WILDLIFE HEALTH AUSTRALIA

NATIONAL GUIDELINES FOR SAMPLE SUBMISSION

TULARAEMIA – DIAGNOSTIC TESTING

Tularaemia is a nationally notifiable disease of terrestrial animals[1]. 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 overseas[2].

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 sheet[3] 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 range[4]. 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 Australia[6]. There is limited further information on the presence, prevalence or epidemiology of tularaemia in the Australian context[7].
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
- Location
- Species
- Number of animals affected
- Presenting signs
- Presence/type of ectoparasites
- Observations on the status of the population
- Body condition
- Weight
- Types of samples submitted
- In-contact persons
- Photographs
- Number of any lesions
- Size of any lesions
- Location of any lesions

[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 pericaridits:

- 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 meat[10]. No human-to-human transmission has been reported[11].

c. Decontamination[12]

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 SSBAs 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) Coordinator[14], 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) Coordinator[14], or contact the corresponding laboratory in your jurisdiction prior to collecting or submitting any samples.

Wildlife Health Australia

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 to 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	• Molecular assays[15] • Bacterial culture • Histopathology	• 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)	• Molecular assays • Bacterial culture (fresh tissue preferred)	• 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	• Molecular assays • Bacterial culture	• 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)	• Molecular assays • Bacterial culture (fresh tissue preferred)	
	Formalin	• 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	• Molecular assays • Bacterial culture	• 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)	• Molecular assays • Bacterial culture (fresh tissue preferred)	
	Formalin	• Histopathology • Immunohistochemistry	
Swab/scrape/FNA of affected areas	4 °C	• Molecular assays • Bacterial culture	• 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	• Molecular assays	• 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 Australia[16].

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*	*Hemibelideus lemuroides*	Lemuroid ringtail possum
			Petauroides	*Petauroides volans*	Greater glider
			Petropseudes	*Petropseudes dahli*	Rock ringtail possum
			Pseudocheirus	*Pseudocheirus occidentalis*	Western ringtail possum
				Pseudocheirus peregrinus	Common ringtail possum[17]
			Pseudochirops	*Pseudochirops archeri*	Green ringtail possum
			Pseudochirulus	*Pseudochirulus cinereus*	Daintree River ringtail possum
Eutheria	*Artiodactyla*	*Bovidae*[18]	*Bos*	*Bos indicus*	Zebu cattle
				Bos javanicus	Banteng
				Bos taurus	European cattle
			Bubalus	*Bubalus bubalis*	Water buffalo
			Capra	*Capra hircus*	Goats
		Camelidae	*Camelus*	*Camelus dromedarius*	Camel[19]
		Cervidae[18]	*Axis*	*Axis axis*	Chital
			Dama	*Dama dama*	Fallow deer
			Cervus	*Cervus elaphus*	Red deer
				Cervus timorensis	Rusa deer
				Cervus unicolor	Sambar
		Suidae	*Sus*	*Sus scrofa*	Pig[20]
Eutheria	*Carnivora*	*Canidae*[21]	*Canis*	*Canis lupus dingo*	Dingo
			Vulpes	*Vulpes vulpes*	Red fox[22]
		Felidae	*Felis*	*Felis catus*	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. Tularemia: 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-Pöltl 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*	*Crocidura attenuate trichura*	Christmas Island shrew
Eutheria	*Lagomorpha*	*Leporidae*	*Lepus*	*Lepus europaeus*	European hare[23]
			Oryctogalus	*Oryctogalus cuniculus*	Rabbit[23]
Eutheria	*Perissodactyla*	*Equidae*[16]	*Equus*	*Equus caballus*	Horse[24]
				Equus asinus	Donkey
Eutheria	*Rodentia*	*Muridae*[17]	*Hydromys*	*Hydromys chrysogaster*	Water rat
			Leporillus	*Leporillus conditor*	Greater stick-nest rat
			Mesembriomys	*Mesembriomys gouldii*	Black-footed tree-rat
				Mesembriomys macrurus	Golden-backed tree-rat
			Mus	*Mus musculus*	Domestic mouse[23]
			Notomys	*Notomys alexis*	Spinifex hopping mouse
			Pseudomys	*Pseudomys australis*	Plains rat
				Pseudomys shortridgei	Heath rat
				Pseudomys fumeus	Smoky mouse
				Pseudomys chapmani	Pebble-mound mouse
				Pseudomys praeconis	Shark Bay mouse
			Rattus	*Rattus* spp.	Rats[23]
			Zyzomys	*Zyzomys argurus*	Common rock rat
				Zyzomys palatilis	Carpentarian rock rat
				Zyzomys pedunculatus	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*	*Ornithorhycidae*	Platypus
		Tachyglossidae	Short beaked echidna
Marsupialia	*Dasyuromorphia*	*Dasyuridae*	Quolls, dibbler, mulgaras, phascogales, antechinuses, planigales, dunnarts
		Myrmecobiidae	Numbat
	Diprotodontia	*Acrobatidae*	Feathertail gliders
		Burramyidae	Pygmy-possums
		Hypsiprymnodontidae	Musky rat-kangaroo
		Macropodidae	Kangaroos, wallabies, quokka, pademelons
		Myrmecobiidae	Numbat
		Petauridae	Gliders
		Phalangeridae	Brushtail possums, cuscus
		Phascolarctidae	Koala
		*Pseudocheiridae**	Ringtail possums, see Appendix 1
		Potoroidae	Bettongs and potoroos
		Tarsipedidae	Honey possum
		Vombatidae	Wombats
	Notoryctemorphia	*Notoryctidae*	Marsupial moles
	Peramelemorphia	*Peramelidae*	Bandicoots
		Thylacomyidae	Bilbies
Eutheria	*Artiodactyla*	*Bovidae^*	Cows, goats. See Appendix 1
		*Camelidae^*	Camels. See Appendix 1
		*Cervidae^*	Deer, chital. See Appendix 1
		*Suidae^*	Pigs. See Appendix 1
	Carnivora	*Canidae^*	Dingo, fox. See Appendix 1
		*Felidae^*	Cats. See Appendix 1
		Otariidae	Fur or eared seals
		Phocidae	'True' seals
	Cetacea	*Baleaenidae*	Southern right whale
		Balaenopteridae	Humpback whale
		Delphinidae	Ocean dolphins
		Kogiidae	Pygmy and dwarf sperm whales
		Neobaleaenidae	Pygmy right whale
		Phocoenidae	Spectacled porpoise
		Physeteridae	Sperm whale
		Ziphiidae	Beaked and bottlenose whales
	Chiroptera	*Pteropodidae*	Flying-foxes, blossom bats
		Megadermatidae	Ghost bat
		Rhinolophidae	Horseshoe bats
		Hipposideridae	Leaf-nosed bats
		Emballonuridae	Sheath-tailed bats
		Molossidae	Free-tailed bats
		Miniopteridae	Bent-winged bats
		Vespertilionidae	Long-eared bats
	Eulipotyphyla	*Soricidae^*	See Appendix 1
	Lagomorpha	*Leporidae^*	See Appendix 1
	Perissodactyla	*Equidae^*	See Appendix 1
	Rodentia	*Muridae^*	See Appendix 1
		Sciuridae	Indian palm squirrel
	Sirenia	*Dugongidae*	Dugong

* = Tularaemia has been reported in Australia

^ = Tularaemia has been reported overseas