cloudy) from the mouth, nose, ears, or vent
- Extensive swelling and/or purple discoloration of the tissues of the head (including the conjunctiva)
- Abnormal feathers with annular constrictions of the shaft, haemorrhages in the shaft, retained waxy sheaths. Rainbow lorikeets may only have loss of the outer three primary wing feathers.

Reception staff at wildlife rehabilitation centres should be made aware of these clinical signs. People submitting birds to wildlife centres should be questioned to determine whether the animal exhibited any such signs. Records should contain the contact details of the person submitting the animal so that they can be contacted for any additional information or case follow-up. Any bird with signs of infectious disease should be kept isolated until it can be examined by a veterinarian.

Once it has been decided to investigate**, try to collect as much information about the animal as possible**. A sick/dead bird event form is included (Appendix 1) in this document to provide the type of information that should be collected and passed on to your state Australian Wildlife Health Network coordinator (Appendix 2), even if animal samples are not collected. Photographs of animals (alive or dead) may also be useful in investigating wildlife disease.

Live Bird Handling

Examine sick or dead animals after all other animals have been handled for the day. If this is not possible, examine the bird in an isolation-type room and wear a lab coat, and latex gloves. Be sure to wash your hands and disinfect your instruments and clothes, as indicated in the section entitled Hygiene Protocols.

If an animal exhibits signs of a respiratory infection, consider wearing a high filtration surgical face mask (preferably a P2/N-95 mask[1]). Training in applying and wearing the face mask should be obtained from a medical professional.

If a live bird being examined has signs of an infectious disease, collect blood into a serum separating tube prior to euthanasing the bird.

[1] P2/N-95 face masks, 3M Brand, part number 3M9320, (Approximately $2.30 per mask). Supplier: BOC Gasses, phone 131262, fax 132427.

If birds are to be euthanased using barbiturates, then this should be undertaken using recommended doses and titrating the dose to effect. Excessive quantities of barbiturates can severely damage tissues that may be required for histological examination.

Dead Bird Collection

Try to minimise direct contact with dead birds. Before handling a dead bird wash your hands thoroughly and put on vinyl or latex gloves. The best method to collect a dead bird is to invert a plastic bag around your hand and then surround the animal with the bag so that you do not touch the animal. Keep the animal away from your face and do not attempt to examine it in the field. Seal the bag tightly (double bag if required for strength or cleanliness) and clearly and indelibly label the bag with the species, date, time and location where the animal was found.

In the event of mass mortalities, collect as many animals as possible (to prevent secondary infections or intoxications in other animals), and complete the mortality log portion of the attached Sick/dead Bird Event Form to retain as much information as possible about the incident. Where possible, try to collect sick animals as well as freshly dead animals.

If there are so many animals present that individual bagging and labelling are not possible, try to bag well preserved animals that will be most useful for diagnostic purposes, and keep these separate from decomposing carcasses. A decomposing carcass is one that is bloated, green, foul smelling, and has feathers that pull out easily.

Be sure to wash your hands thoroughly, and wash and disinfect equipment and clothing after handling dead animals (as per the instructions in the Hygiene Protocols section of this document).

Animal carcasses for diagnostic investigation are best maintained chilled - not frozen. Keep carcasses away from fridges used for animal or human food.

If possible, transport carcasses (in sealed bags) in a separate air space from the vehicle occupants.

Sample Collection

Each sample collected must be properly labelled with the animal's species, a unique identification number, date, and organ involved. Always label the jar rather than the lid to ensure that the identification is not lost when lids are removed during sample handling. Ensure that labels are made with pencil or permanent ink, which will not dissolve in the fixative that you are using (alcohol-based fixatives will dissolve the ink of many "permanent/indelible" markers).

If you don't already have an identification system for your animals, you can create one easily. Just ensure that each animal has a unique number. You might like to use a number that includes the date, type of animal, and a unique identifier (i.e. 2005B125 = the 125th bird sampled in 2005). Only select one identification number per animal, even if you are collecting several samples from the animal. Ensure that your identification number on the sample container and paperwork are the same.

Contact your state agriculture department laboratory, or state Australian Wildlife Health Network coordinator (see appendix 2) prior to sample collection to ensure that the list below is appropriate, to obtain viral transport medium for the swabs, to discuss sample storage and transport, and to establish methods for carcass disposal.

An avian necropsy protocol is attached (Appendix 3) to assist with the sample collection process.

Additional information regarding recommended euthanasia techniques, and animal sample collection is available in the "Wildlife Health Investigation Manual" published through the Australian Registry of Wildlife Health (order form available on http://www.arwh.org/ARWH/OtherPublications/oPublication.aspx).

In the event of mass avian mortalities (5 or more birds), it may be wise to conduct a rapid influenza antigen detection test on cloacal swabs or oropharyngeal (back of the mouth) swabs collected from

animals prior to initiating necropsies.[2] This may help determine the level of occupational health and safety to employ during the necropsy procedure. Ensure that you wear a full range of personal protective equipment to conduct the test, as outlined in the section entitled Hygiene Protocols. The results of these tests must only be viewed as indicative, since their sensitivity and specificity are not as high as for other diagnostic tests available. Any positive test results should remain confidential and be reported only to your state Chief Veterinary Officer. Any animal that tests positive using the rapid antigen detection test should be examined in a PC 2 level laboratory, preferably at a state government veterinary laboratory.

Swabbing Techniques

Swabs taken from the cloaca (vent) and oropharynx (back of the mouth/throat) then stored in viral transport medium can be used for viral culture or real-time polymerase chain reaction (PCR) to detect the presence of a variety of viral pathogens.

Swab Collection Equipment List:

- Latex or vinyl gloves
- Swabs and transport medium
- Esky and ice blocks to transport medium and swabs
- Pencil, pen and sick/dead bird event form to label your samples and record your findings
- Packing tape and courier forms
- Optional: scissors and a large vial of ethanol

Wear disposable latex or vinyl gloves during the swabbing procedure to decrease the risk of contaminating the swabs with bacteria from your skin.

Use either a sterile plain cotton swab on a wooden applicator and then cut or break off the part of the applicator that you touched while depositing the swab into the vial of viral transport medium, or use commercially available sterile swabs that come in a tube containing transport medium.[3]

Handle the cotton swab or applicator stick from the very end, or the plastic handle only. Do not touch the portion of the applicator stick that will enter the tube containing the viral transport medium.

Moisten the swab in the viral transport medium. Insert the swab well inside the cloaca, or oropharynx (different swabs for each site please) and twirl it around before pulling it out (Figures 1 & 2).

If using a plain cotton swab, break off or cut the swab so that the cotton bud falls into the viral transport medium (Figure 3). If scissors are used to cut the swabs, the scissors should be disinfected in a large vial of ethanol between birds.

Label each sample and fill in the relevant information on the attached Sick/dead Bird Event Form as you collect each sample (Figure 4).

[2] Synbiotic Avian Flu Direct Antigen Capture test kits are available (1 kit with 20 tests = approx $350.00). Supplier: Bio-Mediq DPC Pty. Ltd., Phone 03-9840-1800, Fax 03-9840-2767.

[3] Copan ™ Transystem Viral Transport Swab, catalogue number 147C (Approximately $1.30 per swab). Supplier: Interpath Services, www.interpath.com.au, Phone 02-9524-1199, Fax 02-9524-1099.

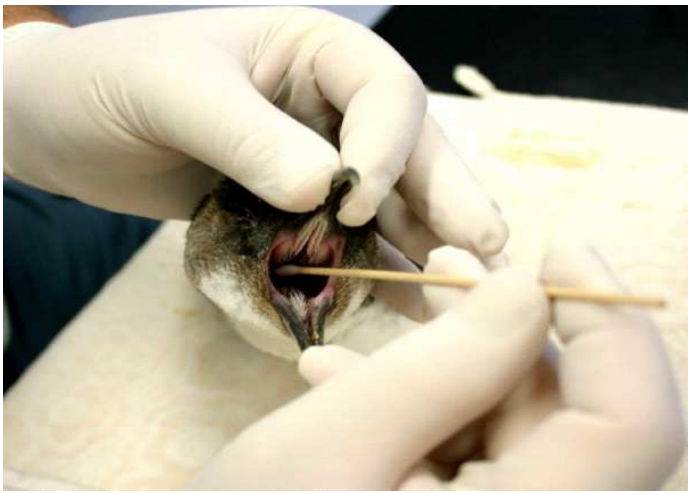

FIGURE 1
OROPHARYNGEAL SWAB

FIGURE 3
BREAKING THE SWAB

FIGURE 2
CLOACAL SWAB

FIGURE 4
SAMPLE LABEL

Sample Handling and Transport

Store viral transport medium at 4°C or in an esky containing ice blocks before and after use. Transport the samples on ice blocks. If samples can not be shipped to an appropriate laboratory within one week of collection, they may be stored in a -80°C freezer or liquid nitrogen and transported on dry ice at a later date.

Keep serum and fresh tissue samples at 4°C until they can be shipped (as soon as possible). Transport the samples on ice blocks. Alternatively freeze the samples in a -80°C freezer or liquid nitrogen and transport these on dry ice.

Please avoid freezing any swabs or tissue samples between 0°C and -20°C (such as in many domestic freezers).

Samples to be fixed in 10% neutral buffered formalin should be no thicker than 1 cm so that the fixative penetrates the entire sample. Fixed samples can be stored at room temperature. Formalin in quantities greater than 50 mL is considered a dangerous good by courier companies, which increases the costs and complexity of shipping. The tissues can be more readily shipped by courier or post if the fixative is decanted after the samples have been fixed for a period of at least 48 hours.

Fresh or frozen tissues that could contain infectious agents should be shipped within a three layer packaging system produced by CSIRO's Australian Animal Health Laboratories.[4] These Biological Product and Diagnostic Transport Containers meet IATA regulations. These containers are suitable for sample transport by courier (road or air transport), but are not suitable for transport by post.

Fresh or frozen tissues must be shipped as quickly as possible to the laboratory. Same day courier service is preferable, but overnight courier delivery is acceptable. Avoid shipping samples on Fridays. It is expensive and difficult to organise Saturday delivery. Samples that go missing in the courier system on a weekend are often of little value when finally found. Always advise the receiving laboratory in advance that specimens are being despatched and the expected time of arrival.

Sample Collection List

PRIORITY RANKED:

- **Oropharyngeal swab**
- **Cloacal swab**
- **Serum** - from a live animal, centrifuged heart blood from a dead animal
- **Fresh tissue** samples collected into sterile vials:

  Liver, kidney, trachea and lung, brain, spleen, pancreas

  Plus:
  Half of any lesion
  Caecae and intestine if the animals exhibit diarrhoea.
  Feathers if the animal had abnormal feathers[+]

- **Formalin fixed tissues (minimum collection list)**:

  Brain, trachea, lung, heart, liver, kidney, spleen, pancreas, bursa of Fabricius if present, proventriculus/ventriculus, duodenum, caecae, skin including feather follicles, half of any lesion.

+ Please consult Dr. Shane Raidal, Murdoch University, regarding the possibility of testing serum and feather samples from wild birds with feather abnormalities that could be infected with psittacine beak and feather disease, Phone 08-9360-2418, Fax 08-9310-4144.

[4] Biological Product and Diagnostic Transport Containers (approximately $30.00 per re-usable box). Supplier: CSIRO's Australian Animal Health Laboratories, Geelong, VIC 3213, Phone (03) 5227-5000, Fax (03) 5227-7555

Hygiene Protocols - Wildlife with Signs of Infectious Disease

Wild birds are the hosts for a number of pathogens that can infect people. It is recommended that wildlife carers, wildlife health professionals and people contacting sick, injured or dead birds undertake standard precautions for infection control. Increased precautions should be taken when animals are suspected to be suffering from respiratory infections.

The following recommendations are extracted from the World Health Organisation's Standard Precautions to minimise droplet, contact and airborne transmission of disease.

Hand washing

The first line of defence against transmitting or contracting an infection is hand washing.

- Hands must be washed appropriately using hot water and soap before putting gloves on and after removing them
- Hands must be checked for cuts and abrasions and clean waterproof dressing applied
- Always wash hands before and after eating, smoking and going to the toilet.

When washing hands make sure the backs and palms of both hands are wet with warm water, apply soap or **hospital grade** antiseptics, (domestic antiseptics offer little protection from infections) lather and wash backs of hands, between fingers of each hand, and palms. Using a twisting/screwing motion clean the thumbs of each hand between the thumb and forefinger of the other hand. Rinse well and dry using paper towel. Be careful when turning taps on and off if there is not an automatic sensor or foot operated system in place. Ensure tap handles are clean.

If clean running water is not available use an alcohol based hand rub and wash hands as described above.

Personal Protective Equipment

There are 4 key items of personal protective equipment (PPE) that will protect against respiratory disease:

- Face mask (P2/N-95 masks are recommended for the examination of animals that have signs of respiratory illness)
- Face shield, glasses or goggles
- Gloves (non sterile)
- Long sleeved gown (plastic apron if splashing is anticipated)

When using this PPE fit your mask first, making sure it is a secure fit around your face, then your goggles and gown and finally your gloves (having washed your hands prior to fitting your mask) When protection is no longer required remove your PPE in the reverse order you put it on, making sure that you wash your hands immediately after removing your gloves and that all PPE is disposed of in a hazardous waste bag.

Waste

All waste produced from animal handling and examination of birds with signs of infectious disease must be treated as infectious. Gloves, gowns and masks should not be used again. Disposable items and carcasses should be disposed of through a biohazard incineration service whenever possible. Gowns, clothing and other equipment should be washed with detergent and hot soapy water and disinfected if possible. Most avian viruses are sensitive to a broad range of detergents and hospital grade disinfectants. It is important that materials are washed thoroughly and rinsed prior to disinfection.

Disinfectants active against avian influenza Viruses include: 2% sodium hypochlorite for 10 - 30 minutes, 4% quaternary ammonium salts, 2% synthetic phenols, sodium carbonate (washing soda) 10% weight/volume for 30 minutes, citric acid 0.2% weight/volume for 30 minutes (good for clothing and body). Austvet plan (2000)

APPENDIX 1:

Sick/Dead Bird Event Form

The Australian Registry of Wildlife Health

Ph 02-9978-4749, Fax 02-9978-4516

Please Note[5]

Submitter Information	Incident Information
Submitter's Name:	Date of Observation:
Dept/Organisation:	Date of Report:
Address:	**Location** (Exact Location - with GPS data if possible):
Phone: Fax:	
Mobile #:	Landowner and land access:
Email:	
Signature:	

Animal Details:

Species Affected (Common name, genus and species):

Total of Each Species: Unaffected/Normal: Sick: Dead:

Approximate Ages of Affected Animals:

Sex of Affected Animals: Unknown: Male: Female:

Description of Incident:

Environmental Conditions: Weather, recent rainfall, sea conditions, recent local use of chemicals, changes in ground water levels, changes in domestic animal management):

Clinical Signs of Animals:

Gross Pathology Findings:

Management Actions Taken:

[5] Some state government veterinary laboratories may prefer that you use their standard specimen advice form when submitting samples. If that is the case, please try to ensure that information from each field above is included on their form (particularly animal common name, genus and species).

APPENDIX 1: Sick/Dead Bird Event Form
Sample Collection Log Sheet

The Australian Registry of Wildlife Health

Species	Animal ID	Location	Alive/Died/ Euthanased	Carcass kept fresh / frozen	Serum	Swabs		Tissues		Photos Yes/No	Sample Collector	Comments
						Oro-pharynx	Cloacal	Fresh	Fixed			

Specimens Stored/Sent Where _____

APPENDIX 2:

Australian Wildlife Health Network
State & Territory Coordinators

State or Territory	Co-ordinators	Notes	Address	Contact details
ACT	**Primary contact** **Murray Evans**	Senior Wildlife Ecologist	Environment ACT PO Box 144, Lyneham, ACT 2602	murray.evans@act.gov.au W: 02 6207 2118 F: 02 6207 2122
	Alternate Will Andrew		ACT Veterinary Services PO Box 144 Lyneham ACT 2602	Will.andrew@act.gov.au W: 02 6207 2357 F: 02 6207 2361 M: 0419 239 073
WA	**Primary contact** **Cleve Main**	Government rep/ pathologist	Animal Health Laboratory Agriculture WA Locked Bag 4 Bentley Delivery Service WA 6983	cmain@agric.wa.gov.au W: 08 9368 3426 F: 08 9474 1881
	Assistant Shane Raidal	Murdoch University Pathologist/ Avian medicine and husbandry	Division of Veterinary and Biomedical Sciences Murdoch University South Street Murdoch WA	Raidal@murdoch.edu.au W: 08 9360 2418 F: 08 9310 4144
	Alternate Cree Monaghan	Perth Zoo	Senior Veterinarian c/o Perth Zoo 20 Labouchere Rd South Perth, WA 6151	cree.monaghan@perthzoo.wa.gov.au W: 08 9474 0438 F: 08 9474 0391
	Conservation contact Tamra Chapman	Conservation and Land Management, WA	Locked Bag 104 Bentley Delivery Centre WA 6983	tamrac@calm.wa.gov.au W: 08 9334-0290 F: 08 9334-0278
TAS	**Primary contact** **Stephen B. Pyecroft**	Government rep/ pathologist	Supervising Veterinary Pathologist Diagnostic Services Department of Primary Industries, Water and Environment, Tasmania Fish Health Unit Mount Pleasant Laboratories PO Box 46 Kings Meadows TAS 7249	Stephen.Pyecroft@dpiwe.tas.gov.au W: 03 63365275 F: 03 63365374
	Assistant Patsy Davies	Government wildlife co-ordinator Tasmania	Dept Primary Industry, Water and Environment Nature Conservation Branch GPO Box 44 Hobart, TAS 7001	Patsy.davies@dpiwe.tas.gov.au W: 03 6233 6556
	Jemma Bergfeld Alternate	Veterinarian	Department of Primary Industries, Water and Environment, Tasmania Animal Health Laboratory PO Box 46 King Meadows TAS 7249	Jemma.Bergfeld@dpiwe.tas.gov.au W: 03 6336 5456 F: 03 6344 3085
QLD	**Primary contact** **Hume Field**	Government rep/ wildlife epidemiologist	QLD Dept Primary Industries Animal Research Institute LMB 4 Moorooka QLD 4105	Hume.Field@dpi.qld.gov.au W: 07 3362 9566 F: 07 3362 9457 M: 0412 556 641
	Assistant Michael Pyne	Currumbin Sanctuary	Senior Veterinarian Currumbin Sanctuary 28 Tomewin Street Currumbin QLD 4223	vets@cws.org.au W: 07 5534 0833 F: 07 5525 0197 M: 0413 967 073
VIC	**Primary contact** **Malcolm Lancaster**	Government rep/ pathologist	Senior Veterinary Pathologist Department of Primary Industries Primary Industries Research Victoria 475-485 Mickleham Rd, Attwood 3049	malcolm.lancaster@dpi.vic.gov.au W: 03 9217 4354 F: 03 9217 4199
	Assistant Peter Holz	Healesville Sanctuary/ wildlife veterinarian and pathologist	Veterinarian Healesville Sanctuary PO Box 248 Healesville VIC 3777	pholz@zoo.org.au W: 03 5957 2864 F: 03 5957 2860

State or Territory	Co-ordinators	Notes	Address	Contact details
SA	**Primary contact Sue Bigwood**	Monarto Zoo/ WDA rep for SA	55 Anderson Avenue Bridgewater SA 5155	andrewb@health.on.net W: 08 8339 2320 M: 0418 877 499
	Assistant Scott Jennings	Government rep/ Ecologist	Ecologist, Wildlife Management Department for Environment and Heritage PO Box 1047 Adelaide SA 5001	jennings.scott@saugov.sa.gov.au W: 08 8222 9427 F: 08 8222 9546
	Alternate David Schultz	WDA and AAVA	Senior Veterinarian Adelaide Zoological Gardens Frome Rd Adelaide, SA 5000	dschultz@adelaidezoo.com.au W: 08 8267 3255 F: 08 8267 4289
NT	**Primary contact Cathy Shilton**	Government rep/ pathologist	Dept of Business, Industry and Resource Development Berrimah Vet Laboratories GPO Box 3000 Darwin, NT 0801	Cathy.shilton@nt.gov.au W: 08 8999 2122 F: 08 8999 2024
	Assistant Jodie Low Choy	Territory Wildlife Park	Supervisor/ Veterinarian Territory Wildlife Park PO Box 771 Palmerston, NT 0831	Jodie.LowChoy@plmbay.pwcnt.nt.gov.au W: 08 8988 7227 F: 08 8988 7201 M: 0401 115 799
NSW	**Primary contact Ian Lugton**	Government rep	Leader, Prevention of Cruelty to Animals Act Animal Welfare Branch NSW Department of Primary Industries 161 Kite St, ORANGE NSW 2800	ian.lugton@dpi.nsw.gov.au W: 02 6391 3688 F: 02 63 913570
	Assistant Karrie Rose	Australian Registry of Wildlife Health	Taronga Zoo Veterinary and Quarantine Centre PO Box 20 Mosman NSW 2088	krose@zoo.nsw.gov.au W: 02 9978 4749 F: 02 9978 4516 M: 0402 553 537
Australian Antarctic Territory	**Primary contact Colin Southwell**	Government rep/ DEH	Australian Antarctic Division Channel Highway Kingston TAS 7050	Colin.southwell@aad.gov.au W: 03 6232 3450 F: 03 6232 3351 M: 0407 768 085
	Assistant Martin Riddle	Government rep/ DEH/ Human health	Program Leader Human Impacts Research Program Australian Antarctic Division Channel Highway Kingston TAS 7050	Martin.riddle@aad.gov.au W: (03) 6232 3573 F: (03) 6232 3351
Head Office	Rupert Woods	National coordinator	AWHN PO Box 20 Mosman NSW 2088	rwoods@zoo.nsw.gov.au W: 02 9978 4788 F: 02 9978 4502
	Amy Jones	Administrative assistant	As above	ajones@zoo.nsw.gov.au W: 02 9978 4788 F: 02 9978 4502

Notes: The State and Territory co-ordinators capture and report wildlife disease events and information primarily to support the NAHIS, but also to support human health and biodiversity agencies. Two co-ordinators have been chosen for each State or Territory: a "primary" contact, preferably from a Regional Veterinary Laboratory (to capture "main-stream" diagnostic information), and an "assistant" (to capture "non-mainstream data). Co-ordinators report quarterly by teleconference, or on an "as needs" basis: a model based on that used by the Communicable Diseases Network of Australia. Data submission is by standard pro forma or direct entry into eWHIS. Information is moderated and assigned different levels of access based on its sensitivity. Reports are generated quarterly for Animal Health Surveillance Quarterly, Wildlife Diseases Association, National Enteric Pathogen Surveillance Scheme, the World Conservation Union Species Survival Commission Veterinary Specialists Group and the AWHN. Reports are produced yearly for the Office of the Chief Veterinary Officer and Animal Health in Australia. The AWHN coordinators liaise closely with the relevant NAHIS coordinator for their jurisdictions (e.g. in NSW Ian Lugton receives "mainstream, government" information from Barbara Maloney, the NAHIS coordinator and Karrie Rose provides "non-government" data).

The focus for disease reporting are: mass or unexpected mortalities/ morbidities of unknown causes; significant clusters of deaths; suspect livestock associated notifiable diseases; undiagnosed syndromes; suspected human/ zoonotic connection; diseases likely to spread and be difficult to eradicate if they become established (eg Pacheco's disease); OIE list diseases; diseases with overseas events or international drivers e.g. chronic wasting disease in deer and; diseases listed as key threatening processes by DEH. Submission of negative data is also required. Each organisation from which the coordinator is drawn receives a small amount of funding from Animal Health Australia to cover any administration costs (e.g. telephone calls, photocopying). (Terms of reference and *modus operandi* are available on request.)

This system was chosen primarily because: 1) NAHIS coordinators are already very busy and a person to support their activities was considered more appropriate and; 2) the majority of wildlife diagnostic data does not currently flow through the normal data pathways i.e. it occurs outside Government laboratories, hence the need for a person who works outside this system to capture these data.

APPENDIX 3:

Avian Necropsy Protocol

The Australian Registry of Wildlife Health

Necropsy Occupational Health & Safety

1. The necropsy room should be a sole purpose isolation-type room. Necropsy equipment, instruments and cutting boards should not be used for other purposes. Necropsy equipment and surfaces must be thoroughly cleaned and then disinfected after each use. Ideally, a footbath should be set up at the doorway(s) of the necropsy room.
2. The necropsy area as well as fridges and freezers used to store pathology samples should be within a human and animal food exclusion zone.
3. Support staff should be thoroughly briefed on the hazards of zoonotic disease, potential methods of disease transmission, and be informed of biohazard and chemical spill management.
4. Individuals conducting or observing gross post-mortem examinations and those cleaning the post-mortem room should wear appropriate protective clothing. Protective clothing should include a face mask (P2/N-95 masks are recommended for the examination of animals that have signs of respiratory illness), disposable (non-sterile) gloves, waterproof splash-aprons, long sleeved gown with tight fitting cuffs, safety glasses, and gumboots. A hand washing station should be accessible within the necropsy room.
5. Animal feathers should be wet down with a very dilute detergent solution and water prior to commencing the examination to reduce the risk of aerosolizing infectious agents.
6. A biohazard safety cabinet should be used to examine birds that exhibited signs suggestive of infectious disease.
7. Referral laboratories should be notified when tissues bearing potential zoonotic agents are submitted (tissues bearing suspected Cryptococcus sp., any tissue from bats, avian tissues where chlamydiosis or avian influenza is suspected). Conducting in-house impression smears or other diagnostic testing in these cases is not recommended unless they may be performed within a biohazard safety cabinet.
8. Carcasses should be maintained frozen until a diagnosis has been established, and then they should be disposed of in a means approved by the local council, preferably through a biohazard incineration service.
9. Animal tissues and remains should be retained frozen until the presence of zoonotic disease is ruled out, prior to being disseminated to museums or other researchers.

Avian Necropsy Protocol:

History

A history should include:

- Species, origin (wild/zoo/rehabilitation/privately owned), date and location of collection
- Diet, food and water sources
- Environmental conditions or housing conditions - ventilation, substrate, cage type, etc
- Exposure to other birds
- Exposure to toxic substances - lead, plants, fumes
- Any recent changes in the environment
- Clinical signs of disease, the onset and progression of these signs
- Treatment offered, including whether the animal was euthanased or died

An examination of the bird's environment can provide invaluable information.

External examination

An external general physical examination of the bird should be conducted following the same systematic method that would be used for a live bird

Collect cloacal and combined oropharyngeal/tracheal swabs prior to beginning the necropsy.

Ensure that you examine the following:

- Verify the carcass species, age, and look for identifying bands
- Plumage and skin for evidence of parasites, moulting, bruising, laceration, punctures, abrasion, swelling, anaemia, dermatitis
- Nostrils, eyes, ears, cloaca, and oral cavity for exudates, parasites, foreign bodies
- Quantity of muscle mass and presence of subcutaneous fat
- Long bones and joints for evidence of fracture, luxation, swelling
- State of feathers around the vent. Are these feathers pasted with faeces or urates?
- Cloacal mucosa
- Feet for evidence of trauma or bumblefoot (thickened or ulcerated plantar surfaces)

Internal Examination

Several protocols for avian necropsy are available. Your protocol should be one that you feel comfortable with and one that is thorough and systematic.

Spray or dip the carcass in a dilute solution of detergent to wet the feathers and reduce the risk of aerosolising infectious particles.

Cut across the upper beak at the level of the oral commissure to examine the nares and sinuses. Cut through the mandible and make an incision in the skin extending from the mandible to the thoracic inlet. Cut the oesophagus from the oral cavity, through the crop and down to the level of the thoracic inlet.

Examine the soft palate and larynx. Longitudinally incise the trachea beginning at the larynx and proceeding to the level of the thoracic inlet. Explore the trachea for parasites, fungal plaques, exudates, foreign bodies, congestion, or blood clots.

Incise the skin from the thoracic inlet to the vent. Disarticulate the coxofemoral joints. Reflect the skin off of the abdomen and breast. Tightly adherent skin and dark tissues may be an indicator of dehydration.
Make serial incisions into the pectoral musculature to rule out the presence of lesions. Palpate the coracoid and furcula for any subtle fractures. Remove the sternum by cutting through the abdominal muscles, ribs and coracoid bones and furcula.

As soon as the internal body cavity is exposed, use clean instruments to collect fresh tissue samples. Do this prior to touching the organs with your gloved hands. Then take the opportunity to examine the position and general appearance of the organs. Pay particular attention to evidence of free coelomic fluid, parasites, abscesses or masses. Carefully lift the ventriculus and intestines to investigate the abdominal air sacs and reproductive organs.

Coelomic surfaces coated with fibrin are consistent with infection caused by bacteria, including *Chlamydia* and *Chlamydophila* species. White chalky material upon the surfaces of the heart, liver and other organs are most often uric acid crystals and are secondary to hyperuricemia from nephritis or urate nephrosis secondary to water deprivation. Excessive quantities of barbiturates used during euthanasia can produce white crystals along surfaces of the heart and greater vessels. Barbiturates often also partially liquefy these tissues, making them soft and brown.

Large blood clots in the abdomen or a haematoma within the liver are often a result of trauma. Blood clots may also be a result of haemorrhage from a large tumour, rupture of the aorta, or fungal vasculitis. Ascites may result from heart disease, liver disease, ingestion of toxins or neoplasia. White-yellow lesions on the air sacs, within the tracheal lumen or lungs are most often due to fungal infection (aspergillosis), but can also be due to bacterial infection, or tumours.

In chicks, check the navel and yolk sac for evidence of infection.

Begin to examine the tissues of the body while collecting 1 cm wedges of each organ into 10% buffered formalin.
If you come across a lesion, place half of the lesion in formalin and half into a sterile vial for culture or freezing back pending histopathological examination.

Examine the circulatory system and immune system. Examine and sample the thyroid glands as they disappear quickly upon dissection of other organs. The thyroid glands are found just at the base of the internal carotid artery. Sampling the whole gland and portions of the blood vessel around it will often provide a sample of the parathyroid gland, ultimobranchial body, artery, vein, air sac, and in a young bird, the thymus.

Remove the heart by severing the major vessels at the base of the heart. Make a transverse cut along the apex of the heart to expose the ventricular chambers and valves. If blood was not collected antemortem, collect heart blood using a syringe and place the fluid into a serum collection tube.

Birds that are anaemic have pale tissues and watery blood. Birds that are hypovolemic often have a conical and contracted appearance of the cardiac ventricles.

Cut the oesophagus at the level of the bifurcation of the trachea. Grasp the caudal oesophagus with forceps and gently lift it as you cut the peritoneal membranes that attach the liver and intestinal tract to the dorsal body wall. Reflect the liver and intestinal tract onto the table beyond the cloaca. Stretch out the intestinal tract and

examine the serosal surface carefully. Examine the pancreas and spleen. The pancreas is the tan tissue located between the descending and ascending loop of the duodenum. The spleen is usually nestled between the liver and the serosa of the stomach, at the junction of the proventriculus and ventriculus.

Test the patency of the bile duct by expressing the gall bladder or bile duct prior to removing the liver from the intestinal mass. Create serial sections through the liver to observe the integrity of the hepatic parenchyma and biliary system.

Yellow discolouration of the liver may be a physiological change in a laying hen or a very young chick when lipid metabolism is occurring at a high rate.

Peel out the lungs. Examine the pulmonary parenchyma and incise several major bronchi.

Examine the adrenal glands and gonads. Open the oviduct if one is present. Confirm the sex of the bird by the shape of the gonads. Most female birds have only a left ovary and oviduct, except for brown kiwi and some birds of prey that have two ovaries.

Examine the kidneys and ureters. Attempt to find the bursa of Fabricius, which is only present in young birds. The bursa is pale white or tan and can be found in the caudal coelomic cavity, just dorsal to the cloaca

Starting at the proventriculus, cut through the wall of the entire intestinal tract, including the caecae (ensure that samples for bacterial and viral culture have been collected prior to opening the intestinal tract). Examine the digestive tract for evidence of normal or abnormal ingesta, haemorrhage, necrosis, ulceration, parasites or vascular accident.

Examine the skin, integument, muscles, bones, and joints. Reduced muscle mass, lack of fat deposits, a small liver, contracted ventricles, a full gall bladder and serous atrophy of fat are indicators of prolonged anorexia. Check bone strength by breaking one of the long bones. Place ½ of the tibiotarsus in formalin to allow examination of bone marrow. Incise the soft tissue surrounding several joints to look for evidence of degenerative change, infection or articular gout.

Disarticulate and remove the head from the cervical spine. Using scissors or bone rongeurs gently snip away the dorsal portions of the cranium beginning at the foramen magnum. Grossly examine the cranial vault and brain. Either place the entire head in formalin, or remove the brain from the cranial vault and place half of the brain in formalin, and freeze the other half.

If the bird was blind, or has an eye lesion, collect an eye into formalin.

If the bird had a drooping wing or lameness, collect samples of femoral nerve and brachial plexus into formalin.

Complete a detailed necropsy report, or a sick/dead bird event form (Appendix 1) to document your observations and list the samples that you have collected. Forward a copy of the report to your AWHN state coordinator (Appendix 2), even if you did not collect any samples.

Avian Necropsy Equipment List

Personal Safety Equipment:

- Tarps and rope to create a tent to ward off rain or sun
- Insect repellent
- Sunscreen, hat, sunglasses
- Drinking water
- Change of clothes
- Coveralls
- PVC apron
- Latex gloves or dish-washing gloves
- Surgical face masks
- Rubber boots and good walking shoes
- Wash bucket, nail brush, antiseptic soap, paper towels
- Torch – hand held and head lamp
- First Aid Kit
- Mobile phone
- Emergency locater beacon if on water or very remote

Carcass Collection Equipment:

- Heavy duty rubbish bags
- String
- Bag tags and pencil or indelible pen
- Sick/Dead Bird Event Form

General Equipment:

- Good pest proof packs for carrying equipment
- Necropsy worksheet or sick/dead bird event form
- Pencils and sharpener
- Clipboard with a clear piece of plastic to keep rain off
- Cooler and ice packs
- Sharps disposal unit
- Camera
- Masking tape and packing tape
- Ruler
- GPS unit and maps

Necropsy Equipment:

- Knives and steel (knife sharpener)
- String and manila labels
- Scalpel handle (# 4) and disposable blades (#24)
- Forceps - various
- Scissors - various
- Poultry shears or large bandage scissors

Clean-up Equipment:

- Tarp
- Water, scrub brush, detergent
- Heavy duty rubbish bags

Sample Collection Equipment:

- Permanent marking pen
- Syringes - 5, 10, 20 mL
- Needles – various gauges
- Serum collection tubes
- Sterile plastic bottles - 90 mL
- Sterile cryovials - 2 mL
- Sterile plastic bags (Whirl-pak® bags)
- Large zip-lock bags
- One litre plastic containers filled with 10% neutral buffered formalin (x 3)
- 100 mL of 70- 90% ethanol
- Bacterial culture swabs
- Dry sterile swabs
- Capillary tubes
- Glass microscope slides, cover slips, and slide storage box
- Microscope (may require mirror as light source if no access to power)
- Centrifuge (manual serum separating devices are available)
- Saline
- Formalin
- Parasite preservative
- Any special media required - Chlamydial culture medium, viral culture medium
- Methanol to fix blood films
- Faecal flotation vials and solution
- Esky and ice bricks or liquid-nitrogen dry shipper