

Tularaemia is a nationally notifiable disease of terrestrial animals¹. This document provides a framework to assist veterinarians with the appropriate collection and submission of samples to facilitate the diagnosis or exclusion of tularaemia in free-ranging mammals within Australia (including feral species).

This document will not specifically address sample collection from birds as few epizootics of tularaemia in wild birds have been documented overseas².

Whilst domestic animals are also beyond the scope of this document, in circumstances where the husbandry applied to domestic species tends to mimic wild conditions (e.g. housed outdoors), the principles of sample submission outlined in this document can be applied.

Veterinarians should always use appropriate personal protection equipment (PPE) when interacting with wildlife (see further details below).

BACKGROUND

The WHA [Tularaemia and Australian wildlife fact sheet³](#) is available on the WHA website and provides detailed information on tularaemia.

a. Species likely to be affected by tularaemia in Australia

Tularaemia (*Francisella tularensis*) is commonly found in a range of wildlife species across the northern hemisphere. Worldwide, *F. tularensis* primarily occurs in rodents, rabbits and hares however it has an extremely broad host range⁴. Until recently, it was believed to be absent from Australian wildlife. In September 2016, one of the four subspecies of *F. tularensis* (*Francisella tularensis* subsp. *holartica*) was detected in archived tissue samples from common ringtail possums (*Pseudocheirus peregrinus*)⁵.

Australia is home to a number of native and feral mammal species that are likely to be susceptible to tularaemia. Species present as free-ranging wildlife populations in Australia **AND** are known to become infected with tularaemia (in Australia or overseas) are highlighted in Appendix 1.

¹ National list of notifiable diseases of terrestrial animals at November 2015. <http://www.agriculture.gov.au/pests-diseases-weeds/animal/notifiable#national-list-of-notifiable-diseases-of-terrestrial-animals-at-november-2015>

² Friend M., 2006. Tularemia: Reston, VA., US Geological Survey, Circular 1297, 68p

³ Tularaemia and Australian wildlife fact sheet: www.wildlifehealthaustralia.com.au/FactSheets.aspx

⁴ Mörner T. and Addison E., 2001. Tularemia. In: Williams ES and Barker IK, editors. Infectious Diseases of Wild Mammals. Iowa State University Press, Ames, 303-312.

⁵ Eden J.S., Rose K., Ng J., Shi S., Wang Q., Sintchenko V., Holmes E.C., 2017. *Francisella tularensis* spp. *holartica* in Australian ringtail possums. Emerging Infectious Diseases. Vol 23, Number 7 – July 2017

b. Clinical signs of tularaemia in wildlife

Detailed and accurate records of the circumstances and clinical state of the animal(s) presented for investigation will assist discussion with your WHA Coordinator to determine if diagnostic testing is indicated. A list of information to record is provided in Table 1.

The causative bacterium *F. tularensis* has only been detected in *P. peregrinus* in Australia⁶. There is limited further information on the presence, prevalence or epidemiology of tularaemia in the Australian context⁷.

Clinical signs are largely non-descript and infection may cause acute septicaemia and death. Diagnostic testing for tularaemia should be considered in animals displaying any of the following clinical signs:

- Sudden death
- Emaciation
- Depression
- Pyrexia
- Local inflammation or ulceration
- Enlargement of lymph nodes draining inflamed or ulcerated areas.

Based on the above information, if you suspect tularaemia in wildlife, please call your local [State / Territory Wildlife Health Australia \(WHA\) Coordinator](#)⁸, or the Emergency Animal Disease Hotline 1800 675 888 prior to collecting or submitting any samples.

TABLE 1 - Information to record

- | | | |
|----------------------------------|--|---------------------------|
| • Date found | • Observations on the status of the population | • Photographs |
| • Location | • Body condition | • Number of any lesions |
| • Species | • Weight | • Size of any lesions |
| • Number of animals affected | • Types of samples submitted | • Location of any lesions |
| • Presenting signs | • In-contact persons | |
| • Presence/type of ectoparasites | | |

⁶ Eden J.S., Rose K., Ng J., Shi S., Wang Q., Sintchenko V., Holmes E.C., 2017. *Francisella tularensis* spp. *holartica* in Australian ringtail possums. Emerging Infectious Diseases. Vol 23, Number 7 – July 2017

⁷ Tularaemia and Australian wildlife fact sheet: www.wildlifehealthaustralia.com.au/FactSheets.aspx

⁸ www.wildlifehealthaustralia.com.au/AboutUs/ContactDetails.aspx

c. Case Selection

PROGRESSION TO DIAGNOSTIC/EXCLUSION TESTING SHOULD ALWAYS BE MADE IN CONSULTATION WITH YOUR WHA COORDINATOR

1. Testing as per this document **should be performed** in cases of:
 - Mass mortalities (as a guide, ≥ 3 animals with or without clinical signs consistent with tularaemia) involving species KNOWN to be susceptible to infection with tularaemia (see highlighted entries in Appendix 1)
2. Testing as per this document **should be considered** in cases of:
 - Sick animals in a species KNOWN to be susceptible to infection with tularaemia (see highlighted entries in Appendix 1)
 - Mass mortalities in animals of the same taxonomic Family as those KNOWN to be susceptible to infection with tularaemia (see non-highlighted entries in Appendix 1)
 - Sick animals or mass mortalities (with or without clinical signs consistent with tularaemia) involving animals that inhabit similar ecological niches to *P. peregrinus* (see highlighted entries in Appendix 2)

SAMPLE SUBMISSION GUIDELINES

a. Human health precautions

Tularaemia is a zoonotic disease, and a number of human health risk from tularaemia have been identified. Depending on the *F. tularensis* subspecies, it can cause serious disease in humans. See below for further information on disease transmission and biosecurity. If bites or scratches occur, people are advised to seek medical advice from their Public Health agency.

If there is a high suspicion of tularaemia infection in an animal, the animal or carcass should be handled with care. **Do not conduct a post mortem examination until first consulting with your State/Territory Wildlife Health Australia Coordinator.**

The Australian Veterinary Association (AVA) [Guidelines for veterinary personal biosecurity](#)⁹ provide general advice regarding personal biosecurity.

Those working closely with wildlife should be aware of tularaemia and take appropriate hygiene and infectious control precautions.

Hygiene and infection control measures include the following:

- Do not perform necropsies on animals with febrile disease and lymphadenopathy and/or ulcerative skin lesions
- Cover cuts and abrasions with a waterproof dressing
- Wear gloves
- Use sedation or appropriate restraint when handling animals to minimize scratches and bites
- Avoid aerosol-generating procedures
- Wash and dry hands after handling potentially infected material
- Do not eat or smoke while handling animals that may be infected. Wash and dry hands before smoking or eating.
- Shower after work

If you begin a necropsy or surgery on a wild animal and find miliary tan to white foci in the liver or spleen, pneumonia or pericarditis:

- ask all nearby staff to make their way out of the vicinity, remove PPE and wash their hands,
- Double bag the animal and place it in a necropsy fridge, or in an esky with ice while you contact your WHA coordinator to organize testing
- Do not submit samples from the animal for in-house or commercial microbiology or other ancillary diagnostic testing.

⁹ www.ava.com.au/biosecurity-guidelines

b. Disease transmission and biosecurity

F. tularensis can be highly infectious and is spread by contact with blood or tissues of infected animals, blood feeding arthropods including ticks, biting insects and mosquitoes, inhalation of aerosols or particles, or ingestion of contaminated water or meat¹⁰. No human-to-human transmission has been reported¹¹.

c. Decontamination¹²

F. tularensis is relatively sensitive to all standard inactivation procedures including hypochlorite, other commonly-used decontaminants and UV radiation. Materials that are potentially contaminated with *F. tularensis* should be sterilized before disposal. Ordinary autoclave cycles are suitable for the inactivation of *F. tularensis*.

d. Security Sensitive Biological Agents

***Francisella tularensis* is a Tier 2 Security Sensitive Biological Agents (SSBA)¹³**. Whilst working with clinical specimens is not handling an SSBA in most situations, when it comes to culture isolates, [handling an SSBA and handling suspected SSBA](#)s have significant implications for veterinary medical laboratory scientists and pathologists. There are specific reporting requirements under the SSBA Regulations and the National Health Security Act 2007. A [SSBA inbox](#) and hotline (02 6289 7477) are available for any queries.

¹⁰ Mörner T. and Addison E., 2001. Tularemia. In: Williams ES and Barker IK, editors. Infectious Diseases of Wild Mammals. Iowa State University Press, Ames, 303-312.

¹¹ Tärnvik A. and Berglund L., 2003. Tularaemia. European Respiratory Journal, 21:361-373.

¹² World Health Organization, 2007. WHO guidelines on tularaemia.

¹³ Australian Department of Health information on Security Sensitive Biological Agents: <http://www.health.gov.au/ssba#list>

e. Sample Collection

To ensure the most appropriate samples are submitted and stored appropriately during transport, please call your local [State / Territory Wildlife Health Australia \(WHA\) Coordinator¹⁴](#), or contact the corresponding laboratory in your jurisdiction prior to collecting or submitting any samples.

Details of samples to be collected and available tests are provided in Table 2.

f. Sample Submission and Testing

Samples must be submitted to respective State/Territory government laboratories. The receiving laboratory **must** be notified of the submission in advance so that health and safety risks to staff receiving/unpacking the samples can be managed.

State/Territory government laboratories may subsequently refer your samples to the Australian Animal Health Laboratory (AAHL) or other laboratories for further testing including specific molecular assays, microbiology and bacterial culture.

Testing available at AAHL includes bacterial culture and molecular testing using a series of PCRs that, when run in parallel, are able to identify the bacterium to the species level. Next generation sequencing is available to provide information on subspecies.

To ensure the collected samples are stored appropriately during transport, please call your local [State / Territory Wildlife Health Australia \(WHA\) Coordinator¹⁴](#), or contact the corresponding laboratory in your jurisdiction prior to collecting or submitting any samples.



Find out more at www.wildlifehealthaustralia.com.au
email admin@wildlifehealthaustralia.com.au
or call +61 2 9960 6333

¹⁴ www.wildlifehealthaustralia.com.au/AboutUs/ContactDetails.aspx

TABLE 2 – Sample collection and testing

Please note:

- To ensure samples are submitted and stored appropriately during transport please call your local State / Territory Wildlife Health Australia (WHA) Coordinator, or contact the corresponding laboratory in your jurisdiction prior to collecting or submitting any samples.
- Samples must be sent to respective State/Territory government laboratories in the first instance, and must not be sent directly to AAHL. Suspect samples may then be forwarded to AAHL for confirmatory testing.

PREFERRED SAMPLES (DEAD ANIMALS)			
The whole carcass should be submitted where possible, to allow histopathology to be conducted and to maximise the opportunity for testing.			
Sample	Storage	Available tests	Notes
Whole carcass	4 °C	<ul style="list-style-type: none"> • Molecular assays¹⁵ • Bacterial culture • Histopathology 	<ul style="list-style-type: none"> • Place the carcass in double plastic bag and then in a rigid, water-proof container and label the outer layer of packaging and sample submission form “Suspect tularaemia”
	Frozen (-20 °C)	<ul style="list-style-type: none"> • Molecular assays • Bacterial culture (fresh tissue preferred) 	<ul style="list-style-type: none"> • If a fresh carcass cannot be investigated within 24 h, please contact your State/Territory government laboratory for advice on how best to store the carcass.
PREFERRED SAMPLES (LIVE ANIMALS)			
Non-lethal sampling techniques may have a reduced reliability of detection as compared to whole carcass evaluation.			
Biopsy	4°C	<ul style="list-style-type: none"> • Molecular assays • Bacterial culture 	<ul style="list-style-type: none"> • Collect both a formalin fixed sample and either a frozen or fresh portion of tissue from each lesion • Ideally as large a sample as possible should be collected. • Biopsies should be collected from affected areas with consideration of the impact to the live animal. • If possible, exclude air from bags containing fresh tissues for bacterial culture.
	Frozen (-20 °C)	<ul style="list-style-type: none"> • Molecular assays • Bacterial culture (fresh tissue preferred) 	
	Formalin	<ul style="list-style-type: none"> • Histopathology • Immunohistochemistry 	
OTHER SAMPLE OPTIONS (LIVE + DEAD ANIMALS)			
Non-lethal sampling techniques may have a reduced reliability of detection as compared to whole carcass evaluation.			
Sample	Storage	Available tests	Notes
Necropsy tissues	4 °C	<ul style="list-style-type: none"> • Molecular assays • Bacterial culture 	<ul style="list-style-type: none"> • Suggested necropsy samples include fresh or frozen liver, spleen, and lung along with formalin fixed liver spleen, lymph node, lung, heart including pericardium, kidney, stomach small and larger intestine. • Any tissues with lesions, including soft or firm tan to white foci, abscesses or granulomas should also be bisected with half submitted in formalin and half being submitted fresh or frozen. Sample larger lesions from the margin of normal and abnormal tissue. • Formalin-fixed specimens must be packaged separately from specimens for bacterial isolation.
	Frozen (-20 °C)	<ul style="list-style-type: none"> • Molecular assays • Bacterial culture (fresh tissue preferred) 	
	Formalin	<ul style="list-style-type: none"> • Histopathology • Immunohistochemistry 	
Swab/scrape/FNA of affected areas	4 °C	<ul style="list-style-type: none"> • Molecular assays • Bacterial culture 	<ul style="list-style-type: none"> • Ideally as large a sample as possible should be collected. • Lymph nodes draining affected areas maybe suitable sites for FNAs • Collect both a cytological impression smear and a bacterial transport media swab to sample lesions.
Ectoparasites	4 °C	<ul style="list-style-type: none"> • Molecular assays 	<ul style="list-style-type: none"> • Ideally collected live in moistened sample tubes. Parasites can live 48-72 hours in the sample vials during transport. Freezing may be appropriate if transport to a lab in that timeframe cannot be accomplished.

¹⁵ Molecular testing available at Australian Animal Health Laboratory (AAHL) include qPCR and next generation sequencing. Four qPCR tests are run in parallel on all samples submitted to identify the bacteria to the species level. Sequencing is required to determine the subspecies.

APPENDIX 1 – Families of the Subclass Marsupialia and Subclass Eutheria that have been found to have tularaemia **AND** are present as free-ranging wildlife populations in Australia¹⁶.

Please note:

- = Tularaemia has been reported in Australia
- = Tularaemia has been reported overseas (and not in Australia)

Subclass	Order	Family	Genus	Species	Common Names	
Marsupialia	Diprotodontia	Pseudocheiridae	Hemibelideus	<i>Hemibelideus lemuroides</i>	Lemuroid ringtail possum	
			Petauroides	<i>Petauroides volans</i>	Greater glider	
			Petropseudes	<i>Petropseudes dahli</i>	Rock ringtail possum	
			Pseudocheirus	<i>Pseudocheirus occidentalis</i>	Western ringtail possum	
				<i>Pseudocheirus peregrinus</i>	Common ringtail possum ¹⁷	
			Pseudochirops	<i>Pseudochirops archeri</i>	Green ringtail possum	
			Pseudochirulus	<i>Pseudochirulus cinereus</i>	Daintree River ringtail possum	
Eutheria	Artiodactyla	Bovidae ¹⁸	Bos	<i>Bos indicus</i>	Zebu cattle	
				<i>Bos javanicus</i>	Banteng	
				<i>Bos taurus</i>	European cattle	
			Bubalus	<i>Bubalus bubalis</i>	Water buffalo	
			Capra	<i>Capra hircus</i>	Goats	
			Camelidae	Camelus	<i>Camelus dromedarius</i>	Camel ¹⁹
		Cervidae ¹⁸	Axis	<i>Axis axis</i>	Chital	
			Dama	<i>Dama dama</i>	Fallow deer	
			Cervus	<i>Cervus elaphus</i>	Red deer	
				<i>Cervus timorensis</i>	Rusa deer	
				<i>Cervus unicolor</i>	Sambar	
			Suidae	Sus	<i>Sus scrofa</i>	Pig ²⁰
			Eutheria	Carnivora	Canidae ²¹	Canis
Vulpes	<i>Vulpes vulpes</i>	Red fox ²²				
Felidae	Felis	<i>Felis catus</i>			Cat ¹⁸	

¹⁶ Van Dyck S., Gynther I., Baker A., 2013. *Field companion to the mammals of Australia*. New Holland Publishers.

¹⁷ NSW Department of Primary Industries. October 2016. Biosecurity Bulletin. Detection of tularaemia infection in NSW wildlife: information for veterinarians.

¹⁸ Friend M., 2006. Tularaemia: Reston, VA., US Geological Survey, Circular 1297, 68p.

¹⁹ Awol, N., Ayelet G., Jenberie S., Gelaye E., Sisay T., Nigussie, H., 2011. Bacteriological studies on pulmonary lesions of camel (*Camelus dromedarius*) slaughtered at Addis Ababa abattoir, Ethiopia. African Journal of Microbiology Research 5(5): 522-527.

²⁰ Hungerford, T. G. (Thomas Gordon) 1990, *Diseases of livestock*, 9th ed, McGraw-Hill, Sydney

²¹ World Health Organization, 2007. WHO guidelines on tularaemia.

²² Höflechner-Pörtl A., Hofer E., Awad-Masalmeh M., Müller M., Steineck, T., 2000. Prevalence of tularaemia and brucellosis in European brown hare (*Lepus europaeus*) and red fox (*Vulpes vulpes*) in Austria. *Tierärztliche Umschau*, 55(5), 264-268.

Subclass	Order	Family	Genus	Species	Common Names
Eutheria	Eulipotyphyla	Soricidae ²³	Crocidura	<i>Crocidura attenuate trichura</i>	Christmas Island shrew
Eutheria	Lagomorpha	Leporidae	Lepus	<i>Lepus europaeus</i>	European hare ²³
			Oryctogalus	<i>Oryctogalus cuniculus</i>	Rabbit ²³
Eutheria	Perissodactyla	Equidae ¹⁶	Equus	<i>Equus caballus</i>	Horse ²⁴
				<i>Equus asinus</i>	Donkey
Eutheria	Rodentia	Muridae ¹⁷	Hydromys	<i>Hydromys chrysogaster</i>	Water rat
			Leporillus	<i>Leporillus conditor</i>	Greater stick-nest rat
			Mesembriomys	<i>Mesembriomys gouldii</i>	Black-footed tree-rat
				<i>Mesembriomys macrurus</i>	Golden-backed tree-rat
			Mus	<i>Mus musculus</i>	Domestic mouse ²³
			Notomys	<i>Notomys alexis</i>	Spinifex hopping mouse
			Pseudomys	<i>Pseudomys australis</i>	Plains rat
				<i>Pseudomys shortridgei</i>	Heath rat
				<i>Pseudomys fumeus</i>	Smoky mouse
				<i>Pseudomys chapmani</i>	Pebble-mound mouse
				<i>Pseudomys praeconis</i>	Shark Bay mouse
			Rattus	<i>Rattus spp.</i>	Rats ²³
			Zyomys	<i>Zyomys argurus</i>	Common rock rat
				<i>Zyomys palatilis</i>	Carpentarian rock rat
				<i>Zyomys pedunculatus</i>	Central rock rat

²³ World Health Organization, 2007. WHO guidelines on tularaemia

²⁴ Hungerford T. G. (Thomas Gordon) 1990, *Diseases of livestock*, 9th ed, McGraw-Hill, Sydney

APPENDIX 2 – All Families of the Class Mammals (excluding humans) that are present as free-ranging wildlife populations in Australia

Please note:

- = Families that include species that inhabit similar ecologically niches to Common ringtail possums *Pseudocheirus peregrinus*

Subclass	Order	Family	Examples	
Prototheria	Monotremata	<i>Ornithorhycidae</i>	Platypus	
		<i>Tachyglossidae</i>	Short beaked echidna	
Marsupialia	Dasyuromorphia	<i>Dasyuridae</i>	Quolls, dibbler, mulgaras, phascogales, antechinuses, planigales, dunnarts	
		<i>Myrmecobiidae</i>	Numbat	
	Diprotodontia	<i>Acrobatidae</i>	Feathertail gliders	
		<i>Burramyidae</i>	Pygmy-possums	
		<i>Hypsiprymnodontidae</i>	Musky rat-kangaroo	
		<i>Macropodidae</i>	Kangaroos, wallabies, quokka, pademelons	
		<i>Myrmecobiidae</i>	Numbat	
		<i>Petauridae</i>	Gliders	
		<i>Phalangeridae</i>	Brush-tail possums, cuscus	
		<i>Phascolarctidae</i>	Koala	
		<i>Pseudocheiridae</i> *	Ringtail possums, see Appendix 1	
		<i>Potoroidae</i>	Bettongs and potoroos	
		<i>Tarsipedidae</i>	Honey possum	
		<i>Vombatidae</i>	Wombats	
		<i>Notoryctemorphia</i>	<i>Notoryctidae</i>	Marsupial moles
		<i>Peramelemorphia</i>	<i>Peramelidae</i>	Bandicoots
	<i>Thylacomyidae</i>	Bilbies		
Eutheria	Artiodactyla	<i>Bovidae</i> [^]	Cows, goats. See Appendix 1	
		<i>Camelidae</i> [^]	Camels. See Appendix 1	
		<i>Cervidae</i> [^]	Deer, chital. See Appendix 1	
		<i>Suidae</i> [^]	Pigs. See Appendix 1	
	Carnivora	<i>Canidae</i> [^]	Dingo, fox. See Appendix 1	
		<i>Felidae</i> [^]	Cats. See Appendix 1	
		<i>Otariidae</i>	Fur or eared seals	
		<i>Phocidae</i>	'True' seals	
		<i>Cetacea</i>	<i>Balaenidae</i>	Southern right whale
		<i>Balaenopteridae</i>	Humpback whale	
		<i>Delphinidae</i>	Ocean dolphins	
		<i>Kogiidae</i>	Pygmy and dwarf sperm whales	
		<i>Neobalaenidae</i>	Pygmy right whale	
		<i>Phocoenidae</i>	Spectacled porpoise	
		<i>Physeteridae</i>	Sperm whale	
		<i>Ziphiidae</i>	Beaked and bottlenose whales	
	Chiroptera	<i>Pteropodidae</i>	Flying-foxes, blossom bats	
		<i>Megadermatidae</i>	Ghost bat	
		<i>Rhinolophidae</i>	Horseshoe bats	
		<i>Hipposideridae</i>	Leaf-nosed bats	
		<i>Emballonuridae</i>	Sheath-tailed bats	
		<i>Molossidae</i>	Free-tailed bats	
		<i>Miniopteridae</i>	Bent-winged bats	
		<i>Vespertilionidae</i>	Long-eared bats	
		<i>Eulipotyphyla</i>	<i>Soricidae</i> [^]	See Appendix 1
		<i>Lagomorpha</i>	<i>Leporidae</i> [^]	See Appendix 1
	<i>Perissodactyla</i>	<i>Equidae</i> [^]	See Appendix 1	
	Rodentia	<i>Muridae</i> [^]	See Appendix 1	
		<i>Sciuridae</i>	Indian palm squirrel	
	Sirenia	<i>Dugongidae</i>	Dugong	

* = Tularaemia has been reported in Australia

[^] = Tularaemia has been reported overseas