

NATIONAL KOALA DISEASE RISK ANALYSIS REPORT

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Dedication



This report is dedicated Dr. Joanna Griffith, a courageous and spirited woman with a passion for all wildlife, but particularly for the koalas that were the focus of her distinguished veterinary career. Right up until the week before her death, Jo was involving herself in the KDRA process, sharing her knowledge and resources, and providing valued feedback. She was a dear friend as well as a respected colleague to many people working with koalas, and her tenacity and spirit have been an inspiration to us all.

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Definitions

Term	Definition
Acceptable level of risk	The maximum overall exposure to <i>risk</i> that should be accepted, based on the benefits and costs involved.
Acute disease	A disease occurring in a short timeframe, regardless of severity of clinical signs.
Antibody	A protein made by the body's immune cells in response to an <i>antigen</i> , which acts to combine with and remove the <i>antigen</i> from the <i>host's</i> body.
Antigen	Any substance that causes the body to mount an adaptive immune response, especially the production of <i>antibodies</i> , against that substance. Antigens from infectious agents are commonly targets in diagnostic tests to detect presence of those agents.
Antigenaemia	The presence of <i>antigen</i> in the blood of the host.
Arthropod	Invertebrate animals (such as insects, spiders and mites) that have a segmented body, jointed appendages and an exoskeleton.
Association	A statistical relationship between two variables. Two variables may be associated without a causal relationship.
Biosecurity	The set of precautions taken to minimise the risk of introducing a <i>parasite</i> or <i>infectious disease</i> into an animal (or human) population, or to a group or individual.
Captive/captivity	An animal that lives under human control or care, either temporarily or permanently.
Carrier	A <i>host</i> that harbours a <i>pathogen</i> in its body without manifesting <i>clinical signs</i> , thus acting as a potential source or distributor of <i>infection</i> .
Causation	A situation where the exposure to a <i>hazard</i> is responsible for the effect.
Chronic disease	A disease occurring over a long timeframe, regardless of the severity of clinical signs.
Clinical sign	Observed, objective changes in the normal healthy state, bodily function or behaviour of an animal.
Colonisation	The presence of <i>pathogens</i> on a body surface (e.g. skin, mouth, intestines or airway) without causing <i>disease</i> in the individual.
Contagious hazard	<i>Infectious diseases</i> that are spread through (direct or indirect) contact with infected individuals e.g. <i>Chlamydia</i> . Not all infectious diseases are contagious.
Critical control point	A key point in a <i>hazard's</i> biological pathway at which practical <i>risk management</i> strategies could be implemented.
Cytokine	Substances such as interferon, interleukin and growth factors, that are secreted by certain cells of the immune system and have an effect on other cells.
Dedicated equipment	Equipment that is dedicated for sole use for an animal, task or area, with the purpose of reducing the risk of cross-contamination.
Diagnostic test	Any procedure used to aid in the characterisation of the cause or nature of a <i>disease</i> .
Disease	Any disturbance in the health or function of an animal or human.
Disseminated intravascular coagulation	A serious <i>disease</i> in which the proteins that control blood clotting become overactive.
DNA (deoxy-ribonucleic acid)	The molecule that carries genetic information for the development and functioning of an organism.
Dyspnoea	Shortness of breath or difficulty breathing.

Term	Definition
Endemic	A <i>disease</i> or <i>parasite</i> regularly found among particular populations or in a specified geographic area.
Endogenous KoRV transmission	The transmission of KoRV within the koala genome by inheritance, which occurs only with the endogenous form of KoRV.
Endogenous retrovirus	Retrovirus which is incorporated into <i>germ cells</i> of the host and therefore is inherited by successive generations.
Exogenous KoRV transmission	The transmission of KoRV from koala to koala by means other than inheritance. This is thought to occur with exogenous forms of KoRV.
Exogenous retrovirus	Retrovirus that is incorporated only into the <i>somatic cells</i> of the host and therefore is not inherited.
Exotic (disease)	A <i>pathogen</i> not known to be present in a specified geographic area.
Fomite	Any inanimate object that can harbour <i>parasites</i> and thereby play a role in <i>transmission</i> of those parasites.
Genotype	The <i>DNA</i> sequence at a specific position within the genome of the <i>pathogen</i> , also known as sequence type, subtype, variant or strain.
Germ cell	A gamete cell (egg or sperm).
Habitat degradation	A reduction in the quality of the habitat available, such that it affects the carrying capacity, health or welfare of koalas in the habitat.
Habitat fragmentation	Loss of connectivity between areas of habitat supporting koalas, independent of the absolute amount of habitat available.
Habitat loss	Any process which results in a reduction of the absolute amount of habitat available to koalas.
Haematogenous dissemination	Spread via the blood system of a <i>host</i> .
Hazard (disease)	Biological, physical or chemical factors in the animal's environment that can have negative impacts on their health. See also <i>disease</i> .
Host	Any animal harbouring a <i>parasite</i> , regardless of whether it plays a role in the further <i>transmission</i> of the parasite.
Hyperoxaluria	Elevated oxalate levels in the urine.
Immune modulation	Changes to the function of the <i>immune system</i> ; may be stimulatory, suppressive or a shift in focus.
Immune system	The collection of organs, cells and molecules that together provide the animal with defence against invading organisms.
Inapparent infection	An infection that is associated with clinical signs that are only identifiable with specialised diagnostic equipment or via internal or microscopic examination. In particular, chlamydial infections where there are no outward signs of disease but internal urogenital pathology is present and detectable via ultrasound examination or histopathology.
Incubation period	The time that elapses between <i>infection</i> with a <i>parasite</i> and the onset of <i>disease</i> .
Infection	The entry and development or multiplication of a <i>parasite</i> in the body of a <i>host</i> , where it may or may not cause <i>disease</i> .
Infectious disease	The debilitating effects of <i>infection</i> or <i>infestation</i> by a <i>parasite</i> . It is possible for a <i>host</i> to be infected by a parasite but to show no <i>clinical signs of disease</i> .
Infectious hazard	A <i>hazard</i> which involves the entry and development or multiplication of a <i>microparasite</i> or <i>macroparasite</i> in the koala, where it may or may not cause <i>disease</i> .
Infestation	When a <i>macroparasite</i> is present on the external surface of a <i>host</i> , regardless of whether the infestation results in <i>disease</i> .

Term	Definition
Integration site	In terms of KoRV, the position within the koala genome where KoRV undergoes <i>integration</i> into the koala <i>DNA</i> .
Integration	The process of incorporation of <i>KoRV</i> into the <i>host koala DNA</i> , also known as <i>insertion</i> .
KoRV traits	The range of characteristics by which KoRV presence in a <i>host</i> may be quantified or described. Includes viral load, <i>proviral load</i> , presence or load of variants and presence or load of certain genetic markers (e.g. <i>pol</i> gene).
Latent infection	A persistent <i>subclinical infection</i> in which the <i>parasite</i> is dormant but has the potential to become active and cause <i>disease</i> or be <i>transmitted</i> in the future.
Latent phase (virus)	The stage of the virus lifecycle when it generally lies dormant within <i>host</i> cells.
Listed koala	Combined populations of koalas in Qld, NSW and the ACT.
Lytic phase (virus)	The active phase during which the virus replicates within the <i>host</i> cell and releases a new generation of viruses when the infected host cell lyses.
Macroparasite	<i>Multicellular parasites</i> such as worms, fleas and <i>arthropods</i> that are typically visible to the naked eye.
Mesopredator	A mid-ranking predator in the middle of a trophic level, that typically preys on smaller animals. In koala habitat, feral cats and foxes are considered to be mesopredators.
Microbiome	The assemblage of living microorganisms present in a particular environment (including the body or a part of the body).
Microparasite	<i>Parasites</i> such as bacteria, viruses and fungi that are typically invisible to the naked eye.
Mitigation	To apply a treatment or action that lessens or decreases the severity or likelihood of a <i>risk</i> .
Necrosis	The death of most or all of the cells in an organ or tissue due to disease, injury, or failure of the blood supply.
Non-infectious hazard	A <i>hazard</i> that is not living and cannot be <i>transmitted</i> to a <i>host</i> from another <i>host</i> or from the environment.
Non-pathogenic	An organism or strain not causing disease.
Northern koala	Koalas found in Qld, NSW and the ACT.
Opisthotonus	A state of severe hyperextension in which an individual's head, neck and spinal column enter into an "arching" position.
Overt	In the context of the KDRA, <i>disease</i> that is plainly apparent without the use of specialised diagnostic equipment.
Parasite	A biological agent that lives on or within a <i>host</i> and survives at the expense of the host regardless of whether a <i>disease</i> state follows. This includes both <i>microparasites</i> (e.g. bacteria, viruses) and <i>macroparasites</i> (e.g. helminths, arthropods).
Pathogen	Any organism causing <i>disease</i> .
Pathognomonic	<i>Clinical signs, diagnostic test</i> results or other findings which are unique to a particular diagnosis.
Personal protective equipment	Anything worn by a person to keep them healthy and safe when undertaking a task.
<i>Pol</i> KoRV	KoRV which possesses the <i>pol</i> gene and is therefore capable of integrating into the koala host DNA and replicating; "replication-competent" KoRV.
<i>Pol</i> -negative koala	A koala that is infected with KoRV that does not possess the <i>pol</i> gene and is therefore incapable of replication.

Term	Definition
<i>Pol</i> -positive koala	A koala that is infected with KoRV possessing the <i>pol</i> gene ("replication-competent" KoRV).
Polyparasitism	The presence of multiple <i>parasites</i> on, or in, the same <i>host</i> .
Precautionary principle	The principle by which, if there is limited information, a <i>risk</i> is assumed to exist (and require management), until proven otherwise.
Prevalence	The proportion of the <i>host</i> population with <i>infection</i> , <i>disease</i> or <i>antibody</i> presence, often expressed as a percentage.
Proviral load	The amount of <i>provirus</i> within a host.
Provirus	A virus genome that is integrated into the DNA of a host cell. In terms of KoRV, refers to the transcribed <i>DNA</i> copy of <i>KoRV</i> which is incorporated into the koala genome.
Pruritus	Severe itching of the skin.
Quarantine	Isolation and observation of an animal in a biosecure setting for a specified period of time to allow <i>diseases</i> of concern to be detected and treated, and to prevent all new exposures to <i>parasites</i> of concern.
RecKoRV	Defective KoRV elements that appear to be endogenous but lack the <i>pol</i> gene and are therefore not capable of <i>replication</i> .
Rehabilitation facility	A facility (large or small) for the treatment and care of injured, orphaned, or sick wild animals so that they can be released back to the wild.
Replication-competent (retrovirus)	In terms of KoRV: KoRV that contains the full gene complement and is therefore capable of integrating into <i>host DNA</i> and replicating to produce additional virus.
Reservoir	A species which can harbour a <i>pathogen</i> indefinitely with no ill effects. Reservoir <i>hosts</i> provide an environmental 'reservoir' for a <i>pathogen</i> and generally don't get sick from <i>infection</i> .
Riparian habitat	Habitat occurring along water courses and water bodies.
Risk	The likelihood of encountering some form of harm, loss or damage, combined with the severity or consequence of the event.
Risk analysis	The process composed of problem description, hazard identification, risk assessment, risk management and risk communication.
Risk assessment	The evaluation of the likelihood and the consequences of entry, establishment or spread of a pathogenic agent within a specified animal population or environment.
Risk management	The process of identifying, selecting and implementing measures that can be applied to reduce the level of <i>risk</i> .
RNA (ribonucleic acid)	A nucleic acid present in all living cells that has structural similarities to DNA.
Screening test	Any procedure used to aid in the identification of individuals in a population that have subclinical infections, so that appropriate action can be taken.
Sensitivity (test)	The likelihood that a truly positive (infected) individual will return a positive test result.
Sequence type	See <i>genotype</i> .
Somatic cell	Any cell of a living organism other than the <i>germ cells</i> .
Southern koala	Koalas originating from SA and Vic free-ranging populations.
Specificity (test)	The likelihood that a truly negative (non-infected) individual will return a negative test result.
Strain (of organism)	See <i>genotype</i> .

Term	Definition
Subclinical infection	A state in which an <i>infectious hazard</i> is present in the <i>host</i> without any clinical signs of illness.
Surveillance	A systematic ongoing program of investigation designed to establish the presence, extent of or absence of a <i>disease</i> , or of <i>infection</i> , or presence of a <i>pathogen</i> . It includes the examination and testing of animals for <i>clinical signs</i> , <i>antibodies</i> or the presence of a <i>pathogen</i> and the timely dissemination of information so that action can be taken.
Trans-faunation	Oral administration of intestinal content to restore bacteria to the gut.
Transmission	The process by which a <i>parasite</i> passes from a source of infection to a new <i>host</i> .
Transparency (of process)	Comprehensive documentation of all data, information, assumptions, methods, results, discussion and conclusions used in the <i>risk analysis</i> . Conclusions are supported by an objective and logical discussion and the document is fully referenced.
Triage	The process of organising patients according to the severity of their condition and treating each patient within an appropriate time frame. Triage also includes an assessment of the viability of the patient, and whether euthanasia should be considered due to the severity of <i>disease</i> and associated welfare concerns.
Trypanosomiasis	Infection of a host by trypanosome <i>parasites</i> .
Vector	A living organism (frequently an arthropod) that transmits an infectious hazard from one host to another.
Virion	The complete, infective form of a virus outside a host cell.
Wet bottom	Chronic staining and wetness of the rump of koalas, associated with urinary incontinence and often caused by urinary tract infections, particularly <i>Chlamydia</i> .
Wildlife hospital	A hospital or clinic that provides veterinary assessment, treatment and care for free-ranging wildlife.
Zoonosis	A <i>disease</i> of animals that can be transmitted to humans.

Abbreviations and Acronyms

ABBREVIATION	
ACT	Australian Capital Territory
CCP	Critical control point
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DNA	Deoxyribonucleic acid
EAG	Expert Advisory Group
eWHIS	(National) electronic wildlife health information system
FelV	Feline leukaemia virus
h	Hours
ha	Hectares
IFN γ	Gamma interferon
IUCN	International Union for the Conservation of Nature
KHH	Koala Health Hub
KoRV	Koala retrovirus
LCAT	Latex cryptococcal antigen test
LFA	Lateral flow immunochromatography assay
MLST	Multi-locus sequence typing
MVA	Motor vehicle accident
NSW	New South Wales
NT	Northern Territory
NZ	New Zealand
OIE	Office International des Epizooties (now World Organisation for Animal Health)
ON	Oxalate nephrosis
PCR	Polymerase chain reaction
PhaHV	Phascolarctid herpesvirus
Qld	Queensland
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
SA	South Australia
SME	Subject matter expert
T _a	Ambient temperature
Tas	Tasmania
T _b	Body temperature
TNZ	Thermoneutral zone
Vic	Victoria
WA	Western Australia
WHA	Wildlife Health Australia
WOAH	World Organisation for Animal Health (formerly OIE)
ZAA	Zoo and Aquarium Association

Executive Summary

- This document details a national disease risk analysis for the koala and identifies the knowledge base, information gaps, risk assessments and critical control points for koala disease hazards. Recommendations are yet to be considered for implementation. Commitment of government funding for implementation of the Koala Disease Risk Analysis (KDRA) is recommended, including a process for ongoing stakeholder communication, review and updating of the KDRA.
- The findings and recommendations of the KDRA should be embedded and operationalised in the context of the broader national Koala Recovery Plan. Resourcing to support national koala disease risk mitigation actions is highly recommended.
- The KDRA should be used as a key guiding document for decisions on koala disease risk prioritisation. It underpins and facilitates, but does not replace local context risk assessment.
- The national KDRA explored disease threats to all koalas in Australia: in captivity, in rehabilitation and in the wild. **Ten diseases** were identified as significant risks to koala populations and require risk mitigation action (see Section 1.1 *Significant Disease Hazards Identified*). In most instances, effective actions can be taken to reduce disease risks in wild koalas and many of the identified risk management options have the capacity to mitigate multiple disease risks to koalas.
- A comprehensive threat analysis found **decline in environmental health** to be the principal driver of disease impacts in koalas. Consequently, preservation and restoration of habitat is a critical component of mitigating disease risks to free-ranging koalas.
- Clear linkage between disease hazards and other non-disease drivers indicates that **integrated strategies** are required, that simultaneously manage and prioritise multiple threats, including disease. The national KDRA will enable the integration of health and disease risk mitigation actions into broader koala conservation efforts nationally.
- The **multiple knowledge gaps** identified through the KDRA should be used to guide research prioritization for koala health and disease. Disease hazards assessed as high risk through the KDRA, or where a low level of confidence in the risk assessment was identified, are high priorities for further research.
- The KDRA provides the basis for the development of a nationally-agreed approach to koala disease management and risk mitigation. This should include **nationally-agreed standards** for diagnosis, triage, investigation, treatment, care and record-keeping of diseased koalas to support best practices in koala health management.
- To build community confidence in koala conservation decisions, funding for koala conservation should address all threats facing koalas in an integrated manner, underpinned by scientifically robust information.

1 Summary of Findings and Recommendations

The national focus of the KDRA provides a clear, evidence-based assessment of koala disease which will be of value in evaluating disease risk at all regional levels and in all management situations (captive, rehabilitation, free-ranging).

Participants in the Koala Disease Risk Analysis (KDRA) project identified their ideal future for koala health in the following Vision Statement:

“Sustainable, resilient and healthy populations of koalas, living in positive welfare within healthy ecosystems across their range. Koalas are well-managed in their local context and populations are supported by robust and consistent legislation, informed community engagement and long-term funding.”

Identification and analysis of risks and the recommendations arising from the KDRA were underpinned by this vision.

This disease risk analysis followed the current gold-standard methodology endorsed by the International Union for the Conservation of Nature (IUCN) and the World Organisation for Animal Health (WOAH). It was conducted over a 10-month period and incorporated a series of facilitated online meetings and workshops involving 44 disease and wildlife management experts, government representatives, non-government organisations and other stakeholders. A feature of the KDRA was the uniformly shared concern for the conservation and welfare of koalas and willingness to collaborate among this wide range of stakeholders.

1.1 Significant Disease Hazards Identified

In the context of the KDRA, disease is defined as “*any disturbance in the health or function of an animal or human*” and includes diseases due to both infectious and non-infectious causes.

The KDRA explored disease threats to all koalas in Australia, whether in captivity, in rehabilitation or in the wild. Through a systematic refinement process, 13 disease hazards were identified requiring detailed risk assessment. Of these, the following 10 disease hazards were assessed as requiring active management because they present significant risks to both koala population viability and resilience *on a broad scale*, and to individual koala health and welfare:

High risk to koala population viability: *Chlamydia* spp., koala retrovirus (northern populations).

Moderate risk to koala population viability: *Cryptococcus* spp., heat stress, koala retrovirus (southern populations) and thermal burn trauma.

Low risk to koala population viability: Motor vehicle trauma, neoplasia, oxalate nephrosis, sarcoptic mange.

Table 1 Overall estimates of risk for koalas for diseases selected for detailed risk assessment. For further details, and for overall risks to humans and other species, see individual disease hazard risk assessments in Section 5.

Mod = moderate risk; Neg = negligible risk; Med = medium confidence. ¹ KoRV overall risk determined separately for northern koalas and southern koalas; ² oxalate nephrosis overall risk to individual koalas determined separately for Mt Lofty Ranges (MLR) population and other populations. Note: Disease hazards assessed as high risk through the KDRA, or where a low level of confidence in the risk assessment was identified, are high priorities for further research.

	Chlamydia	Crypto-coccus	Heat stress	KoRV	Motor vehicle trauma	Neoplasia	Actino-myces	Oxalate nephrosis	Herpes-viruses	Predator attack trauma	Sarcoptic mange	Thermal burn trauma	Trypano-somes
Overall risk estimates for koalas (for risks to humans and other species, see Section 5)													
Koala population resilience & viability	High	Mod	Mod	High (nth) Mod (sth) ¹	Low	Low	Neg	Low	Neg	Mod	Low	Mod	Neg
Koala individual health & welfare	High	Mod	High	High (nth) Mod (sth) ¹	Mod	Mod	Mod	High (MLR) Low (other) ²	Mod	High	Mod	High	Mod
Level of confidence in assessment													
	High	Med	Med	Low	Med	Med	Low	Med	Low	Med	Med	Med	Low

1.2 Recognition of Spatial Variation in Disease Risks

Disease threats to koalas vary across their geographic distribution, and whether a koala is in captivity, in rehabilitation or in the wild. Given the scope and national focus of the KDRA, risk assessments generally took a ‘whole of species’ approach, except where current evidence clearly indicated a significant variation in disease occurrence (e.g. koala retrovirus in northern vs southern koalas).

1.3 Knowledge Gaps and Refinement

In common with the state of knowledge of wildlife disease in general, the KDRA identified substantial gaps in current scientific knowledge of the epidemiology and regional prevalence of many disease hazards (see Section 8 *Key Knowledge Gap Summary* for an overview). For this reason, this qualitative analysis was based on the best available information, combining a comprehensive literature review and expert opinion. It is anticipated that this risk analysis will be continuously refined through future reviews, as further research helps to close the identified knowledge gaps.

1.4 Drivers of Disease

While disease is a natural part of ecosystems, disease impacts are inextricably linked to a number of other threats faced by koalas, and they place significant additional pressure on wild koala populations already suffering the cumulative impacts of severe non-disease threats. To identify and understand the underlying drivers of disease in this wider context, KDRA participants conducted a comprehensive threat analysis. This provided a framework for the identification of causal effect pathways and the points on these pathways where the most effective risk management intervention could take place (critical control points). To successfully manage disease risk to koalas, it is necessary to understand the linkage of disease to these non-disease drivers and to develop integrated strategies that simultaneously manage multiple threats in ways which are appropriate to the local situation and scale. For instance, decline of environmental health – particularly habitat loss, degradation and fragmentation – is recognized as a principal driver of disease in koalas. This is further exacerbated by the effects of climate change, including increased number and severity of bush fires, floods and droughts.

1.5 Disease Risk Management

It may not always be possible to directly address the causes of disease in wild koalas but in most instances, effective actions can be taken to reduce disease risks. Effectively mitigating disease risks in koalas is a complex task, requiring active participation from all levels of government and community over a long term with significant funding and resourcing¹. The

¹ See *National Guidelines for the Management of Disease in Free-ranging Australian Wildlife* https://wildlifehealthaustralia.com.au/Portals/0/Documents/ProgramProjects/National_Guidelines_Management_Disease_Freeranging_Aust_Wildlife_Nov_2020.pdf

KDRA identified a range of risk mitigation options for the 13 disease hazards undergoing detailed risk assessment. Not all risk mitigation options listed in this report may be currently available, feasible, universally applicable, or cost-effective. Proposed by KDRA participants, risk mitigation options are included in this report as a basis for review during a subsequent multi-stakeholder workshop to develop a KDRA risk management implementation plan. Many of the identified risk management options have the capacity to mitigate multiple disease risks to koalas. In particular, the preservation and restoration of habitat and habitat connectivity was identified as a critical component of disease risk mitigation for free-ranging koalas. In addition, preventing introduction of pathogens is more cost effective than eradication; thorough action-specific risk assessment should be conducted for any action involving movement of animals.

1.6 Over-arching Recommendations

The following over-arching recommendations are made based on the KDRA as a whole, including its non-disease threat analysis, review of literature, stakeholder meetings and workshops, hazard refinement and individual disease risk assessments. Detailed recommendations for each of the 13 disease hazards are included in the risk assessments. This includes a list of General Recommendations that apply broadly across most of the disease hazards of concern.

1.6.1 Environmental management

- Decline in environmental health – particularly habitat loss, degradation and fragmentation – is a principal driver of disease in koalas and significantly amplifies disease impacts. This is exacerbated by the effects of climate change including increased number and severity of bush fires, floods and droughts. The importance of habitat conservation and restoration as a principal component of disease risk mitigation in koalas should be recognised in all disease management planning.

1.6.2 Implementation, collaboration, alignment and review of the KDRA

- The concern, collaborative spirit, and diverse expertise harnessed during the KDRA project should continue to be fostered through a KDRA risk management Implementation workshop (currently in the planning stages), and future revisions of the KDRA.
- Disease in wild koalas should be addressed in a holistic and consistent manner, with the greatest disease risks receiving the most focus, both in mitigation actions and in research efforts. The KDRA should be used as a key guiding document for decisions on disease risk prioritisation.
- The development of a national Koala Recovery Team should be supported, to address the need for nation-wide koala management policy and coordination. This will assist in the development of over-arching threat management principles that can be applied across the koala's geographic range. It is recommended that the Recovery

Team include representation from stakeholders of both northern and southern koalas, to allow a truly national approach to managing threats to the koala.

- The national Koala Recovery Team should establish means for consultation with participants who worked on the KDRA and are familiar with its processes. The Recovery Team should use and cite the KDRA as a source of current knowledge on matters relating to koala health and disease risk.
- A process for ongoing review and updating of the KDRA should be established, to enable ongoing national-level collation of expertise on koala disease risk to guide koala management into the future. In order to stay abreast of ongoing developments in knowledge of koala populations and disease, the findings and recommendations of the KDRA should be reviewed within the next 2 years (by July 2024), or earlier if significant new information becomes available.

1.6.3 Incorporation of genetic knowledge into disease risk

- Recent advances in the mapping of the koala genome are likely to provide critical resources to support research on the functional role of genetic diversity in disease expression and population viability in coming years. The incorporation of genomic survey data into management strategies is in its early stages. Once its functional significance is more fully understood, it will be a critical component of holistic decision-making, to enable integration of genetic traits influencing disease and non-disease risks into koala management strategies. A collaborative process for the prompt incorporation of this knowledge into disease risk evaluation should be developed within national initiatives such as the Koala Recovery Team, National Koala Monitoring Project (NKMP) and research strategies.

1.6.4 Community engagement

- There is a need to improve clarity in messaging and public perception of the disease threats to koalas. This includes improved briefing and education of the media, citizen scientists and the wider community on the key disease issues facing koalas, their underlying causes and how the community can effectively respond. It is recommended that this report be used as a reference for developing evidence-based messaging on koala disease.
- Funding to support a communication strategy for the KDRA should be secured, to enable information sharing with stakeholders. This should include webinar communications tailored to different audiences, advice on the use of the KDRA in a variety of jurisdictions and scenarios and cross-reference to ongoing development of strategies and resources through the NKMP, the Koala Recovery Team, and other national initiatives.
- Funding allocations for koala conservation should be accompanied by scientifically robust justifications to build community understanding and confidence of koala conservation decisions.

1.6.5 Standards and consistency

- Through the NKMP (or other national approaches), existing guidelines on disease surveillance in koalas should be unified and gaps in protocols identified, with a view to producing comprehensive, nationally-agreed guidelines on biological sampling, sample storage and diagnostic laboratory contacts, for use by those working with koalas.
- Through the Koala Recovery Plan and engagement with State Governments, a nationally consistent approach to translocation risk assessment should be developed
- A nationally-agreed, standardised and shareable system is required for capturing all data pertaining to health and disease in koalas (captive, wild and rehabilitation). This is likely to involve development of a centralised point of access for existing databases, along with some centralised data evaluation.
- A nationally-agreed approach to diagnosis, triage, investigation, treatment, care and record-keeping of diseased koalas will enable uniform best practices to be established. This will require resources for the development of key protocols and guidelines as outlined in Section 8 *Key Knowledge Gaps Summary* and in the *Recommendations* provided in each disease hazard *Risk Assessment*.
- Veterinary involvement is essential in relation to diagnosis, triage and assessment, treatment and biosecurity in captive and rehabilitated koalas and should be supported by appropriate training, funding, regulatory controls and non-government and rehabilitation organisation policy and procedure.
- Continued and expanded support is recommended for nationally-consistent best practice training in diagnosis, triage, treatment, biosecurity and care of koalas during rehabilitation (e.g. Taronga Conservation Society Australia training, see Section 2.6 *Aligned Research Programs*) for veterinary personnel, scientists and rehabilitators.
- Development of nationally-agreed, post-release identification and monitoring protocols for koalas following rehabilitation is recommended. These should aim to evaluate the short and long term impacts of disease on individuals and populations and to inform further refinement of rescue, assessment, treatment and rehabilitation practices.
- Formal training for veterinary personnel and wildlife carers should be incorporated into emergency incident management to allow the timely integration of wildlife rescue into bushfire (and other emergency) response.
- Caring for sick or dying koalas and making decisions about the survival of individual animals is both mentally and emotionally taxing. Personal mental health and welfare care plans, based on science and experience, are needed for all those involved in rescue, treatment and care of injured wildlife.
- Current processes for reporting information on koala disease to the national wildlife health information system (eWHIS), including key words and disease descriptors, should be reviewed, to maximise the value of koala health and disease data.

1.6.6 Research

- More study of koala disease and more robust population data are needed to better quantify the risks of disease to koalas and improve certainty of disease risk assessments. In particular, scientifically validated national population estimates of koalas, and ongoing population monitoring is vital to better understand the impact of disease and other threats to koalas.
- The knowledge gaps identified in the KDRA should be used as a guide for research prioritization for koala health and disease. Disease hazards assessed as high risk through the KDRA, or where a low level of confidence in the risk assessment was identified, are high priorities for further research.
- There is a need for a national population and habitat viability analysis for koalas². At present there is no robust scientific basis or collective understanding on which to base decisions on the sustainable genetic and demographic management of koalas. There is an urgent need to use evidence-based, rigorous methodologies to evaluate population and habitat viability which incorporate all threats, including disease.

1.6.7 Resourcing

- Ongoing commitment of government funding to implement the recommendations of the KDRA, including the KDRA Implementation Plan, nationally-agreed protocols for koala health and disease and a Communication Strategy for the KDRA is recommended.
- Ongoing financial support is recommended for specific training of veterinarians and rehabilitators and providing resources for those undertaking this work.
- Funding models should be explored to enable rapid, high quality investigation and response to unexpected outbreaks of disease in koalas.
- Funding and support is needed to develop a national framework for emergency preparedness and response for wildlife, in situations such as bushfire, flood, drought and disease outbreak.

The reader is also referred to Section 8 *Key Knowledge Gap Summary* and to the detailed recommendations for each of the 13 disease hazards in Section 5 *Risk Assessments*.

1.7 Next Steps

The following recommendations are proposed as short term priorities (July – December 2022):

1.7.1 Report circulation and familiarisation

- Distribution of the completed KDRA should be coordinated through the Koala Health Hub (KHH, University of Sydney), Wildlife Health Australia (WHA) and IUCN, with

² See www.cbsg.org/our-approach/workshop-processes/phva-workshop-process

support from the Commonwealth government, so that the report is available to stakeholders as soon as possible.

- The final report will be made publicly available on national and international platforms, including the IUCN-SSC Conservation Planning Specialist Group website, the WHA website and the KHH website, and will act as an exemplar of a best-practice national DRA for an Australian wildlife species.
- The development of communication webinars and other presentations to introduce stakeholders to the KDRA should be supported, to enable broad stakeholder familiarisation of the final report ahead of the proposed Implementation Planning workshop.

1.7.2 KDRA implementation workshop and plan

- Funding should be sought to enable a facilitated multi-stakeholder face-to-face Implementation Planning workshop in September or October 2022.
- The Implementation Planning workshop should aim to identify detailed strategies, actions, time frames, assignment of roles and responsibilities, cost estimates and sources of funding together with a monitoring and evaluation framework.
- Risk mitigation options will require evaluation for impact and outcome during the implementation planning, with the findings used to review and update the KDRA.
- A detailed strategic communication plan, incorporating the information needs of the wide range of koala stakeholders, should be developed as part of the KDRA risk management implementation plan.

1.7.3 Additional planning and alignment

- The knowledge gaps identified in the KDRA require development into a Research Plan which can be used to identify and prioritise research needs.
- Discussions should occur between major national stakeholders in koala health and management (e.g. the Koala Recovery Team, government agencies, KHH, WHA and the NKMP) to determine capacity and remit to progress these recommendations.

2 Introduction

2.1 Background and Justification for the Koala Disease Risk Analysis

In the context of the Koala Disease Risk Analysis (KDRA), disease is defined as “any disturbance in the health or function of an animal or human” [1]. Disease has long been recognised as a threat to the viability of koala (*Phascolarctos cinereus*) populations in the wild, and to the welfare of individual koalas. For over 30 years, the impacts of chlamydiosis have been a major challenge for management of the species [1-4], and in the last 20 years, koala retrovirus (KoRV) has been identified as a threat with complexities that are only just beginning to be understood [5-8]. Non-infectious hazards such as motor vehicle and predator trauma are ongoing, and in 2019-20, the suffering of koalas in the Australian bushfires had veterinarians and rehabilitators grappling with the management of burns on an unprecedented scale [2, 3]. The last few years have seen the identification of a number of novel koala pathogens such as *Actinomyces* [4] and *Chromobacterium* [5]. Diseases which are well-recognised in other species, such as sarcoptic mange, now appear to be also establishing in koala populations [6, 7]. This national KDRA provides a means of bringing together the most recent knowledge on disease hazards, determining the most important disease threats for koalas as a species, identifying risk management options and documenting current gaps in knowledge.

The drivers of disease in koalas are complex and often incompletely understood. There is currently no nationally-agreed approach to disease monitoring, investigation, treatment and record keeping for koalas, although work is under way to better incorporate health monitoring into the National Koala Monitoring Program (NKMP - see 2.5 *Aligned Research Programs*). A national KDRA provides a comprehensive evidence base to inform disease monitoring strategies for the NKMP. Identification of knowledge gaps can inform and focus koala research priorities at the national level.

Although disease is a natural phenomenon, population-level impacts of disease on wildlife occur where the natural balance between the health of the environment and animals within it is disturbed. As such, disease impacts are inextricably linked to a number of other threats faced by koalas, such as habitat loss and climate change (see Section 3.6 *Non-disease Threats and Their Impact on Disease*) that drive or exacerbate the negative impacts of disease on koala populations [8, 9]. Identifying the relationship between disease and other threats through the national KDRA highlights the interconnectedness of all threats to koala conservation, and provides the necessary holistic context to support effective management of koala disease risks [9, 10].

There are significant regional differences in the disease manifestations of a number of key disease hazards of koalas, including chlamydiosis and koala retrovirus [11]. The non-disease threats facing koalas also differ in their regional impact across the koala’s distribution [10].

A KDRA which evaluates disease risk at the national level and facilitates input from a broad range of stakeholders with first-hand knowledge and understanding of regional differences in disease manifestations, enables the information sharing needed to inform regional decisions on disease risk mitigation.

To this end, the *National Recovery Plan for the Koala* identifies the importance of building and sharing knowledge, and collaborating with a range of stakeholders to protect koalas [12]. In particular, it identifies the need for a consistent approach and guidance on matters relating to koala health and disease, which incorporates the knowledge of veterinary experts, koala welfare and rehabilitation experts, disease research professionals, Traditional Owners and other stakeholders in koala health. The Commonwealth initiative to develop a national KDRA via the *Bushfire Recovery for Wildlife and their Habitats* Program recognises the importance of developing a nationally-relevant document on koala disease risk, to assess current knowledge, identify gaps, and propose disease management strategies.

2.2 Koala Disease Risk Analysis Purpose

The purpose of the KDRA is to:

- Consolidate available knowledge about koala diseases and disease risks.
- Record gaps in knowledge.
- Identify potential research priorities.
- Inform nationally-agreed guidelines for koala disease risk mitigation, disease risk assessment for koala movements, disease screening protocols, koala health examination, sample collection, interpretation of diagnostic test results and biosecurity practices for koala management.
- Communicate risks of disease in koalas, and outputs of the KDRA, to stakeholders.

2.3 Koala Disease Risk Analysis Questions

The KDRA addresses the following questions:

- Where is disease risk placed in the context of other threats facing koalas?
- What are the known and possible disease threats to the koala?
- Which of these diseases are the priorities for action?
- What disease risk mitigation actions will best improve the future viability of koala populations regionally and nationally?
- What are the gaps in current disease knowledge and how can these be addressed to increase confidence in risk assessments?

2.4 Koala Disease Risk Analysis Scope, Focus and Vision

The KDRA was confined to the following scope and focus:

- The koala, *Phascolarctos cinereus*, a single species with no close relatives.

- All koala populations in Australia, both free-living and captive.
- Non-disease threats with potential to directly or indirectly impact disease risk.
- Disease hazards only identified in koalas located overseas were excluded.

For the purpose of the KDRA, a “disease” was defined as “any disturbance in the health or function of an animal or human” [1]. The KDRA considered all infectious and non-infectious disease hazards to which koalas may be susceptible, including diseases only known to be associated with the captive state, and disease syndromes which did not have a clear identified cause.

Stakeholders developed a *Vision Statement* to express the shared aspirations for the health and conservation management of koalas:

Sustainable, resilient and healthy populations of koalas, living in positive welfare within healthy ecosystems across their range. Koalas are well-managed in their local context and populations are supported by robust and consistent legislation, informed community engagement and long-term funding.

2.5 Aligned Research Programs

The KDRA was funded by the Australian Government as part of a suite of projects aimed at improving conservation outcomes for koalas. The purposes and outputs of the KDRA are achieved in alignment and collaboration with these other koala conservation initiatives, which are summarised in Figure 1.

Improved Conservation Outcomes for Koalas		
Koala Health Research & Veterinary Support <small>Department of Climate Change, Energy, the Environment and Water Bushfire Recovery and Communications Section Biodiversity Conservation Division</small>	Koala Health Hub I: National Koala Health Research Initiative <small>University of Sydney</small>	Assess significance, distribution & diagnostic test availability for key pathogens/traits Develop & validate testing techniques for faecal analysis Evaluate biosecurity practices for capture and rehabilitation
	Koala Health Hub II: National Koala Disease Risk Analysis <small>University of Sydney</small>	Identify & assess disease hazards for koala populations Identify consequences for koala health of disease hazards Develop recommendations for disease risk mitigation
	Koala Health Monitoring <small>National Koala Monitoring Program, CSIRO</small>	Integrate koala health monitoring into the National Koala Monitoring Plan Critical assessment of koala health monitoring methods Koala health monitoring protocol design & pilot trials Koala health monitoring implementation plan
	Veterinary Support <small>Taronga Conservation Society Australia</small>	Training for veterinary professionals in treatment and care of Australian wildlife (including koalas) Interactive training workshops in the states where koalas are found
Time Frame 1 August 2021- 30 June 2022		

Figure 1 Koala conservation initiatives supported by the Department of Climate Change, Energy, the Environment and Water, August 2021 - June 2022

The Koala Health Hub (KHH) (<https://koalahealthhub.org.au>) is an initiative of the University of Sydney that benefits koala welfare and conservation by providing research services and expertise, and connecting researchers across the country with those responsible for koala care and management. The KDRA was conducted through the Koala Health Hub. In addition to the KDRA, the KHH led a number of nationally collaborative, priority, research investigations supported by the *Bushfire Recovery for Wildlife and their Habitats* program. The findings and progress of these investigations were incorporated into the KDRA.

The *National Koala Monitoring Program* (www.csiro.au/en/research/indigenous-science/Managing-Country/Koala-monitoring-program) is an initiative led by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in consultation with government (state, territory and local), natural resource management organisations, Indigenous Australians, community and industry groups, and researchers. Koala population monitoring is critical to understanding the impact of disease on populations. Local population disease surveillance programs are undertaken at various times, and in association with other koala monitoring activities. However, such programs are reliant on engagement by particular research groups and, where health screening is not part of project planning from the outset, the effectiveness of the surveillance effort can be limited. An initiative to incorporate health surveillance into the NKMP was undertaken as part of the *Bushfire Recovery for Wildlife and their Habitats* program and will also be informed by this KDRA.

The need for improved training of veterinary professionals in the treatment and care of wildlife, including koalas, was identified as a priority following the 2019-20 bushfires in eastern Australia. With the support of the *Bushfire Recovery for Wildlife and their Habitats* program, the Taronga Conservation Society Australia (TCSA) is providing training for veterinary professionals to address this need (<https://taronga.org.au/education/veterinary-professional-training>). The TCSA course, *Veterinary Professional Training in Wildlife Treatment and Care*, is aligned with the KDRA purpose of informing nationally-agreed guidelines for koala health and disease practices that may then be passed on to veterinarians in practice.

2.6 The Disease Risk Analysis Approach

2.6.1 DRA Framework

The disease risk analysis (DRA) framework and tools used in this KDRA are described in the *Manual of Procedures for Wildlife Disease Risk Analysis* endorsed by the IUCN-Species Survival Commission (IUCN-SSC) and the World Organisation for Animal Health (WOAH) [13]. This is a transparent, evidence-based process designed specifically for application to wildlife disease. It is underpinned by One Health: the recognition that the health of people, animals and their shared environment are interconnected [14]. As such, it promotes and facilitates the collaborative involvement in the analysis of multiple stakeholders who could be impacted by a wildlife disease issue or who can have a significant impact on the outcome

of the analysis. This includes decision makers as well as subject matter experts that can help ensure that decisions are based on the best available information. *Appendix 1* lists the stakeholders consulted through online meetings and workshops in the development of this KDRA.

The KDRA was undertaken by a small Project Team in consultation with a DRA Specialist Member of the IUCN-SSC's *Conservation Planning Specialist Group* to guide effective stakeholder engagement and neutral facilitation of the DRA process. The KDRA Project Team membership is listed in *Appendix 1*.

The overall DRA framework is comprised of six interlinked steps as shown in Figure 2.



Figure 2 The steps of the disease risk analysis process Jakob-Hoff et al. 2014 [13]

2.6.2 The “precautionary principle”

In many cases, current knowledge of the epidemiology and prevalence of koala diseases is incomplete or lacking (see Section 8 *Key Knowledge Gap Summary*), creating uncertainty regarding the likelihood or consequences of risk of a disease hazard. Where such limitations on available evidence were encountered, the “precautionary principle” was invoked [13], whereby a risk was assumed to exist (and require management), until proven otherwise.

2.6.3 Disease association vs causation

Confirming causative links between infectious disease hazards and disease is often a challenge in wildlife medicine. Knowledge is commonly based on studies in which researchers look for statistical associations between the presence of a disease agent and various disease manifestations. While these studies are valuable in identifying avenues for future research, they do not necessarily provide evidence for causation of the disease manifestation by the hazard in question. This is particularly the case for infectious disease

hazards, where identification of statistical associations with disease commonly precedes evidence of a mechanism whereby the infection causes disease.

Each detailed risk assessment within this report contains a section on *Associations with other disease hazards of koalas* where information on disease associations is summarised.

2.7 References

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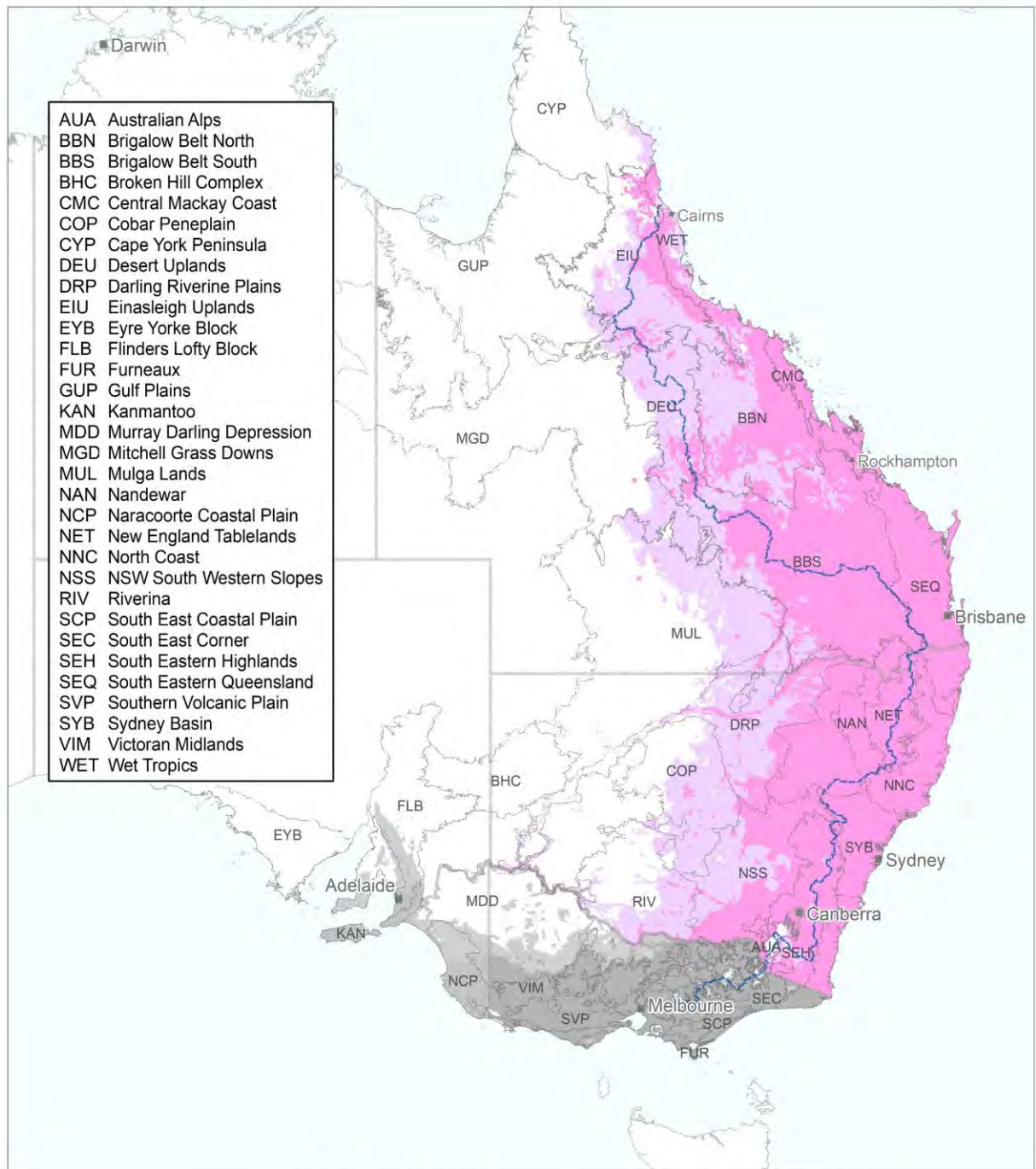
3 Problem Description

3.1 The Koala Species

The koala is the only surviving member of the marsupial family *Phascolarctidae* [1, 2]. It is a tree-dwelling, leaf-eating marsupial which feeds on trees of the genera *Eucalyptus*, *Corymbia* and *Angophora* [3, 4]. It is recognised globally as one of Australia's most distinctive and iconic wildlife species and has major cultural and emotional significance to both Indigenous and non-Indigenous Australians [5-10]. Its iconic status makes it a significant tourism drawcard benefiting Australia's economy [11].

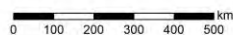
3.2 Koala Geographic Range and Habitats

The koala is widely, but patchily, distributed across eastern and southern mainland Australia (Figure 3), associated with the distribution of the tree species which are its predominant food source [12].



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Contextual data sources:
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and PSMA Australia.



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- Great Dividing Range
- Listed species distribution**
- Species known or likely to occur
- Species may occur
- Non-listed species distribution**
- Species known or likely to occur
- Species may occur

11/03/2021

Figure 3 Modelled 2021 distribution (geographic range) of listed and unlisted koalas [13]

3.3 Conservation Status of the Koala

The koala presents a challenge for conservation because it is not uniformly classified as threatened throughout its range [14]. In Qld and NSW most populations are declining rapidly [12, 15], whereas populations in Vic and SA are relatively stable [13], or in some cases experiencing localised overcrowding in the available habitat [16]. The koala’s conservation status reflects these regional differences in threats and conservation status, with the northern population of koalas (Qld, NSW and the ACT) determined to be a listed ‘species’ in 2012 under Section 517 of the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act). These koala populations are currently listed as Endangered [15]. The southern population (Vic and SA) is currently unlisted under the EPBC Act and under the legislation of those states. The conservation status of koalas is summarised in Table 2.

Table 2 International, national, state and territory conservation status of the koala [13]

Legislation	Conservation status
<i>Environment Protection and Biodiversity Conservation Act 1999</i> <i>Phascolarctos cinereus</i> (combined populations in Queensland, New South Wales and the Australian Capital Territory)	Endangered
<i>Nature Conservation Act 1992</i> (Qld) Koala (<i>Phascolarctos cinereus</i>)	Vulnerable
<i>Biodiversity Conservation ACT 2016</i> (NSW) Koala (<i>Phascolarctos cinereus</i>)	Vulnerable
<i>Nature Conservation Act 2014</i> (ACT) Koala (<i>Phascolarctos cinereus</i>)	Vulnerable
<i>Flora and Fauna Guarantee Act 1988</i> (VIC) Koala (<i>Phascolarctos cinereus</i>)	Not listed
<i>National Parks and Wildlife Act 1972</i> (SA) Koala (<i>Phascolarctos cinereus</i>)	Not listed
IUCN Red List of Threatened Species Koala (<i>Phascolarctos cinereus</i>)	Vulnerable

The KDRA adapted the terminology of the 2022 *National Recovery Plan for the Koala* [13] in referring to the various koala populations as follows:

- when referring to individuals or populations, the terms ‘koala’ or ‘koalas’ is used.
- the total population of the species in Australia is referred to as ‘the koala’ or ‘the species’.
- the terms ‘listed koala’ or ‘northern populations’ are used when referring explicitly to the EPBC Act-listed koala (combined populations of Qld, NSW and the ACT). These may also be referred to individually by their state origin.
- the terms ‘unlisted koala’ or ‘southern populations’ are used for the collective populations of Vic and SA. These may also be referred to individually by their state of origin.

3.4 Jurisdictions for Koala Management

Across Australia, biodiversity conservation and protection are delivered through the combined efforts of the Australian Government, local, state, and territory governments, along with the actions of landholders, communities, Traditional Owners, the private sector, and non-government organisations [13].

The EPBC Act is the Australian Government's key piece of environmental legislation that provides a legal framework to protect and manage nationally and internationally important flora, fauna, ecological communities and heritage places. These entities (including the listed koala) are defined in the EPBC Act as Matters of National Environmental Significance (MNES). Consequently, the listed koala is subject to regulatory decision making under the EPBC Act, which is triggered when an action has, will have, or is likely to have, a significant impact. These actions require referral to the Australian Government for assessment and approval under the EPBC Act to be carried out lawfully [13].

Management of koala health is influenced by a variety of cross-jurisdictional policies, legislation, regulations and programs, working through Commonwealth, state, territory and local government agencies. A range of non-government organisations, wildlife organisations, community groups, veterinary organisations and citizen scientists support koala health management (see below).

3.5 Stakeholders in Koala Health

A wide range of stakeholders (see *Appendix 1*) are involved in the investigation, recording and management of koala health [14].

Research is undertaken on the biology, epidemiology and clinical significance of various koala diseases. Disease research is undertaken principally by university academics, both within Australia and overseas, and often in partnership with rehabilitation facilities, koala hospitals and koala monitoring programs. Research priorities for koala health and disease are not currently coordinated at the national level.

A large number of **rehabilitation facilities and wildlife hospitals** across Australia care for sick, injured and displaced koalas. Most are privately run, but may receive funding from government sources. There is communication and collaboration within the koala rehabilitation community, and willingness by many to share their knowledge and experiences. However, there is limited capacity for strategic centralisation and coordination of experience. Consequently, most rehabilitation facilities develop their own protocols for biosecurity, disease management, diagnosis and treatment, based on local priorities and constraints, with variable levels of involvement of veterinarians or other disease experts. Most rehabilitators are volunteers who may not have scientific training; facilities collect data on koala admissions, but the level and quality of data varies significantly. In NSW and Qld, admission data are collated into online databases that are publicly available, and contain some information on the health status of cases [17, 18]. Veterinary involvement in

diagnosis, assessment and treatment varies between rehabilitation facilities, as does the capacity and funding to conduct diagnostic testing and disease screening. Rehabilitation facilities and veterinary hospitals receiving sick and injured free-ranging koalas largely maintain individual control of their data which is not centrally compiled or managed. However, this information has been used in collaboration with scientific researchers to inform disease prevalence, impacts on morbidity and mortality, and geographic disease distribution (e.g. [19-22]).

Zoos and other institutions that house koalas in captivity generally maintain individual medical records that are a potential source of detailed information on captive disease incidence and management. In the last 10 years, many of these institutions have converted to using the Species360 Zoo Information Management System (ZIMS) database for managing their medical records (www.species360.org). This system enables rapid and reliable information management and sharing between institutions to enhance understanding of disease in captive populations. Zoo veterinary personnel are often involved in an advisory or clinical capacity in support of rehabilitators, some through zoo-based wildlife hospitals, and often also have a role in emergency response within their jurisdiction.

Wildlife Health Australia (WHA; <https://wildlifehealthaustralia.com.au/Home.aspx>) is the national coordinating body for wildlife health in Australia. WHA administers the national electronic Wildlife Health Information System (eWHIS) database, a web-enabled, secure database capturing information relating to wildlife health surveillance and disease investigation in Australia. WHA receives wildlife health data from sources both within governments (including state and territory agricultural, environment and health agencies) and from sources outside of governments (such as university veterinary clinics and pathology departments, zoo wildlife hospitals and private veterinary practitioners). Other sources include the Australian Registry of Wildlife Health. WHA collates and moderates the data in eWHIS to ensure that it is as accurate as possible. WHA has a wide range of fact sheets on wildlife disease issues, with several focused on disease in koalas (<https://wildlifehealthaustralia.com.au/ProgramsProjects/eWHIS-WildlifeHealthInformationSystem.aspx>).

The **Australian Registry of Wildlife Health** (ARWH; <https://arwh.org/about>) is hosted by Taronga Zoo and is a diagnostic and resource centre for Australian wildlife health and disease. The Registry focuses on detecting and diagnosing endemic, emerging and exotic diseases of wildlife that could have impacts on Australia's trade/economy, biodiversity, tourism and human health.

The **Koala Health Hub** (KHH; <https://koalahealthhub.org.au>) is an initiative of the University of Sydney to benefit koala welfare and conservation by connecting researchers across the country with people undertaking koala care and management. Their aim is to create an inclusive, diverse and innovative source of support for koala health management in

Australia. The KHH has developed a range of fact sheets, protocols and guidelines for koala health and disease investigation work.

Wildlife scientists who are not directly involved in koala health research are based in universities, government agencies and non-government organisations. They may encounter diseased koalas when undertaking field work in koala habitat and may collaborate with wildlife health researchers to sample koalas for disease surveillance or diagnostic purposes. They are also involved in studies where disease is directly linked to ecological processes, such as predation and bushfire.

A range of **land managers** (private land owners, Traditional Owners, industry employees or local government, state and federal government officers) may be responsible for managing land on which koalas live. Traditional Owners of lands play an important role in bringing Traditional Knowledge to scientific practice to maximise outcomes for biodiversity [23].

Other organisations that undertake activities, fundraising and advocacy on behalf of wild koalas are a vital to raising community awareness of koala health and disease issues. Many of these groups also support scientific research and koala rehabilitation efforts. Groups include animal welfare agencies (e.g. RSPCA, International Fund for Animal Welfare), animal conservation agencies (e.g. World Wildlife Fund) and other non-government organisations.

A non-exhaustive list of stakeholders in koala health is included in *Appendix 1*.

3.6 Non-disease threats and their impact on disease

There are many factors other than disease which pose threats to the long-term viability of koalas. Disease is intimately connected to threats such as habitat destruction and fragmentation, habitat-altering natural phenomena (bushfires, drought, flooding), low genetic diversity, climate change and localised overcrowding.

To successfully manage disease risk to the koala, it is necessary to understand the nature of non-disease threats and to develop integrated strategies that simultaneously manage multiple threats in ways which are appropriate to the local situation and scale [13, 24, 25]. For this reason, non-disease threat statements were developed, in consultation with stakeholders, as part of the KDRA (see *Appendix 4 Non-disease Threat Descriptions*). The interactions of non-disease threats with disease are illustrated in a causal flow diagram (Figure 4), developed with stakeholder input. The interactions summarised by the threat statements and the causal flow diagram were considered in the development of disease hazard flow charts, critical control points and disease risk mitigation strategies.

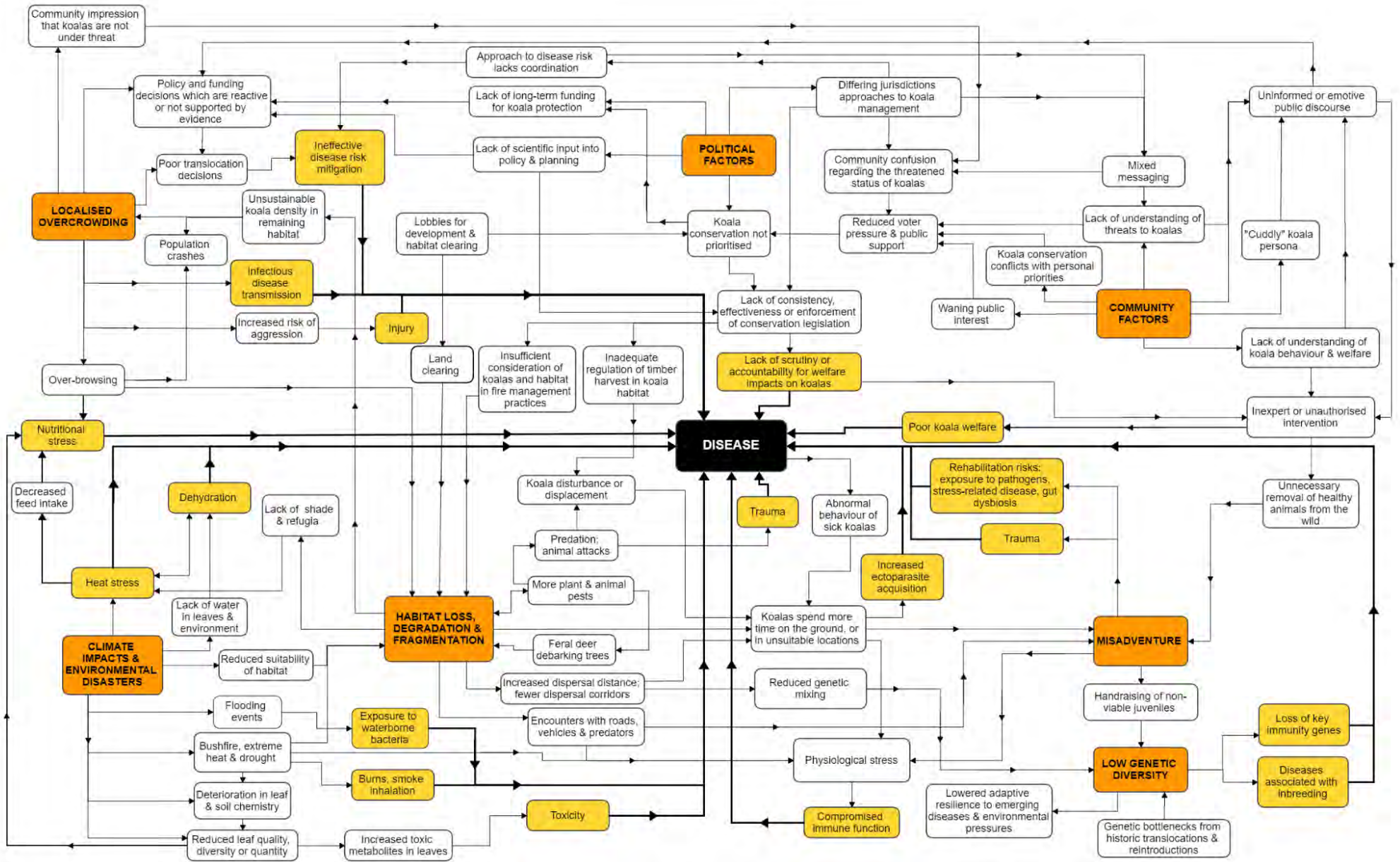


Figure 4 Causal flow chart showing interactions of non-disease threats (orange) with disease in koalas; yellow boxes are proximate causes for disease

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4 Hazard Identification and Refinement

Details of the hazard identification and refinement methodology are in *Appendix 2*. The hazard identification process identified 91 disease hazards of koalas, listed in Table 3.

Table 3 List of disease hazards of koalas identified through the KDRA process

INFECTIOUS HAZARDS (n=56)
VIRUSES
Barmah Forest virus
Encephalomyocarditis virus
Koala retrovirus (KoRV)
Papillomaviruses
Phascolarctid herpesviruses
Ross River virus
BACTERIA
<i>Acinetobacter lwoffii</i>
<i>Aeromonas hydrophila</i>
<i>Bacteroides</i> spp.
<i>Bordetella bronchiseptica</i>
<i>Burkholderia pseudomallei</i>
<i>Chlamydia</i> spp. (<i>C. pecorum</i> ; <i>C. pneumoniae</i> ; novel <i>Chlamydiales</i>)
<i>Chromobacterium violaceum</i>
<i>Clostridium piliforme</i>
<i>Clostridium septicum</i>
<i>Corynebacterium</i> spp.
<i>Coxiella burnetti</i>
<i>Enterococcus faecalis</i>
<i>Escherichia coli</i>
<i>Helicobacter</i> spp.
<i>Klebsiella</i> spp. (including <i>K. pneumoniae</i> , <i>K. oxytoca</i>)
<i>Leptospira</i> spp. (<i>L. interrogans</i> serovars)
<i>Morganella morganii</i>
<i>Mycobacteria</i> spp. (including <i>M. ulcerans</i> , <i>M. scrofulaceum</i>)
<i>Mycoplasma</i> spp.
<i>Nocardia asteroides</i>
Novel <i>Actinomyces</i> sp.
<i>Proteus</i> spp.
<i>Pseudomonas aeruginosa</i>
<i>Salmonella</i> spp. (including <i>S. typhimurium</i> , <i>S. sachsenwald</i> , <i>S. bovismoribificans</i>)
<i>Serratia marcescens</i>
<i>Staphylococcus</i> spp. (including <i>S. epidermidis</i>)
<i>Streptobacillus moniliformis</i>
<i>Streptococcus</i> spp. (including α - and β -haemolytic <i>Streptococcus</i> spp.)
<i>Ureaplasma</i> spp.
<i>Yokenella regensburgei</i>
FUNGI
<i>Aspergillus</i> spp.
<i>Candida</i> spp. (including <i>C. catenulata</i>)
<i>Coccidioides</i> spp.

Table 3 (continued)

FUNGI (cont.)
<i>Cryptococcus</i> spp. (<i>C. gattii</i> , <i>C. neoformans</i>)
<i>Encephalitozoon intestinalis</i>
Ringworm fungi (<i>Trichophyton mentagrophytes</i> ; <i>Microsporium gypseum</i>)
PROTOZOA
<i>Cryptosporidium</i> spp.
<i>Giardia</i> spp.
<i>Toxoplasma gondii</i>
<i>Trypanosoma</i> spp. (including <i>T. copemani</i> , <i>T. gilletti</i> , <i>T. irwini</i> , <i>T. vegrandis</i> , <i>T. noyesi</i>)
INTERNAL MACROPARASITES
<i>Bertiella obesa</i>
<i>Durikainema phascolarcti</i>
Nematodes (including <i>Breinlia mundayi</i> ; <i>Johnstonema</i> , <i>Marsupostrongylus</i> & <i>Ophidascaris</i> spp.)
EXTERNAL MACROPARASITES
<i>Ctenocephalides</i> spp.
<i>Demodex</i> spp.
Fly strike (<i>Lucilia cuprina</i>)
<i>Koalachirus perkinsi</i>
Paralysis ticks (<i>Ixodes holocyclus</i> , <i>I. cornuatus</i> , <i>I. hirsti</i>)
Sarcoptic mange (<i>Sarcoptes scabiei</i>)
Ticks other than paralysis ticks (including <i>Haemaphysalis</i> spp., <i>Ixodes tasmani</i>)
NON-INFECTIOUS HAZARDS (n = 35)
DEGENERATIVE
Degenerative joint disease
Degenerative ocular lesions
Hip and shoulder dysplasia
Periodontal disease
Tooth wear
NEOPLASTIC
Neoplasia (includes lymphoid and non-lymphoid neoplasia)
TOXICOSIS
Aluminium toxicosis
Envenomation - snake bite
Fluorosis
DEVELOPMENTAL
Developmental cardiac disease
Developmental urogenital disease
Hydrocephalus
Iris cysts
Malocclusion
Scoliosis and kyphosis
ENVIRONMENTAL
Ballistics trauma
Entanglement trauma
Heat stress
Ocular disease secondary to trauma
Motor vehicle trauma
Predator attack trauma

Table 3 (continued)

ENVIRONMENTAL (cont.)
Reproductive disease secondary to trauma
Thermal burn trauma
Trauma through falling from trees
Trauma from intraspecific aggression
OTHER
Colloid goitre
Diabetes mellitus
Gastrointestinal torsion, intussusception or entrapment
Lithiasis (including struvite; calcium oxalate; uric acid)
Microchip transponder reactions
Oxalate nephrosis
Phytobezoars
OTHER CLINICAL SYNDROMES
Gut dysbiosis (caeco-colic dysbiosis/typhlocolitis syndrome)
Putative KoRV-associated disease syndromes
Wasting syndromes

Appendix 2 shows the detailed information captured during the hazard refinement process and the rationale for hazard prioritisation. The hazard refinement process identified 13 disease hazards for detailed risk assessment, and these are listed in alphabetical order in Table 4.

Table 4 Disease hazards selected for detailed risk assessment

Shortlisted Disease Hazards (in alphabetical order)
<i>Chlamydia</i> spp.
<i>Cryptococcus</i> spp.
Heat stress
Koala retrovirus
Motor vehicle trauma
Neoplasia
Novel <i>Actinomyces</i> sp.
Oxalate nephrosis
Phascolarctid herpesviruses
Predator attack trauma
Sarcoptic mange
Thermal burn trauma
<i>Trypanosoma</i> spp.

Three other disease hazards (gut dysbiosis, wasting syndromes and putative KoRV-associated disease syndromes) warranted further discussion due to their potential impact on koala populations. Because they lacked a clear single aetiology and case definition, they did not lend themselves to the formal IUCN risk assessment process. These syndromes are discussed in more detail in Section 7 *Other Disease Hazards*.

5 Risk Assessments for Selected Hazards

Detailed risk assessments were conducted for the 13 selected hazards listed in Table 4. Reviews of current knowledge and literature, that formed the basis for each risk assessment, are provided in *Appendix 5 Hazards for Detailed Risk Assessment – Literature Reviews*.

5.1 *Chlamydia* spp. in Koalas - Risk Assessment

The literature review which supports this risk assessment is in *Appendix 5.1*.

5.1.1 Hazard summary

- *Chlamydia* spp. are widespread pathogens of koalas. Chlamydial infection in koalas may be ‘subclinical’ (no signs of disease) or may cause disease which is ‘inapparent’ (detectable only using specialist equipment or internal examination) or ‘overt’ (obvious external clinical signs) [1-4].
- *Chlamydia pecorum* is the cause of almost all infections and most serious disease caused by *Chlamydia* spp. [1, 2, 5-7].
- *Chlamydia pneumoniae* infection in koalas is rarely detected and less pathogenic than *C. pecorum* [2, 8, 9]. Several other *Chlamydia*-like bacteria also infect koalas [10, 11], but their significance is unknown.
- *Chlamydia* is probably primarily transmitted via sexual contact [1, 6], with direct contact between dam and joey likely to be important in transmission to juveniles [1, 12-15]. Transmission from other species could potentially occur, but its significance to *Chlamydia* epidemiology in koalas is unknown [16-18].
- Prevalence of chlamydial infection and expression of chlamydial disease (chlamydiosis) are variable throughout the koala’s geographic range [1, 3, 5, 9, 12, 19-26].
- Pockets of zero prevalence may be present [1, 3, 4, 27], but the factors leading to this circumstance are not well understood. The ubiquitous nature of this organism and the extensive sampling needed to declare freedom of infection in populations means that claims of *Chlamydia*-free status should be accompanied by an indication of the level of certainty of the claim.
- Longitudinal monitoring data suggest that progression from chlamydial infection to disease may be more common than previously thought [20].
- Typical clinical presentations are ocular disease [1, 28-30], urinary tract disease [28, 31-35], reproductive disease [1, 3, 11, 25] or combinations of the three. Disease is characterised by marked inflammation and tissue fibrosis [36] and can have severe welfare implications for affected individuals.
- Chlamydial disease can cause reduced fertility or infertility in male and female koalas [1, 3, 11, 25], posing significant threats to population viability [37]. Population impact will likely vary depending on other pressures and threats [38-45].
- Chlamydial disease occurs throughout the koala’s range [1-3, 5, 8, 9, 19, 22, 24-26, 46-48], although disease is generally considered less severe in southern koala populations [3, 5, 9, 25].
- Chlamydial disease is initially inflammatory, and chlamydial shedding is likely to be at its greatest in the early phase [20, 36]. Disease progresses in many cases to permanent scarring of the urinary and reproductive tract [32, 33], which can persist without

outward signs of disease or chlamydial shedding. Ultrasonography is generally required to detect later phases of urogenital disease [34].

- The development of disease in an individual koala is likely due to a complex combination of host [6, 20, 25, 36, 49-55], pathogen [1, 2, 5-7, 11, 12, 20, 23, 44, 51, 56], and environmental factors [4, 6, 42, 45, 57, 58], which remain poorly understood and probably vary among populations. Co-infection with other agents [26, 41, 44, 59-65], particularly KoRV [60, 61, 66-69] and herpesviruses [62, 63], potentially also plays a role.
- Effective treatment of chlamydiosis currently requires hospitalisation and antibiotic therapy. Treated animals released back to the wild may relapse or become reinfected [70].
- The control of *Chlamydia* at the population level, through coordinated programs to capture, treat and release individuals, can be an effective tool for reversing population decline in defined populations [43, 71].
- Vaccination could eventually be a valuable tool for preventing and controlling chlamydial infection and disease [72-83], but further research is needed to identify an effective product.
- For further detail on current knowledge of this hazard, refer to *Appendix 5.1 Chlamydia spp. in Koalas - Literature Review*.

5.1.2 Justification for hazard selection

Chlamydia is a common and widespread pathogen of koalas that can cause severe illness and death [44, 45, 84-87] and may have a significant role in koala population declines by increasing mortality and reducing fertility [37, 43, 58, 88].

5.1.3 Identified gaps in knowledge

- Understanding of chlamydial virulence traits and strain diversity is needed to clarify the importance of genotype to pathogenicity, and to better define what constitutes a “novel” strain for management purposes [3, 9, 25, 89, 90].
- The role of co-infections, particularly KoRV and PhaHV, in predisposing koalas to chlamydial disease and infertility needs to be elucidated [26, 44, 60, 63, 91, 92].
- An understanding of the role of koala genetics in determining immune response to chlamydial infection is needed to improve understanding of host differences in chlamydial susceptibility, disease severity and vaccination response [93].
- The relative importance of chlamydiosis, among other threatening processes, to koala population dynamics has yet to be elucidated over the koala's distributional range [58, 94]. This requires long-term, longitudinal monitoring for chlamydial infection and disease, fecundity and other indices of population viability, in conjunction with comprehensive host, environment and pathogen data [58, 94].
- The capacity for cross-species transmission of *Chlamydia* between koalas and livestock or other wildlife species is incompletely understood [3].

- More evidence-based investigation of treatment regimens and post-release outcomes for chlamydiosis is needed to improve the efficacy of treatment options and release decisions [95-97]. This may involve longitudinal monitoring of koalas after treatment and further pharmacokinetic and clinical efficacy studies of medications commonly used to treat *Chlamydia* infection (antibiotics, anti-inflammatories etc.).
- Further development of non-antimicrobial treatment options, including vaccination against *C. pecorum*, is required.
- Validation of sensitivity and reliability of techniques for detection of *Chlamydia* in scats is needed to assist in the rapid and accurate determination of chlamydial prevalence in free-ranging populations.
- Nationally-agreed guidelines for treatment, triage, assessment and biosecurity in relation to *Chlamydia* infection are needed.

5.1.4 Risk assessment

Chlamydia pecorum is the most commonly detected species of *Chlamydia* in sick and injured koalas and is the primary cause of chlamydial disease in this species [19, 30]. For this reason, this risk assessment considered only *C. pecorum*. However, risk mitigation options are also likely to be valid for other *Chlamydia* species.

Below is a schematic of the hazard pathways and critical control points (CCPs) identified for this hazard. Critical control points are *key* points in the hazard pathway where risk mitigation methods could be most effective (see *Appendix 2.3 Risk Assessment Methods* for further information). Nineteen CCPs (CCP1-CCP19) were identified across all hazards, but only those applicable to this hazard are described below. See Section 6 *Critical Control Points by Disease Hazard* for a summary of CCPs across all hazards selected for detailed assessment.

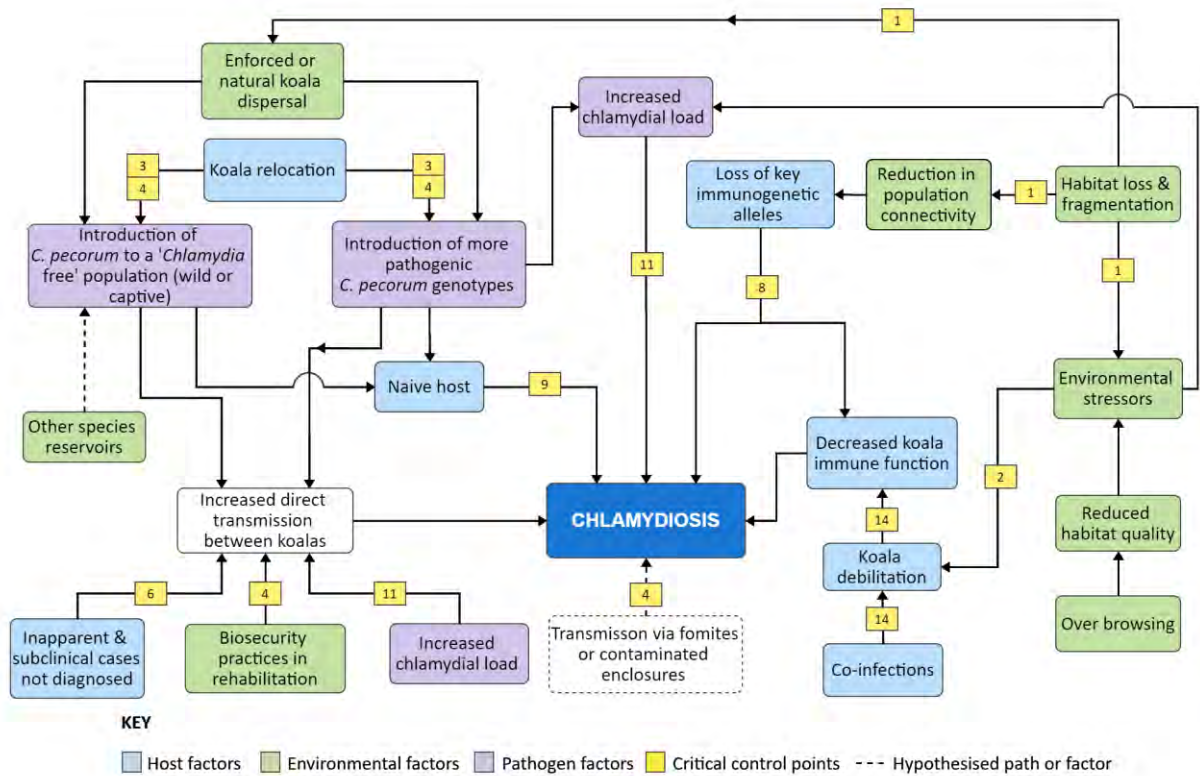


Figure 5 Hazard pathways and critical control points for chlamydia in koalas

Critical control points

CCP1 Habitat loss, fragmentation and reduction in quality

Habitat loss and reduction in habitat quality are major environmental stressors due to the koala's reliance on particular tree species for food, shelter and thermoregulation [98, 99]. Habitat fragmentation reduces connectivity between suitable koala habitats, forcing dispersal and potentially contributing to the loss of important genetic alleles in populations that can no longer interbreed [100-102]. Causes include urban expansion, land clearing for production, inappropriate fire management practices, the introduction of pests, weeds and plant pathogens, over-browsing by koalas as a result of local overcrowding, and the habitat-altering effects of climate change [40, 103-108].

The relationship between development of chlamydial disease and habitat factors is complex and is likely to reflect a range of co-factors operating over different scales of time [44, 45].

CCP2 Environmental stressors

Stressors in the koala's environment (in the wild or in captivity) contribute to general debilitation of the host, reduced immune function, and increased susceptibility to disease or severity of disease expression [99, 109-111]. Major environmental stressors for koalas include habitat loss, fragmentation and reduction in quality; nutritional stress; climate change; extremes of weather and disturbance related to human activities [98, 112].

A variety of environmental stressors including overcrowding (in the wild or in captivity), declining food resources and extremes of weather could affect the prevalence of chlamydial disease in koala populations [40-42, 113]. Stress and malnutrition have been implicated in exacerbating chlamydial disease in other species [114-116].

CCP3 Koala relocation

Human-mediated movement of koalas may increase disease risk by introducing pathogens, or new pathogen varieties [117]. This may occur when koalas are brought into rehabilitation or released from rehabilitation (especially if not returned to their point of capture), when captive koalas are transferred between facilities [118], when koalas are translocated from one wild population to another [119], or when habitat corridors are created to reconnect long-separated populations without consideration of disease control.

In particular, relocated koalas infected with *C. pecorum* pose a risk of introducing more pathogenic molecular types to a population or introducing *C. pecorum* to a 'Chlamydia-free' population [37, 120].

CCP4 Biosecurity practices

Biosecurity practices aim to ensure that infected koalas (or those of unknown infection status) do not pose a risk to other koalas in the population (captive or wild). Biosecurity practices may include quarantine of incoming or outgoing animals, appropriate use of personal protective equipment, general hygiene, prophylactic treatment and disinfection practices [117].

In rehabilitation, koalas infected with *C. pecorum* may infect other animals if biosecurity practices are inadequate [117]. In the wild, lack of consideration of biosecurity in translocations may lead to introduction or spread of *C. pecorum*.

CCP6 Diagnostics

Insufficient or inadequate diagnostic methods for *Chlamydia*, or poor application of diagnostic tests, may lead to failure to detect *Chlamydia*-infected animals [58], resulting in an increased risk of transmission to uninfected koalas.

CCP8 Genetics

The individual koala may have a genetic predisposition to the development of disease or increased severity of disease.

In particular, koalas that develop more severe chlamydial disease may lack critical alleles that would enable them to mount a more effective immune response [51, 52, 54, 121]. Improving or at least maintaining genetic diversity in koala populations is likely to conserve adaptive potential [122] and encourage the retention of the most robust koala genetic profiles to avoid disease consequences of *Chlamydia* infection.

CCP9 Naïve host

An infectious pathogen may be introduced to a previously unexposed population or a previously uninfected individual.

Introduction of *Chlamydia pecorum*, or novel genotypes, to naïve individuals or populations, may have a detrimental impact on individual health and welfare or population viability [3, 23, 37, 123].

CCP11 Increased pathogen load

An increase in the load of an infectious pathogen that a koala is exposed to, or that a koala carries, influences the severity of disease caused by that pathogen as well as the likelihood of transmission.

The chlamydial load in infected koalas is highest during early infection and declines in chronic infections [20, 36]. Progression of urogenital tract disease (with its associated detrimental effects on koala fertility and welfare) is significantly associated with an increased chlamydial load [20].

CCP14 Concurrent infections and debilitation

Concurrent infections and debilitation can compromise immune function and general health in ways that increase the risk or severity of disease. The pathogenicity of an infectious hazard may be potentiated by co-infections.

Chlamydial disease is likely to be exacerbated by opportunistic infections from a range of microbes [33, 41, 64, 124]. The presence of koala gammaherpesviruses (PhaHV-1 and PhaHV-2) has been associated with infection with *C. pecorum* in both male and female koalas in Victoria [62, 63]. An association between reproductive disease caused by *C. pecorum* and PhaHV co-infection has been reported in SA koalas (Stephenson, 2021). Associations between chlamydial disease severity and increased KoRV proviral or viral load have been identified in a number of studies [66-69, 125], although there is currently no clear causative evidence of KoRV inducing more severe chlamydial disease in koalas.

Likelihood assessments

Likelihood of entry and exposure for koalas was considered for the transmission pathways “koala population to koala population” and “other animal population to koala population”. *Chlamydia pecorum* is not known to be zoonotic [126, 127], and fomites were not considered a likely major source of transmission, so the “human to koala” and “environment to koala” pathways were not considered. The likelihood of an infected koala causing a hazard to other animals (via their exposure to koalas or koala environments) was also evaluated.

Entry assessment - koalas

Chlamydial infection has been recorded in virtually all wild koala populations [30] and is commonly detected in apparently healthy free-ranging koalas [2, 20, 25, 42, 57, 59, 128].

Chlamydia pecorum sequence types identical or very similar to those found in koalas are also present in a range of other species in koala environments, including Australian marsupials, native birds and livestock [16, 17, 24, 129-131]. A small number of koala populations may be “*Chlamydia*-free” [3, 132, 133], but they are commonly found near infected koala populations or other potentially infected species, particularly livestock [24, 131].

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of *C. pecorum* entering, or being present, in a koala population is considered **HIGH**.

Exposure assessment - koalas

Chlamydial infection is likely to be transmitted sexually between koalas [1, 6] with direct contact between dam and joey probably the most important transmission route to juveniles [1, 12-15]. *Chlamydia* can survive outside of the host [134], which may allow for transmission via fomites, but this has not been confirmed.

The proportion of koalas exposed to the bacterium that go on to develop an infection is not known. Prevalence of chlamydial infection is variable throughout the koala’s geographic range [1, 3, 5, 9, 12, 19-26], from over 80% in certain populations in Qld [1], Vic [22] and SA [1, 25] to 0% in a few populations [1, 3, 4, 27]. Based on the precautionary principle, the assessment of exposure likelihood was made on the assumption that *Chlamydia* is present in most, if not all, populations.

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of exposure to *C. pecorum* for an individual koala is considered **HIGH**.

Using the principles of combining entry and exposure likelihood as outlined in *Appendix 2.3 Risk Assessment Methods*, the combined likelihood of entry and exposure of *C. pecorum* for koalas is considered **HIGH**.

Likelihood assessments for other species exposed to infected koalas

Spillover of *C. pecorum* from koalas to other species such as livestock and other wild marsupials has been hypothesised [16, 17]. By the precautionary principle, spillover was considered possible in this assessment, although there are no confirmed reports of this occurring, and it is considered very unlikely to occur.

Using the principles of combining entry and exposure likelihood as outlined in *Appendix 2.3 Risk Assessment Methods*, the combined likelihood of entry and exposure of *C. pecorum* to other species due to infection in koalas is considered **LOW**.

Consequence assessments

Koala population resilience and viability

Prevalence of chlamydial infection in free-ranging koala populations can be over 80% [1, 22, 25], and very few populations have zero prevalence. Not all infected koalas develop disease, and the reported prevalence of chlamydial disease is lower than infection [2, 20, 25, 42, 57, 59, 128]. Disease prevalence reported in free-ranging populations ranges from 4% to 43% [9, 12, 69, 135]. A study in south-east Qld found that 66% of koalas diagnosed with chlamydial infection via PCR progressed to overt disease within 3-4 years [20]. On the precautionary principle, the consequence to population viability was evaluated based on the highest reported figures of >80% prevalence of infection and 44% prevalence of disease.

Chlamydial infection can impact fertility in male and female koalas [1, 3, 11, 25] and free-ranging koala population viability [37, 43, 58, 88]. Modelling studies indicate that eradicating chlamydial infection can be critical to reversing population declines in koalas [38, 43]. However, as with almost all disease states in wild populations, the relative importance of chlamydiosis as a driver of population dynamics is difficult to measure [49, 58] and probably varies across the koala's distribution [94, 136].

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of *C. pecorum* to koala population resilience and viability is considered **MODERATE**.

Koala individual health and welfare

As noted above, not all cases of chlamydial infection progress to disease. However, severe chlamydial disease in koalas is painful, debilitating, and without treatment, can result in death [44, 45, 84-87]. Chlamydial disease accounted for up to 52% of admissions in NSW and Qld rehabilitation facilities and was present in up to 63% of post mortem cases in two SA studies [6, 7, 25, 49, 87].

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of *C. pecorum* to koala individual health and welfare is considered **MAJOR**.

Health and welfare of other species

Exposure to infected koalas (or their environments) has not been reported to lead to chlamydial disease in other species.

Disease due to *C. pecorum* occurs commonly in livestock [137] and is reported occasionally in Australian native marsupials other than koalas [16, 126, 138]. Subclinical infections in livestock are common, but disease manifestations can include conjunctivitis, meningitis and polyarthritis [137]. Disease manifestations in Australian native species are less common, but ocular and possibly urogenital signs have been reported [16, 138].

It is highly unlikely that exposure to an infected koala would have measurable consequences for other species. Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of *C. pecorum* infection to the health and welfare of other species is considered **NEGLIGIBLE**.

Overall risk estimate

The overall risk of *C. pecorum* to koala populations, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **HIGH**.

The overall risk of *C. pecorum* to individual koalas, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **HIGH**.

(Since the consequence of *C. pecorum* in koalas to the health and welfare of other animal species was evaluated as negligible, the estimate of risk for this hazard for the health and welfare of other animals was also considered **NEGLIGIBLE** - see *Appendix 2.3 Risk Assessment Methods*).

This assessment exceeds the acceptable risk thresholds outlined in *Appendix 2.3 Risk Assessment Methods* for koala populations and for individual koalas. Therefore, risk management for *C. pecorum* is recommended for both koala populations and individual koalas.

Level of confidence in risk assessment

Factors associated with koala-to-koala transmission of *Chlamydia* are well recognised and well-studied. There is information on *Chlamydia* prevalence in most populations. The capacity for transmission from other species to koalas and vice versa is unknown. Overall, the level of confidence in the likelihood assessment is **HIGH**.

The impact of *C. pecorum* on population viability is not well understood in all populations throughout the koala's range and details of population impacts are not widely documented in peer-reviewed literature [43, 58]. Therefore, the level of confidence in the consequence assessment for koala populations is **MEDIUM**.

The clinical manifestations of chlamydial disease are well studied and well recognised. Therefore, the level of confidence in the consequence assessments for individual koalas is **HIGH**.

Overall, the confidence in the risk estimate for *C. pecorum* is considered **HIGH**.

5.1.5 Risk mitigation options

The following risk mitigation measures have been identified for *C. pecorum*. Further information and justification can be found in the *Treatment and Prevention and control* sections of the relevant literature review chapter. Not all risk mitigation options will be currently available, feasible, universally applicable or cost-effective, but are listed here for completeness.

Free-ranging populations

Conserve and improve the quality, quantity, diversity and complexity of koala habitat (CCP1)

- Re-vegetation, restoration and preservation of habitat, including in urban landscapes.
- Strengthen regulatory controls against habitat clearing in koala habitat and dispersal corridors.

These measures will reduce the impact of habitat-related environmental stress on chlamydial disease expression. Conservation and improvement of habitat supports larger wild koala populations which have stronger potential to adapt genetically to avoid disease consequences of *Chlamydia* infection.

Manage concurrent infections, debilitation and environmental stressors (CCP2 and CCP14)

- Minimise concurrent infections, debilitation and other environmental stressors (e.g. predators, disturbance related to human activities) in individual koalas and populations, to reduce the risk of pathogenic consequences for koalas infected with *Chlamydia*.

Risk mitigation in translocations (CCP3, CCP4 and CCP9)

- Repopulate viable habitat with koalas free of *C. pecorum*.
- Establish biosecurity and koala relocation protocols to prevent the introduction of *Chlamydia* into populations with low or zero prevalence.
- Minimise stress in koalas being translocated.

Maintain genetic diversity and population sizes in koalas (CCP8)

- Support genetic diversity in koala populations to encourage the retention of the most robust koala genetic profiles to enable effective immune response and avoid disease consequences of *Chlamydia* infection.

Pathogen monitoring, detection, and response in free-ranging populations (CCP6, CCP9, CCP11 and CCP14)

- Early diagnosis, triage and treatment (or euthanasia) of diseased individuals.
- Develop protocols for robust, systematic testing to accurately identify *Chlamydia*-positive and -negative individuals and reduce the risk of transmission to uninfected individuals.
- Implement biosecurity practices to avoid spread of *Chlamydia* when conducting field work on wild koalas e.g. dedicated equipment, disinfection.

Reduce disease consequences of hazard exposure in the wild (CCP11)

- Incorporate vaccination (once available) into population management to reduce *Chlamydia* prevalence, load and severity.

Rehabilitation populations

Manage concurrent infections, debilitation and environmental stressors (CCP2 and CCP14)

- Minimise concurrent infections, debilitation and other stressors in the rehabilitation environment, to reduce the risk of pathogenic consequences in koalas infected with *Chlamydia*.

Biosecurity practices in rehabilitation (CCP4)

- Implement biosecurity practices specific to *Chlamydia* for koalas entering rehabilitation.
- Practise barrier hygiene between koalas of different *Chlamydia* status.
- Prevent cross-contamination through appropriate disinfection, personal protective equipment and equipment use.

Pathogen monitoring, detection and response in rehabilitation (CCP6, CCP9 and CCP11)

- Reduce the risk of introducing *Chlamydia*, or novel genotypes, to free-ranging populations after rehabilitation by adopting pre-release testing protocols (especially if animals are not returning to their location of origin).

Reduce disease consequences of hazard exposure in rehabilitation (CCP11)

- Incorporate *Chlamydia* vaccination of koalas (when available) into rehabilitation settings to reduce severity of disease and assist in clearing infection.

Captive populations

Manage concurrent infections, debilitation and environmental stressors (CCP2 and CCP14)

- Minimise concurrent infections, debilitation and other stressors in the captive environment, to reduce the risk of pathogenic consequences in koalas infected with *Chlamydia*.

Pathogen monitoring, detection and response in captive care (CCP3, CCP4, CCP6, CCP9 and CCP11)

- Maintain *Chlamydia*-negative captive populations of koalas through quarantine and diagnostic testing of new arrivals and routine diagnostic testing of existing captive koala populations.

Reduce disease consequences of hazard exposure in captivity (CCP11)

- Incorporate *Chlamydia* vaccination of koalas (when available) into collection management to reduce likelihood of disease development.

5.1.6 Recommendations

Hazard-specific recommendations

The following recommendations are specific to this hazard:

- Continue investigations to determine the importance of chlamydial genotypes to pathogenicity.
- Incorporate *Chlamydia* risk assessment and mitigation into koala relocation protocols, including general biosecurity practices, pre-release clinical evaluation and *Chlamydia* testing.
- Develop nationally-agreed guidelines for chlamydial diagnostic testing for *Chlamydia* and *Chlamydia*-specific biosecurity protocols for koalas in free-living, captive and rehabilitation environments.
- Develop nationally-agreed protocols for triage and assessment of koalas with *Chlamydia* infection, including criteria for consideration of euthanasia.
- Support pharmacokinetic and clinical studies for treatment of *Chlamydia* infection (antibiotics, anti-inflammatories etc.)
- Develop nationally-agreed protocols for treatment for chlamydiosis.
- Share information with rehabilitators on the biosecurity risks of *Chlamydia* transmission in rehabilitation.
- Support research and development of vaccines against *Chlamydia* in koalas.
- Develop nationally-agreed protocols for use of vaccination as a tool for controlling *Chlamydia*.

General recommendations

The following general recommendations apply to all 13 koala disease hazards identified for detailed risk assessment:

- Evaluate and prioritise the identified *Risk Management Options* for feasibility, completeness and effectiveness at a national KDRA Implementation Workshop.
- Identified knowledge gaps for this hazard should be prioritised and developed into a *Research Plan*.
- Prioritise protection and improvement of koala habitat as a key risk mitigation process for koala disease.
- Improve national population estimates and ensure ongoing population health monitoring for koalas.
- Develop robust, longitudinal and nationally-agreed data sets on causes of illness and death in free-living koalas.
- Maintain or increase genetic diversity and population size for koalas, so that koalas have the most robust genetic profiles for avoiding disease consequences.
- Minimise factors that might lead to stress in the koala's environment (in the wild or in captivity).
- Minimise co-infections and other causes of debilitation to reduce cumulative risk of disease.

- Increase awareness and implementation of good biosecurity practices (in accordance with the *National Wildlife Biosecurity Guidelines* [117]) when interacting with koalas during field work, treatment and rehabilitation.
- Develop clinical and research collaborative working groups to establish nationally-agreed best practice protocols for koala disease diagnosis, treatment and biosecurity and implement these in collaboration with managers of koala populations across all jurisdictions.
- Develop collaborative relationships between managers of koala populations (state and Commonwealth) and koala clinical and research working groups, including co-participation in the Koala Recovery Team.
- Develop communication tools, using the iconic status of the koala, to raise awareness and encourage behaviour change around factors contributing to disease (e.g. climate change, vehicle driver behaviours, dog ownership and management, land clearing, fire management).

5.1.7 References

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5.2 *Cryptococcus* spp. in Koalas – Risk Assessment

The literature review which supports this risk assessment is in *Appendix 5.2*.

5.2.1 Hazard summary

- The fungal organism *Cryptococcus gattii* is the major cause of cryptococcal infection in koalas [1], probably because of its strong association with tree species commonly used by koalas for food and shelter [2-4].
- *Cryptococcus gattii* is widely distributed throughout Australia and koala habitats, with different molecular types predominating in different regions [5].
- Although the various molecular types of *C. gattii* are commonly associated with eucalypts and other native Australian trees, the tree species is likely to be less important than the capacity of the tree to form decayed hollows as an effective substrate for the organism [6-8].
- Koalas acquire *C. gattii* by inhaling the organisms from the environment [9]. Nasal colonisation with *C. gattii* occurs commonly in healthy koalas subsequent to exposure, and in most cases will either resolve spontaneously, or persist indefinitely without progressing to disease [10].
- In some koalas, nasal colonisation may progress to subclinical infection [9, 11]. In subclinical infection, early, limited invasion of the respiratory system occurs without clinical signs or identifiable lesions but with cryptococcal antigen detectable in the host blood (known as antigenaemia). Subclinical infection may resolve or progress to cryptococcal disease [9, 11, 12].
- Cryptococcal disease is much less prevalent than nasal colonisation or subclinical infection. It is not an inevitable sequel of these earlier phases of infection [7, 9, 11, 12], suggesting that in most cases of nasal colonisation or subclinical infection, the host immune response is sufficient to contain or eliminate the pathogen [13].
- Cryptococcal disease (cryptococcosis) in koalas has been most studied in NSW, and there is a strong regional bias to NSW in reported cases [11, 12, 14]. Cryptococcosis also occurs in captive and wild koalas in Qld and sporadically elsewhere in captive koalas [15-17]. There are no reports of cryptococcosis in free-ranging koalas in SA or Vic.
- Cryptococcal disease is likely to be precipitated by stress, including poor nutrition, starvation, illness due to other causes, and transport [5, 10, 18].
- Cryptococcal disease most often occurs in captivity, but outbreaks in free-ranging animals occur [14, 15]. It is thought that koalas can amplify *Cryptococcus* in their immediate environment [11, 18], which could be a factor in the higher incidence of disease in captivity.
- Signs of cryptococcal disease are commonly respiratory (sneezing, nasal discharge, nose bleed, facial distortion, pneumonia), but the organism may spread to other

organs, particularly the nervous system, with associated clinical signs. Affected koalas are generally unwell and have depression, inappetence and weight loss [10, 14, 19].

- Treatment of cryptococcal disease is very challenging and is generally only attempted in captive koalas [10, 20-23]. Serological screening tools are highly effective in detecting antigenaemia in the subclinical stages when disease management is more likely to succeed [10].
- For further detail on current knowledge of this hazard, refer to *Appendix 5.2 Cryptococcus spp. in Koalas - Literature Review*.

5.2.2 Justification for hazard selection

Cryptococcal disease is a significant cause of morbidity and mortality in some captive colonies of koalas [17]. It can also cause morbidity and mortality in wild koalas [14].

5.2.3 Identified gaps in knowledge

- Comprehensive environmental surveillance for *C. gattii* is needed to understand its environmental niche and clarify how molecular type and genotype influence environmental colonisation [6].
- Information on the prevalence of both *C. gattii* and cryptococcal disease in koala populations outside of NSW (both wild and captive) is needed to better understand the significance of this hazard in koalas.
- Understanding of the mechanisms through which koalas amplify *Cryptococcus* load in the environment is needed to mitigate the risk of environmental build-up of *Cryptococcus* in captive koala colonies.
- Understanding of the role of koala immune function in preventing the progression of nasal colonisation to cryptococcal disease is needed.
- Investigation of the role of co-infections such as KoRV in the progression to cryptococcal disease in koalas is required.
- The risks posed by introducing novel cryptococcal molecular types to a koala population, particularly for captive koalas, require further investigation.
- Knowledge of the pharmacokinetics of suitable drugs in koalas is needed to improve treatment options for diseased koalas.

5.2.4 Risk assessment

The great majority of cases of *Cryptococcus* infection in koalas are caused by *C. gattii* [1]. For this reason, this risk assessment considered only *C. gattii*. However, risk mitigation options are also likely to be valid for other *Cryptococcus* species.

Below is a schematic of the hazard pathways and critical control points (CCPs) identified for this hazard. Critical control points are *key* points in the hazard pathway where risk mitigation methods could be most effective (see *Appendix 2.3 Risk Assessment Methods* for further information). Nineteen CCPs (CCP1-CCP19) were identified across all hazards, but only those applicable to this hazard are described below. See Section 6 *Critical Control*

Points by Disease Hazard for a summary of CCPs across all hazards selected for detailed assessment.

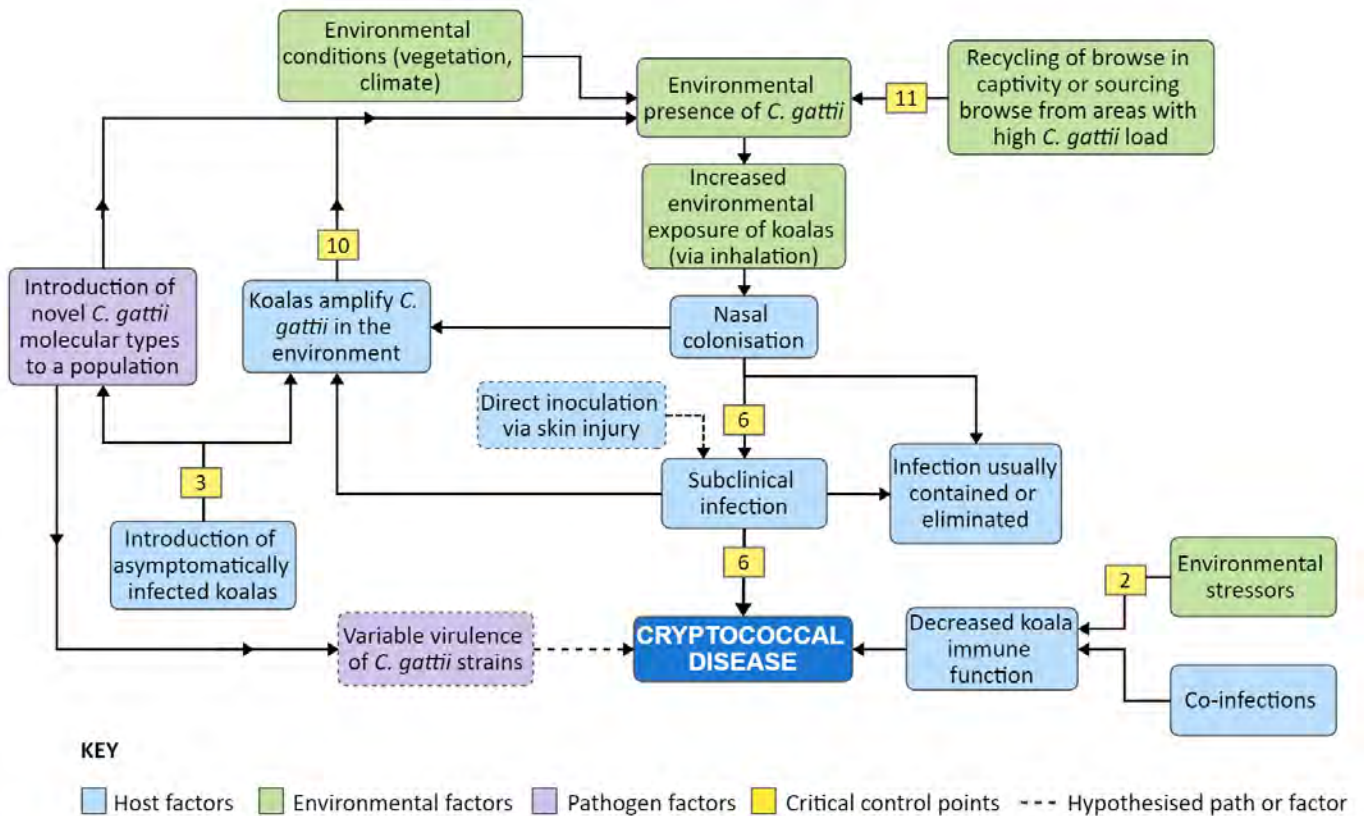


Figure 6 Hazard pathways and critical control points for cryptococcal disease in koalas

Critical control points

CCP2 Environmental stressors

Stressors in the koala's environment (in the wild or in captivity) contribute to general debilitation of the host, reduced immune function, and increased susceptibility to disease or severity of disease expression [24-27]. Major environmental stressors for koalas include habitat loss, fragmentation and reduction in quality; nutritional stress; climate change; extremes of weather and disturbance related to human activities [28, 29].

In particular, environmental stressors may play a role in the progression from nasal colonisation to subclinical infection to cryptococcal disease in koalas [10, 12, 18]. Transport stress is an important consideration for the development of cryptococcal disease in captive koalas [5].

CCP3 Koala relocation

Human-mediated movement of koalas may increase disease risk by introducing pathogens, or new pathogen varieties [30]. In the case of *Cryptococcus*, this is most likely to be

significant when captive koalas are transferred between regions where the molecular types of *C. gattii* endemic in the environment are different (e.g. [5]).

CCP6 Diagnostics

Detection of nasal colonisation and subclinical cryptococcal infection cases through diagnostic testing may allow early management or treatment and halt progression to cryptococcal disease [10].

CCP10 Pathogen amplification

Cryptococcal load in the immediate environment may be increased due to the amplification of *C. gattii* by infected koalas [11, 18]. This is primarily a consideration for captive koalas or koalas in rehabilitation.

CCP11 Increased pathogen load

An increase in the load of an infectious pathogen that a koala is exposed to, or that a koala carries, influences the severity of disease caused by that pathogen.

Contaminated browse may increase the environmental load of *C. gattii* for koalas in the captive environment [10].

Likelihood assessments

The likelihood of entry and exposure of koalas was considered for “environment to koala population”, “koala population to koala population” and “other animal population to koala population” transmission pathways. Likelihood of an infected koala causing a hazard to humans or other animals (via their exposure to koalas or koala environments) was also evaluated.

Entry assessment - koalas

Cryptococcus organisms are widespread throughout the environment in Australia [31]. *Cryptococcus gattii* is present in tropical, subtropical and temperate regions worldwide and has been detected throughout the free-ranging and captive distribution of the koala in Australia [5, 10]. *Cryptococcus gattii* VGI, the cause of most cases of cryptococcal disease in koalas, has a strong association with *Eucalyptus* spp., the main food trees of koalas [2]. Heavy environmental loads of *C. gattii* may occur in the wild in the absence of koalas [11]. In captive environments, high koala density may be important to maintaining a high environmental presence of *C. gattii* [32], and koalas may amplify *C. gattii* in their immediate environment [11, 18].

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of *C. gattii* entering, or being present, in a koala population is considered **HIGH**.

Exposure assessment - koalas

The likelihood of exposure to *C. gattii* refers to the likelihood that an individual koala will be exposed to *C. gattii*, regardless of whether the individual becomes infected.

Exposure of koalas to *C. gattii* occurs via inhalation of environmental cryptococcal organisms [9, 10], which accumulate in tree hollows [33]. *Cryptococcus gattii* molecular types are present in all environments where Australian koalas live [5, 10].

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of exposure to *C. gattii* for an individual koala is considered **HIGH**.

Using the principles of combining entry and exposure likelihood as outlined in *Appendix 2.3 Risk Assessment Methods*, the combined likelihood of entry and exposure for *C. gattii* in koalas is considered **HIGH**.

Likelihood assessments for other species exposed to koalas

Cryptococcus gattii is acquired from the environment rather than via transmission between hosts. There are no reported cases where amplification of the cryptococcal environmental load by koalas (either in the wild or in captivity) was thought to have led to *C. gattii* infection in humans.

In captivity, the amplification of *Cryptococcus* by koalas may increase the cryptococcal load on browse and in the environment. This has been associated with the development of *C. gattii* disease in other species in the same facility when koala browse was re-used as substrate for other species [10].

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of exposure to *C. gattii* for humans due to koalas is considered **NEGLIGIBLE**. Therefore, as outlined in *Appendix 2.3 Risk Assessment Methods*, the risk estimate for humans is classified as **NEGLIGIBLE** and the risk analysis for humans is concluded at this point.

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of exposure to *C. gattii* for other animal species due to koalas is considered to be **NEGLIGIBLE** in wild populations and **LOW** in captive and rehabilitation facilities. Therefore, as outlined in *Appendix 2.3 Risk Assessment Methods*, the risk estimate for wild populations of other species is classified as **NEGLIGIBLE** and the risk analysis for wild populations of other species is concluded at this point. Risk analysis for other species in captive and rehabilitation facilities continues below.

Consequence assessments

Not all exposure to *C. gattii* will result in infection of koalas. If infection of koalas does occur, this can progress in consequence from nasal colonisation (which is common and generally self-resolving) to subclinical infection (early, limited respiratory infection with antigenaemia)

and ultimately to clinical cryptococcal disease [9, 11]. The low reported prevalence of cryptococcal disease, given the ubiquity of *Cryptococcus* in the environment, suggests that in the majority of cases of infection, the koala's immune response is sufficient to contain or eliminate the pathogen [13], and disease does not ensue.

Most cases of nasal colonisation in both captive and free-ranging koalas do not progress to disease, although disease is more common in the captive setting [8, 11, 14, 18, 34]. In retrospective post mortem studies, only 3–4% of koalas (mostly from NSW) had cryptococcal lesions [18]. There are no reports of cryptococcal disease in free-ranging koalas in SA and Vic [35].

Koala population resilience and viability

There is little information on the prevalence of cryptococcal colonisation, infection and disease in free-ranging koala populations outside of NSW. In one NSW free-ranging population, the prevalence of nasal colonisation was 6%, and the prevalence of antigenaemia due to subclinical infection was 7% [18]. While clusters of cryptococcal disease have been reported in free-ranging koala populations [14, 18], there is no suggestion that these resulted in a population decline. The majority of nasal colonisation and subclinical infection cases will not progress to cryptococcal disease in either the free-ranging or captive environment. It therefore will not have an impact on koala population health and viability.

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of *C. gattii* infection to koala population resilience and viability is considered **MINOR**.

Koala individual health and welfare

Most koalas infected with *C. gattii* will resolve the infection at the nasal colonisation or subclinical infection stage without progressing to clinical disease [8, 11, 14, 18, 34]. The progression to clinical disease is a more common consequence in the captive environment. However, it is still much less prevalent than infection without disease, given nasal colonisation may have close to 100% prevalence and subclinical infection >50% prevalence in captive facilities [11, 12, 23]. This evaluation of the consequence of *C. gattii* disease for individual koalas incorporated the low rates at which exposure has been shown to progress to disease and the significant consequences for individual health and welfare for the small proportion of animals in which severe disease occurs.

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of *C. gattii* infection to koala individual health and welfare is considered **MINOR** in the free-ranging environment and **MODERATE** in the captive and rehabilitation setting.

Health and welfare of other species

Evaluation of the overall consequences of *C. gattii* disease in koalas for individuals of other species incorporated the low reported rates at which exposure to koalas (or their environments) has been shown to lead to disease, as well as the consequences for individual health and welfare for the small proportion in which severe disease occurs.

Most other animal species exposed to *C. gattii* will not develop disease. However, when it occurs, cryptococcal disease can have severe and fatal consequences in many species of Australian wildlife [1, 10, 36-41]. Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of exposure to *C. gattii* for the health and welfare of other species is considered **MINOR**.

Overall risk estimate

The overall risk of *C. gattii* infection to koala populations, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **MODERATE**.

The overall risk of *C. gattii* infection to individual koalas, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **MODERATE** in free-ranging koalas and **HIGH** for koalas in captivity or rehabilitation.

The overall risk of *C. gattii* infection to the health and welfare of other species, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods* is **LOW** for animals in captivity where koalas are present.

This assessment exceeds the acceptable risk thresholds outlined in *Appendix 2.3 Risk Assessment Methods* for koala populations, individual koala health and welfare, and health and welfare of other species in captivity. Therefore, risk management for *C. gattii* is recommended for these groups.

Level of confidence in risk assessment

Factors associated with *C. gattii* in the koala environment are well recognised and well-studied. Therefore, the level of confidence in the likelihood assessment is **HIGH**.

Cryptococcal disease is not well studied outside of NSW in free-ranging populations. There is little information on the proportion of koalas exposed to *C. gattii* that progress to infection and disease. The level of confidence in the consequence assessments for populations is **LOW**.

The clinical effects of *C. gattii* infection on the health and welfare of koala individuals are well documented. The proportion of koalas that develop serious disease consequences due to exposure is not well understood, and the consequences for humans and other species of exposure to koalas are not well studied. The level of confidence in the consequence assessments for individual koalas is **MODERATE**.

The effects of *C. gattii* on the health and welfare of other species are well documented. However, the role of koalas in amplifying *C. gattii* in their immediate environment, and the

importance of this amplification in increasing the risk to other animals sharing their environment, is not well understood. The confidence level in the consequence assessments for humans and other species is **MODERATE**.

Overall, the confidence in the risk estimate for *C. gattii* is considered **MODERATE**.

5.2.5 Risk mitigation options

The following risk mitigation measures have been identified for *Cryptococcus* spp. Further information and justification can be found in the *Treatment* and *Prevention and control* sections of the relevant literature review chapter.

Pathogen monitoring, detection and response (CCP 6)

- Early and consistent management of captive koalas with nasal colonisation or subclinical infection to prevent progression to cryptococcal disease.
- Regular testing of captive koalas to monitor for cryptococcal colonisation and subclinical infection, to allow early treatment if needed.

Manage environmental and physiological stress (CCP2)

- Minimise stressful events and circumstances to reduce the risk of pathogenic consequences in koalas with *Cryptococcus*, particularly where nasal colonisation or antigenaemia has been identified.

Risk mitigation in translocations and relocations (CCP3)

- Avoid transport of koalas where nasal colonisation or antigenaemia has been identified.
- Minimise stress in koalas being translocated or relocated.

Minimise the risks of cryptococcal amplification in the captive setting (CCP10 and CCP11)

- Undertake regular decontamination of the captive environment to reduce cryptococcal load.
- Avoid re-use of koala browse for other captive animals.
- Avoid collecting browse from locations where cryptococcal disease outbreaks have been reported in free-ranging koalas.

5.2.6 Recommendations

Hazard-specific recommendations

The following recommendations are specific to this hazard:

- Review current protocols on sampling, storage and testing of samples for *Cryptococcus* spp. infection, with a view to creating nationally-agreed protocols.
- Develop nationally-agreed guidelines for the diagnosis, prevention and treatment of cryptococcal disease in captive populations.

General recommendations

The following general recommendations apply to all 13 koala disease hazards identified for detailed risk assessment:

- Evaluate and prioritise the identified *Risk Management Options* for feasibility, completeness and effectiveness at a national KDRA Implementation Workshop.
- Identified knowledge gaps for this hazard should be prioritised and developed into a *Research Plan*.
- Prioritise protection and improvement of koala habitat as a key risk mitigation process for koala disease.
- Improve national population estimates and ensure ongoing population health monitoring for koalas.
- Develop robust, longitudinal and nationally-agreed data sets on causes of illness and death in free-living koalas.
- Maintain or increase genetic diversity and population size for koalas, so that koalas have the most robust genetic profiles for avoiding disease consequences.
- Minimise factors that might lead to stress in the koala's environment (in the wild or in captivity).
- Minimise co-infections and other causes of debilitation to reduce cumulative risk of disease.
- Increase awareness and implementation of good biosecurity practices (in accordance with the *National Wildlife Biosecurity Guidelines* [30]) when interacting with koalas during field work, treatment and rehabilitation.
- Develop clinical and research collaborative working groups to establish nationally-agreed best practice protocols for koala disease diagnosis, treatment and biosecurity and implement these in collaboration with managers of koala populations across all jurisdictions.
- Develop collaborative relationships between managers of koala populations (state and Commonwealth) and koala clinical and research working groups, including co-participation in the Koala Recovery Team.
- Develop communication tools, using the iconic status of the koala, to raise awareness and drive behaviour change around factors contributing to disease (e.g. climate change, vehicle driver behaviours, dog ownership and management, land clearing, fire management).

5.2.7 References

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5.3 Heat Stress in Koalas - Risk Assessment

The literature review which supports this risk assessment is in *Appendix 5.3*.

5.3.1 Hazard summary

- Heat stress has been reported as a cause of morbidity and mortality in koalas across their geographic range [1-4].
- Heat stress can occur in koalas when the ambient temperature exceeds the upper limit of their thermoneutral zone (24.5°C) and becomes more apparent when ambient temperature exceeds 35°C [5].
- Cooler night temperatures allow koalas to dissipate the body heat they accumulate during the day; warm night temperatures inhibit this process, and their body temperature may rise sequentially over several hot days and nights [5].
- Koalas use physiological cooling strategies such as sweating, panting and peripheral vasodilation to dissipate heat [5, 6]. They also use behavioural cooling strategies such as seeking cooler refugia within their habitat, adapting their body posture and increasing free water intake to offset heat accumulation [7-10].
- Climate change is likely to increase the frequency, intensity and duration of heat events in Australia [11, 12].
- Koalas have a greater chance of surviving heat stress if it is identified and treated in the early stages, and before it progresses to severe stages of illness with multiple organ involvement [13].
- During heat events, increasing the availability of cooler microclimates and providing strategic access to free water may reduce the effects of increased ambient temperature, but are unlikely to fully mitigate them [13].
- Using weather forecasts to predict heat event risk for koalas, as occurs for other heat-susceptible wildlife such as flying-foxes [14], may help to manage the impact of these events on koala populations.
- For further detail on current knowledge of this hazard, refer to *Appendix 5.3 Heat Stress in Koalas - Literature Review*.

5.3.2 Justification for hazard selection

Heat stress has been reported as a cause of morbidity and mortality in koalas across their geographic range, therefore this hazard has been retained for risk assessment.

5.3.3 Identified gaps in knowledge

- Documented reports and protocols for clinical signs, triage, treatment, survival rates and prognostic indicators of heat stress in koalas are lacking, even though heat stress is a relatively common presentation in koalas.
- Effective and efficient ways to reduce the risks of heat stress in free ranging koala populations need to be developed.

- More information is needed on thermal tolerance in koalas (as has been developed for flying-foxes in Australia). This would allow development of a koala heat stress prediction tool, based on weather forecasting.
- Information is needed on differences in physiology between northern and southern koalas and whether genetic management of koala populations for heat tolerance is a viable management tool.

5.3.4 Risk assessment

Below is a schematic of the hazard pathways and critical control points identified for this hazard. Critical control points (CCPs) are defined as *key points in the hazard pathway where risk mitigation methods could be most effective* (see *Appendix 2.3 Risk Assessment Methods* for further information). Nineteen CCPs (CCP1-CCP19) were identified across all hazards, but only those applicable to this hazard are described below. See Section 6 *Critical Control Points by Disease Hazard* for a summary of CCPs across all hazards selected for detailed assessment.

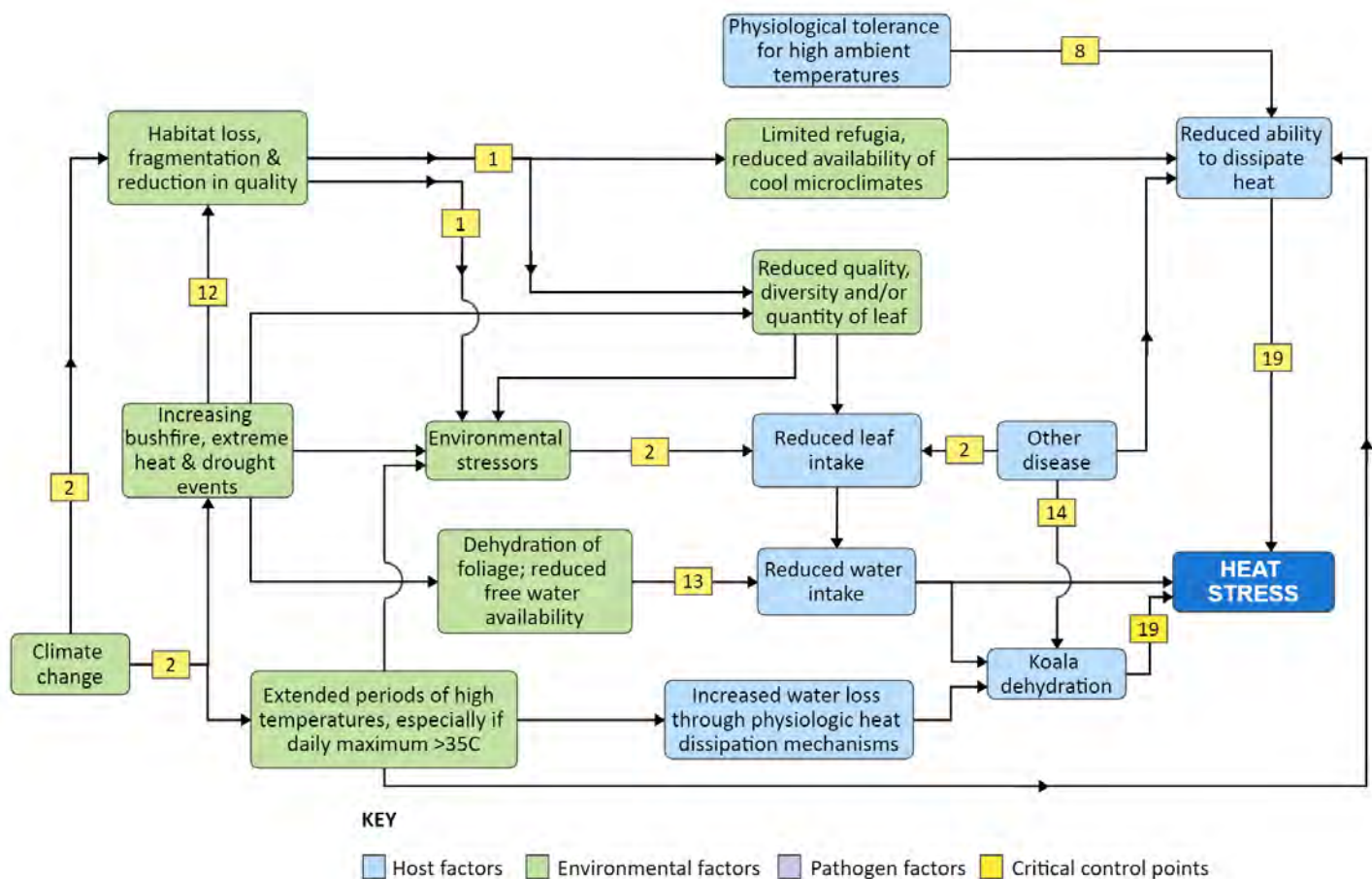


Figure 7 Hazard pathways and critical control points for heat stress in koalas

Critical control points

CCP1 Habitat loss, fragmentation and reduction in quality

Habitat loss and reduction in habitat quality are major environmental stressors due to the koala's reliance on particular tree species for food, shelter and thermoregulation [1, 15]. Habitat loss, reduction and fragmentation reduce connectivity between suitable koala habitats. The resultant loss of refugia for koalas increases the risk of dehydration and exposure to high ambient temperatures [10, 16, 17], which can promote the development of heat stress. Habitat loss and reduction of habitat quality lead to reduced quality, diversity and quantity of leaf for koala consumption [16, 17], causing nutritional stress, decreasing leaf intake and reducing the koala's hydration status.

Causes include urban expansion, land clearing for production, inappropriate fire management practices, the introduction of pests, weeds and plant pathogens, over-browsing by koalas as a result of local overcrowding, and the habitat-altering effects of climate change [18-24].

CCP2 Environmental stressors

Stressors in the koala's environment (in the wild or in captivity) contribute to general debilitation, reduced immune function and increased susceptibility to disease or severity of disease expression [1, 2, 25, 26]. Major environmental stressors for koalas include habitat loss, fragmentation and reduction in quality; nutritional stress; climate change; extremes of weather; and disturbance related to human activities [15, 27].

Koalas obtain about 75% of their water intake from foliage [28]. A reduction in food (and therefore water) intake due to ill health, injury or other stress, may increase the risk of dehydration (which in itself is a source of physiological stress) [27], and reduce heat dissipation through evaporative means.

CCP8 Genetics

An individual koala, or koala populations may have a genetic susceptibility or resistance to developing heat stress [29]. Improving or at least maintaining genetic diversity in koala populations is likely to conserve adaptive potential [30] and encourage the retention of the most robust koala genetic profiles that favour thermal tolerance.

CCP12 Fire management practices

Inadequate planning, inappropriate methodology or flawed execution of fire management practices may contribute to habitat loss and reduction of habitat quality, reducing refugia options for koalas during heat events [31, 32].

CCP13 Reduced water intake

In hot weather, koalas may experience reduced water intake due to lower leaf moisture content and lower free water availability (artificial or natural sources) for drinking [18, 33].

Reduced water intake may hamper physiologic heat dissipation mechanisms and cause dehydration [10, 33].

CCP14 Concurrent infections and debilitation

Concurrent infections and debilitation can compromise immune function and general health in ways that increase the risk or severity of disease. Any disease process that inhibits convection, evaporative or behavioural means of thermoregulation would likely decrease the koala's ability to cope with elevated ambient temperatures. Compromised immunity, hydration status and general health may increase the risk and severity of heat-related illness [13, 34].

CCP19 Emergency response to heightened risk

In situations where environmental conditions are associated with increased disease threats (e.g. bushfires, high ambient temperatures), *in situ* preparedness and response activities may significantly reduce risk to koalas. Actions may include community awareness and behaviour change campaigns (e.g. [35, 36]) as well as advance planning for emergencies, and deployment of rapid response teams (e.g. [37, 38]).

Likelihood assessments

Heat stress is a non-infectious hazard and therefore likelihood assessments were not made in relation to humans or other species.

Ambient temperatures above 30°C commonly occur over much of the koala's natural range [39]. Loss of habitat quality and complexity makes koalas more vulnerable to heat stress events when they occur [18, 40]. The frequency and intensity of heat events in koala habitat is likely to increase with climate change [21, 41]. There are no accurate data on the prevalence of heat stress in koala populations that are experiencing high ambient temperatures. Not all areas of the koala's natural distribution experience extremes of temperature likely to result in heat stress.

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of a koala experiencing prolonged ambient temperatures sufficient to induce heat stress, during its lifetime is considered **MODERATE**.

Consequence assessments

Heat stress is a non-infectious hazard and therefore consequence assessments were not made in relation to humans or other species.

Koala population resilience and viability

The impact of heat stress events on koala populations is not well-studied. There are no accurate data on the prevalence of heat stress in wild koala populations experiencing extended periods of high temperature. The impact of persistent drought conditions on koala populations has been shown to be significant [18, 40, 42, 43]. Heat stress often develops under drought conditions, however the impacts of prolonged drought are not the same as

the impacts of heat stress *per se*, so it is not possible to extrapolate data on drought effects and apply it to heat stress impacts on koala populations.

It is assumed that koalas will vary in their susceptibility to developing heat stress, based on physiological tolerance and on their individual environmental circumstances (availability of refugia, water sources, hydration status of available browse). The proportion of koalas experiencing heat stress, when placed in an environment of ongoing high ambient temperature, is not known. It is assumed that not all koalas at risk will develop clinical heat stress.

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of heat stress to koala population resilience and viability is considered **MODERATE**.

Koala individual health and welfare

The proportion of koalas experiencing heat stress, when placed in an environment of ongoing high ambient temperature, is not known. It is assumed that not all koalas at risk will develop clinical heat stress. However, it is well-documented that individual koalas experiencing heat stress can become extremely unwell and die as a result [18, 42].

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of heat stress to koala individual health and welfare is considered **MAJOR**.

Overall risk estimate

The overall risk of heat stress to koala population resilience and viability, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **MODERATE**.

The overall risk of heat stress to individual koala health and welfare, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **HIGH**.

This assessment exceeds the acceptable risk thresholds for koala populations and individual koalas as outlined in *Appendix 2.3 Risk Assessment Methods*, therefore risk management for heat stress is recommended.

Level of confidence in risk assessment

The level of confidence in the likelihood assessment for heat stress is **MEDIUM**. Although there are excellent weather data available across the koala's natural range, there is limited information on how many koalas experiencing extremes of ambient temperature progress to clinical heat stress. The level of confidence in the consequence assessment for koala populations is **LOW** as there are no data on the prevalence of heat stress in wild koala populations experiencing extremes of ambient temperature. The level of confidence in the consequence assessment for individual koalas is **MEDIUM**. Although the severe impacts of heat stress on individual koala health and welfare have been documented, there is no data

on the proportion of koalas experiencing heat stress, when placed in an environment of ongoing high ambient temperature.

Overall, the confidence in the risk estimate for heat stress is considered **MEDIUM**.

5.3.5 Risk mitigation options

The following risk mitigation measures have been identified for heat stress. Further information and justification can be found in the *Treatment and Prevention and control* sections of the relevant literature review chapter. Not all risk mitigation options will be currently available, feasible, universally applicable or cost-effective, but are listed here for completeness.

Act to reduce the impacts of climate change and a warming, drying climate (CCP2)

- Act to bring about “climate-friendly” policies at a local, state and federal level.
- Use koalas and their plight as a focus-point for community education about climate change and a “call to action” for associated behaviour change.
- Consider climate change projections when undertaking habitat assessment and planning for koalas.

Conserve and improve the quality, quantity, diversity and complexity of koala habitat (CCP1)

- Re-vegetation, restoration and preservation of habitat, including in urban landscapes.
- Protect or develop climate refugia, such as valleys, understory, midstory and fast-growing shade-producing non-browse vegetation that provide good thermal protection for koalas.
- Strengthen existing habitat corridors and restore habitat connectivity which has been lost, through re-vegetation and restoration projects.
- Strengthen regulatory controls against habitat clearing and road development in koala habitat and dispersal corridors.

These measures will increase habitat refugia for koalas.

Support the retention and availability of water in koala environments (CCP1, CCP2, CCP13 and CCP19)

- Select species for koala habitat revegetation that are best suited to changing climate in the bioregion and that are the most drought- and heat-tolerant.
- Support soil quality and diversity, and better manage storm water run-off, to retain moisture in the soil and vegetation.
- Support the capacity of koalas to move to available water in their environment. In the wild, this includes improving water catchment and storage via artificial or natural wetlands and slowing water outflow to increase tree hydration.
- For captive and rehabilitation koalas, supply free water, optimise management of cut browse to maintain leaf moisture (including regular misting), and feed koalas in the

evening rather than in the morning when weather forecasts indicate high ambient temperatures.

Maintain genetic diversity and population sizes in koalas (CCP8)

- Support genetic diversity in koala populations to encourage the retention of the most robust genetic profiles for adapting to a changing climate and coping with the impacts of high ambient temperatures and drought.
- Translocate koalas shown to have higher thermal tolerance into heat-vulnerable populations, to increase genetic resilience to the threat of heat stress.

Translocation and relocation (CCP3)

- Translocate koalas from marginal habitat significantly compromised by climate change to more suitable locations (if available).

Emergency response to heightened risk (CCP19)

- Develop metrics for advance warning of heat stress risk.
- Develop and apply early intervention measures when weather forecasts or conditions indicate heat stress risk to wild koalas (e.g. provision of free water, monitoring of at-risk koala populations or individuals, with identification and removal for treatment of those with clinical heat stress).

Managing concurrent infections, environmental stress and debilitation (CCP14)

- Minimise environmental stressors, concurrent infections and other causes of stress (such as general debilitation) in individual koalas, and populations, to maintain hydration, appetite and organ function and thereby reduce the risk of clinically significant consequences of exposure to high ambient temperatures.

Fire management practices (CCP12)

- Adopt fire management practices that minimise impacts on koala habitat quantity, quality and connectivity, to retain refugia from hot weather and minimise hydration stress on koalas and trees (see *Risk mitigation options in Appendix 5.12 Thermal Burn Trauma* for more details).

5.3.6 Recommendations

Hazard-specific recommendations

The following recommendations are specific to this hazard:

- Develop nationally-agreed protocols for diagnosis, assessment, treatment and care of heat stressed koalas.
- Undertake research on thermal tolerance in koalas, including whether this (or other indicators of a koala's ability to survive increased ambient temperatures) vary genetically or over the koala's range (north-south or east-west). This will help to inform the option of translocation to mitigate climate change associated risks.

- Develop and apply a nationally-applicable koala heat stress prediction tool, based on weather forecasting.
- Develop community and first responder early intervention and emergency response protocols for koala populations during extreme heat events.
- Support fire management practices that minimise the loss of koala refugia.

General recommendations

The following general recommendations apply to all 13 koala disease hazards identified for detailed risk assessment:

- Evaluate and prioritise the identified *Risk Management Options* for feasibility, completeness and effectiveness at a national KDRA Implementation Workshop.
- Identified knowledge gaps for this hazard should be prioritised and developed into a *Research Plan*.
- Prioritise protection and improvement of koala habitat as a key risk mitigation process for koala disease.
- Improve national population estimates and ensure ongoing population health monitoring for koalas.
- Develop robust, longitudinal and nationally-agreed data sets on causes of illness and death in free-living koalas.
- Maintain or increase genetic diversity and population size for koalas, so that koalas have the most robust genetic profiles for avoiding disease consequences.
- Minimise factors that might lead to stress in the koala's environment (in the wild or in captivity).
- Minimise co-infections and other causes of debilitation to reduce cumulative risk of disease.
- Increase awareness and implementation of good biosecurity practices (in accordance with the *National Wildlife Biosecurity Guidelines* [44]) when interacting with koalas during field work, treatment and rehabilitation.
- Develop clinical and research collaborative working groups to establish nationally-agreed best practice protocols for koala disease diagnosis, treatment and biosecurity and implement these in collaboration with managers of koala populations across all jurisdictions.
- Develop collaborative relationships between managers of koala populations (state and Commonwealth) and koala clinical and research working groups, including co-participation in the Koala Recovery Team.
- Develop communication tools, using the iconic status of the koala, to raise awareness and drive behaviour change around factors contributing to disease (e.g. climate change, vehicle driver behaviours, dog ownership and management, land clearing, fire management).

5.3.7 References

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5.4 Koala Retrovirus – Risk Assessment

The literature review which supports this risk assessment is in *Appendix 5.4*.

5.4.1 Hazard summary

- Koala retrovirus (KoRV) is an RNA virus that replicates (like other retroviruses) by inserting DNA copies of itself (proviruses) into the genome of host cells [1, 2].
- KoRV is a relatively young retrovirus that exists in both endogenous and exogenous forms [3, 4]. Endogenous KoRV is incorporated into koala gamete cells and therefore is inherited genetically. Exogenous KoRV integrates into the genome of somatic (non-reproductive) cells, and is not inherited by the next generation [1]. Endogenous KoRV-A retains many features commonly associated with pathogenesis by exogenous retroviruses [5, 6].
- The complete KoRV genome consists of three genes (gag, pol and env). KoRV variants (KoRV-A to KoRV-M) are based on phylogenetic groupings of the env gene [7]. The pol gene is required for transcription [1] and KoRV types that lack all or part of the pol gene are not replication-competent [8-10].
- KoRV-A occurs both endogenously and exogenously and is replication-competent [8]. It is endogenous in 100% of northern koalas [6, 11] but in southern populations, endogenous KoRV-A has not been detected [6, 12, 13].
- All KoRV variants other than KoRV-A are exogenous and are only present in koalas that also harbour KoRV-A [11, 14-16]. Prevalence and distribution of exogenous variants appear variable [5, 16-24]. Some exogenous variants are not replication-competent [2, 10, 11, 25, 26].
- Transmission of exogenous KoRV variants appears to occur through close contact, most likely mother-to-offspring [14, 24, 27, 28] though some experts dispute this.
- Defective retroviral elements known as recKoRV appear to be endogenous and are widespread in koala populations, including southern populations where endogenous KoRV has not been detected [8].
- KoRV might increase koala susceptibility to disease by increasing mutagenic load, thus predisposing koalas to neoplastic disease [29] and by causing immune modulation [30-32], which could increase disease susceptibility of the koala and severity of disease expression for a range of disease hazards.
- Various KoRV traits (such as viral load, proviral load, variant presence or load and pol presence or load) have been associated with a range of disease states and co-infections in koalas [15, 18, 20, 21, 33-41]. It is generally considered likely that all of these variables actually reflect increased viral replication in the host. In most cases, it is unclear whether the relationship is causative (i.e. that KoRV causes disease) or secondary (i.e. disease permits or induces amplification of KoRV).
- It is unclear if replication-competent KoRV variants differ in their ability to cause disease.

- Presence in both endogenous and exogenous forms, and replication competence of endogenous forms, indicate a relatively new host-pathogen relationship that is likely to cause morbidity and mortality until equilibrium is achieved. The ability of the koala species to survive that process depends in part on mitigating mortality from other threats, while maintaining the species adaptive potential through sufficient population size and genetic diversity.
- For further detail on current knowledge of this hazard, refer to *Appendix 5.4 Koala Retrovirus - Literature Review*.

5.4.2 Justification for hazard selection

KoRV is retained for risk assessment because of its potential to cause or exacerbate disease states in koalas.

5.4.3 Identified gaps in knowledge

- Associations between KoRV and other disease hazards require further investigation, particularly with neoplasia, chlamydiosis, koala herpesviruses, *Trypanosoma* spp., novel *Actinomyces* sp., and general debilitating states that are considered the hallmarks of the “KoRV koala” (see also Section 7.1 *Clinical Syndromes with Undefined or Multiple Aetiologies*). In most cases, it remains to be determined whether KoRV plays a causative role in disease manifestations or susceptibilities, or if conversely these other hazards alter KoRV traits in the affected individual.
- The transmissibility and transmission mechanisms of exogenous KoRV variants requires investigation.
- Further investigation of KoRV profiles of southern koala populations is needed, to determine the prevalence of recKoRV in populations previously thought to be “KoRV free”; to resolve contradictory evidence over presence or absence of variants other than KoRV-A; and to understand the drivers for regional differences in KoRV load.
- The hypothesis that recKoRV elements protect southern koalas from replication-competent KoRV endogenisation requires investigation.
- More comprehensive and coordinated knowledge of the KoRV status of koalas in captivity is needed, including recommendations on how best to quantify and manage KoRV status in captive breeding and insurance populations.
- A more complete assessment of the potential for cross-species transmission of KoRV is required, including zoonotic transmission.
- Regimens for treatment of koalas with KoRV, including anti-retroviral drug options and pharmacokinetics, require investigation.
- Further investigation of the viability and safety of vaccination for the treatment, prevention and control of KoRV is needed.

5.4.4 Risk assessment

Below is a schematic of the hazard pathways and critical control points (CCPs) identified for this hazard. Critical control points are defined as *key points* in the hazard pathway where risk mitigation methods could be most effective (see *Appendix 2.3 Risk Assessment Methods* for further information). Nineteen CCPs (CCP1-CCP19) were identified across all hazards, but only those applicable to this hazard are described below. See Section 6 *Critical Control Points by Disease Hazard* for a summary of CCPs across all hazards selected for detailed assessment.

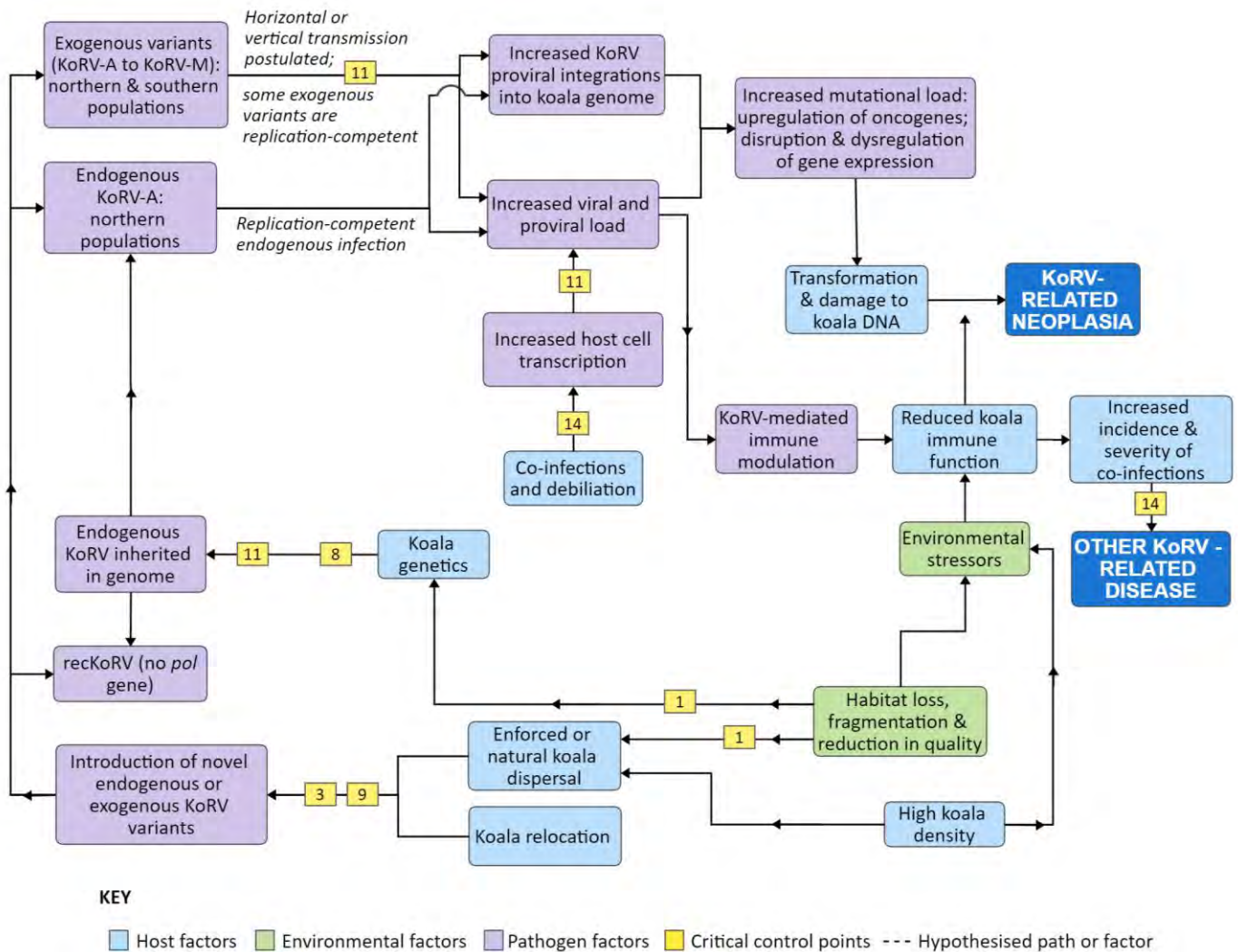


Figure 8 Hazard pathways and critical control points for KoRV

Critical control points

CCP1 Habitat loss, fragmentation and reduction in quality

Habitat loss and reduction in habitat quality are major environmental stressors due to the koala's reliance on particular tree species for food, shelter and thermoregulation [42, 43]. Habitat fragmentation reduces connectivity between suitable koala habitats, forcing

dispersal and potentially contributing to the loss of important genetic alleles in populations that can no longer interbreed [44-46]. Causes include urban expansion, land clearing for production, inappropriate fire management practices, the introduction of pests, weeds and plant pathogens, over-browsing by koalas as a result of local overcrowding, and the habitat-altering effects of climate change [47-53].

In particular, habitat loss and fragmentation limits effective population size [44-46], increasing the likelihood of genetic establishment of deleterious KoRV integrations, or of heritable traits which encourage KoRV to escape containment, so that extinction may be reached before host adaptation can be achieved.

CCP3 Koala relocation

Human-mediated movement of koalas may increase disease risk by introducing pathogens, or new pathogen varieties [54]. This may occur when koalas are brought into rehabilitation or released from rehabilitation (especially if not returned to their point of capture), when captive koalas are transferred between facilities [55], when koalas are translocated from one wild population to another [56], or when habitat corridors are created to reconnect long-separated populations without consideration of disease control.

In particular, *pol*-positive koalas (koalas infected with replication-competent KoRV) moved to *pol*-negative populations could pass on replication-competent KoRV to offspring and descendants within the new population [1, 3, 14, 57]. Relocation of individuals may pose a risk of introducing novel exogenous variants to a naïve population, if exogenous transmission occurs [14, 23, 24, 58].

CCP8 Genetics

The individual koala may have a genetic predisposition to the development of disease or increased severity of disease. Improving or at least maintaining genetic diversity in koala populations is likely to encourage the retention of the most robust koala genetic profiles to avoid disease consequences of KoRV and facilitate virus-host co-evolution.

Based on fundamental dynamics of recessive genetic traits, inherited KoRV integrations are likely to become fixed or more readily expressed if inbreeding increases [44-46]. In particular, inherited KoRV integrations in the koala genome are associated with increased neoplasia risk to koalas [20, 22], and may be responsible for a hereditary pattern for some neoplastic conditions [59].

CCP9 Naïve host

An infectious pathogen may be introduced to a previously unexposed population or a previously uninfected individual.

Replication-competent (*pol*-positive) KoRV-A has a prevalence of 100% in northern koalas [31] but appears to be absent in its endogenous form from southern populations [6, 12, 13], where competent KoRV-A behaves as an exogenous virus and endogenous *pol*-negative

recKoRV appears widespread [8]. The introduction of replication-competent KoRV into the genome of southern populations (via breeding between *pol*-positive and *pol*-negative koalas) could increase the risk of KoRV-related disease in southern populations and potentially increase the risk of KoRV endogenization.

Exogenous *env* gene variants appear to vary in prevalence throughout the koala's distribution [5, 16-24]. There may be the potential for transmission of new variants into a population via close contact between koalas [23, 58]. The likelihood and impact of this is not understood.

CCP11 Increased pathogen load

An increase in the load of an infectious pathogen that a koala is exposed to, or that a koala carries, influences the severity of disease caused by that pathogen as well as the likelihood of transmission.

Associations between disease states and KoRV viral and proviral load have been identified in many studies [20, 33, 34, 36, 38, 58, 60]. The variety of associations between KoRV traits and disease states suggests that disease is most associated with escape and proliferation of KoRV in any competent form [23].

CCP14 Concurrent infections and debilitation

Concurrent infections and debilitation can compromise immune function and general health in ways that increase the risk or severity of disease. The pathogenicity of an infectious hazard may be potentiated by co-infections.

In particular, koala host cells that are stimulated by inflammation to increase transcription or proliferate (in this case as a result of pathogen infection or general debilitation of the host) likely increase KoRV viral and proviral load [61], resulting in more opportunities for the pathogenic effects of KoRV to be manifest in the host. Host-pathogen co-evolution requires resilient populations. Additional disease threats increase risk of local host extinction occurring before co-evolutionary equilibrium between KoRV and the host [62] can be achieved.

Approach to assessment of KoRV

Likelihood and consequence were assessed only for replication-competent (*pol*-positive) KoRV (hereafter called "*pol* KoRV") in both its exogenous and endogenous forms. RecKoRV, and many exogenous KoRV variants, are not replication-competent and in the absence of *pol* KoRV should be capable of only limited disruption of the koala genome [2, 10], which may limit their pathogenicity.

Likelihood assessments

There is a clear disparity between the prevalence of *pol* KoRV in northern and southern koala populations [5, 19, 37, 63] which affects the assessment of likelihood. For this reason, separate likelihood assessments have been made for northern and southern populations.

Entry assessment

The likelihood of *pol* KoRV entering or being present in a koala population was considered only for the transmission pathway of “koala population to koala population”, as KoRV is not known to be acquired from the environment or present in human or other animal species.

Endogenous (inherited) *pol* KoRV has been detected in 100% of northern koalas [5, 6]. In contrast, endogenous *pol* KoRV is absent in southern populations [6, 12, 13] and would only enter via interbreeding with northern koalas. This would require human-assisted movement of koalas, or significant increases in the geographic spread of koalas, as northern and southern populations currently have minimal overlap in geographic distribution [49]. However, exogenous *pol* KoRV is present with variable prevalence in both northern and southern populations [5, 16-24].

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of *pol* KoRV entering, or being present in northern populations is considered **HIGH**.

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of *pol* KoRV entering, or being present, in a southern koala population is considered **HIGH**.

Exposure assessment

The likelihood of exposure to *pol* KoRV was only considered for the “koala-to-koala” transmission pathway, as KoRV is not considered to be present in the environment, or in human or other animal species.

An individual koala acquires endogenous *pol* KoRV through Mendelian inheritance; the transmissibility of exogenous *pol* KoRV has not been established. *Pol* KoRV has been detected in 100% of northern koalas and is endogenised in northern populations (although exogenous *pol* KoRV is also present). Exogenous *pol* KoRV is present with variable prevalence in southern populations and its transmissibility is not confirmed. Endogenous *pol* KoRV has not been detected in southern populations [8].

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of exposure to *pol* KoRV in northern populations is **HIGH**.

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of exposure to *pol* KoRV in southern koala populations is **MODERATE**.

Using the principles of combining entry and exposure likelihood outlined in *Appendix 2.3 Risk Assessment Methods*, the combined likelihood of entry and exposure for *pol* KoRV in northern populations is **HIGH**. The combined likelihood of entry and exposure to *pol* KoRV in southern populations is **MODERATE**.

Consequence assessments

As *pol* KoRV is not considered a hazard for humans or other animal species, the consequence of *pol* KoRV infection in other species, including humans, has not been evaluated.

Although disease manifestations associated with KoRV are considered more significant in northern than southern populations, there are also associations between presence of *pol* KoRV and disease in southern koalas [37] and there is no clear evidence that southern koalas are intrinsically less susceptible to the detrimental effects of KoRV than their northern counterparts. Therefore, consequence assessments for *pol* KoRV for individual koalas and populations were considered to be the same in northern and southern koalas.

Koala population resilience and viability

The consequences of *pol* KoRV infection on koala populations are not clear. There are no reliable estimates of the prevalence of KoRV-related disease in KoRV populations. Fertility and mortality rates in koala populations might be negatively affected by *pol* KoRV status as this might result in increased prevalence and severity of neoplasia and of infectious diseases such as chlamydiosis [15, 18, 20, 21, 33-41, 58, 60]. The precautionary principle dictates that we assume that *pol* KoRV does have a causal influence on these disease states.

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of *pol* KoRV to koala population resilience and viability in northern and southern populations is considered **MODERATE**.

Koala individual health and welfare

The consequences of *pol* KoRV infection in individual koalas are not clear. KoRV probably increases susceptibility to neoplasia [29], and might influence development or increased severity of many other disease states [15, 18, 20, 21, 33-41, 58, 60]. These outcomes could lead to significant illness or death, or severe welfare impacts in individual koalas. The precautionary principle dictates that we assume that *pol* KoRV does have a causal influence on the disease states outlined above. Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of *pol* KoRV infection to individual koala health and welfare is considered **MODERATE**.

Overall risk estimate

The overall risk of *pol* KoRV infection to northern koala populations, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **HIGH**.

The overall risk of *pol* KoRV infection to individual northern koalas, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **HIGH**.

The overall risk of *pol* KoRV infection to southern koala populations, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **MODERATE**.

The overall risk of *pol* KoRV infection to individual southern koalas, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **MODERATE**.

These assessments exceed the acceptable risk thresholds as outlined in *Appendix 2.3 Risk Assessment Methods* for both koala populations and individual koalas. Therefore, risk management for *pol* KoRV infection is recommended for both northern and southern koala populations.

Level of confidence in risk assessment

The confidence in the entry assessment is **HIGH**, as there is considerable data on the prevalence of *pol* KoRV infection in free-living northern and southern koala populations. The confidence in the exposure assessment is **MEDIUM** as there are uncertainties about the modes and effectiveness of *pol* KoRV transmission between koalas. The confidence in the consequence assessment for KoRV, for both individuals and populations, is **LOW** as there are many gaps in knowledge regarding the pathogenicity and initiating factors for KoRV-related health issues.

Overall, the confidence in the risk estimate for KoRV is **LOW**.

5.4.5 Risk mitigation options

The following risk mitigation measures have been identified for KoRV. Further information and justification can be found in the *Treatment and Prevention and control* sections of the relevant literature review chapter. Approaches to risk mitigation of KoRV are drawn from the currently limited knowledge base. Not all risk mitigation options will be currently available, feasible, universally applicable or cost-effective, but are listed here for completeness.

Conserve and improve the quality, quantity, diversity and complexity of koala habitat (CCP1 and CCP8)

- Re-vegetation, restoration and preservation of habitat, including in urban landscapes
- Strengthen regulatory controls against habitat clearing and road development in koala habitat and dispersal corridors.

These measures will encourage the maintenance of larger wild koala populations with more robust genetic profiles, which will have stronger potential to avoid fixing of undesirable KoRV integrations.

Pathogen detection and response (CCP3 and CCP9)

- Identify *pol*-negative captive and free-living southern koala populations, and protect them from the entry of replication-competent KoRV by managed isolation of these populations, to prevent breeding with *pol*-positive koalas.

Risk mitigation in breeding, translocations and relocations (CCP3, CCP8 and CCP9)

- Select individuals with low KoRV viral loads for breeding and translocation, and avoid breeding and translocation of koalas with high viral loads, so that koalas have the most robust genetic profiles for avoiding disease consequences of KoRV.
- Preferentially breed from koalas with the lowest number of replication-competent exogenous variants, to minimise the spread of exogenous variants through populations.
- Test koalas for KoRV variants and select those for relocation based on the number of KoRV variants detected, to minimise the risk of introducing novel exogenous variants to a naïve population.
- Minimise breeding from koala populations or family lines with a high prevalence of neoplasia or other disease states recognised to be potentially KoRV-associated (e.g. severe chlamydiosis, wasting syndrome, persistent or multiple opportunistic infections) to avoid heritable deleterious KoRV integrations, or other genetic traits that allow KoRV to “escape” containment and interfere with cell function.
- Prevent local extinctions from other causes to enable co-evolution of KoRV to continue with the minimum of impact on koala health and population resilience.

Reduce disease consequences of exposure to the hazard (CCP11)

- Use of vaccination against KoRV in koalas (when available) to:
 - ◆ develop and maintain populations free from endogenous KoRV-A.
 - ◆ reduce KoRV viral and proviral loads (and hence associated disease risk to the individual koala).
 - ◆ reduce risk of exogenous transmission (if this occurs) to in-contact koalas.
- Use anti-retroviral medication to treat individual koalas with persistently high viral and proviral KoRV loads, to reduce the likelihood of disease development.
- Treat dams raising joeys with anti-retroviral medication to reduce the risk of exogenous KoRV transfer to the joey.

Manage concurrent infections, debilitation and environmental stressors (CCP14)

- Minimise concurrent infections, debilitation and other environmental stressors (e.g. predators, disturbance related to human activities) in individual koalas and populations to:
 - ◆ reduce the risks of disease in koalas with KoRV-associated immunosuppression.
 - ◆ reduce stimulation of koala host cells that could increase cell transcription or proliferation and lead to increased KoRV viral and proviral load resulting in more opportunities for the pathogenic effects of KoRV to arise.

5.4.6 Recommendations

Hazard-specific recommendations

The following recommendations are specific to this hazard:

- Define thresholds or consistent methodologies for quantifying proviral and viral KoRV load and use these to develop consistent protocols to enable meaningful incorporation of KoRV status into koala management.
- Continue research into KoRV with a focus on determining the extent to which it causes disease. This will require a combination of longitudinal and *in vitro* studies to determine causation, and field studies to determine contribution to disease outcomes, relative to other drivers of disease.
- Develop nationally-agreed guidelines for biosecurity, control and prevention of KoRV risk in the management, breeding and movement of free-ranging and captive koalas. Prevention and control strategies should be revised regularly, given the rapid rate of development of knowledge of KoRV.
- Support increased testing and improved data sharing between captive koala populations to allow an evidence-based, scientifically coordinated approach to managing KoRV status of Australia’s captive breeding koala population.

General recommendations

The following general recommendations apply to all 13 koala disease hazards identified for detailed risk assessment:

- Evaluate and prioritise the identified *Risk Management Options* for feasibility, completeness and effectiveness at a national KDRA Implementation Workshop.
- Identified knowledge gaps for this hazard should be prioritised and developed into a *Research Plan*.
- Prioritise protection and improvement of koala habitat as a key risk mitigation process for koala disease.
- Improve national population estimates and ensure ongoing population health monitoring for koalas.
- Develop robust, longitudinal and nationally-agreed data sets on causes of illness and death in free-living koalas.
- Maintain or increase genetic diversity and population size for koalas, so that koalas have the most robust genetic profiles for avoiding disease consequences.
- Minimise factors that might lead to stress in the koala’s environment (in the wild or in captivity).
- Minimise co-infections and other causes of debilitation to reduce cumulative risk of disease.
- Increase awareness and implementation of good biosecurity practices (in accordance with the *National Wildlife Biosecurity Guidelines* [54]) when interacting with koalas during field work, treatment and rehabilitation.
- Develop clinical and research collaborative working groups to establish nationally-agreed best practice protocols for koala disease diagnosis, treatment and biosecurity

and implement these in collaboration with managers of koala populations across all jurisdictions.

- Develop collaborative relationships between managers of koala populations (state and Commonwealth) and koala clinical and research working groups, including co-participation in the Koala Recovery Team.
- Develop communication tools, using the iconic status of the koala, to raise awareness and drive behaviour change around factors contributing to disease (e.g. climate change, vehicle driver behaviours, dog ownership and management, land clearing, fire management).

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5.5 Motor Vehicle Trauma in Koalas – Risk Assessment

The literature review which supports this risk assessment is in *Appendix 5.5*.

5.5.1 Hazard summary

- Motor vehicle accidents (MVA) cause severe and often fatal trauma to koalas [1, 2].
- Urbanisation and habitat fragmentation increase the likelihood of koalas encountering roads, which in turn increases the risk of motor vehicle trauma [3, 4].
- Male koalas during breeding season, and dispersing young, are more likely to cross roads, and are overrepresented in MVA trauma admissions and fatality records [5].
- The prognosis for motor vehicle trauma is generally poor, with high fatality and euthanasia rates reported in koalas [6, 7].
- Motor vehicle trauma tends to affect otherwise healthy koalas [6, 8], and is likely to result in the loss of the most viable individuals in a population.
- The effectiveness of prevention and control strategies for koala MVA requires more study. Effective reduction of motor vehicle trauma will likely involve a combination of habitat restoration [9-12] and specific MVA mitigation methods [10, 13-18].
- For further detail on this hazard, refer to *Appendix 5.5 Motor Vehicle Trauma in Koalas - Literature Review*.

5.5.2 Justification for hazard selection

Motor vehicle trauma is the most common cause of trauma-related koala admissions to rehabilitation facilities. In many cases it is also the most common cause of death among koalas entering rehabilitation [3, 6, 8, 19, 20]. With ongoing habitat loss and land development, koalas are increasingly exposed to roads and motor vehicles [11]. For dispersing juveniles and breeding males which travel significant distances as a normal activity, encounters with roads are likely. Normal activity patterns mean koalas are most likely to be active during the times of peak traffic and diminished visibility, increasing the risks of MVA.

5.5.3 Identified gaps in knowledge and information needs

- The effectiveness of prevention and control strategies for koala MVA is insufficiently studied and poorly understood. Given the potentially high economic cost of mitigation strategies such as traffic modification, physical barriers and road-crossing structures [10, 11], this knowledge is critical to appropriate risk management.
- Better understanding of koala movement patterns is needed. Longitudinal tracking studies would assist in informing development plans, habitat preservation and rehabilitation efforts [12].
- The identification of “black spots” for koala MVA across their range would assist in targeting mitigation practices [14, 21, 22].

- Robust, longitudinal data sets on causes of mortality in free-living koalas throughout their range are required to better understand the prevalence, source, and population level impacts of motor vehicle trauma.
- More consistent and effective post-release monitoring is needed as it would assist in determining the success of treatment of injured koalas, as well as more clearly evaluating the impact of motor vehicle trauma on populations.

5.5.4 Risk assessment

Below is a schematic of the hazard pathways and critical control points identified for this hazard. Critical control points are defined as *key points* in the hazard pathway where risk mitigation methods could be most effective (see *Appendix 2.3 Risk Assessment Methods* for further information). Nineteen CCPs (CCP1-CCP19) were identified across all hazards, but only those applicable to this hazard are described below. See Section 6 *Critical Control Points by Disease Hazard* for a summary of CCPs across all hazards selected for detailed assessment.

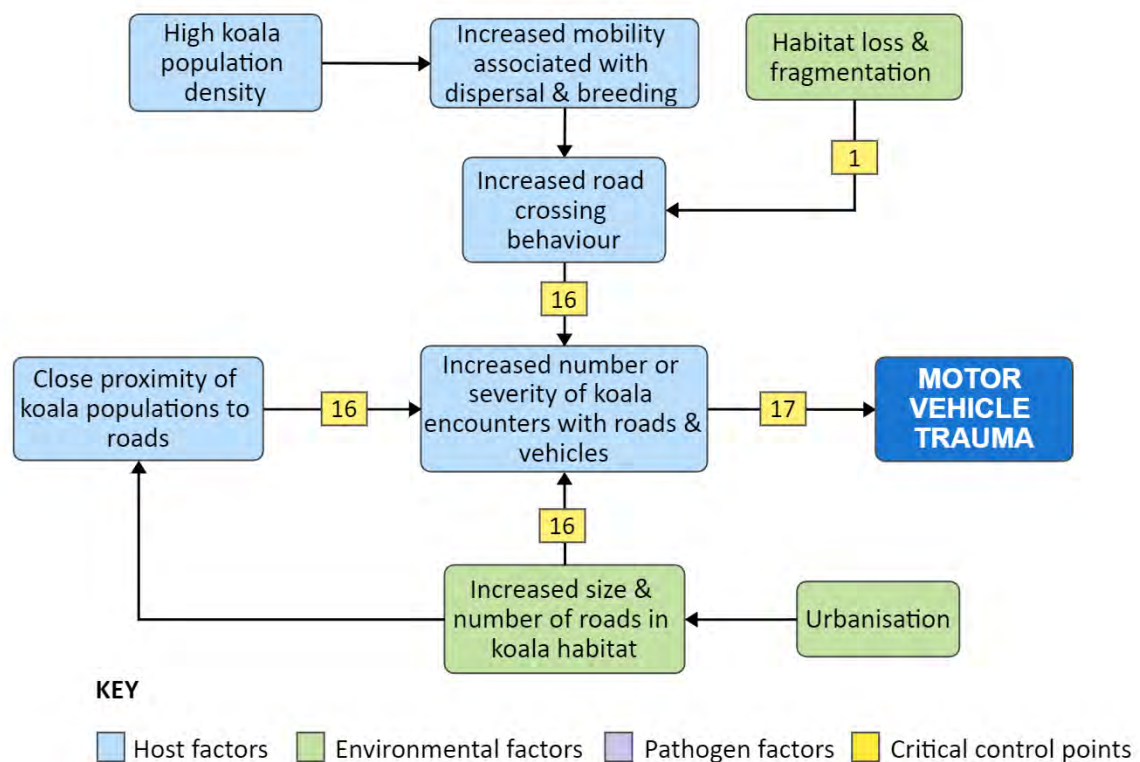


Figure 9 Hazard pathways and critical control points for motor vehicle trauma in koalas

Critical control points

CCP1 Habitat loss, fragmentation and reduction in quality

Habitat loss and reduction in habitat quality are major environmental stressors due to the koala's reliance on particular tree species for food, shelter and thermoregulation [23, 24].

Habitat fragmentation reduces connectivity between suitable koala habitat, forcing dispersal and causing koalas to spend more time on the ground, moving between trees and increasing the likelihood of encountering roads and vehicles [3, 9, 13, 21]. Poor quality habitat may force koalas to move greater distances in search of food and shelter, increasing the likelihood of crossing more roads during their travels [12].

Causes of habitat loss and fragmentation include urban expansion, land clearing for production, inappropriate fire management practices, introduction of pests, weeds and plant pathogens, and the habitat-altering effects of climate change [4, 25-29]. In areas of localised overcrowding of koalas in remaining habitat, over-browsing by koalas can be a contributing factor [30].

CCP16 Exposure to roads

Land clearing as a result of urbanisation increases the likelihood that koalas will encounter roads within their habitat [3, 9, 13, 21]. Larger roads and more roads in a koala's habitat increase the likelihood of MVA and associated trauma [31]. Koalas that cross roads more frequently, or that have unimpeded access to roads, will be at higher risk of motor vehicle trauma [5, 16].

CCP17 Severity of trauma from vehicle impact

Higher vehicle speeds may increase the severity of trauma or the likelihood of fatality to koalas involved in MVA [14]. Koalas usually cross roads at night, when there may be low visibility and reduced avoidance behaviour due to driver fatigue [31, 32].

Likelihood assessments

A koala's need to travel on the ground will frequently bring it into contact with roads. Koalas are generally slow moving and are most likely to cross roads at twilight or in darkness when driver visibility is poor [31, 32].

Motor vehicle accidents account for a variable percentage of koala hospital admissions in Australia (see Table 17 in *Appendix 5.5 Motor Vehicle Trauma - Literature Review*), although these figures probably underestimate the overall impact of motor vehicle trauma, as data on fatalities which are not admitted to care are not captured [33]. It is not possible at this time to estimate the proportion of the free-ranging koala population that is affected by motor vehicle trauma. The likelihood of MVA will vary significantly across koala populations and between individuals. Populations situated near busy roads will be at increased likelihood of MVA.

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of an individual free-ranging koala experiencing MVA during the course of its lifespan is considered **LOW** (however, see level of confidence in this assessment below).

Consequence assessments

Motor vehicle trauma is a non-infectious hazard and therefore consequence assessments were not made in relation to the health and welfare of other species.

Koala population resilience and viability

Because habitat fragmentation leads to small islands of koala populations, often hemmed in by roads, motor vehicle trauma may cause sufficient mortality to contribute to koala population decline. This may make small populations vulnerable to local extirpation [34]. In addition, MVA trauma more commonly affects healthy animals, eliminating otherwise viable animals from populations [6, 8]. Deaths from koala MVA may result in small to moderate population level declines in vulnerable areas.

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of motor vehicle trauma to koala population resilience and viability is considered **MODERATE**.

Koala individual health and welfare

Koala MVAs have a high fatality rate and a high rate of euthanasia [3, 7, 12, 20, 21, 35].

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of vehicle trauma to koala individual health and welfare is considered **MAJOR**.

Overall risk estimate

The overall risk of motor vehicle trauma to koala populations, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods* is **LOW**.

The overall risk of motor vehicle trauma to individual koalas, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods* is **MODERATE**.

This assessment exceeds the acceptable risk thresholds as outlined in *Appendix 2.3 Risk Assessment Methods*, therefore risk management for motor vehicle trauma is recommended.

Level of confidence in risk assessment

The confidence in the likelihood assessment is **MEDIUM** because estimates of free-ranging koala populations are uncertain, and the overall number of koalas affected by motor vehicle trauma cannot be extrapolated from hospital admissions data. The confidence in the consequence assessment for populations is **MEDIUM** for the same reason. The confidence in the consequence assessment for individual koalas is **HIGH** as it is based on detailed documentation of hospital admissions.

Overall, the confidence in the risk estimate for motor vehicle trauma is considered **MEDIUM**.

5.5.5 Risk mitigation options

The following risk mitigation options have been identified for motor vehicle trauma. Further information and justification can be found in the *Treatment and Prevention and control* sections of the relevant literature review chapter. Not all risk mitigation options will be currently available, feasible, universally applicable or cost-effective, but are listed here for completeness.

Conserve and improve the quality, quantity, diversity and complexity of koala habitat (CCP1)

- Re-vegetation, restoration and preservation of habitat, including in urban landscapes
- Strengthen existing habitat corridors and restore habitat connectivity which has been lost, through re-vegetation and restoration projects.
- Strengthen regulatory controls against habitat clearing and road development in koala habitat and dispersal corridors.

Incorporate koala considerations into road planning and design (CCP16 and CCP17)

- Seek timely, location-specific input from koala advisors on koala behaviour and movement patterns for road design projects.
- Focus on modifying existing roads to carry more traffic rather than building more roads.
- Avoid building new roads close to existing high-value koala habitat.

Traffic modification (CCP17)

- Apply speed limits or build speed modification structures such as roundabouts in identified “black spot” areas, to significantly slow approaching traffic to reduce likelihood and severity of koala road trauma.
- Use koala road warning signs. “Smart” signs, which illuminate in response to motorist speed, or in the presence of tagged koalas in the area, may be more effective than “static” signs.
- Alter patterns of road use to reduce overlap of high traffic with high frequency of koala road crossing.

Road-crossing structures and physical barriers (CCP16)

- Use structures such as climbing partitions, wildlife bridges, escape ramps and underpasses, to enable safer road crossing for koalas.
- Use exclusion fencing, alone or in conjunction with road-crossing structures, to prevent koalas from crossing roads.

Community education and advocacy (CCP17)

- Undertake education programs to inform and engage the community about road risks to koalas and promote change of vehicle driver behaviours to reduce risk.

- Advise on strategies to reduce risks of koala dispersal towards roads during timber harvesting.

5.5.6 Recommendations

Hazard-specific recommendations

The following recommendations are specific to this hazard:

- Support nationally-agreed monitoring for koalas following rehabilitation and release, to inform the long-term outcomes for koalas, and to evaluate the impact of vehicle trauma on population levels.
- Incorporate considerations of koala ecology and behaviour into road planning and design at the onset of projects.
- Implement studies to investigate the effectiveness of vehicle strike prevention strategies, including traffic modification, road-crossing structures, physical barriers and driver education and awareness.
- Develop nationally-agreed protocols for clinical evaluation, triage and treatment for koala trauma injuries.
- Identify “black spots” for koala MVA across their range.
- Implement nationally-agreed and long-term longitudinal tracking studies to improve understanding of koala movement patterns with respect to roads.

General recommendations

The following general recommendations apply to all 13 koala disease hazards identified for detailed risk assessment:

- Evaluate and prioritise the identified *Risk Management Options* for feasibility, completeness and effectiveness at a national KDRA Implementation Workshop.
- Identified knowledge gaps for this hazard should be prioritised and developed into a *Research Plan*.
- Prioritise protection and improvement of koala habitat as a key risk mitigation process for koala disease.
- Improve national population estimates and ensure ongoing population health monitoring for koalas.
- Develop robust, longitudinal and nationally-agreed data sets on causes of illness and death in free-living koalas.
- Maintain or increase genetic diversity and population size for koalas, so that koalas have the most robust genetic profiles for avoiding disease consequences.
- Minimise factors that might lead to stress in the koala’s environment (in the wild or in captivity).
- Minimise co-infections and other causes of debilitation to reduce cumulative risk of disease.

- Increase awareness and implementation of good biosecurity practices (in accordance with the *National Wildlife Biosecurity Guidelines* [36]) when interacting with koalas during field work, treatment and rehabilitation.
- Develop clinical and research collaborative working groups to establish nationally-agreed best practice protocols for koala disease diagnosis, treatment and biosecurity and implement these in collaboration with managers of koala populations across all jurisdictions.
- Develop collaborative relationships between managers of koala populations (state and Commonwealth) and koala clinical and research working groups, including co-participation in the Koala Recovery Team.
- Develop communication tools, using the iconic status of the koala, to raise awareness and encourage behaviour change around factors contributing to disease (e.g. climate change, vehicle driver behaviours, dog ownership and management, land clearing, fire management).

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5.6 Neoplasia in Koalas – Risk Assessment

The literature review which supports this risk assessment is in *Appendix 5.6*.

5.6.1 Hazard summary

- There is a high incidence of neoplasia in koalas, relative to most other species [1-7], with the most common neoplasms being lymphoid (lymphoma, leukaemia and lymphosarcoma), craniofacial osteochondroma and mesothelioma [8-11].
- There is epidemiological and mechanistic evidence for an association between KoRV and neoplasia [1, 6, 12-19], but the association is complex, and causation has yet to be confirmed. The most likely mechanism appears to be genetic interference from KoRV genomic integrations (some of which are heritable), although KoRV-induced immune modulation may play a role.
- Neoplasia is more prevalent in northern koala populations than southern, which may reflect differences in the genetic expression and epidemiology of KoRV between northern and southern koala populations [19].
- The role of causal factors other than KoRV in neoplasia has not been explored in koalas.
- Signs of neoplasia depend on the organs involved, but commonly include lethargy, anorexia and loss of body condition [8, 11, 20].
- The prognosis is poor for the common types of neoplasia seen in koalas [3, 5, 7, 8, 11, 20, 21]. Treatment for neoplasia has mostly been ineffective in koalas, except in a small number of cases where early surgical resection of localised tumours has been successful.
- The options for prevention and control of neoplasia in koalas are focused on reducing the likely impacts of KoRV-associated effects and maintaining genetic diversity, including in restricted, inbred populations. Future work may identify additional methods of prevention and control.
- For further detail on current knowledge of this hazard, refer to *Appendix 5.6 Neoplasia in Koalas - Literature Review*.

5.6.2 Justification for hazard selection

Neoplasia is commonly reported in koalas and appears to occur at a higher prevalence than in other marsupials/ eutherians [1-7]. Neoplasia is often fatal in koalas, and treatment, control and prevention strategies have not been confirmed.

5.6.3 Identified gaps in knowledge

- Further studies are required to clarify the role of oncogenes and their interaction with KoRV viral proteins in neoplasia.
- Additional surveillance data are required to develop a more accurate picture of neoplasia prevalence in koalas.
- Other than KoRV, there is little information on the possible predisposing or causative factors for neoplasia in koalas.

5.6.4 Risk assessment

Below is a schematic of the hazard pathways and critical control points (CCPs) identified for this hazard. Critical control points are *key* points in the hazard pathway where risk mitigation methods could be most effective (see *Appendix 2.3 Risk Assessment Methods* for further information). Nineteen CCPs (CCP1-CCP19) were identified across all hazards, but only those applicable to this hazard are described below. See Section 6 *Critical Control Points by Disease Hazard* for a summary of CCPs across all hazards selected for detailed assessment.

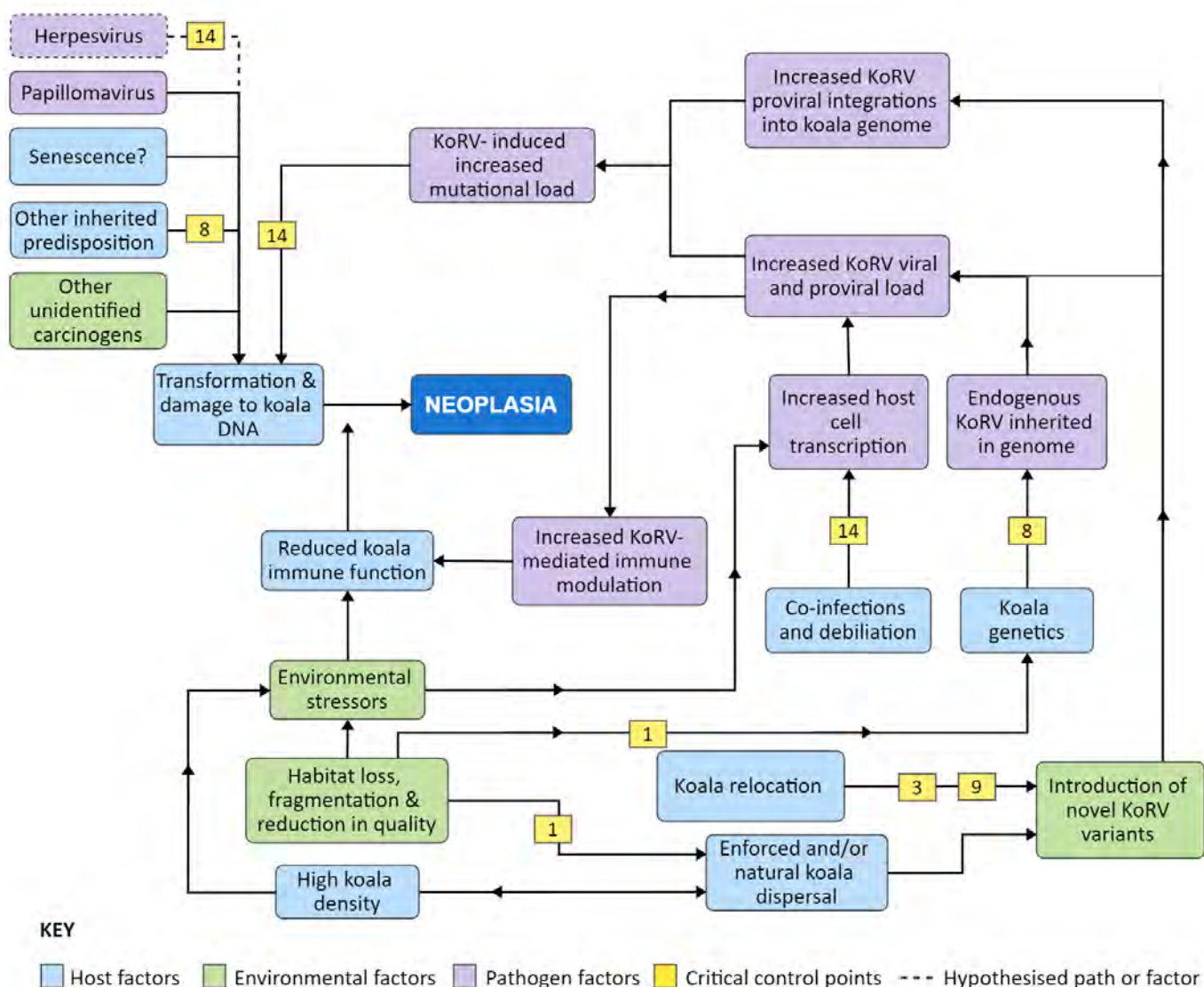


Figure 10 Hazard pathways and critical control points for neoplasia in koalas

Critical control points

CCP1 Habitat loss, fragmentation and reduction in quality

Habitat loss and reduction in habitat quality are major environmental stressors due to the koala's reliance on particular tree species for food, shelter and thermoregulation [22, 23]. Habitat fragmentation reduces connectivity between suitable koala habitat, potentially contributing to the loss of important genetic alleles in populations which can no longer interbreed [24-26] and promoting inherited predispositions to neoplasia. Causes include urban expansion, land clearing for production, inappropriate fire management practices, the introduction of pests, weeds and plant pathogens, over-browsing by koalas as a result of local overcrowding, and the habitat-altering effects of climate change [27-33].

CCP3 Koala relocation

Human-mediated movement of koalas may increase disease risk by introducing pathogens, or new pathogen varieties [34]. This may occur when koalas are brought into rehabilitation or released from rehabilitation (especially if not returned to their point of capture), when captive koalas are transferred between facilities [35], when koalas are translocated from one wild population to another [36], or when habitat corridors are created to reconnect long-separated populations without consideration of disease control.

In particular, relocated koalas that have KoRV integrations in different locations in their genomes to the resident population could increase integration site diversity in the resident population [17, 37, 38]. If those novel integrations are oncogenic, it could increase the risk of neoplasia in the population.

CCP8 Genetics

The individual koala may have a genetic predisposition to development of neoplasia. Inherited KoRV integrations in the koala genome are associated with increased neoplasia risk to koalas [12, 19], and may be responsible for a hereditary pattern for some neoplastic conditions [6]. Improving or at least maintaining genetic diversity in koala populations is likely to conserve adaptive potential [39] and encourage the retention of the most robust koala genetic profiles for avoiding inherited (including KoRV-related) susceptibility to neoplasia.

CCP9 Naïve host

An infectious pathogen may be introduced to a previously unexposed population or a previously uninfected individual.

The endogenous form of replication competent (*pol*-positive) KoRV-A has a prevalence of 100% in northern koalas [40] but appears to be absent from southern populations [41]. The introduction of replication-competent KoRV into the genome of southern populations (via breeding between *pol*-positive and *pol*-negative koalas) could increase the risk of KoRV-related neoplasia in southern populations.

Exogenous KoRV variants appear to vary in their prevalence throughout the koala's distribution [12, 15, 19, 37, 38, 42-46]. There may be the potential for transmission of exogenous variants into a population via close contact between koalas [17, 37, 47-49].

CCP14 Concurrent infections and debilitation

Concurrent infections and debilitation can compromise immune function and general health in ways that increase the risk or severity of disease.

In particular, koala host cells that are stimulated by inflammation to increase transcription or proliferate (as a result of pathogen infection or general debilitation of the host) likely increase KoRV viral and proviral load [19], resulting in more opportunities for the oncogenic effects of KoRV to be manifest in the host.

Likelihood assessment

Assessments for the likelihood of neoplasia were made only for koalas, and were not made in relation to the health and welfare of other species.

Neoplasia occurs spontaneously in all animal species. Both northern and southern koala populations report cases of neoplasia, although neoplasia is generally more prevalent in northern populations [6, 10, 19, 50-52]. Although rates of neoplasia in koalas are considered higher than expected in other mammal species [1-7], most koalas do not develop neoplasia in their lifetime.

The prevalence of neoplasia in wild koala populations has not been determined, in part because there are no nationally-accepted denominator data on the size of koala populations. Studies of admissions to wildlife hospitals in Qld found 1% to 3.2% of koalas presented with neoplasia [7, 53], although these figures are likely to under-represent the numbers in the wild as most koalas with neoplasia would not be expected to come into care. Neoplasia was reported to cause approximately 55% of disease in captive koalas in Australian facilities [6].

Endogenous, replication competent KoRV (which is probably a risk factor for koala neoplasia and is present in 100% of northern koalas but appears absent in southern populations) is inherited by subsequent generations [54]. Inherited neoplasia due to factors other than KoRV may also occur in koalas. Exogenous KoRV variants may also have a mutagenic effect on the koala genome, although they are not subsequently inherited [1]. Based on neoplasia prevalence figures in northern koalas, most koalas with replication-competent (whether exogenous or endogenous) KoRV in their genome do not develop KoRV-associated neoplasia [7, 53].

There may be other unconfirmed risk factors (host, environment or pathogen related) that increase the likelihood of neoplasia developing in individual koalas, but these have not been extensively studied. The likelihood of neoplasia may be higher in genetically restricted koala populations (both in captivity and in the wild) [6].

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of a koala experiencing neoplasia during its lifetime is considered **LOW**.

Consequence assessments

Assessments for the consequence of neoplasia were made only for koalas, and were not made in relation to the health and welfare of other species.

Koala population resilience and viability

Most, but not all, cases of neoplasia in koalas are expected to result in death [3, 5, 7, 8, 11, 20, 21]. Many neoplastic conditions may occur as animals grow older and may be a part of the natural process of senescence. The lack of information on prevalence of neoplasia in wild koala populations hampers the assessment of consequence of neoplasia on koala population resilience and viability. Inherited tendency to neoplasia is likely to be more readily expressed in small, fragmented populations where inbreeding is more likely to occur [24-26, 55].

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of neoplasia to koala population resilience and viability is considered **MINOR**.

Koala individual health and welfare

Most koalas with neoplasia would be unwell. Most, but not all, cases of neoplasia in koalas are expected to result to death. Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of neoplasia to koala individual health and welfare is considered **MAJOR**.

Overall risk estimate

The overall risk of neoplasia to koala population resilience and viability, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **LOW**.

The overall risk of neoplasia to individual koalas, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **MODERATE**.

This assessment exceeds the acceptable risk thresholds for both koala populations and individual koalas as outlined in *Appendix 2.3 Risk Assessment Methods*, therefore risk management for neoplasia is recommended.

Level of confidence in risk assessment

The level of confidence in the likelihood assessment is considered **MEDIUM**. Neoplasia is known to spontaneously occur in all animals and is well documented in both northern and southern koala populations. However, the prevalence of neoplasia in wild koala populations has not been determined and there are no accurate denominator data on the size of koala populations. The level of confidence in the consequence assessment is considered **MEDIUM**.

The disease and welfare impacts of neoplasia on the individual koala are well documented, however, there is limited data on the demographics of neoplasia in wild koala populations, which will influence the population effects of this disease.

Overall, the confidence in the risk estimate for neoplasia is considered **MEDIUM**.

5.6.5 Risk mitigation options

The following risk mitigation measures have been identified for neoplasia. Further information and justification can be found in the *Treatment and Prevention and control* sections of the *Neoplasia - Literature Review* chapter. Not all risk mitigation options will be currently available, feasible, universally applicable or cost-effective, but are listed here for completeness.

Conserve and improve the quality, quantity, diversity and complexity of koala habitat (CCP1 and CCP8)

- Re-vegetation, restoration and preservation of habitat, including in urban landscapes.
- Strengthen regulatory controls against habitat clearing and road development in koala habitat and dispersal corridors.

These measures encourage the maintenance of larger wild koala populations with more robust genetic profiles, which are more likely to avoid inherited predisposition to neoplasia.

Maintaining genetic diversity and population sizes in koalas (CCP8)

- Support genetic diversity in koala populations to encourage the retention of the most robust koala genetic profiles for avoiding inherited neoplastic predisposition.
- Minimise breeding or translocating from koala populations or family lines with a high prevalence of neoplasia.

Manage neoplasia risks associated with KoRV (CCP3, CCP8, CCP9 and CCP14)

- Given the likely role of KoRV as a risk factor for koala neoplasia, risk mitigation options applicable to KoRV may also mitigate neoplasia risk (see Section 5.4 *Koala Retrovirus – Risk Assessment*).

5.6.6 Recommendations

Hazard-specific recommendations

The following recommendations are specific to this hazard:

- Further investigate association between neoplasia and KoRV.
- Investigate associations between neoplasia and other potential oncogenic agents, particularly PhaHV.
- Investigate the role of host genetics in the development of neoplasia and develop breeding and translocation protocols to minimise inherited predisposition to neoplasia development.

- Recommendations applicable to KoRV are also relevant for neoplasia.

General recommendations

The following general recommendations apply to all 13 koala disease hazards identified for detailed risk assessment:

- Evaluate and prioritise the identified *Risk Management Options* for feasibility, completeness and effectiveness at a national KDRA Implementation Workshop.
- Identified knowledge gaps for this hazard should be prioritised and developed into a *Research Plan*.
- Prioritise protection and improvement of koala habitat as a key risk mitigation process for koala disease.
- Improve national population estimates and ensure ongoing population health monitoring for koalas.
- Develop robust, longitudinal and nationally-agreed data sets on causes of illness and death in free-living koalas.
- Maintain or increase genetic diversity and population size for koalas, so that koalas have the most robust genetic profiles for avoiding disease consequences.
- Minimise factors that might lead to stress in the koala's environment (in the wild or in captivity).
- Minimise co-infections and other causes of debilitation to reduce cumulative risk of disease.
- Increase awareness and implementation of good biosecurity practices (in accordance with the *National Wildlife Biosecurity Guidelines* [34]) when interacting with koalas during field work, treatment and rehabilitation.
- Develop clinical and research collaborative working groups to establish nationally-agreed best practice protocols for koala disease diagnosis, treatment and biosecurity and implement these in collaboration with managers of koala populations across all jurisdictions.
- Develop collaborative relationships between managers of koala populations (state and Commonwealth) and koala clinical and research working groups, including co-participation in the Koala Recovery Team.
- Develop communication tools, using the iconic status of the koala, to raise awareness and encourage behaviour change around factors contributing to disease (e.g. climate change, vehicle driver behaviours, dog ownership and management, land clearing, fire management).

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5.7 Novel *Actinomyces* sp. in Koalas – Risk Assessment

The literature review which supports this risk assessment is in *Appendix 5.7*.

5.7.1 Hazard summary

- Bacterial pneumonia caused by a novel species of *Actinomyces* has been reported in at least 20 wild koalas from SA since 2016 [1-3]. Actinomycotic pneumonia has been reported in one koala from Qld [4].
- *Actinomyces* are anaerobic or facultative aerobic bacteria, believed to form part of the healthy gastrointestinal microbiome of many animal species including humans [5-11].
- *Actinomyces* spp. can cause slowly developing, chronic soft tissue, skeletal and pulmonary infections in humans and animals when the normal immune defence mechanisms of the host are disrupted, and the bacterium penetrates the soft tissues of the host [5, 6, 8, 10, 12-19].
- The initiating factors for actinomycotic pneumonia in SA koalas are not known, but aspiration of plant material (perhaps alimentary tract contents) may be involved, and dental disease has been proposed as a contributing factor [3].
- Treatment of affected koalas has not been attempted and all koalas have died or been euthanased [3].
- Treatment of actinomycosis in other species requires prolonged antibiotic treatment and carries a guarded prognosis [20, 21].
- Methods of prevention and control for pulmonary actinomycosis in koalas have not been identified [3].
- For further detail on current knowledge of this hazard, refer to *Appendix 5.7 Novel Actinomyces sp. in Koalas – Literature Review*.

5.7.2 Justification for hazard selection

Novel *Actinomyces* sp. is a recently identified pathogen of koalas and has caused severe pneumonia, generally progressing to death, in a small number of SA animals [3].

5.7.3 Identified gaps in knowledge

- Confirmation of novel *Actinomyces* sp. as a part of the healthy gastrointestinal microbiome of koalas, and an understanding of whether this organism is present in all koala populations.
- Understanding of the initiating factors which allow development of actinomycotic pneumonia in koalas is needed. The possible association with dental disease needs investigation.
- Identification of possible prevention, control and treatment methods for actinomycotic pneumonia in koalas is needed.
- Investigation is required to determine possible host genetic predispositions in affected koala populations to development of actinomycotic pneumonia.

- Identification of associations or causality between actinomycotic pneumonia and other recognised diseases of koalas is required.

5.7.4 Risk assessment

Below is a schematic of the hazard pathways and critical control points identified for this hazard. Critical control points (CCPs) are defined as *key points* in the hazard pathway where risk mitigation methods could be most effective (see *Appendix 2.3 Risk Assessment Methods* for further information). Nineteen CCPs (CCP1-CCP19) were identified across all hazards, but only those applicable to this hazard are described below. See Section 6 *Critical Control Points by Disease Hazard* for a summary of CCPs across all hazards selected for detailed assessment.

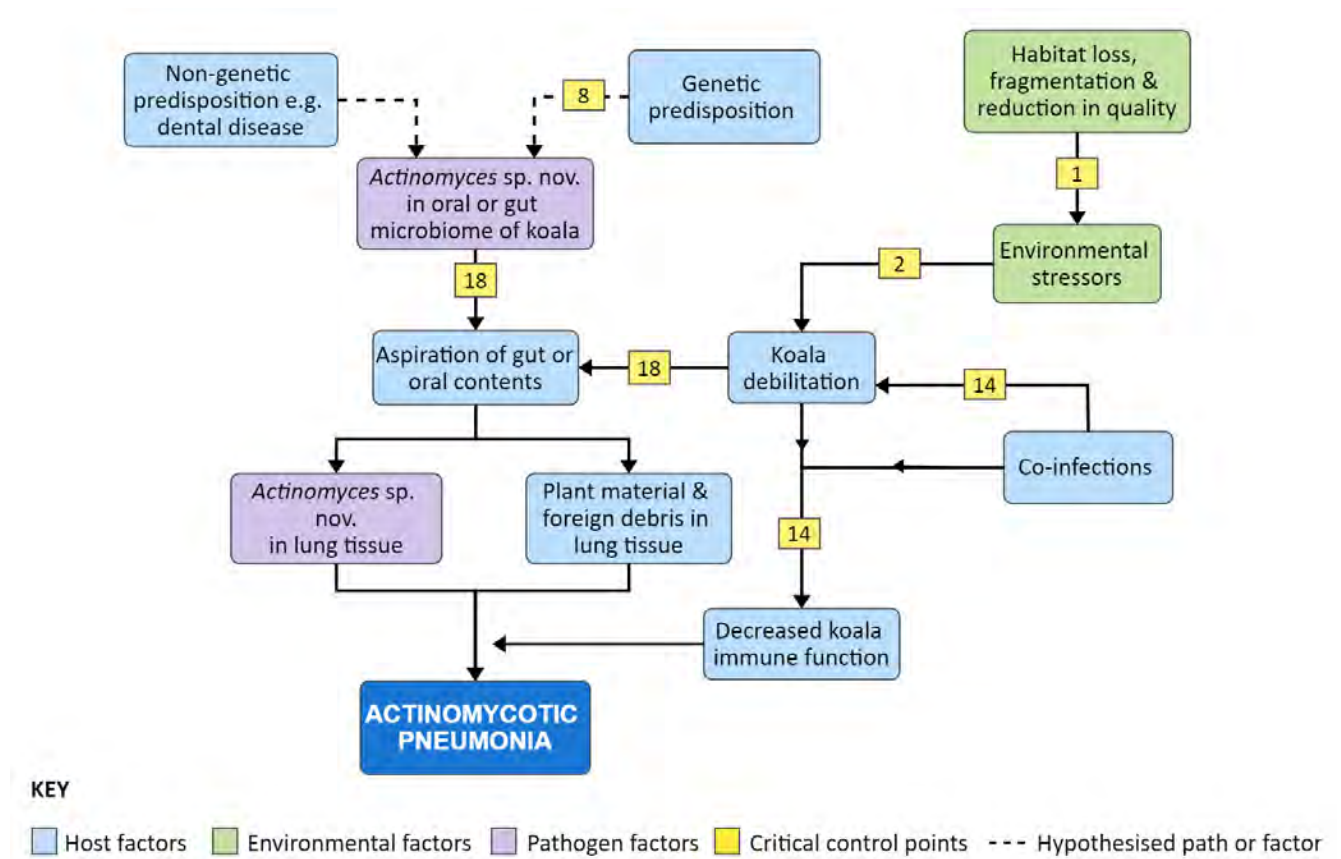


Figure 11 Hazard pathways and critical control points for actinomycotic pneumonia in koalas

Critical control points

CCP1 Habitat loss, fragmentation and reduction in quality

Habitat loss and reduction in habitat quality are major environmental stressors due to the koala's reliance on particular tree species for food, shelter and thermoregulation [22, 23]. Habitat fragmentation reduces connectivity between suitable koala habitats, forcing dispersal and potentially contributing to the loss of important genetic alleles in populations that can no longer interbreed [24-26]. Causes include urban expansion, land clearing for

production, inappropriate fire management practices, the introduction of pests, weeds and plant pathogens, over-browsing by koalas as a result of local overcrowding, and the habitat-altering effects of climate change [27-33].

CCP2 Environmental stressors

Stressors in the koala's environment (in the wild or in captivity) contribute to general debilitation, reduced immune function and increased susceptibility to disease or severity of disease expression [23, 34-36]. Major environmental stressors for koalas include habitat loss, fragmentation and reduction in quality; nutritional stress; climate change; extremes of weather and disturbance related to human activities [22, 37].

In particular, environmental stressors may contribute to general debilitation, reduced immune function and probably will increase susceptibility to *Actinomyces* infection and disease. Particular environmental stressors may increase the likelihood of koalas developing disease with *Actinomyces* if alimentary tract contents are inhaled.

CCP8 Genetics

The individual koala may have a genetic predisposition to development of disease or to increased severity of disease. Improving or at least maintaining genetic diversity in koala populations is likely to conserve adaptive potential [38] and encourage the retention of the most robust koala genetic profiles to avoid disease consequences.

Possible genetic predisposition to actinomycotic pneumonia include a lack of key immunogenetic alleles; a genetic predisposition to carrying novel *Actinomyces* sp. in the microbiome; and genetically-based anatomical differences predisposing koalas to aspiration [3].

CCP14 Concurrent infections and debilitation

Concurrent infections and debilitation can compromise immune function and general health in ways that increase the risk or severity of disease. In particular, co-infection with other pathogens might be a contributing factor to development of pulmonary actinomycosis.

CCP18 Pathogen access to target tissue

Dental disease may contribute to the initiation of pulmonary actinomycosis, as has been seen in humans [3]. Events that cause a koala to inhale gut or mouth contents or soil are believed necessary for actinomycotic pneumonia to develop. Inhalation of alimentary tract content appears likely to deposit a large load of novel *Actinomyces* sp. bacteria into the lower respiratory tract of the koala, along with a large amount of foreign organic material (plant matter). The increased load of pathogen in an abnormal site, along with foreign material, will be a strong trigger for development of bacterial pneumonia.

Likelihood assessments

As novel *Actinomyces* sp. is not considered a contagious disease, the risk of infection in other species, including humans, has not been considered.

Entry assessment

Novel *Actinomyces* sp. is assumed to be part of the normal alimentary tract flora of koalas. However, no studies have been done to confirm this, nor have studies investigated the possibility that novel *Actinomyces* sp. may be present in SA koalas but not in koalas from other populations. An unspiciated *Actinomyces* sp. was found in a koala with pneumonia in Qld. Therefore, and using the precautionary principle, this entry assessment considers that novel *Actinomyces* sp. is present, as a part of normal flora, in all koala populations.

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of actinomycotic pneumonia entering, or being present, in a koala population is considered **HIGH**.

Exposure assessment

In the circumstance of novel *Actinomyces* sp., we assume the exposure pathway is that of an individual koala being exposed to *Actinomyces* in its lung tissue. The circumstances by which this exposure comes about are not known, but are believed to be associated with inhalation of alimentary tract contents. The reported cases of actinomycotic pneumonia in koalas are low, and it has only been detected in one state (SA) [3]. The likelihood of an individual koala being exposed to novel *Actinomyces* sp. in the lung tissue appears to vary according to geographic location, and the reasons for this are not understood. It is considered very unlikely that a healthy koala would inhale food material, and the reason this occurs, in the development of actinomycotic pneumonia, is not known.

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of exposure to novel *Actinomyces* sp. in the lung tissue for an individual koala is considered **LOW**.

Using the principles of combining entry and exposure likelihood as outlined in *Appendix 2.3 Risk Assessment Methods*, the combined likelihood of entry and exposure for actinomycotic pneumonia is considered **LOW**.

Consequence assessments

Most, if not all, koalas exposed to novel *Actinomyces* sp. in the lung tissue are likely to develop actinomycotic pneumonia, but the severity of disease in all cases is not known. By the precautionary principle, we assume that all koalas that inhale novel *Actinomyces* sp. into lung tissue will develop pneumonia.

Koala population resilience and viability

There is no information available on the likely impact of novel *Actinomyces* sp. and actinomycotic pneumonia on koala population resilience and viability. Given the low numbers of case of actinomycotic pneumonia reported, in just one small area of the koala's geographic range, the population level impacts of this disease are likely to be unnoticeable.

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of actinomycotic pneumonia to koala population resilience and viability is considered **NEGLIGIBLE**.

Koala individual health and welfare

If novel *Actinomyces* sp. is inhaled in sufficient quantity and possibly with accompanying foreign material, severe bacterial pneumonia ensues. Reported cases of actinomycotic pneumonia cause significant ill health and death in koalas [3].

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of actinomycotic pneumonia to koala individual health and welfare is considered **MAJOR**.

Overall risk estimate

Since the consequence of novel *Actinomyces* sp. was evaluated as negligible for koala population resilience and viability, the estimate of risk for this hazard for koala populations was also considered **NEGLIGIBLE** (see *Appendix 2.3 Risk Assessment Methods*). Note, however that the level of confidence in this risk estimate (see below) is **LOW**. The overall risk of actinomycotic pneumonia to individual koalas, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **MODERATE**.

This assessment exceeds the acceptable risk thresholds for individual koalas, as outlined in *Appendix 2.3 Risk Assessment Methods*, therefore risk management for actinomycotic pneumonia is recommended for individual koalas.

Level of confidence in risk assessment

The level of confidence in the likelihood assessment is **LOW** as there are many gaps in knowledge about novel *Actinomyces* sp., whether it is a part of the normal flora of the koala, and whether this differs geographically (or due to other variables). It is also not known how novel *Actinomyces* sp. gains entry to the lung tissue, and if by inhalation of gastrointestinal contents, what the initiating factors are for this process, or if SA koalas have a genetic predisposition to carrying novel *Actinomyces* sp., or developing actinomycotic pneumonia.

The level of confidence in the consequence assessment for populations is **LOW**, as there are few data available. The impacts on individual koalas are better understood.

Overall, the confidence in the risk estimate for actinomycotic pneumonia is **LOW**.

5.7.5 Risk mitigation options

Few specific risk mitigation measures have been identified for novel *Actinomyces* sp. Further information and justification can be found in the *Treatment and Prevention and control* sections of the relevant literature review chapter. Not all risk mitigation options will be currently available, feasible, universally applicable or cost-effective, but are listed here for completeness.

Currently, no prevention or control methods have been identified that are specific for actinomycotic pneumonia in koalas. A greater understanding of the pathogen epidemiology and distribution, likely initiating factors in disease development, and treatment options will assist in more effective prevention and control strategies for pulmonary actinomycosis in koalas.

General risk mitigation options will also be applicable for novel *Actinomyces* sp. These include:

Conserve and improve the quality, quantity, diversity and complexity of koala habitat (CCP1 and CCP8)

- Re-vegetation, restoration and preservation of habitat, including in urban landscapes.
- Strengthen regulatory controls against habitat clearing and road development in koala habitat and dispersal corridors.

These measures will encourage the maintenance of larger wild koala populations with more robust genetic profiles which are more likely to withstand the pathogenic consequences of novel *Actinomyces* infection.

Manage concurrent infections, debilitation and environmental stressors (CCP2, CCP14 and CCP18)

- Minimise concurrent infections, debilitation and other environmental stressors (e.g. predators, disturbance related to human activities) in individual koalas and populations, as sick and debilitated koalas are likely to be more at risk of aspiration.
- Promote good dental health, as dental disease may be a contributing factor to actinomycotic pneumonia.
- Minimise risk of aspiration in koalas.

Maintain genetic diversity and population sizes in koalas (CCP8)

- Support genetic diversity in koala populations to encourage the retention of the most robust koala genetic profiles to enable effective immune response to novel *Actinomyces* sp. and avoid genetically-based anatomical differences which may predispose koalas to aspiration.

5.7.6 Recommendations

Hazard-specific recommendations

The following recommendations are specific to this hazard:

- Further investigate the biology and epidemiology of novel *Actinomyces* sp. in koalas, including the geographic distribution of the organism, whether it is a normal part of the koala microbiome, or present in the environment, and initiating genetic and environmental factors.
- Investigate early detection and treatment options for actinomycotic pneumonia.

General recommendations

The following general recommendations apply to all 13 koala disease hazards identified for detailed risk assessment:

- Evaluate and prioritise the identified *Risk Management Options* for feasibility, completeness and effectiveness at a national KDRA Implementation Workshop.
- Identified knowledge gaps for this hazard should be prioritised and developed into a *Research Plan*.
- Prioritise protection and improvement of koala habitat as a key risk mitigation process for koala disease.
- Improve national population estimates and ensure ongoing population health monitoring for koalas.
- Develop robust, longitudinal and nationally-agreed data sets on causes of illness and death in free-living koalas.
- Maintain or increase genetic diversity and population size for koalas, so that koalas have the most robust genetic profiles for avoiding disease consequences.
- Minimise factors that might lead to stress in the koala's environment (in the wild or in captivity).
- Minimise co-infections and other causes of debilitation to reduce cumulative risk of disease.
- Increase awareness and implementation of good biosecurity practices (in accordance with the *National Wildlife Biosecurity Guidelines* [39]) when interacting with koalas during field work, treatment and rehabilitation.
- Develop clinical and research collaborative working groups to establish nationally-agreed best practice protocols for koala disease diagnosis, treatment and biosecurity and implement these in collaboration with managers of koala populations across all jurisdictions.
- Develop collaborative relationships between managers of koala populations (state and Commonwealth) and koala clinical and research working groups, including co-participation in the Koala Recovery Team.
- Develop communication tools, using the iconic status of the koala, to raise awareness and encourage behaviour change around factors contributing to disease (e.g. climate change, vehicle driver behaviours, dog ownership and management, land clearing, fire management).

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5.8 Oxalate Nephrosis in Koalas – Risk Assessment

The literature review which supports this risk assessment is in *Appendix 5.8*.

5.8.1 Hazard summary

- Oxalate nephrosis (ON) is a significant cause of morbidity and mortality in some wild koala populations, notably the Mount Lofty Ranges (MLR) population in SA [1, 2].
- A genetic predisposition to the development of ON is likely in the populations where it is most common [3, 4], with disease expression potentially affected by a range of factors, including patient hydration status and gastrointestinal microflora [5-7], and oxalate and moisture content of browse [8].
- Additional regions of increased prevalence of ON in free-ranging koalas have recently been identified in Vic [4]. Oxalate nephrosis in Qld and NSW most commonly develops in wild koalas during rehabilitation and is rarely diagnosed in free-ranging animals [9, 10].
- Oxalate nephrosis also occurs in captive koalas [4, 10-14].
- Animals with ON have compromised renal function which can progress to renal failure [15].
- Treatment is generally unsuccessful in advanced cases of ON, however episodes of illness in captive animals can be managed long-term with fluid therapy, optimisation of nutrition and minimization of stress [7].
- Reduction of risk of ON in captive and rehabilitation koalas involves supportive strategies to promote hydration and food intake and to minimise stress [7, 16].
- For further detail on current knowledge of this hazard, refer to *Appendix 5.8 Oxalate Nephrosis in Koalas - Literature Review*.

5.8.2 Justification for hazard selection

Oxalate nephrosis is a leading cause of disease in the MLR population of SA [1], and may be increasing in prevalence in other parts of the koala's distribution [4]. The disease is often fatal, and its development is associated with a number of non-disease risk factors which threaten koalas, including loss of genetic diversity, loss of habitat quality and quantity, and environmental stress.

5.8.3 Identified gaps in knowledge

- Further investigation into the genetic basis for ON is required [3, 4].
- Investigation of ON in koala populations beyond MLR is required, both within and outside SA, to better understand the risk factors associated with disease development in the wild and in rehabilitation.
- Controlled pharmacokinetic studies and clinical trials are required to determine safe and effective therapeutic agents for treating ON cases.

5.8.4 Risk assessment

Below is a schematic of the hazard pathways and critical control points (CCPs) identified for this hazard. Critical control points are *key* points in the hazard pathway where risk mitigation methods could be most effective (see *Appendix 2.3 Risk Assessment Methods* for further information). Nineteen CCPs (CCP1-CCP19) were identified across all hazards, but only those applicable to this hazard are described below. See Section 6 *Critical Control Points by Disease Hazard* for a summary of CCPs across all hazards selected for detailed assessment.

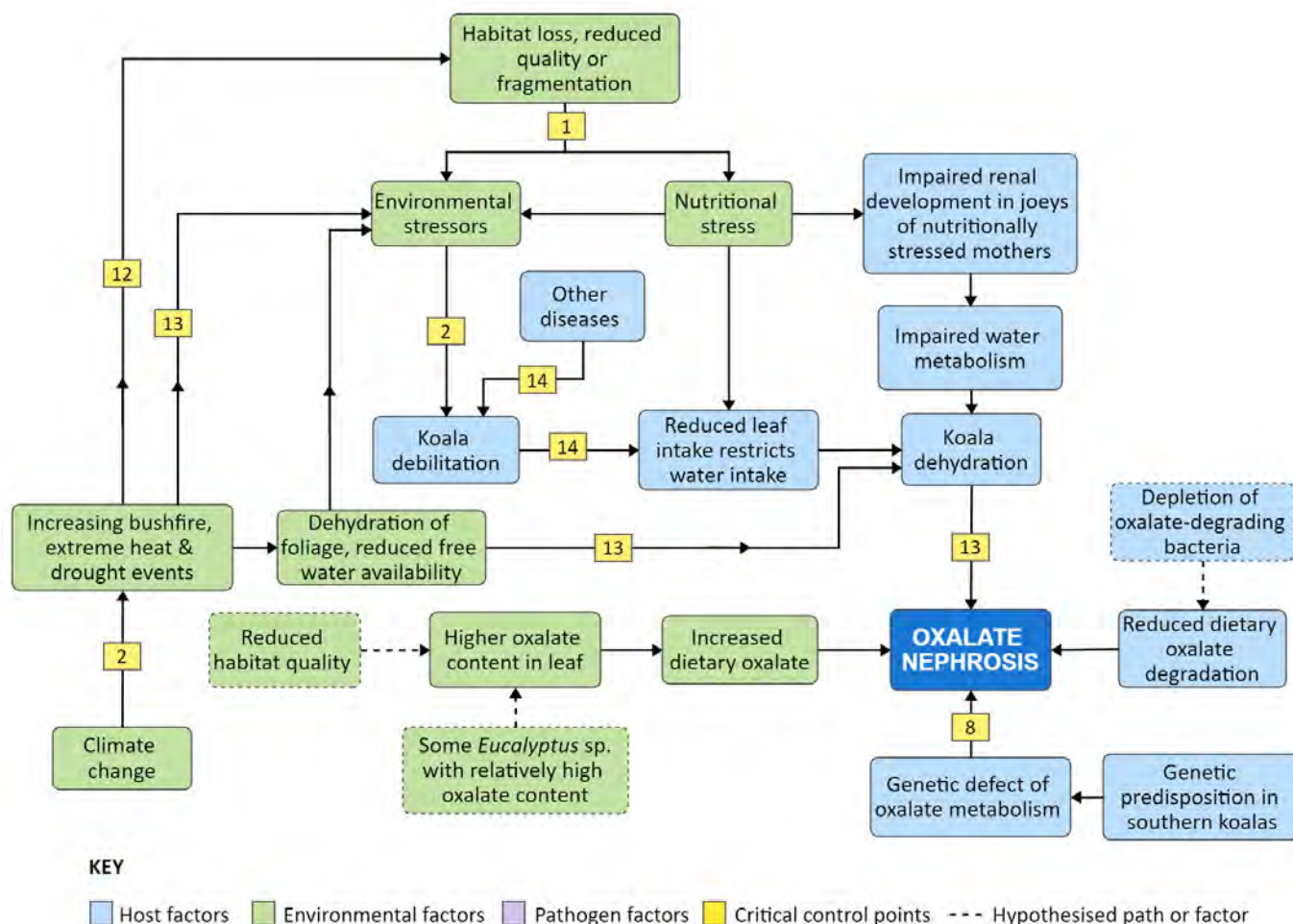


Figure 12 Hazard pathways and critical control points for oxalate nephrosis in koalas

Critical control points

CCP1 Habitat loss, fragmentation and reduction in quality

Habitat loss and reduction in habitat quality are major environmental stressors due to the koala's reliance on particular tree species for food, shelter and thermoregulation [17, 18]. Habitat fragmentation reduces connectivity between suitable koala habitats, forcing dispersal and potentially contributing to the loss of important genetic alleles in populations

that can no longer interbreed [19-21]. Causes include urban expansion, land clearing for production, inappropriate fire management practices, the introduction of pests, weeds and plant pathogens, over-browsing by koalas as a result of local overcrowding, and the habitat-altering effects of climate change [22-28].

Habitat loss, reduction and fragmentation result in loss of refugia for koalas, increasing the risk of dehydration which can promote the development of ON, particularly in hot weather [5].

CCP2 Environmental stressors

Stressors in the koala's environment (in the wild or in captivity) contribute to general debilitation of the host, reduced immune function, and increased susceptibility to disease or severity of disease expression [18, 29-31]. Major environmental stressors for koalas include habitat loss, fragmentation and reduction in quality; nutritional stress; climate change; extremes of weather and disturbance related to human activities [17, 32].

Koalas obtain about 75% of their water intake from the foliage they feed on [33]. A reduction in food (and therefore water) intake due to ill health, injury or other stress, may increase the risk of dehydration, which in itself is a source of physiological stress [32], as well as increasing the risks of ON [5].

CCP8 Genetics

The individual koala may have a genetic predisposition to development of disease or to increased severity of disease. Improving or at least maintaining genetic diversity in koala populations is likely to conserve adaptive potential [34] and encourage the retention of the most robust koala genetic profiles to avoid disease consequences.

In particular, koalas predisposed to developing ON are postulated to have genetic predisposition to abnormal oxalate metabolism [4, 35].

CCP12 Fire management practices

Inadequate planning, inappropriate methodology or flawed execution of fire management practices may contribute to habitat loss and reduction of habitat quality, reducing refugia options for koalas during heat events [36, 37] which may predispose koalas to dehydration and ON.

CCP13 Reduced water intake

In hot weather, koalas may experience reduced water intake due to lower leaf moisture content and lower free water availability (artificial or natural sources) for drinking [22, 38].

In particular, reduced water intake exacerbates renal stress and promotes the development of ON in both free-ranging and captive koalas [5, 16].

CCP14 Concurrent infections and debilitation

Concurrent infections and debilitation can compromise immune function and general health in ways that increase the risk or severity of disease. Any disease process that leads to debilitation and inappetence will contribute to koala dehydration and potentially increase susceptibility to ON [5, 16].

Likelihood assessments

Due to the higher reported prevalence of oxalate nephrosis [1, 2], the MLR koala population is considered separately to the rest of the koala population in assessment of likelihood for this hazard.

The prevalence of oxalate nephrosis appears variable across the koala's distribution. It is commonly reported in the MLR koala population [1, 39, 40] and recent studies suggest a high prevalence in some wild Vic populations [4]. In northern populations, it arises most commonly in wild koalas after they enter rehabilitation [10, 12, 15, 41-45].

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of an individual koala being affected by oxalate nephrosis is considered **LOW**, with the exception of the MLR population, where the likelihood is considered **MODERATE**.

Consequence assessments

Oxalate nephrosis is a non-infectious hazard and therefore consequence assessments were not made in relation to the health and welfare of other species or humans.

Koala population resilience and viability

Oxalate nephrosis is a leading cause of mortality in koala admissions to rehabilitation from the MLR koala population. The levels of mortality due to oxalate nephrosis could be sufficient to drive decline in the MLR population.

Koalas predisposed to developing oxalate nephrosis are hypothesised to have a genetic predisposition to abnormal oxalate metabolism [4, 35]. This is likely a factor in the high prevalence of oxalate nephrosis in MLR koalas. Other regions of increased prevalence, that may also have a genetic basis, are emerging in Vic [4]. The impact of oxalate nephrosis to population resilience and viability is likely to be significantly lower in other koala populations.

Adopting the precautionary principle, the prevalence of oxalate nephrosis in the MLR population has been used as the basis of the consequence assessment.

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of oxalate nephrosis to koala population resilience and viability is considered **MINOR**.

Koala individual health and welfare

Oxalate nephrosis causes serious, and often fatal, clinical disease in affected koalas. Prognosis for recovery, even with treatment, is guarded.

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of oxalate nephrosis to koala individual health and welfare is considered **MAJOR**.

Overall risk estimate

The overall risk of oxalate nephrosis to the koala populations, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **LOW**.

The overall risk of oxalate nephrosis to individual koalas, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **LOW** for individual koalas other than the MLR population. The risk of oxalate nephrosis to individual koalas in the MLR population is **HIGH**.

These risk estimates exceed the acceptable risk thresholds as outlined in *Appendix 2.3 Risk Assessment Methods* for the koala populations and for individual koalas in the MLR population.

Risk management for oxalate nephrosis is recommended for individual koalas. Given the low level of confidence in population consequence assessments (see below), risk management at the population level is recommended for the MLR population and for other koala populations where a high prevalence of oxalate nephrosis is detected in the future.

Level of confidence in risk assessment

Oxalate nephrosis is a well-recognised and readily diagnosed disease of koalas but its prevalence in free-ranging koalas is not well understood. Therefore, the level of confidence in the likelihood assessments is considered **MEDIUM**.

The impact of oxalate nephrosis to population numbers is not well studied. The level of confidence in the consequence assessments for koala populations is **LOW**.

The clinical impacts of oxalate nephrosis in koalas are well documented and understood. The level of confidence in the consequence assessments for individual koalas is **HIGH**.

Overall, the confidence in the risk estimate for oxalate nephrosis is considered **MEDIUM**.

5.8.5 Risk mitigation options

The following risk mitigation measures have been identified for oxalate nephrosis in koalas. Further information and justification can be found in the *Treatment and Prevention and control* sections of the relevant literature review chapter. Not all risk mitigation options will be currently available, feasible, universally applicable or cost-effective, but are listed here for completeness.

Conserve and improve the quality, quantity, diversity and complexity of koala habitat (CCP1, CCP2 and CCP13)

- Protect or develop climate refugia, such as valleys, understory, midstory and fast-growing shade-producing non-browse vegetation that provide good thermal protection for koalas and promote high moisture and nutrient content in foliage.
- Re-vegetation, restoration and preservation of habitat, including in urban landscapes.
- Strengthen regulatory controls against habitat clearing and road development in koala habitat and dispersal corridors.

These measures will reduce the impact of habitat-related environmental stress on the development of oxalate nephrosis and encourage the maintenance of larger wild koala populations with more robust genetic profiles that are more capable of adapting to a changing climate and coping with the impacts of high ambient temperatures and drought.

Support the retention and availability of water in koala environments (CCP1, CCP2, CCP13 and CCP19)

- Plant water-retaining browse trees and tree species that are drought- and heat-tolerant.
- Revegetate with browse and non-browse species that are best suited to changing climate in the bioregion.
- Support soil quality and diversity, and better manage storm water run-off, to retain moisture in the soil and vegetation.
- Support the capacity of koalas to move to available water in their environment. This includes improving water catchment and storage via artificial or natural wetlands and slowing water outflow to increase hydration of trees, maintain moisture content of foliage and possibly reduce oxalate concentration in leaf consumed by koalas.
- For captive and rehabilitation koalas, supply free water, misting browse, and optimal management of cut browse to maintain leaf moisture.

Act to reduce the impacts of anthropogenic climate change and a warming, drying climate (CCP1 and CCP2)

- Act to bring about “climate-friendly” policies at a local, state and federal level.
- Use koalas and their plight as a focus-point for community education about climate change and a “call to action” for associated behaviour change.

Manage concurrent infections, debilitation and environmental stressors (CCP2 and CCP14)

- Minimise concurrent infections, debilitation and other environmental stressors (e.g. predators, disturbance related to human activities) in koalas in captivity and in rehabilitation, which could predispose them to inappetence and dehydration and precipitate oxalate nephrosis.

Maintain genetic diversity in koala populations (CCP8)

- Support genetic diversity in koala populations to encourage the retention of the most robust koala genetic profiles for adapting to a changing climate and coping with the impacts of high ambient temperatures and drought. If a genetic predisposition to ON is confirmed, support of genetic diversity may reduce the perpetuation of undesirable genetic traits in koala populations.

Fire management practices (CCP12)

- Adopt fire management practices that minimise impacts on koala habitat quantity, quality and connectivity, to retain refugia from hot weather and minimise hydration stress on koalas and trees (see *Risk mitigation options* in Section 5.12 *Thermal Burn Trauma* for more details).

5.8.6 Recommendations

Hazard-specific recommendations

The following recommendations are specific to this hazard:

- Protect and improve koala habitat to conserve refugia, and improve the quality and hydration status of koala food.
- Prioritise water retention and availability in koala environments in approach to land care, including water catchment and storage, and tree species selection.
- Monitor the prevalence of ON across the koala's range to allow early recognition of increased prevalence outside the MLR population.
- Further investigate the causes of ON in koalas including genetic risk factors and relationship to other infections and co-morbidities.
- Develop nationally-agreed protocols for prevention, assessment, treatment and care of koalas with ON in captivity and rehabilitation, including criteria for possible euthanasia.

General recommendations

The following general recommendations apply to all 13 koala disease hazards identified for detailed risk assessment:

- Evaluate and prioritise the identified *Risk Management Options* for feasibility, completeness and effectiveness at a national KDRA Implementation Workshop.
- Identified knowledge gaps for this hazard should be prioritised and developed into a *Research Plan*.
- Prioritise protection and improvement of koala habitat as a key risk mitigation process for koala disease.
- Improve national population estimates and ensure ongoing population health monitoring for koalas.
- Develop robust, longitudinal and nationally-agreed data sets on causes of illness and death in free-living koalas.

- Maintain or increase genetic diversity and population size for koalas, so that koalas have the most robust genetic profiles for avoiding disease consequences.
- Minimise factors that might lead to stress in the koala's environment (in the wild or in captivity).
- Minimise co-infections and other causes of debilitation to reduce cumulative risk of disease.
- Increase awareness and implementation of good biosecurity practices (in accordance with the *National Wildlife Biosecurity Guidelines* [46]) when interacting with koalas during field work, treatment and rehabilitation.
- Develop clinical and research collaborative working groups to establish nationally-agreed best practice protocols for koala disease diagnosis, treatment and biosecurity and implement these in collaboration with managers of koala populations across all jurisdictions.
- Develop collaborative relationships between managers of koala populations (state and Commonwealth) and koala clinical and research working groups, including co-participation in the Koala Recovery Team.
- Develop communication tools, using the iconic status of the koala, to raise awareness and encourage behaviour change around factors contributing to disease (e.g. climate change, vehicle driver behaviours, dog ownership and management, land clearing, fire management).

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5.9 Phascolarctid Herpesviruses – Risk Assessment

The literature review which supports this risk assessment is in *Appendix 5.9*.

5.9.1 Hazard summary

- Phascolarctid herpesviruses (PhaHV)-1 and -2 are gammaherpesviruses that have likely co-evolved with koalas [1].
- An alphaherpesvirus has been detected in one captive female koala with pneumonia, hepatitis and adrenalitis and concurrent lung infection with *Cryptococcus gattii* [2], tentatively named PhaHV-3.
- PhaHV have only been detected in koalas and are not considered a risk for other wildlife or humans [1].
- Once a koala is infected with herpesvirus, infection persists for life [1].
- Infections may be latent or lytic, and re-activation of latent herpesvirus in other species has been associated with immunosuppression and stress [3, 4].
- Transmission routes for PhaHV are unconfirmed, although prevalence studies suggest that PhaHV-2 may be acquired in infancy, while PhaHV-1 may be acquired through activities related to maturation, such as breeding behaviour [5].
- While PhaHV-1 and -2 have been detected in all koala populations tested to date [5, 6], large areas of koala distribution are as yet untested, particularly in NSW and Qld, although a study still in progress has so far identified PhaHV-1 in both Qld and NSW koala populations [7].
- Both PhaHV-1 and -2 detection are strongly associated with concurrent chlamydial infection [5, 8], and with signs of urogenital disease in male and female koalas [5, 9, 10].
- PhaHV-1 detection is associated with increased detection of KoRV, particularly in female koalas [5].
- Direct prevention and control of PhaHV infections are not considered possible in free-living koala populations because it is unlikely that exposure can be prevented [1, 5].
- Minimising stress and managing concurrent infections are important aspects of controlling herpesvirus-associated disease in koalas [1].
- For further detail on current knowledge of this hazard, refer to *Appendix 5.9 Phascolarctid Herpesviruses in Koalas - Literature Review*.

5.9.2 Justification for hazard selection

PhaHV-1 and -2 have been associated with disease states and increased severity of other diseases in wild and captive koalas in Australia. Only a single case of PhaHV-3 has been reported and it is not known if the koala is the natural host of this novel virus, or if in this instance the virus spilled over from another host [2]. Therefore, PhaHV-3 was not considered further in this risk assessment.

5.9.3 Identified gaps in knowledge

- Study of the prevalence of PhaHV-1 and -2 in northern koala populations is needed.
- The expression of disease in relation to herpesvirus infection in koalas is poorly understood, including the impact of infection with more than one herpesvirus.
- Further investigation of the biology and epidemiology of herpesviruses is needed, including sites of latent and lytic infection and transmission pathways.
- Given the high incidence of lymphomas and other neoplasia in koalas, the possible association between herpesviruses and neoplasia, as seen in humans [11], requires further exploration.
- Improved understanding of the associations between viral shedding and clinical disease could be achieved through combined studies of serological assays and PCR detection of PhaHV-1 and -2. The relationships between herpesviruses and other infectious pathogens, particularly *C. pecorum* and KoRV, require investigation [5, 8].

5.9.4 Risk assessment

Below is a schematic of the hazard pathways and critical control points identified for this hazard. Critical control points are defined as *key points in the hazard pathway where risk mitigation methods could be most effective* (see *Appendix 2.3 Risk Assessment Methods* for further information). Nineteen CCPs (CCP1-CCP19) were identified across all hazards, but only those applicable to this hazard are described below. See Section 6 *Critical Control Points by Disease Hazard* for a summary of CCPs across all hazards selected for detailed assessment.

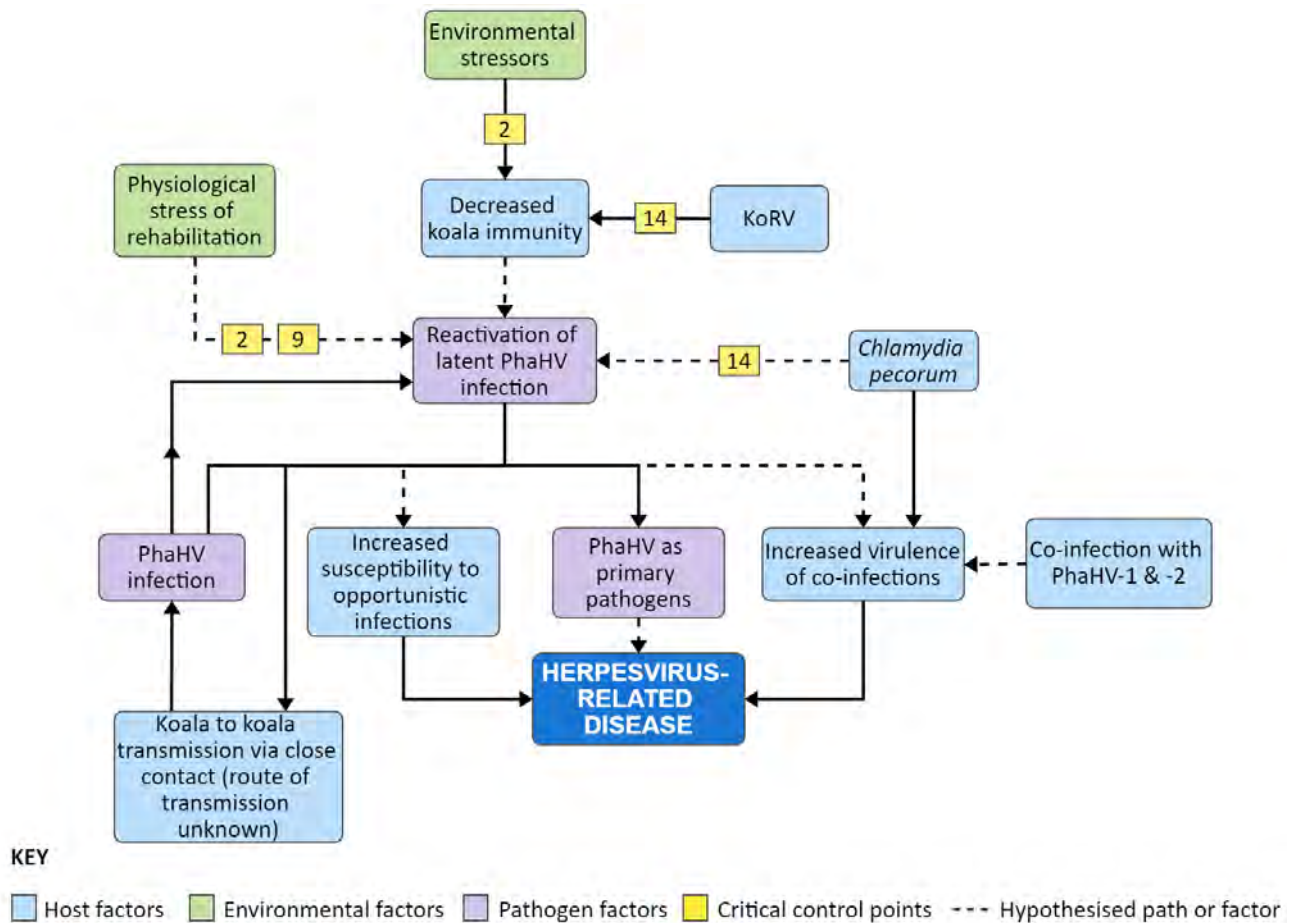


Figure 13 Hazard pathways and critical control points for phascolarctid herpesvirus-1 and -2

Critical control points

CCP2 Environmental stressors

Stressors in the koala's environment (in the wild or in captivity) contribute to general debilitation of the host, reduced immune function and increase susceptibility to disease or severity of disease expression [12-15]. Major environmental stressors for koalas include habitat loss, fragmentation and reduction in quality; nutritional stress; climate change; extremes of weather and disturbance related to human activities [16, 17].

Environmental stressors that contribute to general debilitation and reduced immune function might increase the likelihood of activation of latent PhaHV infection, with resultant disease.

CCP9 Naïve host

An infectious pathogen may be introduced to a previously unexposed population or a previously uninfected individual.

Isolation of PhaHV-positive animals in the rehabilitation environment may be advisable because of the likelihood of physiological stress in rehabilitation, which may increase likelihood of virus shedding.

CCP14 Concurrent infections and debilitation

Concurrent infections and debilitation can compromise immune function and general health in ways that increase the risk or severity of disease. The pathogenicity of an infectious hazard may be potentiated by co-infections.

Chlamydia pecorum infection could potentially reactivate latent herpesvirus infection. There are close associations between some retroviral infections and herpesvirus diseases in humans (e.g. [18]).

Likelihood assessments

Herpesviruses are host specific, so the risk of infection in other species, including humans, has not been considered.

Entry assessment

Phascolarctid herpesviruses have been found in all koala populations studied, including those which have not had introductions for over 100 years. Herpesviruses in general, and PhaHV in particular, are thought to have co-evolved with their host.

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of PhaHV-1 and -2 entering, or being present, in a koala population is considered **HIGH**.

Exposure assessment

The reported prevalence of PhaHV is moderate to high in studied koala populations, and it is assumed that this will also be the case for northern populations. It is assumed (as with other herpesviruses) that PhaHV are shed intermittently, and infection is life-long.

It is not known how frequently, for how long, and under what circumstances individual infected koalas actively shed PhaHV. It is also not known how the virus is transmitted between koalas. Both these factors (shedding, transmission route) will influence the likelihood of an individual, uninfected koala being exposed to PhaHV, or an infected koala being exposed to a different species of PhaHV.

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of exposure to PhaHV-1 and -2 for an individual koala is considered **HIGH**.

Using the principles of combining entry and exposure likelihood as outlined in *Appendix 2.3 Risk Assessment Methods*, the combined likelihood of entry and exposure for PhaHV-1 and -2 is considered **HIGH**.

Consequence assessments

Very little is known about the potential consequence of PhaHV infection at either a population or individual level. Whilst most koala populations studied to date are infected with PhaHV, ensuing disease states are not known.

Koala population resilience and viability

There is no data suggesting PhaHV causes measurable declines in koala populations, or otherwise impacts koala population resilience and viability.

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of PhaHV-1 and -2 to koala population resilience and viability is considered **NEGLIGIBLE**.

Koala individual health and welfare

The impact of PhaHV-1 and -2 infection on individual koala health and welfare is not known. Several associations with clinical disease have been identified for one or both herpesviruses, including wet bottom, uterine and ovarian cysts, testicular malformation, lowered fertility in females, incidence of neoplasia, increased tooth wear and low body condition score [5, 6, 10], but causative relationships have not been established. These associations could represent reactivation and subsequent detection of herpesvirus secondary to other disease processes, rather than a primary role for herpesviruses in causing disease [5].

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of PhaHV-1 and -2 to koala individual health and welfare is considered to be **MINOR**.

Overall risk estimate

Since the consequence of PhaHV-1 and -2 was evaluated as negligible for koala population resilience and viability, the estimate of risk for this hazard for koala populations was also considered **NEGLIGIBLE** (see *Appendix 2.3 Risk Assessment Methods*). Note, however that the level of confidence in this risk estimate (see below) is LOW. The overall risk of PhaHV-1 and -2 to individual koalas, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **MODERATE**.

This assessment exceeds the acceptable risk thresholds as outlined in *Appendix 2.3 Risk Assessment Methods*, therefore risk management for PhaHV-1 and -2 is recommended for individual koalas.

Level of confidence in risk assessment

There is a **MEDIUM** level of confidence in the likelihood assessment as southern koala populations have been relatively well studied for PhaHV. However, there are no data for northern koala populations, and it is not known how frequently, for how long, and under what circumstances individual infected koalas actively shed PhaHV, nor how the virus is transmitted between koalas. There is a **LOW** level of confidence in the consequence

assessment as very little is clearly known about the potential consequence of PhaHV infection at either a populations or individual level.

Overall, the confidence in the risk estimate for PhaHV-1 and -2 is **LOW**.

5.9.5 Risk mitigation options

Few risk mitigation measures have been identified for PhaHV-1 and -2. Further information and justification can be found in the *Treatment and Prevention and control* sections of the relevant literature review chapter. As PhaHV-1 and -2 have been detected in all free-ranging and captive populations of koalas tested to date, the options for preventing exposure to the virus in wild koalas may be limited. Not all risk mitigation options will be currently available, feasible, universally applicable or cost-effective, but are listed here for completeness.

Manage concurrent infections, debilitation and environmental stressors (CCP2 and CCP14)

- Minimise concurrent infections, debilitation and other environmental stressors (e.g. predators, disturbance related to human activities) in individual koalas and populations, to minimise the likelihood of activation of latent PhaHV infections and any resultant disease.

Testing and isolating koalas in rehabilitation or captive care (CCP9)

- In regions where the presence of herpesviruses has not yet been confirmed, test koalas entering rehabilitation for herpesvirus and manage individuals while in care based on their herpesvirus status.

5.9.6 Recommendations

Hazard-specific recommendations

The following recommendations are specific to this hazard:

- Further investigate the biology and epidemiology of koala herpesviruses including expression of disease, the prevalence of PhaHV-1 and -2 in northern koala populations and relationship to other infections, co-morbidities and neoplasia.
- Develop additional diagnostic methods for herpesviruses.
- Develop nationally-agreed protocols for PhaHV testing of koalas in care.

General recommendations

The following general recommendations apply to all 13 koala disease hazards identified for detailed risk assessment:

- Evaluate and prioritise the identified *Risk Management Options* for feasibility, completeness and effectiveness at a national KDRA Implementation Workshop.
- Identified knowledge gaps for this hazard should be prioritised and developed into a *Research Plan*.

- Prioritise protection and improvement of koala habitat as a key risk mitigation process for koala disease.
- Improve national population estimates and ensure ongoing population health monitoring for koalas.
- Develop robust, longitudinal and nationally-agreed data sets on causes of illness and death in free-living koalas.
- Maintain or increase genetic diversity and population size for koalas, so that koalas have the most robust genetic profiles for avoiding disease consequences.
- Minimise factors that might lead to stress in the koala's environment (in the wild or in captivity).
- Minimise co-infections and other causes of debilitation to reduce cumulative risk of disease.
- Increase awareness and implementation of good biosecurity practices (in accordance with the *National Wildlife Biosecurity Guidelines* [19]) when interacting with koalas during field work, treatment and rehabilitation.
- Develop clinical and research collaborative working groups to establish nationally-agreed best practice protocols for koala disease diagnosis, treatment and biosecurity and implement these in collaboration with managers of koala populations across all jurisdictions.
- Develop collaborative relationships between managers of koala populations (state and Commonwealth) and koala clinical and research working groups, including co-participation in the Koala Recovery Team.
- Develop communication tools, using the iconic status of the koala, to raise awareness and encourage behaviour change around factors contributing to disease (e.g. climate change, vehicle driver behaviours, dog ownership and management, land clearing, fire management).

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5.10 Predator Attack Trauma in Koalas – Risk Assessment

The literature review which supports this risk assessment is in *Appendix 5.10*.

5.10.1 Hazard summary

- Predator attacks are a common cause of severe and often fatal trauma to koalas.
- Domestic and wild dogs³ are the most common cause of koala predator trauma in developed areas, and wild dogs are opportunistic predators in protected and bushland habitat [1-4].
- Non-canid predators, particularly pythons, may cause significant juvenile mortality in some koala populations [3, 5-12].
- Foxes, domestic and feral cats and raptors may also predate juvenile koalas, but the population level impacts are likely to be low.
- Koala predation may be an under-reported cause of morbidity and mortality due to the difficulties in retrieving carcasses [2, 6, 9, 10] and the challenges of recognising subtle signs of predator attack [10, 13].
- Koalas are most susceptible to predator attack during the breeding season and during dispersal, when they are more mobile and spend more time on the ground [2, 14-17]. Sick and debilitated koalas are more likely to spend time on the ground and may be at increased risk of predation [3, 7].
- The prognosis for predator trauma cases in koalas is generally poor, with mortality rates of hospital admissions as high as 69% for dog attacks [2].
- Prevention and control strategies focus on domestic dog risk mitigation [3, 18, 19], reducing wild dog impact [4, 8, 10] and ensuring habitat connectivity to minimise the amount of time koalas spend on the ground [15, 20-22].
- For further detail on current knowledge of this hazard, refer to *Appendix 5.10 Predator Attack Trauma in Koalas - Literature Review*.

5.10.2 Justification for hazard selection

Predator attacks cause significant health and welfare impacts to individual koalas [1-3, 17]. Although predation is a natural biological occurrence in wild populations, koalas are exposed to predation risks with increasing habitat loss and urbanisation because they spend more time on the ground, and are more commonly in close proximity to domestic dogs [2]. The impacts of predation can be an additional burden for koalas already debilitated by other disease [3, 7].

³ includes dingoes, dingo-dog crosses and domestic dogs which are no longer living in a domesticated environment (as opposed to stray or unsecured pets).

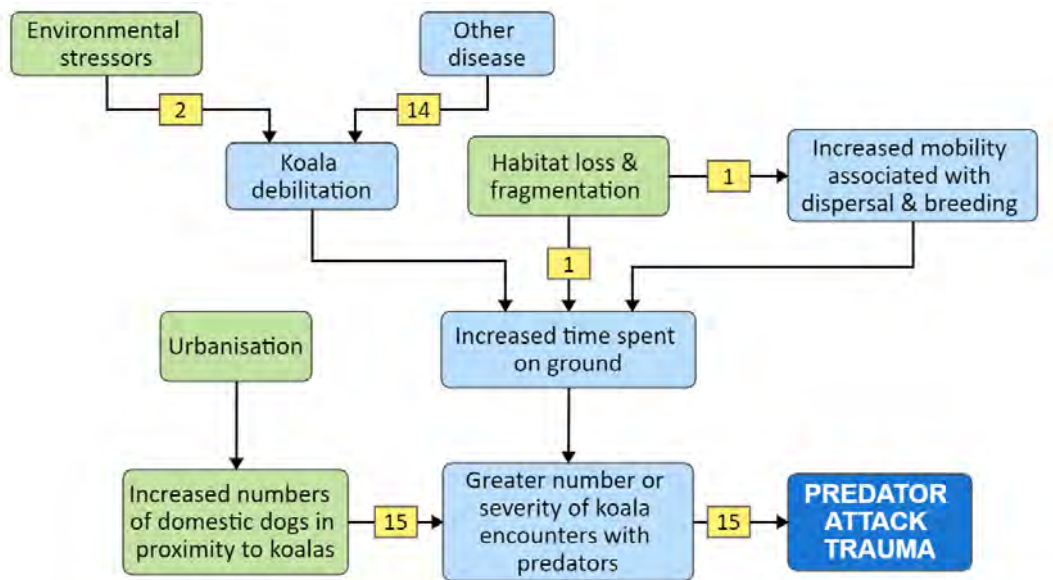
5.10.3 Identified gaps in knowledge

- Robust, longitudinal data sets on causes of mortality in free-living koalas throughout their range are required to better understand predation prevalence, source of predation, and population level impacts [6].
- More consistent and effective post-release monitoring is required to determine the long-term outcomes of injured koalas, and to better evaluate the impact of predator attack on population declines [2, 4].
- Researchers, rescuers and field workers may not have the necessary knowledge to recognise predator attack signs in live and dead koalas, resulting in underreporting of this hazard [23].
- More studies are needed on the effectiveness of prevention strategies, particularly the effectiveness of community education in reducing encounters between koalas and domestic dogs [19, 24], and strategies for controlling free-ranging wild dog populations [4, 10].

5.10.4 Risk assessment

Hazard justification

Below is a schematic of the hazard pathways and critical control points identified for this hazard. Critical control points are defined as *key* points in the hazard pathway where risk mitigation methods could be most effective (see *Appendix 2.3 Risk Assessment Methods* for further information). Nineteen CCPs (CCP1-CCP19) were identified across all hazards, but only those applicable to this hazard are described below. See Section 6 *Critical Control Points by Disease Hazard* for a summary of CCPs across all hazards selected for detailed assessment.



KEY

■ Host factors
 ■ Environmental factors
 ■ Pathogen factors
 ■ Critical control point

Figure 14 Hazard pathways and critical control points for predator attack trauma in koalas

Critical control points

CCP1 Habitat loss, fragmentation and reduction in quality

Habitat loss and reduction in habitat quality are major environmental stressors due to the koala's reliance on particular tree species for food, shelter and thermoregulation [25, 26]. Habitat fragmentation reduces connectivity between suitable koala habitat, increasing the amount of time koalas must spend on the ground and thereby increasing the likelihood of predator attack [2, 15, 21, 22, 27-30].

Causes include urban expansion, land clearing for production, inappropriate fire management practices, the introduction of pests, weeds and plant pathogens, over-browsing by koalas as a result of local overcrowding, and the habitat-altering effects of climate change [30-36].

CCP 2 Environmental stressors

Stressors in the koala's environment (in the wild or in captivity) contribute to general debilitation of the host, reduced immune function, and increased susceptibility to disease or severity of disease expression [26, 37-39]. Major environmental stressors for koalas include habitat loss, habitat fragmentation and reduction in quality, nutritional stress, weather extremes, and disturbance related to human activities [25, 28].

Environmental stressors increase the likelihood of koala debilitation and disease, thereby increasing the likelihood of koalas going to ground, where they are more susceptible to

predator attack and have reduced capacity to escape or defend themselves from predators [1, 3, 15, 40].

CCP14 Concurrent infections and debilitation

Concurrent infections and debilitation can compromise immune function and general health in ways that increase the risk or severity of disease.

In the case of predator attack, koalas debilitated from infection or other causes are more likely to be on the ground and less likely to successfully escape or defend themselves from a predator [1, 3, 15, 40].

CCP 15 Exposure to predators and severity of attack

Increased numbers of domestic dogs in closer proximity to koalas increase the likelihood of koala trauma due to predation. Urbanisation increases the absolute number of pet dogs in an area, and also increases the likelihood of koalas moving through back yards where dogs are kept [18]. Attack severity depends on the size and attack style of the predator [3, 6, 13, 23, 41, 42].

Likelihood assessments

The prevalence of predation is difficult to determine, in part because of the absence of accurate population size estimates for koalas, and it is likely to be an under-recognised hazard. Available data indicates 10-29% of koala mortalities recorded by wildlife hospitals and rehabilitation facilities may be due to dog attack [2]. The likelihood of predator attack will vary based on the abundance and behaviour of predators in an area and it is likely that a significant number of cases go unreported [2, 6, 9, 10, 13].

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of a koala being attacked by a predator during its lifetime is considered **MODERATE**.

Consequence assessments

Predator trauma is a non-infectious hazard and therefore consequence assessments were not made in relation to the health and welfare of other species.

Koala population resilience and viability

Predation by both dogs and non-canid species has been demonstrated to account for 30-50% of mortalities in longitudinal studies of free-living populations in Qld and NSW [1, 10, 43]. Reported mortality rate for dog attack cases admitted to rehabilitation is over 50% and can be as high as 69% [2, 17, 44].

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of predator trauma to koala population resilience and viability is considered **MODERATE**.

Koala individual health and welfare

The reported mortality rate due to predator trauma in koala hospital and rehabilitation facility admissions is as high as 69% [17]. Severe injuries and welfare impacts are common outcomes for koalas that survive predator attack [13, 16], and they may require prolonged treatment and rehabilitation before return to the wild.

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of predator trauma to koala individual health and welfare is considered **MAJOR**.

Overall risk estimate

The overall risk of predator trauma to koala populations, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **MODERATE**.

The overall risk of predator trauma to individual koalas, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **HIGH**.

This assessment exceeds the acceptable risk thresholds as outlined in *Appendix 2.3 Risk Assessment Methods* for individual koalas and koala populations, therefore risk management for predator trauma is recommended.

Level of confidence in risk assessment

The level of confidence in the likelihood assessment is **MEDIUM** because the overall number of koalas affected by predation is difficult to determine, due to a lack of information on koala population numbers, and likely underreporting of predator trauma in wild koalas. The level of confidence in the consequence assessment for koala populations is **MEDIUM** because there are limited monitoring data for koala populations across their range. The level of confidence in the consequence assessment for individual koalas is **HIGH** as it is based on detailed documentation of hospital admissions.

Overall, the confidence in the risk estimate for predator trauma is considered **MEDIUM**.

5.10.5 Risk mitigation options

The following risk mitigation measures have been identified for predator trauma. Further information and justification can be found in the *Treatment and Prevention and control* sections of the relevant literature review chapter. Not all risk mitigation options will be currently available, feasible, universally applicable or cost-effective, but are listed here for completeness.

Conserve and improve the quality, quantity, diversity and complexity of koala habitat (CCP1)

- Strengthen existing habitat corridors and restore habitat connectivity which has been lost, through re-vegetation and restoration projects, including revegetation in urban landscapes.

- Identify key dispersal corridors and prioritise habitat investment, including land purchase, incentive mechanisms for habitat preservation and provision of development and housing planning guidelines for key corridor areas.
- Strengthen regulatory controls against habitat clearing and road development in koala habitat and dispersal corridors.

Manage concurrent infections, debilitation and environmental stressors (CCP2 and CCP14)

- Minimise concurrent infections, debilitation and other environmental stressors (e.g. predators, disturbance related to human activities) in individual koalas and populations, as these may increase likelihood of koalas going to ground and being attacked, and may weaken the koala’s ability to defend itself from attack.

Domestic dog risk mitigation (CCP2 and CCP15)

- Encourage responsible dog ownership, including selecting dogs of a size, breed and nature which are less likely to attack koalas.
- Provide information to dog owners on strategies to prevent backyard attacks.
- Restrict domestic dog movements in koala habitat.
- Educate public on the risks to koalas of keeping domestic dogs.
- Apply regulations on dog size and numbers for residents near koala habitat.

Reduction in wild dog impact (CCP15)

- Employ strategies in koala habitat to reduce wild dog numbers or remove identified individual wild dogs known to specifically predate on koalas (noting the *Hazard-specific recommendations* below, and recognising changing understanding of the genetic provenance of “wild dogs”).

5.10.6 Recommendations

Hazard-specific recommendations

The following recommendations are specific to this hazard:

- Develop nationally-agreed monitoring for koalas following rehabilitation and release, to determine the long-term outcomes of koalas, and to better evaluate the impact of predator attack on population declines.
- Study the effectiveness of predation prevention strategies, particularly the effectiveness of community education in reducing encounters between koalas and domestic dogs, and strategies for controlling free-ranging wild dog populations, in order to inform more effective mitigation strategies.
- Develop training resources for researchers, rescuers and field workers on recognition of signs of predator attack in live and dead koalas, to improve reliability of predator attack data.
- Educate dog owners on responsible and “koala-friendly” dog ownership and consider restrictions on dog size in areas adjacent to key koala habitat.

- Consider restrictions on dog size and number in areas in proximity to koala habitat.
- Review strategies for controlling free-ranging wild dog populations in the context of the current understanding of the role of the dingo as an apex predator and the high level of dingo ancestry in most “wild dogs”, in order to inform more effective mitigation strategies.

General recommendations

The following general recommendations apply to all 13 koala disease hazards identified for detailed risk assessment:

- Evaluate and prioritise the identified *Risk Management Options* for feasibility, completeness and effectiveness at a national KDRA Implementation Workshop.
- Identified knowledge gaps for this hazard should be prioritised and developed into a *Research Plan*.
- Prioritise protection and improvement of koala habitat as a key risk mitigation process for koala disease.
- Improve national population estimates and ensure ongoing population health monitoring for koalas.
- Develop robust, longitudinal and nationally-agreed data sets on causes of illness and death in free-living koalas.
- Maintain or increase genetic diversity and population size for koalas, so that koalas have the most robust genetic profiles for avoiding disease consequences.
- Minimise factors that might lead to stress in the koala’s environment (in the wild or in captivity).
- Minimise co-infections and other causes of debilitation to reduce cumulative risk of disease.
- Increase awareness and implementation of good biosecurity practices (in accordance with the *National Wildlife Biosecurity Guidelines* [45]) when interacting with koalas during field work, treatment and rehabilitation.
- Develop clinical and research collaborative working groups to establish nationally-agreed best practice protocols for koala disease diagnosis, treatment and biosecurity and implement these in collaboration with managers of koala populations across all jurisdictions.
- Develop collaborative relationships between managers of koala populations (state and Commonwealth) and koala clinical and research working groups, including co-participation in the Koala Recovery Team.
- Develop communication tools, using the iconic status of the koala, to raise awareness and encourage behaviour change around factors contributing to disease (e.g. climate change, vehicle driver behaviours, dog ownership and management, land clearing, fire management).

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5.11 Sarcoptic Mange in Koalas – Risk Assessment

The literature review which supports this risk assessment is in *Appendix 5.11*.

5.11.1 Hazard summary

- Sarcoptic mange is a skin disease caused by the parasitic mite *Sarcoptes scabiei* [1].
- Sarcoptic mange is an emerging disease issue for free-ranging koalas [2].
- Sarcoptic mange has been identified most frequently in koalas in Vic and SA [3-6], although there are also unpublished reports of sporadic cases in NSW and Qld [7].
- Sarcoptic mange can have severe impact on the health and welfare of individual koalas [3].
- The epidemiology of sarcoptic mange in Australian wildlife is variably understood among impacted species [1] and no systematic studies of the epidemiology in koalas have been undertaken.
- Because treatment of sarcoptic mange for koalas in rehabilitation is based on extrapolation from treatment in other species or humans, there are some unknown aspects of safety and efficacy for koalas [8-10]. Pharmacokinetic research into the use of fluralaner for the treatment of sarcoptic mange in koalas is underway [7].
- Strategies for treatment of free-ranging koala individuals and populations have not been explored. More information on the parasite's epidemiology in relation to koalas is needed to advise the development of effective protocols.
- For further detail on current knowledge of this hazard, refer to *Appendix 5.11 Sarcoptic Mange in Koalas - Literature Review*.

5.11.2 Justification for hazard selection

Sarcoptic mange is a disease of emerging significance in an increasing number of wildlife species, including koalas in Australia [2, 11, 12]. Outbreaks are reported with increasing frequency in free-ranging koalas, particularly in southern populations, and affected animals can suffer severe, debilitating illness [3, 4].

5.11.3 Identified gaps in knowledge

- Information on the prevalence and distribution of *Sarcoptes scabiei* is variable among host species in Australia. A nationally coordinated approach to investigations of this emerging disease is required [1].
- The transmission pathways leading to *S. scabiei* infection in koalas are poorly understood. More epidemiological investigation is required to confirm whether sustained koala-to-koala transmission occurs, or if cases in koalas arise from repeated transmission from other species or the environment [13].
- Specific information on the prevalence and distribution of sarcoptic mange in free-ranging koalas, and the natural progression of *S. scabiei* infection in koalas, is required to enable evaluation of associated risk.

- Study of disease associations is required to determine whether co-infections such as KoRV, PhaHV and *Chlamydia* might affect the koala's susceptibility to sarcoptic mange, or conversely, whether debilitation caused by sarcoptic mange increases disease expression or susceptibility to other koala disease hazards.
- A scientifically validated, systematic approach to treatment of mange in koalas is required [1]. There is a need for pharmacokinetic research, integration of the experiences of wildlife volunteers and rehabilitators, and investigation of post-treatment monitoring strategies for koalas, drawing on knowledge of mange treatment in other wildlife species.
- Strategies for control and prevention of sarcoptic mange in free-ranging koala individuals and populations require development [14].

5.11.4 Risk assessment

Below is a schematic of the hazard pathways and critical control points (CCPs) identified for this hazard. Critical control points are *key* points in the hazard pathway where risk mitigation methods could be most effective (see *Appendix 2.3 Risk Assessment Methods* for further information). Nineteen CCPs (CCP1-CCP19) were identified across all hazards, but only those applicable to this hazard are described below. See Section 6 *Critical Control Points by Disease Hazard* for a summary of CCPs across all hazards selected for detailed assessment.

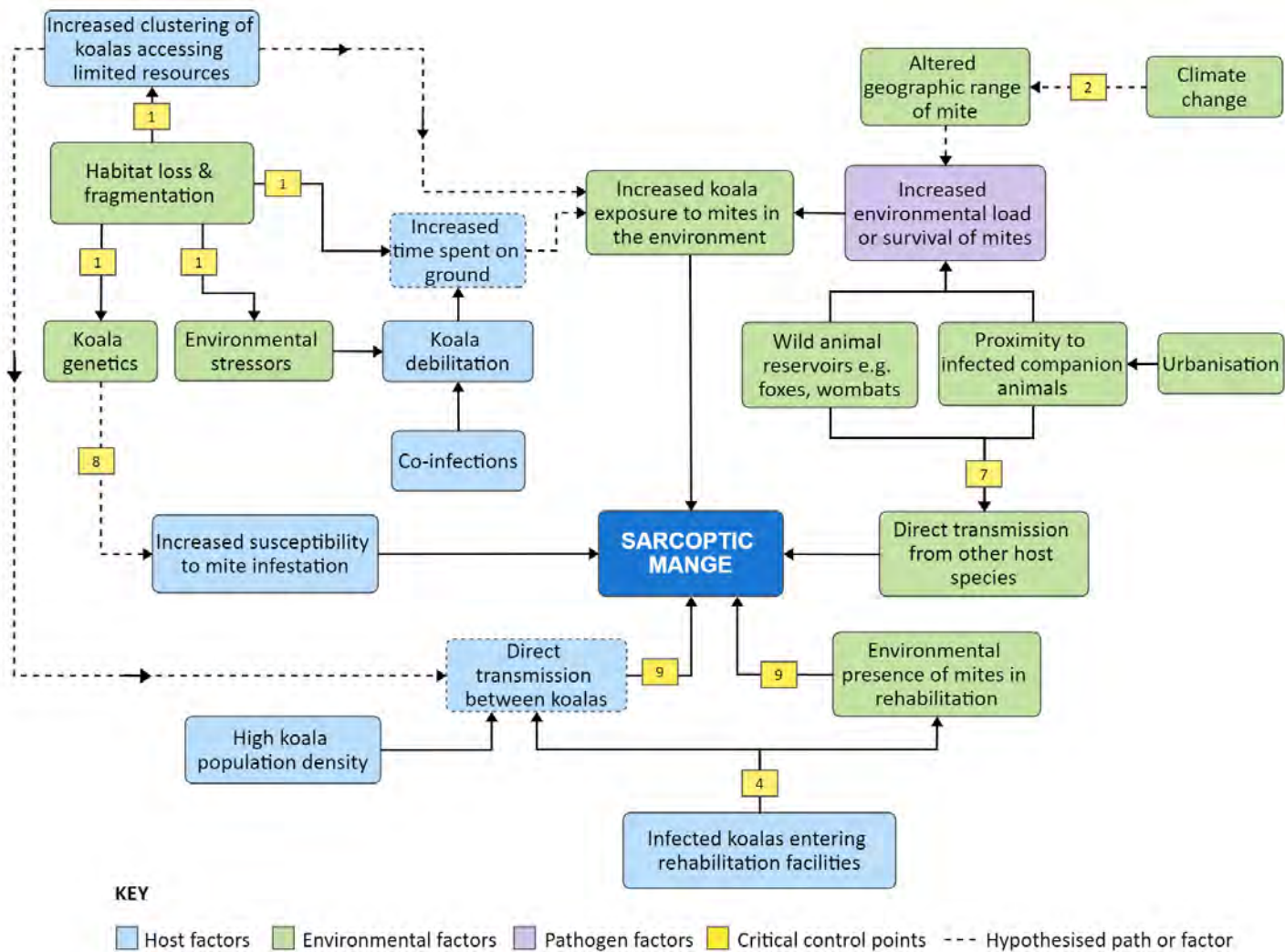


Figure 15 Hazard pathways and critical control points for sarcoptic mange in koalas

Critical control points

CCP1 Habitat loss, fragmentation and reduction in quality

Habitat loss and reduction in habitat quality are major environmental stressors due to the koala's reliance on particular tree species for food, shelter and thermoregulation [15, 16]. Habitat fragmentation reduces connectivity between suitable koala habitats, forcing dispersal and potentially contributing to the loss of important genetic alleles in populations that can no longer interbreed [17-19]. This may increase koala exposure to mites in the environment, by increasing the amount of time spent on the ground, in contact with substrates infected by reservoir species. Loss of habitat connectivity also leads to increased clustering of koalas [20], which could increase mite transmission if direct koala-to-koala contact is a significant transmission pathway [3]. Causes include urban expansion, land clearing for production, inappropriate fire management practices, the introduction of pests, weeds and plant pathogens, over-browsing by koalas as a result of local overcrowding, and the habitat-altering effects of climate change [20-26].

CCP2 Environmental stressors

Stressors in the koala's environment (in the wild or in captivity) contribute to general debilitation of the host, reduced immune function, and increased susceptibility to disease or severity of disease expression [16, 27-29]. Major environmental stressors for koalas include habitat loss, fragmentation and reduction in quality; nutritional stress; climate change; extremes of weather and disturbance related to human activities [15, 30].

Changing climate may result in an increased or altered geographic range for the *Sarcoptes* mite in Australia.

CCP4 Biosecurity practices

Biosecurity practices aim to ensure that infected koalas (or those of unknown infection status) do not pose a risk to other koalas in the population (captive or wild). Biosecurity practices may include quarantine of incoming or outgoing animals, appropriate use of personal protective equipment, general hygiene, prophylactic treatment and disinfection practices [31].

Koalas which are infected with mange may infect other animals in rehabilitation if biosecurity practices are inadequate.

CCP7 Disease reservoirs in other species

Pathogens may be present through other host species with a risk of spillover to koalas. Foxes and wombats are possible (but unconfirmed) reservoirs of sarcoptic mange infection to koalas [3, 13, 32].

CCP8 Genetics

The individual koala may have a genetic predisposition to the development of disease or increased severity of disease. Improving or at least maintaining genetic diversity in koala populations is likely to conserve adaptive potential [33] and encourage the retention of the most robust koala genetic profiles to avoid disease consequences of sarcoptic mange.

CCP9 Naïve host

An infectious pathogen may be introduced to a previously unexposed population or a previously uninfected individual. Koalas may contract *S. scabiei* through environmental presence of the mite or through direct contact with infected koalas [34].

Likelihood assessments

Likelihood of entry and exposure of koalas was considered for “koala population to koala population” and “other animal population to koala population” transmission pathways. Likelihood of an infected koala causing a hazard to humans or other animals (via their exposure to koalas or koala environments) was also evaluated.

Entry assessment - koalas

Sarcoptic mange cases have been reported in koalas or other species throughout the koala's distribution [35-39], suggesting that the mites are potentially present wherever koalas are found.

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of *Sarcoptes scabiei* entering, or being present, in environments where koalas are found is considered to be **HIGH**.

Exposure assessment - koalas

The means by which koalas are exposed to *S. scabiei* mites is unknown. Contact with trees or substrate containing mites has been suggested [3]. It has not been determined if koala-to-koala transmission occurs, but transmission through sharing of habitat with reservoir species such as foxes and wombats is suspected to be a means of exposure [3, 32, 35].

The prevalence of *S. scabiei* in koala populations is not known and reports have been limited to localised outbreaks in a small number of koala populations in Vic and SA [3, 4]. Prevalence in one outbreak in SA was estimated at 8% [4], with a 3-4% prevalence of sarcoptic mange in mortality studies of Vic and SA koalas [5, 40]. These figures are similar to prevalence reported in wombats, where *S. scabiei* is endemic [41].

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of exposure to *S. scabiei* for an individual koala is considered **LOW**.

Using the principles of combining entry and exposure likelihood as outlined in *Appendix 2.3 Risk Assessment Methods*, the combined likelihood of entry and exposure for *S. scabiei* for koalas is considered **LOW**.

Likelihood assessments - other species exposed to koalas

Koalas have not been identified as a reservoir species for *S. scabiei* in the wild. The sporadic and isolated nature of sarcoptic mange outbreaks in koalas suggests that they do not significantly increase the risk of sarcoptic mange entry or exposure to other species (including humans) in the free-ranging environment. In the captive or rehabilitation environment, affected koalas could be responsible for exposure of humans and other captive animals to *S. scabiei*, although this is considered unlikely. There are no reports of koalas passing *S. scabiei* infection on to other non-human species, and there has only been one report of a human possibly acquiring *S. scabiei* infection (scabies) from exposure to an infected koala [42]. While reports of cross-infection from koalas are limited, evidence from all other species suggests that transmission to humans is possible.

The likelihood of a human or other animal species being exposed to *S. scabiei* as a result of free-ranging koala infection is considered **NEGLIGIBLE** unless koalas are being handled directly by humans during field procedures, where the risk is considered **LOW**. The

likelihood of a human or other animal species being exposed to *S. scabiei* as a result of koala infection is considered **LOW** for the captive or rehabilitation environment. Since the likelihood of a human or other animal species being exposed to *S. scabiei* as a result of koala infection, in free-ranging populations, was evaluated as negligible in all cases except humans undertaking direct handling of koalas in the field, the risk estimate for this aspect of the hazard analysis was classified as negligible (see *Appendix 2.3 Risk Assessment Methods*) and risk assessment was only continued for the specific scenario where humans are handling koalas directly in the field. Note, however that the level of confidence in this aspect of the risk estimate (see below) is LOW.

Consequence assessments

It is not known how many cases of exposure to *S. scabiei* result in infection, nor how many cases of infection with *S. scabiei* result in clinical disease, in koalas. However, based on what is known of *S. scabiei* epidemiology in other species, and using the precautionary principle, we assume that most cases of exposure result in infection and most cases of infection result in clinical disease.

Koala population resilience and viability

Population impacts of sarcoptic mange in koalas are not known. In southern populations, outbreaks of sarcoptic mange in wild koalas appear to be increasing in frequency and distribution but appear localised in their effect [3, 4, 40]. Cases of sarcoptic mange in northern populations appear to be sporadic and isolated in nature and population-level outbreaks have not been reported [7].

The consequences for populations were evaluated based on the most severe impacts observed in koalas to date, consistent with the precautionary principle.

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of *S. scabiei* infection to koala population resilience and viability is considered **MINOR**.

Koala individual health and welfare

Chronic sarcoptic mange in koalas is associated with severe debilitation and often death [3]. It is not known how many cases of exposure to *S. scabiei* result in infection and it is not known whether koalas can mount an effective immune response to clear *S. scabiei* infection, rather than developing sarcoptic mange. However, on the basis of the precautionary principle, it is assumed that koalas which are exposed to *S. scabiei* generally develop sarcoptic mange.

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of *S. scabiei* infection to koala individual health and welfare is considered to be **MAJOR**.

Health and welfare – humans and other species

Evaluation of the overall consequences of sarcoptic mange in koalas for individuals of other species incorporated the fact that exposure to koalas (or their environments) has rarely been reported to lead to disease in other species, as well as the consequences for individual health and welfare for the small proportion in which disease might occur.

Scabies in humans is generally a mild and self-limiting condition, although severe disease can occur [43-45]. Sarcoptic mange in other species can range from mild to severe [11].

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of koala *S. scabiei* infection to health and welfare of humans and of other species is considered to be **MINOR**.

Overall risk estimate

The overall risk of *S. scabiei* infection to koala population resilience and viability, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **LOW**.

The overall risk of *S. scabiei* infection to individual koala health and welfare, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **MODERATE**.

The overall risk of *S. scabiei* infection of koalas to human health and welfare, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **LOW** in the captive and rehabilitation environment, and also **LOW** if humans are directly handling free-ranging koalas.

The overall risk of *S. scabiei* infection of koalas to health and welfare of other species, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **LOW** in the captive and rehabilitation environment.

This assessment exceeds the acceptable risk thresholds as outlined in *Appendix 2.3 Risk Assessment Methods* for koala populations and individual koalas, and also for other species and human health in the captive and rehabilitation environment. Therefore, risk management for sarcoptic mange in these situations is recommended.

Level of confidence in risk assessment

Knowledge of the epidemiology of *S. scabiei* in koalas is limited. The level of confidence in the entry assessment for koalas is considered **HIGH** because *S. scabiei* has been identified regularly throughout koala habitats. The level of confidence in the exposure assessment for koalas is considered **LOW** because there is little information on the ways by which koalas become exposed to the mite. The role of koalas in the maintenance of *S. scabiei* load in environments has not been explored and therefore the level of confidence in the likelihood of exposure of other species as a result of koala infection is considered **LOW**.

It is not known how many cases of exposure to *S. scabiei* result in infection in koalas, nor how many cases of infection with *S. scabiei* result in clinical disease. There is no information on prevalence of *S. scabiei* in koala populations, nor on the ability of koalas to clear infection

naturally. Therefore, the level of confidence in the consequence assessments for koala populations is considered **LOW**.

The clinical and health impacts of sarcoptic mange on individual koalas, humans and other species have been well described. Therefore, the level of confidence in the consequence assessments for the health and welfare of individual koalas, humans and other animal species is considered **HIGH**.

Overall, the confidence in the risk estimate for *S. scabiei* infection in koalas is considered **MEDIUM**.

5.11.5 Risk mitigation options

The following risk mitigation measures have been identified for *S. scabiei* infection in koalas. Further information and justification can be found in the *Treatment and Prevention and control* sections of the relevant literature review chapter. Not all risk mitigation options will be currently available, feasible, universally applicable or cost-effective, but are listed here for completeness.

Conserve and improve the quality, quantity, diversity and complexity of koala habitat (CCP1)

- Re-vegetation, restoration and preservation of habitat, including in urban landscapes, to reduce clustering of koalas around limited resources which may increase mite transmission.

Act to reduce the impacts of anthropogenic climate change and a warming, drying climate (CCP 2)

- Reduce impact of climate change on mite geographic range and persistence by supporting actions to bring about “climate-friendly” policies at a local, state and federal level.
- Use koalas and their plight as a focus-point for community education about climate change and a “call to action” for associated behaviour change.

Minimise risk posed by reservoir species (CCP7)

- Control feral animal reservoirs (particularly foxes).
- Support regulations and education programs to control domestic dog interactions with koalas.
- Control sarcoptic mange in susceptible species, such as wombats, that share koala habitat.

Pathogen monitoring, detection and response in free-ranging populations (CCP9)

- Undertake surveillance for sarcoptic mange in koala populations where outbreaks are known to occur, allowing early detection of clinical cases and treatment in rehabilitation or while remaining in the wild.

Biosecurity practices (CCP4)

- Implement biosecurity practices specific to sarcoptic mange risk for koalas entering rehabilitation, and for field procedures where koalas are being directly handled.
- Practise barrier hygiene between animals of different disease status in captivity and in rehabilitation.
- Prevent cross-contamination, zoonotic transmission and transmission to other species through appropriate disinfection, personal protective equipment and equipment use.

Maintain genetic diversity and population sizes in koalas (CCP8)

- Support genetic diversity in koala populations to encourage the retention of the most robust koala genetic profiles for avoiding serious consequences of infection with *S. scabiei*.

5.11.6 Recommendations

Hazard-specific recommendations

The following recommendations are specific to this hazard:

- Develop nationally-agreed protocols for triage and treatment of koalas with sarcoptic mange.
- Investigate the pharmacokinetics of antiparasitic medications in koalas, to enable selection of the safest and most effective drugs for treating sarcoptic mange.
- Standardised records of clinical signs and response to treatment of sarcoptic mange in koalas should be collected, and collated information used for evidence-based evaluation of treatment regimens.
- Investigate the relationship between sarcoptic mange and other infections and co-morbidities of koalas.

General recommendations

The following general recommendations apply to all 13 koala disease hazards identified for detailed risk assessment:

- Evaluate and prioritise the identified *Risk Management Options* for feasibility, completeness and effectiveness at a national KDRA Implementation Workshop.
- Identified knowledge gaps for this hazard should be prioritised and developed into a *Research Plan*.
- Prioritise protection and improvement of koala habitat as a key risk mitigation process for koala disease.
- Improve national population estimates and ensure ongoing population health monitoring for koalas.
- Develop robust, longitudinal and nationally-agreed data sets on causes of illness and death in free-living koalas.

- Maintain or increase genetic diversity and population size for koalas, so that koalas have the most robust genetic profiles for avoiding disease consequences.
- Minimise factors that might lead to stress in the koala's environment (in the wild or in captivity).
- Minimise co-infections and other causes of debilitation to reduce cumulative risk of disease.
- Increase awareness and implementation of good biosecurity practices (in accordance with the *National Wildlife Biosecurity Guidelines* [31]) when interacting with koalas during field work, treatment and rehabilitation.
- Develop clinical and research collaborative working groups to establish nationally-agreed best practice protocols for koala disease diagnosis, treatment and biosecurity and implement these in collaboration with managers of koala populations across all jurisdictions.
- Develop collaborative relationships between managers of koala populations (state and Commonwealth) and koala clinical and research working groups, including co-participation in the Koala Recovery Team.
- Develop communication tools, using the iconic status of the koala, to raise awareness and encourage behaviour change around factors contributing to disease (e.g. climate change, vehicle driver behaviours, dog ownership and management, land clearing, fire management).

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5.12 Thermal Burn Trauma in Koalas – Risk Assessment

The literature review which supports this risk assessment is in *Appendix 5.12*.

5.12.1 Hazard summary

- Bushfires are an inevitable occurrence in koala habitat in Australia [1], with the extent and severity of fires predicted to increase as the effects of anthropogenic climate change are felt [2-4].
- Fire has been recognised as a contributing factor in koala population declines [4-7], and population extirpations [7-10]. However, koala populations may recover from fire events provided an adequate quality and quantity of unburnt adjacent habitat is present [11, 12].
- Burns to koalas are a common occurrence when habitat is burnt, with the risk of injury and death related to the nature and intensity of the fire (particularly the extent of forest canopy burning), the extent of koala travel over a burnt landscape, the degree of pre-existing debilitation of koalas and the interval between burn injury, rescue and treatment [1, 2, 4, 8, 13-15].
- Prompt and expert triage are essential for good welfare outcomes for burnt koalas [16, 17].
- Prevention and control strategies focused on minimising the impact of fire in the landscape will also be relevant to minimising burn risk for koalas, but risk mitigation specific for koalas is also required [1, 18].
- For further detail on current knowledge of this hazard, refer to *Appendix 5.12 Thermal Burn Trauma in Koalas - Literature Review*.

5.12.2 Justification for hazard selection

Bushfires will continue to be a feature in the Australian landscape, and burn trauma is a common and debilitating outcome for koalas when bushfires occur in their habitat.

5.12.3 Identified gaps in knowledge

- Ongoing longitudinal study is needed to fully understand the impacts of the devastating fire events of 2019-2020, and other fires, on koala populations [4, 14].
- More comprehensive data on the location and size of koala populations would assist in predicting the impact of fire on populations, targeting preventative measures and monitoring recovery [1].
- More research is needed in understanding the mortality effects and movement patterns of koalas during and after prescribed burning events, to reduce the impact of burns on koala populations [1].
- The integration of traditional Indigenous fire management knowledge with other fire management practices is an area for development in the pursuit of effective fire mitigation which also preserves ecosystem function [1, 19].

- More data are needed to quantify the impact of thermal burns on the health and welfare of individual koalas [10, 13, 20, 21].
- There are currently no consistent nation-wide guidelines for the first aid, triage and clinical evaluation of burnt koalas. Consolidation of the learnings of the 2019-2020 bushfires in terms of first aid, triage, treatment and prognostic indicators for burnt koalas is needed to inform national guidelines which will improve consistency of approach and welfare-oriented outcomes for koalas.
- Robust, longitudinal data sets on causes of mortality in free-living koalas throughout their range are required to better understand the prevalence and population level impacts of thermal burn trauma.

5.12.4 Risk assessment

Below is a schematic of the hazard pathways and critical control points identified for this hazard. Critical control points are defined as *key points in the hazard pathway where risk mitigation methods could be most effective* (see *Appendix 2.3 Risk Assessment Methods* for further information). Nineteen CCPs (CCP1-CCP19) were identified across all hazards, but only those applicable to this hazard are described below. See Section 6 *Critical Control Points by Disease Hazard* for a summary of CCPs across all hazards selected for detailed assessment.

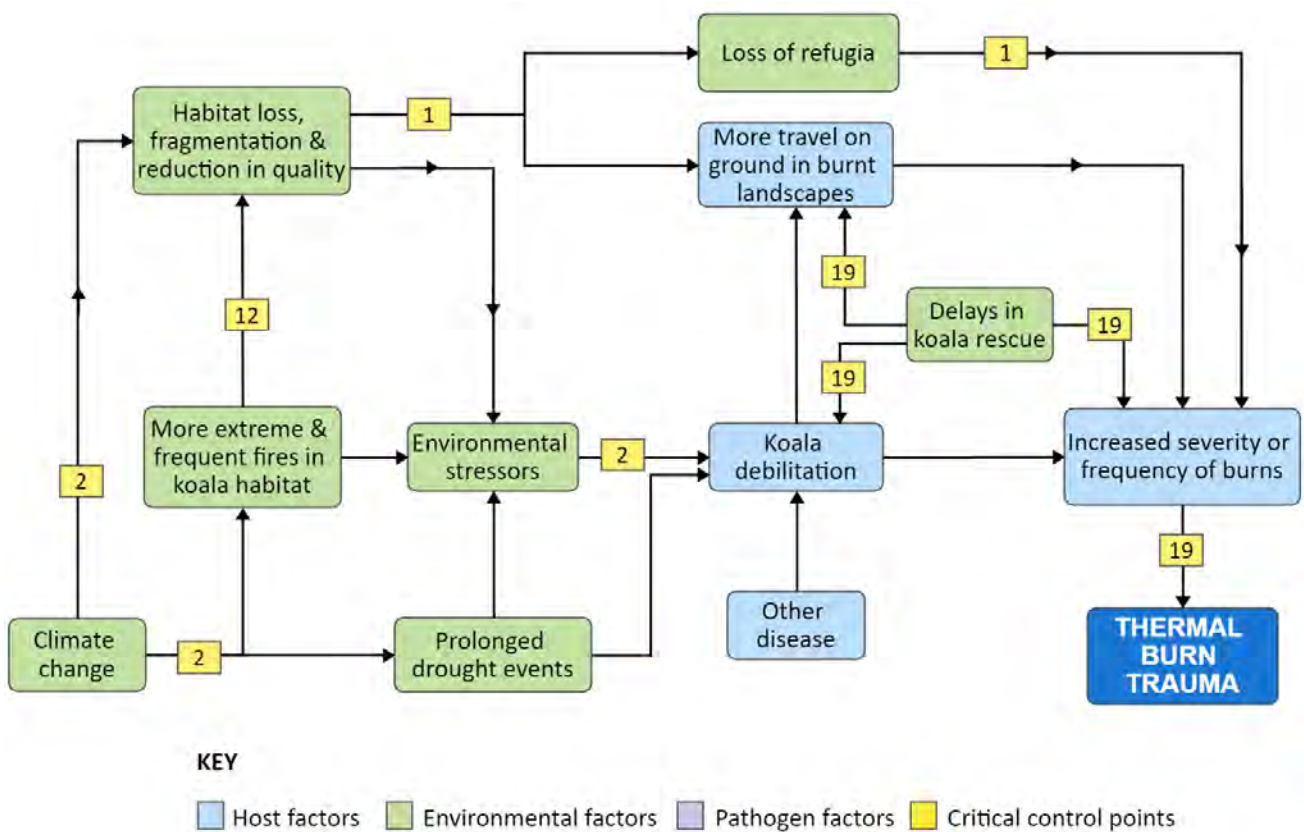


Figure 16 Hazard pathways and critical control points for thermal burn trauma in koalas

Critical control points

CCP1 Habitat loss, fragmentation and reduction in quality

Habitat loss and reduction in habitat quality are major environmental stressors due to the koala's reliance on particular tree species for food, shelter and thermoregulation [22, 23]. Habitat fragmentation reduces connectivity between suitable koala habitats, forcing dispersal and potentially contributing to the loss of important genetic alleles in populations that can no longer interbreed [24-26]. Causes include urban expansion, land clearing for production, inappropriate fire management practices, the introduction of pests, weeds and plant pathogens, over-browsing by koalas as a result of local overcrowding, and the habitat-altering effects of climate change [27-33].

Habitat loss, reduction and fragmentation all result in loss of refugia, increasing likelihood of burns in the event of a fire as koalas are unable to find shelter or food, and are more likely to travel over burnt ground in search of suitable habitat [11, 12, 34, 35].

CCP2 Environmental stressors

Stressors in the koala's environment (in the wild or in captivity) contribute to general debilitation of the host, reduced immune function, and increased susceptibility to disease or severity of disease expression [23, 36-38]. Major environmental stressors for koalas include habitat loss, fragmentation and reduction in quality; nutritional stress; climate change; extremes of weather and disturbance related to human activities [22, 39].

Climate change is a key environmental stressor which leads to more extreme and frequent fires in koala habitat, resulting in greater likelihood of burn trauma [1, 8]. Many significant bushfires are preceded by prolonged drought and heat events [14, 27, 40-42].

CCP12 Fire management practices

Inadequate planning, inappropriate methodology or flawed execution of fire management practices may lead to increased disease risks for koalas [1, 19].

The risk of burns to koalas is related to the nature and intensity of the fire (particularly the extent of forest canopy burning) and the extent of koala travel over a burnt landscape [1, 13, 15]. The extent to which planning and execution of fire management incorporates koala risk mitigation will affect the likelihood and consequences of burns to koalas.

CCP19 Emergency response to heightened risk

In situations where environmental conditions are associated with increased disease threats (e.g. bushfires, high ambient temperatures), *in situ* preparedness and response activities may significantly reduce risk to koalas. Actions may include community awareness and behaviour change campaigns (e.g. [43, 44]) as well as advance planning for emergencies, and deployment of rapid response teams (e.g. [13, 15]).

In the case of burns, actions include fire warning protocols to enable consideration of koala rescue before fires reach populations, early deployment of koala rescue teams into burnt

areas and establishment of best practice protocols for first aid, triage and treatment of burnt koalas [1, 13, 15, 45].

Likelihood assessments

It appears certain that there will be increasing severity and frequency of bushfire events in koala habitat, as a result of climate change [1, 8]. The ability of koalas to escape the effects of fire is affected by a range of factors, including the nature of the burn [1, 4, 8], the availability of adjacent unburnt habitat [1, 11, 12], and the availability of timely, expert treatment [13, 15].

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of a koala being burnt in a bushfire during its lifetime is considered **MODERATE**.

Consequence assessments

Thermal burn trauma is a non-infectious hazard and therefore consequence assessments were not made in relation to the health and welfare of other species.

Koala population resilience and viability

The potentially devastating consequence of koala burns to population viability was highlighted during the 2019-20 fires, when an estimated 60,000 koalas perished and over 10% of total wild koala habitat was burned [8, 46]. Despite these estimates, more data is required on the impacts of fire on free-living koala populations, in both the short and long term. The effects of climate change are likely to increase bushfire severity [1, 8] and the small size and high fragmentation of current koala populations will increase the likelihood of population declines and extirpation as a result of fire [4]. However, koala populations have been shown to recover from fire events under certain conditions [11, 12].

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of thermal burns to koala population resilience and viability is considered **MODERATE**.

Koala individual health and welfare

Koalas that are burnt often suffer injuries which are fatal or require euthanasia [13, 21]. Poor body condition, dehydration and the effects of smoke inhalation commonly occur along with burn trauma and reduce the koala's chances for recovery [13]. Koalas that survive thermal burns may require extended periods of care and treatment [15]. Burn recovery is known to be a painful process in humans [47], and the same may be true for koalas.

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of thermal burn trauma to koala individual health and welfare is considered **MAJOR**.

Overall risk estimate

The overall risk of thermal burn trauma to koala population resilience and viability, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **MODERATE**.

The overall risk of thermal burn trauma to individual koalas, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods* is **HIGH**.

This assessment exceeds the acceptable risk thresholds as outlined in *Appendix 2.3 Risk Assessment Methods* for both koala populations and individuals, therefore risk management for thermal burn trauma in koalas is recommended.

Level of confidence in risk assessment

There is considerable data on the likely impacts of climate change on bushfire events in the Australian context. Data on the absolute numbers of koalas which experience burn trauma is often incomplete because of logistic and safety constraints to timely access to fire grounds to enable koala rescue [13]. Uncertainties regarding free-ranging koala population numbers also limit full evaluation of the impact of fires [1]. The level of confidence in the assessment of likelihood for this hazard is therefore assessed as **MEDIUM**.

There are knowledge gaps in our understanding of the resilience of koala populations to fire [1]. Separation of the impact of thermal burns *per se* from other effects of fire such as habitat loss and nutritional stress is rarely attempted [13]. Therefore, the level of confidence in the assessment of consequence of thermal burns on koala population resilience and viability is **MEDIUM**. The level of confidence in the assessment of consequence for individual koalas is **HIGH** as the clinical and welfare impacts of burns in koalas are well documented.

Overall, the confidence in the risk estimate for burns trauma is **MEDIUM**.

5.12.5 Risk mitigation options

The following risk mitigation measures have been identified for thermal burn trauma. Some of these focus on mitigating the impacts of bushfires in general and others focus on mitigating the impacts of burn trauma on individual koalas. Further information and justification can be found in the *Treatment and Prevention and control* sections of the relevant literature review chapter. Not all risk mitigation options will be currently available, feasible, universally applicable or cost-effective, but are listed here for completeness.

Conserve and improve the quality, quantity, diversity and complexity of koala habitat (CCP1)

- Re-vegetation, restoration and preservation of habitat, including in urban landscapes, to restore habitat connectivity and strengthen existing habitat corridors.
- Protect or develop climate refugia, such as valleys, understory, midstory and fast-growing shade-producing non-browse vegetation that provide good thermal protection for koalas.

- Strengthen existing habitat corridors and restore habitat connectivity which has been lost, through re-vegetation and restoration projects.
- Strengthen regulatory controls against habitat clearing and road development in koala habitat and dispersal corridors.
- Improve fire resilience of habitat by selecting rapid-growing shelter trees and heat-tolerant species.

Act to reduce the impacts of anthropogenic climate change and a warming, drying climate (CCP2)

- Act to bring about “climate-friendly” policies at a local, state and federal level.
- Use koalas and their plight as a focus-point for community education about climate change and a “call to action” for associated behaviour change.
- Promote practices which reduce the impacts of climate change.

Fire management practices (CCP12)

- Adopt fire management practices that minimise impacts on koala habitat quantity, quality and connectivity, including:
 - Limiting the frequency, intensity, nature or extent of burning in koala habitat.
 - Use strategic burn patterns which reduce fire intensity or provide a mosaic of unburnt habitat.
 - Identify important koala areas to be excluded from burning.
 - Incorporate alternatives to burning in landscape management.
 - Incorporate knowledge on habitat-preserving fire management practices of Traditional Owners.
- Adopt specific koala risk mitigation options for fire management, including:
 - Identify and map koala populations to facilitate location and response in the event of a fire.
 - Incorporate koala response and rescue into fire emergency response planning at all jurisdictional levels.
 - Use of technologies to locate free-living koalas, then protect or evacuate them prior to, or during, fires.
 - Time burning to avoid koala breeding and dispersal periods.
 - Reduce fuel load around tree bases and wet trunks of trees known to be used by koalas.

Emergency response to heightened fire risk (CCP12 and CCP19)

- Support early intervention by personnel experienced and trained in koala rescue, triage and treatment.
- Enable timely access to firegrounds by experienced wildlife rescue teams (embedded within the existing state emergency response) to evacuate, triage or rescue koalas.

- Develop decision trees and protocols to improve the consistency and effectiveness of koala triage for positive koala welfare and successful treatment outcomes.
- Support the training of rehabilitators and veterinary personnel in incident management and moving within burnt landscapes to improve the safe and timely integration of animal rescue into bushfire response.
- Support personal mental health and welfare care plans, based on science and experience, for all personnel involved in rescue, treatment and care of injured wildlife.

5.12.6 Recommendations

Hazard-specific recommendations

The following recommendations are specific to this hazard:

- Continue long-term longitudinal studies to fully understand the impacts of the 2019-2020 (and other) fire events on koala populations.
- Develop nationally-agreed protocols and decision trees for triage and assessment, prognosis, treatment and rehabilitation of burnt or fire-affected koalas, including criteria for possible euthanasia.
- Develop nationally-agreed monitoring protocols for koalas following rehabilitation and release, to evaluate the short- and long-term impacts of thermal trauma on individuals and populations and to inform further refinement of rescue, treatment and rehabilitation practices.
- Study movement patterns of koalas during and after prescribed burning events.
- Integrate traditional and Indigenous fire management knowledge into current fire management practices
- Training of wildlife rehabilitators and veterinary personnel during “peacetime” in incident management and safe access to fire fields to allow timely integration of animal rescue into bushfire and other emergency response.

General recommendations

The following general recommendations apply to all 13 koala disease hazards identified for detailed risk assessment:

- Evaluate and prioritise the identified *Risk Management Options* for feasibility, completeness and effectiveness at a national KDRA Implementation Workshop.
- Identified knowledge gaps for this hazard should be prioritised and developed into a *Research Plan*.
- Prioritise protection and improvement of koala habitat as a key risk mitigation process for koala disease.
- Improve national population estimates and ensure ongoing population health monitoring for koalas.

- Develop robust, longitudinal and nationally-agreed data sets on causes of illness and death in free-living koalas.
- Maintain or increase genetic diversity and population size for koalas, so that koalas have the most robust genetic profiles for avoiding disease consequences.
- Minimise factors that might lead to stress in the koala's environment (in the wild or in captivity).
- Minimise co-infections and other causes of debilitation to reduce cumulative risk of disease.
- Increase awareness and implementation of good biosecurity practices (in accordance with the *National Wildlife Biosecurity Guidelines* [48]) when interacting with koalas during field work, treatment and rehabilitation.
- Develop clinical and research collaborative working groups to establish nationally-agreed best practice protocols for koala disease diagnosis, treatment and biosecurity and implement these in collaboration with managers of koala populations across all jurisdictions.
- Develop collaborative relationships between managers of koala populations (state and Commonwealth) and koala clinical and research working groups, including co-participation in the Koala Recovery Team.
- Develop communication tools, using the iconic status of the koala, to raise awareness and encourage behaviour change around factors contributing to disease (e.g. climate change, vehicle driver behaviours, dog ownership and management, land clearing, fire management).

5.12.7 References

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5.13 *Trypanosoma* spp. in Koalas – Risk Assessment

The literature review which supports this risk assessment is in *Appendix 5.13*.

5.13.1 Hazard summary

- Six trypanosome species are known to infect koalas [1-4].
- The number of species and geographic distribution of trypanosome infections in koalas is likely to increase with further investigation [5].
- Most trypanosome species infecting koalas are present in other Australian species, including marsupials, bats and rodents [2, 4, 6-13].
- No Australian trypanosome species have been known to infect humans, although trypanosome species elsewhere are zoonotic [4, 6].
- Concurrent infection with as many as five trypanosome species has been reported in koalas [2, 4].
- The trypanosome life cycle generally involves an intermediate host vector [5]. The vectors of the trypanosomes parasitising koalas and other Australian wildlife are not known, but ticks are likely candidates [4, 8].
- Trypanosome infection has been commonly reported in koalas in NSW and Qld [2, 4, 6-13] and studies still in progress have also detected trypanosomes in SA koalas [14]. Very few koalas have been tested elsewhere in Australia. Several of the trypanosome species known to infect koalas have been detected in other Australian mammals elsewhere in Australia (see *Appendix 5.13 Trypanosoma spp. - Literature Review*).
- The majority of wildlife trypanosomes are considered benign to their vertebrate hosts [6].
- Most koalas infected with trypanosomes are clinically healthy, but some associations with poor body condition, poor survival in rehabilitation, severe, strongly regenerative anaemia and neurological disorders have been observed.
- The ability of trypanosome infections to exacerbate immunosuppression in koalas or increase clinical severity of other infections (particularly chlamydia and KoRV) is not known. Trypanosomes are known to compromise immunity in other species [6].
- Trypanosomes are diagnosed by identifying the parasites on blood smears, but more sensitive results can be obtained using molecular methods [1-5, 9, 11, 15].
- There are no specific treatment recommendations for trypanosome infection in koalas.
- Prevention and control of trypanosome infections in general depend on breaking the cycle of transmission, which requires knowledge of competent vectors [16]. Reduction of stressors is likely to be important to limiting disease risks.
- For further detail on current knowledge of this hazard, refer to *Appendix 5.13 Trypanosoma spp. in Koalas - Literature Review*.

5.13.2 Justification for hazard selection

Trypanosome infections in koalas are commonly found in clinically normal koalas, but have also been associated with poor body condition, poor survival in rehabilitation, severe anaemia and neurological disease, and there is little understanding of the possible disease risks for koalas. Trypanosome infections in other species can cause severe disease.

5.13.3 Identified gaps in knowledge

- More research is needed to confirm the vectors and mode of transmission of trypanosomes in koalas, and in other Australian wildlife species [17, 18].
- The association between trypanosome presence in koalas and observable clinical signs consistent with trypanosomiasis is yet to be thoroughly investigated.
- Further investigation is needed to determine whether trypanosome polyparasitism affects the clinical impact of infection in koalas [4].
- The contribution of trypanosomes (both single and mixed infections) to clinical disease in koalas, particularly in the presence of co-infections such as *Chlamydia* and KoRV, has yet to be elucidated [4].

5.13.4 Risk assessment

Below is a schematic of the hazard pathways and critical control points (CCPs) identified for this hazard. Critical control points are *key points* in the hazard pathway where risk mitigation methods could be most effective (see *Appendix 2.3 Risk Assessment Methods* for further information). Nineteen CCPs (CCP1-CCP19) were identified across all hazards, but only those applicable to this hazard are described below. See Section 6 *Critical Control Points by Disease Hazard* for a summary of CCPs across all hazards selected for detailed assessment.

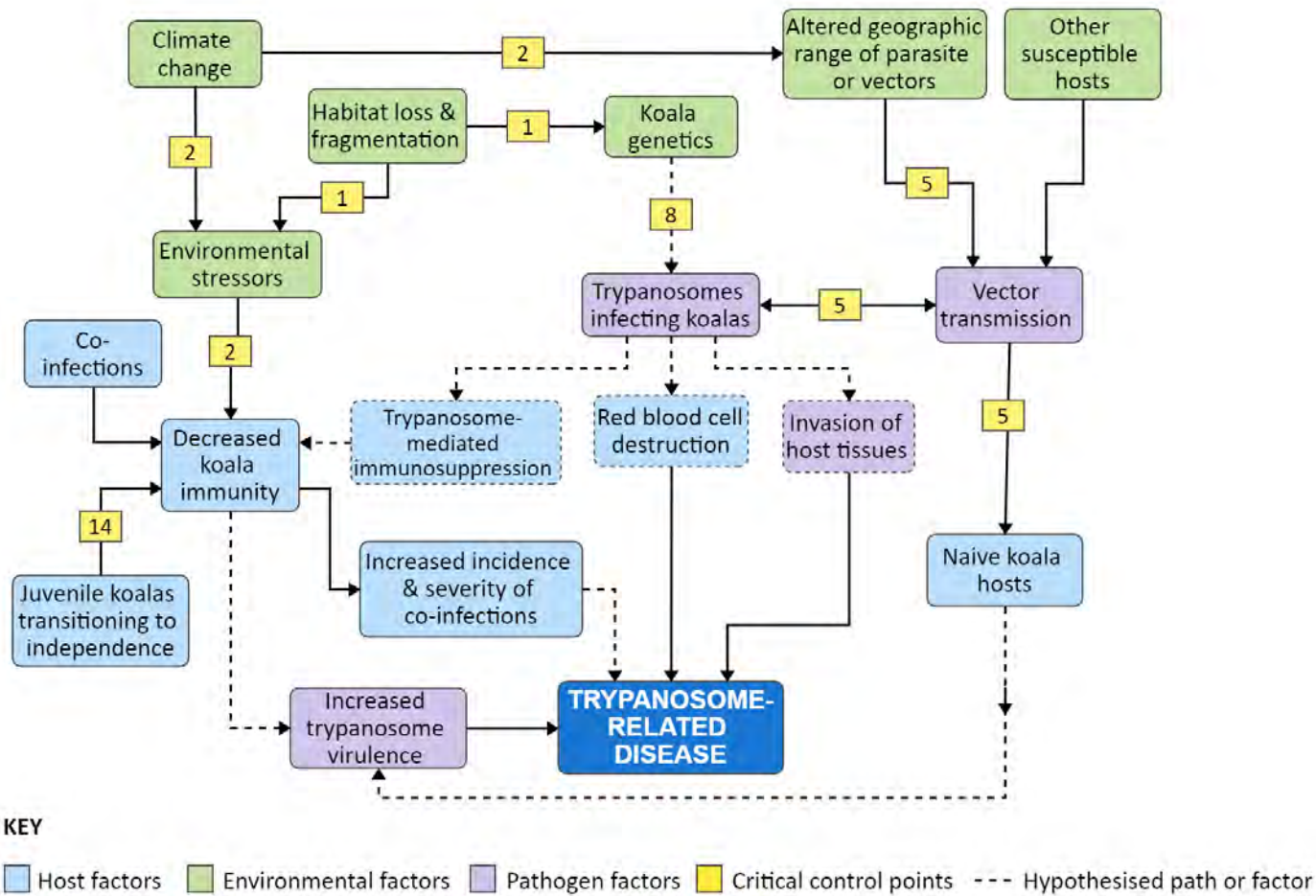


Figure 17 Hazard pathways and critical control points for trypanosome infection in koalas

Critical control points

CCP1 Habitat loss, fragmentation and reduction in quality

Habitat loss and reduction in habitat quality are major environmental stressors due to the koala's reliance on particular tree species for food, shelter and thermoregulation [19, 20]. Habitat fragmentation reduces connectivity between suitable koala habitats, forcing dispersal and potentially contributing to the loss of important genetic alleles in populations that can no longer interbreed [21-23]. Causes include urban expansion, land clearing for production, inappropriate fire management practices, the introduction of pests, weeds and plant pathogens, over-browsing by koalas as a result of local overcrowding, and the habitat-altering effects of climate change [24-30].

CCP2 Environmental stressors

Stressors in the koala's environment (in the wild or in captivity) contribute to general debilitation of the host, reduced immune function, and increased susceptibility to disease or severity of disease expression [20, 31-33]. Major environmental stressors for koalas include

habitat loss, fragmentation and reduction in quality; nutritional stress; climate change; extremes of weather and disturbance related to human activities [19, 34].

Environmental stressors may contribute to general debilitation and reduced immune function and may increase the likelihood of co-infections.

Trypanosome-related disease may be most likely to arise where immunosuppression occurs due to other stressors [35]. Changing climate may result in an increased or altered geographic range for trypanosomes or their vectors in Australia.

CCP5 Vectors

Some infectious diseases are transferred to the vertebrate host (in this case, the koala) by vectors. Koalas that are exposed to potential vectors (e.g. ticks, other invertebrates) may be at higher risk of infection with trypanosomes [12, 36].

CCP8 Genetics

The individual koala may have a genetic predisposition to the development of disease or increased severity of disease. Improving or at least maintaining genetic diversity in koala populations is likely to conserve adaptive potential [37] and encourage the retention of the most robust koala genetic profiles to avoid disease consequences of trypanosome infection.

CCP14 Concurrent infections and debilitation

Concurrent infections and debilitation can compromise immune function and general health in ways that increase the risk or severity of disease. The pathogenicity of an infectious hazard may be potentiated by co-infections.

Outside of Australia, infection with trypanosome species has been associated with severe, sometimes fatal, disease in the presence of co-infections [10]. It has been suggested that co-infection of *Trypanosoma* spp. with pathogens such as *Chlamydia* and KoRV may be associated with poor health and decreased survival of koalas [4].

Likelihood assessments

Trypanosomes are not transmitted directly between hosts [5] and there is no evidence that Australian trypanosomes are zoonotic [4, 6]. Therefore, the likelihood of entry and exposure for trypanosomes was considered only for koalas, and not for humans or other species.

Entry assessment

Trypanosomes have not been reported in koalas outside NSW and Qld, but very little testing of southern koalas has been undertaken for this pathogen. Most trypanosome species infecting koalas are present in other Australian mammals, including marsupials, bats and rodents [2, 4, 6-13] and have been detected in other parts of the koala's geographic range (see *Appendix 5.13 Trypanosoma spp. - Literature Review*).

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, the likelihood of trypanosomes entering, or being present, in a koala population is considered **HIGH**.

Exposure assessment

The prevalence of trypanosomes in koala populations is generally not known. Almost 74% of koalas in one Qld study were infected with at least one trypanosome species [10]. The means by which koalas acquire trypanosome infection is not known.

Based on the review of available information and using Table 7 in *Appendix 2.3 Risk Assessment Methods*, and applying the precautionary principle, the likelihood of exposure to trypanosomes for an individual koala is considered **HIGH**.

Using the principles of combining entry and exposure likelihood as outlined in *Appendix 2.3 Risk Assessment Methods*, the combined likelihood of entry and exposure for trypanosomes in koalas is considered **HIGH**.

Consequence assessments

Most koalas infected with trypanosomes are clinically healthy and the majority of wildlife trypanosomes are considered benign to their vertebrate hosts [1, 6, 10]. The presence of trypanosomes in koalas has been associated with poor body condition, poor survival in rehabilitation, severe anaemia and (in rare cases) neurological disease, but no causality has been shown [1, 10, 38].

Koala population resilience and viability

There are no data to show that the presence of trypanosomes in koalas has a population level effect. Most koalas with trypanosomes are considered clinically healthy and most trypanosomes in wildlife are considered benign.

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of trypanosomes to koala population resilience and viability is considered **NEGLIGIBLE**.

Koala individual health and welfare

Most koalas infected with trypanosomes are clinically healthy and the disease states associated with trypanosome infection in koalas appear to be uncommon [1, 10, 38]. There are no data on the proportion of koalas infected with trypanosomes that display signs of ill health, but based on clinical reports, it is assumed to be very low.

Based on the review of available information and using Table 8 in *Appendix 2.3 Risk Assessment Methods*, the consequence of trypanosomes to koala individual health and welfare is considered **MINOR**.

Overall risk estimate

Since the consequence of trypanosome infection was evaluated as negligible for koala population resilience and viability, the estimate of risk for this hazard for koala populations was also considered **NEGLIGIBLE** (see *Appendix 2.3 Risk Assessment Methods*). Note, however that the level of confidence in this risk estimate (see below) is **LOW**.

The overall risk of trypanosomes to individual koala health and welfare, as defined using Table 9 in *Appendix 2.3 Risk Assessment Methods*, is **MODERATE**.

This assessment exceeds the acceptable risk thresholds as outlined in *Appendix 2.3 Risk Assessment Methods* for individual koalas, therefore risk management for trypanosomes is recommended.

However, the level of confidence in this assessment is **LOW** (see below) and there are significant gaps in knowledge concerning the impact of this parasite in koalas. It is recommended that this assessment be revised when new information becomes available.

Level of confidence in risk assessment

The level of confidence in the likelihood assessment is **LOW** as many koala populations have not been tested for trypanosomes and the method by which koalas acquire trypanosome infection is not known. The level of confidence in the consequence assessment is **LOW** as there is no clear understanding of the possible impacts of trypanosome infection on either individual koalas or populations. Therefore, the confidence in the risk estimate for trypanosomes is considered **LOW**.

5.13.5 Risk mitigation options

Few risk mitigation measures have been identified for trypanosomes. Further information and justification can be found in the *Treatment and Prevention and control* sections of the relevant literature review chapter. Not all risk mitigation options will be currently available, feasible, universally applicable or cost-effective, but are listed here for completeness.

Conserve and improve the quality, quantity, diversity and complexity of koala habitat (CCP1 and CCP8)

- Re-vegetation, restoration and preservation of habitat, including in urban landscapes.
- Strengthen regulatory controls against habitat clearing and road development in koala habitat and dispersal corridors.

These measures will reduce the impact of environmental stress on trypanosome disease expression and encourage the maintenance of larger wild koala populations, so that koalas have the most robust genetic profiles for avoiding disease consequences of trypanosome infections.

Manage concurrent infections, debilitation and environmental stressors (CCP2 and CCP14)

- Minimise concurrent infections, debilitation and other environmental stressors (e.g. predators, disturbance related to human activities) in individual koalas and populations, to reduce the risk of pathogenic consequences of trypanosome infections and co-infections.

Manage risk of vector transmission (CCP5)

- Undertake vector control either on the animal or in the koala's environment, once vectors for trypanosomes infecting koalas are confirmed.

Maintain genetic diversity and population sizes in koalas (CCP8)

- Support genetic diversity in koala populations to encourage the retention of the most robust koala genetic profiles for avoiding possible disease consequences of trypanosome infection.

5.13.6 Recommendations

Hazard-specific recommendations

The following recommendations are specific to this hazard:

- Clinically abnormal cases where trypanosomes are identified should receive a full work-up to identify associations with other causes of illness or mortality.
- Limiting exposure to vectors may be indicated for koalas in captivity and rehabilitation.

General recommendations

The following general recommendations apply to all 13 koala disease hazards identified for detailed risk assessment:

- Evaluate and prioritise the identified *Risk Management Options* for feasibility, completeness and effectiveness at a national KDRA Implementation Workshop.
- Identified knowledge gaps for this hazard should be prioritised and developed into a *Research Plan*.
- Prioritise protection and improvement of koala habitat as a key risk mitigation process for koala disease.
- Improve national population estimates and ensure ongoing population health monitoring for koalas.
- Develop robust, longitudinal and nationally-agreed data sets on causes of illness and death in free-living koalas.
- Maintain or increase genetic diversity and population size for koalas, so that koalas have the most robust genetic profiles for avoiding disease consequences.
- Minimise factors that might lead to stress in the koala's environment (in the wild or in captivity).
- Minimise co-infections and other causes of debilitation to reduce cumulative risk of disease.
- Increase awareness and implementation of good biosecurity practices (in accordance with the *National Wildlife Biosecurity Guidelines* [39]) when interacting with koalas during field work, treatment and rehabilitation.

- Develop clinical and research collaborative working groups to establish nationally-agreed best practice protocols for koala disease diagnosis, treatment and biosecurity and implement these in collaboration with managers of koala populations across all jurisdictions.
- Develop collaborative relationships between managers of koala populations (state and Commonwealth) and koala clinical and research working groups, including co-participation in the Koala Recovery Team.
- Develop communication tools, using the iconic status of the koala, to raise awareness and encourage behaviour change around factors contributing to disease (e.g. climate change, vehicle driver behaviours, dog ownership and management, land clearing, fire management).

5.13.7 References

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7 Other Disease Hazards

7.1 Clinical Syndromes with Undefined or Multiple Aetiologies

The hazard refinement process (see *Appendix 2* for methodology) identified three disease hazards of koalas that did not lend themselves to the formal IUCN risk assessment process, because they lacked a clear single aetiology and case definition:

- wasting syndromes
- gut dysbiosis
- putative KoRV-associated disease syndromes.

These hazards are significant health issues for koalas, due to their impact on the rehabilitation process for koalas and the knowledge gaps in understanding of their impact. Their significance for koala health is summarised in this section.

7.1.1 Wasting syndromes

Terms such as “ill thrift” and “koala stress syndrome” are commonly used to describe the lethargy, depression and inappetence that characterise the sick koala [1-3]. With an improved understanding of predisposing mechanisms for these conditions, and in the context of this report, the term “wasting” is used to refer to debilitation associated with loss of appetite and body condition in koalas.

Koalas are susceptible to debilitating loss of body condition, because they have limited fat reserves and their eucalypt diet is intrinsically low in nutrients [4, 5]. Once koalas have lost body condition, it is difficult for them to regain it, and they may progress rapidly to severe emaciation and death [1, 6, 7].

Factors which can lead to wasting include:

- Nutritional stress caused by inadequate or poor quality habitat, where the availability of good quality nutrition is compromised [5, 8, 9].
- Dental attrition and disease which reduces digestive efficiency [10, 11].
- Sources of physiological stress such as water deprivation [12].
- Debilitation and other diseases that lead to inappetence [13].
- Long-term hospitalisation, frequent interventions and treatments [1].

Figure 18 shows a koala with signs of wasting. A diagram showing the contributing factors for wasting in koalas is provided in Figure 19.



Figure 18 A koala in rehabilitation showing signs of wasting (credit: Adelaide Koala and Wildlife Centre)

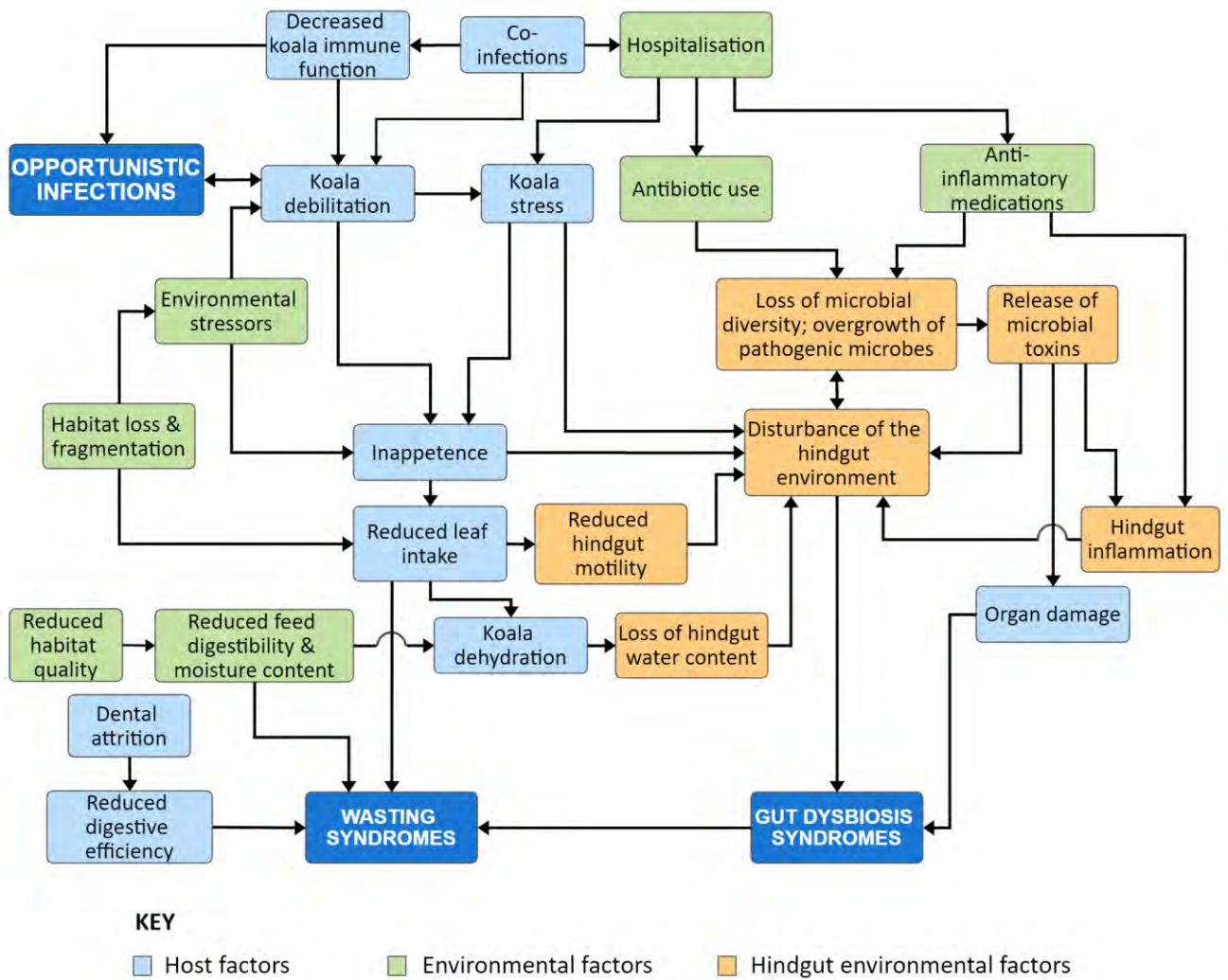


Figure 19 Key contributing factors for wasting syndromes, gut dysbiosis syndromes and opportunistic infections in koalas

Wasting syndromes may increase the severity of disease and have negative impacts on rehabilitation success. The likelihood of some non-infectious disease hazards (such as predator attack trauma) may be increased in koalas with wasting if their debilitation causes them to come to ground [6, 7]. Wasting is commonly associated with other infectious diseases hazards in koalas, although it is often difficult to determine whether loss of body condition is the cause or the effect of concurrent illness [7].

7.1.2 Gut dysbiosis syndromes

Koalas are hindgut fermenters, which means food is mostly digested by microbial fermentation in the large intestine and caecum [5]. The koala hindgut contains a diverse population of bacterial and fungal microbes that help to digest and detoxify the eucalypt diet [14, 15]. Circumstances that adversely affect the hindgut environment (e.g. changes in hindgut motility, water content, microbial number and diversity) disturb the balance and function of these microbes, leading to a spectrum of disorders collectively termed “caecocolic dysbiosis and typhlocolitis syndrome” [16]. For the purposes of this report, these disorders are collectively referred to as “gut dysbiosis”.

A range of factors can disturb the koala hindgut environment and lead to gut dysbiosis. Key factors include:

- Physiological stress, particularly stress associated with hospitalisation [16].
- Antibiotic medication, which can reduce hindgut microbial diversity and encourage the overgrowth of pathogenic microbes [17].
- Combining steroidal and non-steroidal anti-inflammatory medication, which may affect the function and health of the koala hindgut [16].
- Nutritional stress, such as reduced quality and hydration status of browse [18].
- Wasting, as hindgut environment and function are compromised when food intake is reduced [16].
- Dehydration [16].

Conditions arising from dysbiosis can range from mild disease (such as inappetence) and minor opportunistic microbial overgrowth (such as mild candidiasis) through to severe illness, such as hindgut ulceration, haemorrhage, perforation and severe loss of intestinal motility. Severe gut dysbiosis is commonly fatal in koalas [16].

Gut dysbiosis is a potential sequel for all conditions that cause stress and disease in koalas, particularly when these are associated with the use of antibiotics or prolonged captive care. Figure 19 shows the contributing factors for gut dysbiosis in koalas.

7.1.3 Putative KoRV-associated disease syndromes

Koala retrovirus (KoRV) poses risks to koala health through the effects of its insertion into the koala genome, which in broad terms leads to increased mutagenic and immunomodulatory effects (see *Appendix 5.4 Koala Retrovirus – Literature Review*). The link

between mutagenic effects of KoRV and neoplasia is relatively well defined [19] and is discussed in detail in the *Neoplasia – Literature Review*. The replication of KoRV in the koala’s white blood cells is associated with a range of negative impacts on immune cell function [20, 21] which could manifest in a variety of ways, depending on factors such as the presence of co-infections and associated disease states.

Terms such as “koala immunodeficiency syndrome” (“KIDS”) and “KoRV koala” have been coined in an attempt to summarise the wide range of signs associated with poor general health, high incidence of opportunistic infections and poor response to treatment which are presumptively attributed to immunodeficiency resulting from KoRV [22].

Clinicians and researcher have recognised the need to develop a clear case definition, based on clinical signs and laboratory testing, for KoRV-associated immunosuppressive disease syndromes. It has been suggested that it may be possible to identify a group of risk factors which are likely to indicate that KoRV is involved in the manifestation of disease. The identification of such risk factors may help to determine the role of KoRV in a clinical case [23].

The pathways leading to KoRV-associated disease are illustrated in Section 5.4 *Koala Retrovirus – Risk Assessment*.

7.2 Opportunistic Infections

Koalas are susceptible to opportunistic microbial infections that may occur when they become immunocompromised as a result of physiological stress, in the presence of immunosuppressive pathogens such as KoRV and herpesviruses, or which arise due to adverse environmental conditions. Figure 19 shows the contributing factors for opportunistic infections in koalas.

Opportunistic infections may cause health complications for individual koalas during rehabilitation. Multiple cases of opportunistic infections in a free-ranging population can have a disproportionate impact on small or fragmented free-ranging populations, as has been seen with *Chromobacterium violaceum* (see below) [24].

The risk mitigation options identified in the *Risk Assessment* chapters are likely to also be applicable for opportunistic infections. Examples of some opportunistic infections are given below.

7.2.1 Candidiasis

Candidiasis, caused by *Candida* fungal species (usually *C. albicans*) is a very common opportunistic infection of hospitalised and hand-reared koalas [13, 16]. Likely contributing factors include antibiotic use, poor hygiene, inappropriate diet, sudden diet transitions, gut dysbiosis, debilitation from concurrent disease, and physiological stress. The most common sites of candidiasis are the oral cavity and hindgut [16]. Candidiasis in koalas is amenable to

treatment in the early stages, but advanced cases carry a poor prognosis, so early detection and vigilance are necessary in the captive and rehabilitation environments [16, 25].

7.2.2 Opportunistic bacterial infections

A range of bacteria have been reported to cause opportunistic infections among koalas in the wild, in captivity or in rehabilitation [13, 16, 24, 26]. Many of these have been associated with outbreaks of disease, some resulting in high morbidity and mortality. Cases are commonly associated with environmental stressors such as overcrowding, poor hygiene, cold or wet weather and co-infections. Examples include:

- *Bordetella bronchiseptica* causing pneumonia [27, 28].
- *Pseudomonas aeruginosa* causing multisystemic disease and mortality [29].
- *Salmonella* spp. causing septicaemia and sudden death [13, 25].
- *Chromobacterium violaceum* causing septicaemia (see below) [24].

Chromobacterium violaceum septicaemia

Heavy rainfall in summer and autumn of 2022 resulted in extensive flooding in northern NSW and south-east Qld [30]. Koala populations at Petrie and Coomera in south-east Qld were among many that experienced sustained heavy rainfall over several months. Both populations experienced acute mortalities during the flooding, resulting in the deaths of over 10% (n=13) of koalas in the Petrie population over a two week period and 5% (n=4) of the koalas in the Coomera population over approximately three months [31].

Chromobacterium violaceum, a water-borne bacterium, was identified as a cause of septicaemia and acute death in five of these cases [31]. These koala populations had retreated to low-lying riparian habitat due to the loss of more suitable habitat, and were probably in contact with wet conditions for a protracted period during the flooding [24]. Other water-borne pathogens (*Aeromonas* and *Plesiomonas* spp.) were also identified, but this was the first known identification of *C. violaceum* in koalas. Both populations were monitored (in the case of the Petrie population, for several years) and koalas were otherwise considered healthy and in good condition [24]. This example illustrates the vulnerability of fragmented koala populations to acute disease events and opportunistic infectious agents.

7.3 Recommendations

The following are recommendations for the management of the other disease hazards discussed in this section.

- Develop standardised, nationally-agreed terminology and case definitions for wasting syndrome, gut dysbiosis syndrome and putative KoRV-associated disease syndromes that are reviewed regularly as more information and knowledge comes to hand.

- Develop nationally-agreed guidelines for veterinarians and rehabilitators on the use of antibiotics in koalas that clearly summarise the risks and risk mitigation strategies.

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8 Key Knowledge Gap Summary

8.1 Koala Population Data

- More comprehensive and accurate data on the size and location of koala populations is required [1]. The assessment of disease impacts on populations is dependent on the quality of these data. The National Koala Monitoring Project (see Section 2.6 *Aligned Research Programs*) will be instrumental in addressing this knowledge gap.

8.2 Epidemiology

- The relative importance of disease (both generally and for specific disease hazards), among other threatening processes in koala populations, is incompletely understood and needs more investigation [1-5].
- Robust, longitudinal data sets on causes of morbidity and mortality in free-living koalas throughout their range are required. This will contribute to understanding of prevalence and population level impacts of various diseases. Compilation of data sets occurs in Qld and NSW at a state level [6, 7] but is lacking in Vic and SA.
- Basic epidemiological understanding is lacking for a number of infectious hazards in koalas. This includes KoRV, *Cryptococcus* spp., novel *Actinomyces* sp., phascolarctid herpesviruses, sarcoptic mange and *Trypanosoma* spp.. Improved epidemiological knowledge for these disease hazards will enhance the level of certainty of risk assessments.
- Co-infections with KoRV, phascolarctid herpesviruses and trypanosomes are suspected, but not confirmed, to affect the severity of disease caused by other hazards. Verification of this including an understanding of the mechanisms of causation and their degree of influence on disease state relative to other drivers is needed.

8.3 Genetics

- The role of koala genetics in disease susceptibility and expression is poorly understood for many disease hazards, particularly those demonstrating marked regional differences in clinical presentation and prevalence such as KoRV, *Chlamydia*, oxalate nephrosis and novel *Actinomyces* sp. (see Section 5 *Risk Assessments for Selected Hazards* for further information).

8.4 Koala Disease Management

- Nationally-agreed guidelines and protocols are required for many aspects of disease management in koalas, including diagnosis; triage, assessment and prognostic indicators; treatment and care; and record-keeping. Specific hazards with an identified need for such nationally-agreed guidelines and protocols include *Chlamydia*, heat stress, KoRV, motor vehicle trauma, predator attack trauma, sarcoptic mange and

thermal burns (see Section 5 *Risk Assessments for Selected Hazards* for further information).

- The short- and long-term outcomes of rehabilitation require ongoing evaluation and analysis to better inform decisions on triage, assessment and treatment, and assist in refinement of guidelines and protocols for disease hazards (see e.g. [8, 9]).
- Pharmacokinetic studies and clinical trials are required for therapeutic agents that may assist in the treatment of disease in koalas. Specific needs are identified in risk assessment chapters for *Chlamydia*, KoRV, cryptococcal disease, sarcoptic mange and oxalate nephrosis.
- Non-antimicrobial treatment options for disease prevention and control require exploration. In particular, there is a need for further work on vaccination options for KoRV and *C. pecorum*.

8.5 References

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9 Conclusions

The KDRA is the first disease risk analysis to examine disease risk for an Australian wildlife species at the national level. The nationwide approach was made possible with the enthusiastic participation and engagement of a range of stakeholders who each brought their unique perspective to evaluation of disease in this iconic species. The ongoing momentum of good will and collaboration between stakeholders will be an essential factor in implementing the findings of the KDRA and enabling the achievement of the shared vision for koala health and welfare.

The comprehensive, evidence-based threat analysis process clearly identified the links between disease and other drivers of koala endangerment. Through its recognition of the inter-relatedness of koala threats, the KDRA has identified the need for a more unified approach to koala conservation throughout Australia. The KDRA will be a resource for integration of koala health and disease actions into broader conservation efforts through ongoing alignment with other national initiatives.

The KDRA process has systematically identified multiple gaps in current scientific knowledge of koala disease. Further research should address these gaps, to enable further refinement of risk assessments and their level of confidence.

Many risk management options identified through the KDRA have the capacity to mitigate multiple disease risks to koalas, particularly options to preserve, increase and restore habitat and habitat connectivity. An Implementation Planning workshop, now in preparation, will prioritise these options according to their feasibility, applicability and cost-effectiveness.

NATIONAL KOALA DISEASE RISK ANALYSIS - APPENDICES



Version 1.1. August 2022

This document contains the Appendices to the *National Koala Disease Risk Analysis Report* (Version 1.1. August 2022) and should not be considered as a stand-alone document. Refer to the parent document for Acknowledgments, Dedication, Disclaimer and Suggested Citation.

Cover photo: two wild koalas recovering in a rehabilitation facility (credit: Yasmine Muir)

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Appendix 1 Contributors to the Koala Disease Risk Analysis Project

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Tim Portas	RSPCA Queensland
Tracey Wilson	Mosswood Wildlife
Vickii Lett	NSW Wildlife Information, Rescue & Education Service (WIRES)

1.4 Stakeholders in Koala Health

Stakeholders in koala health include people with knowledge relevant to koala health, people who direct conservation action associated with koala health, and those who are affected by those actions. Below are the 193 institutional and government agency stakeholders in koala health. They were kept informed of the progress of the KDRA through a website dedicated to the project (<https://sites.google.com/view/koaladra/home>).

1300KOALAZ

ACT Dept. Environment, Planning & Sustainable Development

Adelaide Bird & Exotics Vet

Adelaide Koala & Wildlife Centre

Adelaide Koala Rescue

AgForce Queensland

Animal Health Australia
Animal Welfare NSW
Animals Australia
Association of Mining & Exploration Companies
Atlas of Living Australia
Aust Wildlife Health Institute
Australia & New Zealand College of Veterinary Scientists
Australia Zoo Wildlife Hospital
Australia Zoo Wildlife Hospital
Australian Bluegum Plantations
Australian Coal Association
Australian Companion Animal Council
Australian Conservation Foundation
Australian Environmental Defenders Office Network
Australian Forest Growers/Institute of Foresters of Australia
Australian Forest Products Association
Australian Forests & Climate Alliance
Australian Institute of Petroleum
Australian Koala Foundation
Australian Livestock & Property Agents Association
Australian Museum
Australian National Council for Fire & Emergency Services
Australian National University
Australian Petroleum Production & Exploration Association
Australian Regional Tourism Network
Australian Registry of Wildlife Health
Australian Tourism Industry Council
Australian Veterinary Association (AVA)
Australian Wildlife Conservancy
Australian Wool Innovation
AVA Veterinary Conservation Biologists Special Interest Group
Bangalow Koalas NSW
BioLink Australia
Bush Heritage Australia
Byron Bay Wildlife Hospital
Central Qld University
Chief Environmental Biosecurity Officer
Chief Veterinary Officers - Commonwealth & State
Clarence Environment Centre
Climate Council of Australia
Coffs Harbour & District Local Aboriginal Land Council

Community Environment Network
Conservation Ecology Centre, Cape Otway
CSIRO
Currumbin Wildlife Sanctuary
Deakin University
Department of Climate Change, Energy, the Environment & Water (formerly Department of
Agriculture, Water & the Environment)
Department of Environment & Science Qld
Disney Animal Kingdom
Dreamworld
Dubbo Zoo
Dutch Thunder Wildlife Shelter
Ecological Society of Australia
EcoPlan Australia
Endeavour Veterinary Ecology
Eurobodalla Koala Project NSW
Federation University
Fire Rescue Victoria
First Nations Legal & Research Services
Forest & Wood Products Australia
Forest Industry Advisory Council SA
Forestry Industry Council Vic
Foundation for Australia's Most Endangered Species
Foundation for National Parks & Wildlife
Frasers Property Australia
Friends of the Earth Australia
Friends of the Koala Inc. NSW
Grain Growers Ltd
Gunaikurnai Land Council
Healthy Land & Water
Higashiyama zoo
Horticulture Innovation Australia
Indigiscapes Centre
International Fund for Animal Welfare
IUCN
IUCN SSC Australasian Marsupial & Monotreme Specialist Group
Jali Local Aboriginal Land Council
James Cook University
Koala & wildlife hospital Northern Rivers, Byron Bay, Lismore
Koala Action Group Qld Inc.
Koala Clancy Foundation

Koala Conservation Australia
Koala Gardens, Tuckurimba, NSW
Koala Island Foundation Vic
Koala Life SA
Koala Recovery Partnership
Koala Rescue Qld
Koala Rescue SA
Kogarah Residents Association, NSW
Land for Wildlife south-east Qld
Land for Wildlife Victoria
Local Government Associations of Qld, NSW, Vic & SA
Lone Pine Koala Sanctuary
Magnetic Island Koala Hospital
Mary River Catchment Coordinating Committee
Milton Thomas Group
Minerals Council of Australia
Moreton Bay Koala Rescue Inc
Mornington Peninsula Koala Conservation Vic
Mosswood Wildlife
National Association of Forest Industries
National Environmental Science Program
National Farmers Federation
National Geographic
National Indigenous Australians Agency
National Landcare Australia
National Parks & Wildlife Service SA
National Parks Association: Qld, NSW & ACT
National Timber Councils Association
National Zoo & Aquarium
Natural Resources Commission NSW
Nature Conservation Council NSW
North Coast Environment Council NSW
North Qld Land Council
Northern Rivers Wildlife Hospital
NSW Aboriginal Land Council
NSW Biodiversity Conservation Trust
NSW Department of Planning, Industry & Environment
NSW Farmers Association
NSW Rural Fire Service
NTSCORP Ltd
OWAD Environment

Perth Zoo
Port Macquarie Koala Hospital
Port Stephens Koala Sanctuary
Prime Minister & Cabinet Regional Network
Property Council of Australia (Qld)
Qld Farmers Federation
Qld Fire & Emergency Services
Qld Resources Council
Qld South Native Title Services
Qld Tourism Industry Council
Qld Trust for Nature
Qld University of Technology
Reptile Rehabilitation Qld Inc.
Responsible Wood
Royal Zoological Society NSW
RSPCA NSW
RSPCA Qld
SA Country Fire Services
SA Department of Environment & Water
SA Dept Primary Industries & Regions
SA Metropolitan Fire Services
SA Native Title Services
San Diego Zoo
Saunders Havill Group
Save Mt Gilead Inc. NSW
Science for Wildlife
South West Fibre
Southern Koala Rescue
Sydney Metropolitan Wildlife Service
Taronga Zoo
Taronga Zoo Conservation Society
The Nature Conservancy, Australia
Timber Communities Australia
Timber NSW
Timber Qld
Torres Strait Regional Authority
Tourism & Transport Forum Australia
Tourism Industry Council SA
Tourism Industry Council, ACT & Region Incorporated
University of Adelaide
University of Melbourne

University of Newcastle
University of Nottingham
University of Queensland
University of Southern Queensland
University of Sydney
University of the Sunshine Coast
University of Western Sydney
Urban Development Institute of Australia
Vets Beyond Borders
Vic Department of Environment, Land, Water & Planning
Vic National Parks Association
Vic Tourism Industry Council
WA Dept Biodiversity, Conservation & Attractions
Wildlife Health Australia wildlife coordinators
Wildlife Disease Association - Australasia
Wildlife Drones
Wildlife Health Australia (WHA)
Wildlife Preservation Society of Qld
Wildlife Victoria
WIRES
World Wide Fund for Nature (WWF)
Zoo & Aquarium Association (ZAA)
Zoos SA
Zoos Victoria

Appendix 2 Koala Disease Risk Analysis Project Methods

2.1 Koala Disease Risk Analysis Stakeholder Consultation and Communication

The Koala Disease Risk Analysis (KDRA) was developed within a collaborative, multi-stakeholder-inclusive workshop environment. Published and unpublished information was combined with expert opinion to reach the best consensus of knowledge and provide direction for both immediate action and targeted research.

The KDRA Project Team established and worked in collaboration with a 17-member Expert Advisory Group (EAG) which assisted in the design, development, and progress review of the KDRA through a series of four online meetings (*Appendix 1.2*). The EAG provided a consultative and collaborative forum for discussion, as well as technical and strategic review of, and input into, draft components of this report.

The KDRA was supported by the expertise of 24 additional selected stakeholders who formed a group of “Workshop Stakeholders” along with the EAG (*Appendix 1.3*). The Workshop Stakeholders participated via a series of six additional facilitated online project workshops, to provide additional perspectives from the large community of people with interest or expertise in koala health and conservation.

Due to constraints on face to face meeting opportunities caused by the COVID-19 pandemic, all workshops and meetings were undertaken in an online environment. The KDRA workshops and EAG meetings were structured, facilitated sessions through which the Project Team drew on the combined knowledge, expertise and understanding of the stakeholders to increase the level of certainty of the risk analysis, obtain consensus on the priority health hazards and identify means of managing associated risks. Meetings were conducted via Microsoft Teams, and the online collaborative tool MURAL[®] was used as an interactive platform. The timeline and topics for the various workshops and meetings are shown in Table 6. Figure 20 is an example of the MURAL[®] workspace.

Table 6 KDRA stakeholder schedule of workshops and meetings

Group	Date	Duration	Subjects discussed
Expert Advisory Group	2 Nov 2021	1.5 h	Introduction to KDRA and EAG
Stakeholder Workshop	16 Nov 2021	2 h	Introduction to KDRA
Expert Advisory Group	17 Nov 2021	2 h	Threat analysis and hazard review Part I
Expert Advisory Group	23 Nov 2021	1 h	Threat analysis and hazard review Part II
Stakeholder Workshop	7 Dec 2021	2 h	Problem description, vision and measuring success
Stakeholder Workshop	14 Dec 2021	2 h	Acceptable risk Part I
Expert Advisory Group	3 Feb 2022	2 h	Risk assessment
Stakeholder Workshop	17 Feb 2022	2 h	Acceptable risk Part II
Stakeholder Workshop	5 Apr 2022	2 h	Hazard pathways and critical control points
Expert Advisory Group	21 April 2022	2 h	Risk management
Stakeholder Workshop	26 May 2022	1.5 h	Overview and project completion

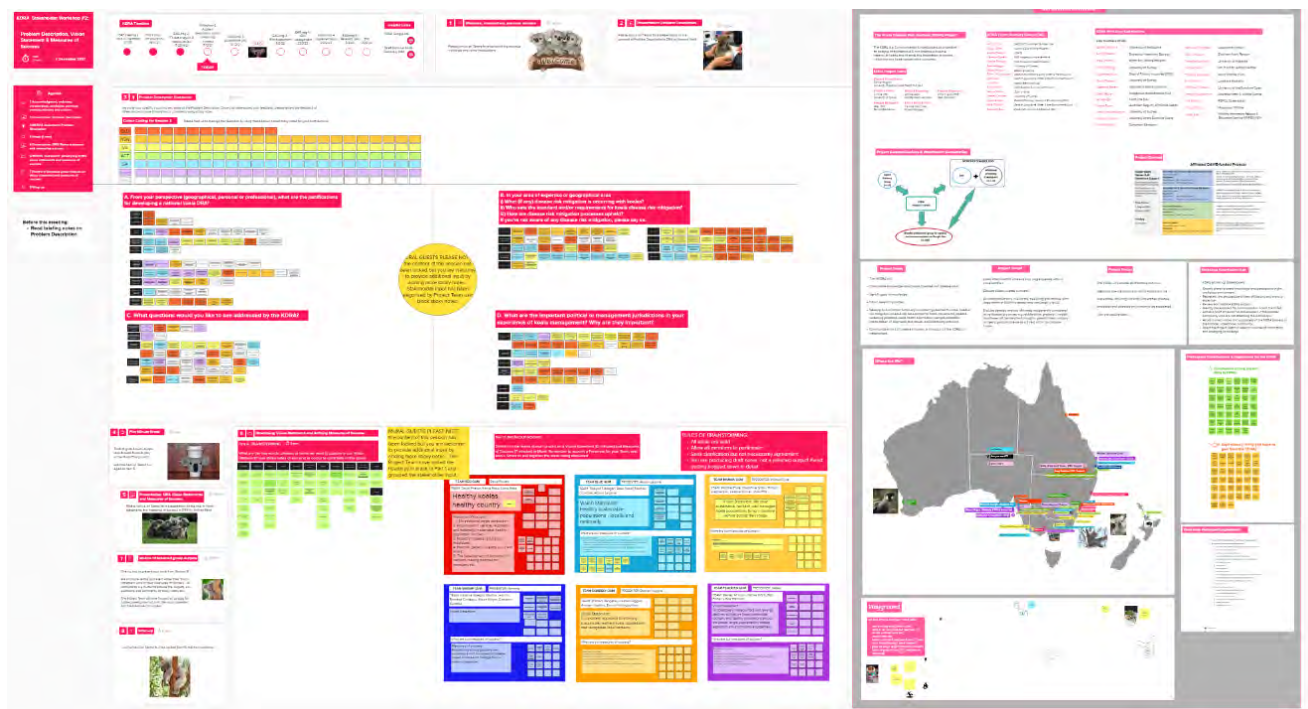


Figure 20 Sample of the MURAL® collaborative workspace used during KDRA stakeholder workshops and meetings

Additional Subject Matter Experts (SMEs) were identified as the project progressed, and invited to help in reviewing chapters of the KDRA relevant to their area of expertise.

The KDRA Project Team established a list of over 300 stakeholders in koala health, from almost 200 organisations and agencies (*Appendix 1.4*). The list included people with interest or expertise in koala health and conservation such as government officers, researchers, veterinarians, rehabilitators, Indigenous representatives, land use managers, non-government conservation representatives, primary industry proponents, communications advisors and policy makers. The development and progress of the KDRA was communicated to the stakeholders through a Google website dedicated to the project that was updated regularly as the project progressed (<https://sites.google.com/view/koaladra/home>). The website includes biographies of the project team and workshop participants, the KDRA bibliography and selected workshop presentations, the background on the project and information on the DRA process.

2.2 KDRA Hazard Identification and Refinement

2.2.1 Information sourcing

A process of literature review, stakeholder consultation and investigation of databases was implemented to collate and synthesise information on diseases of koalas. An online search for relevant peer-reviewed literature was conducted between September 2021 and March 2022 using the PubMed, Google Scholar and Web of Science (Clarivate Analytics) databases. Relevant literature was identified by searching with terms (“koala” OR “koalas”) AND a list of search terms compiled manually from citations and information in the following publications:

- *Current Therapy in Medicine of Australian Mammals* (Vogelnest and Portas 2019 [1])
- *Medicine of Australian Mammals* (Vogelnest and Woods 2008 [2])
- *Pathology of Australian Native Wildlife* (Ladds 2009 [3])
- National Recovery Plan for the Koala *Phascolarctos cinereus* (combined populations of Queensland, New South Wales and the Australian Capital Territory) (Australian Government Department of Agriculture and the Environment 2022 [4])

Publications were excluded if they discussed disease only relating to humans, non-mammals or non-Australian endemic mammals. Searches were conducted without limits on publication dates or geographical location.

The following disease surveillance databases were consulted in compiling information on koala disease:

- Wildlife Health Australia national electronic wildlife health information system database (eWHIS) (<https://wildlifehealthaustralia.com.au/ProgramsProjects/eWHIS-WildlifeHealthInformationSystem.aspx>): all cases in koalas, from beginning of database entry to 15 June 2022.

- Australian Registry of Wildlife Health (<https://arwh.org>): all cases in koalas, from beginning of database entry to 11 November 2021.

Cases reported to these databases represent a small proportion of the overall cases of disease occurring in koalas in Australia, and the datasets are not comprehensive nor spatially or temporally representative. They do not provide a representative picture of the current situation for disease in Australian koalas and there are limitations in the use of these data to make deductions regarding the specific risk of a disease hazard to koalas.

Additional literature and unpublished data were identified through discussion with the EAG and Workshop Stakeholders.

2.2.2 Hazard identification

A list of disease hazards of koalas (see Section 4 *Hazard Identification and Refinement* in KDRA report) was compiled using the information sources outlined in *Appendix 2.2.1*. The EAG and Workshop Stakeholders provided input into the development of the hazard list and confirmed the findings of the Project Team.

Diseases that were only mentioned in a single publication or as a single case in a koala were not included, if subsequent scientific evaluation called the significance of the original finding into doubt (e.g. *Notoedres cati* infestation reported in 1968 [5] but subsequently suspected to have been *Sarcoptes scabiei* infestation [3]). Disease reports were not included if they were isolated occurrences defined only in terms of pathologic abnormalities (e.g. meningitis; enteritis with inclusion bodies; volar hyperkeratosis) rather than causative processes. All other disease hazards that had been reported in more than one koala were included in the list.

2.2.3 Hazard refinement

Each identified disease hazard of koalas was assessed against the following criteria to determine the hazards to be included for detailed risk assessment:

- Known to occur in koalas in Australia.
- Known to cause disease in koalas.
- Known to cause significant population level disease in wild or captive koalas.
- A lack of epidemiological knowledge of the disease in koalas.

The criteria were applied in a stepwise series of questions as shown in Figure 21 (for Results, see Section 4 *Hazard Identification and Refinement*).

The EAG and Workshop Stakeholders provided input into the development of the refined list of disease hazards and confirmed the findings of the Project Team.

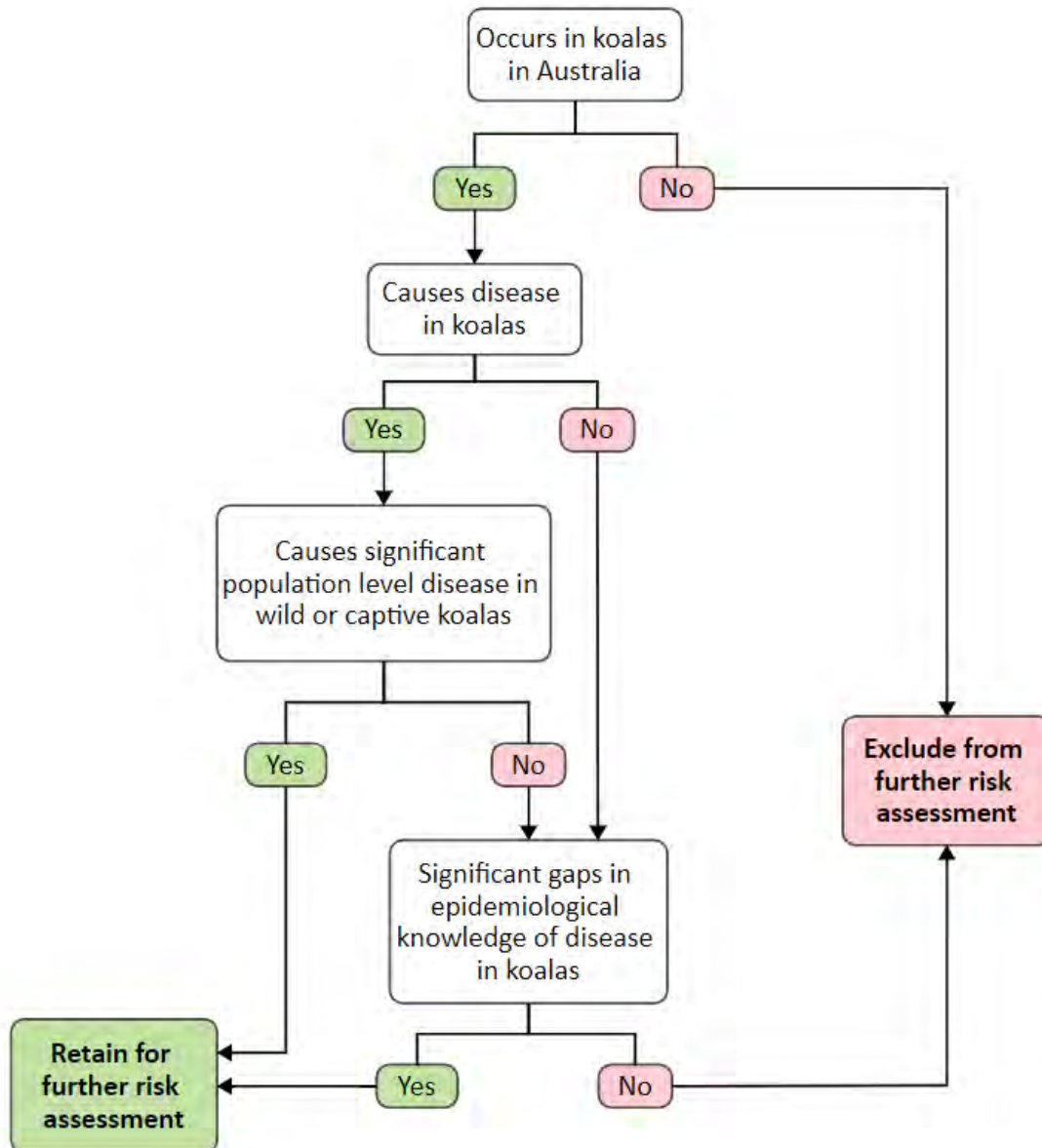


Figure 21 Disease hazard refinement decision tree for KDRA

2.3 Risk Assessment Methods

2.3.1 Hazard transmission pathways and critical control points

Hazard pathways are the means through which a disease hazard poses a risk to the target individuals or populations.

Critical control points (CCPs) are key points in the hazard’s biological pathway, where risk mitigation strategies could be implemented most feasibly and effectively, to reduce the overall risk to the species/population of concern. An understanding of the hazard pathways and CCPs enables consideration and evaluation of the most appropriate risk management options.

The first step in the *Hazard Risk Assessment* process for the KDRA involved the creation of a flow chart or model depicting the transmission pathways for each of the selected hazards. In consultation with stakeholders, hazard transmission pathways were scrutinised to identify CCPs, which were then described in the context of each hazard examined.

Nineteen CCPs were identified across all hazards taken forward for risk assessment, although not all CCPs were of relevance to each hazard. Table 4 in Section 6 *Critical Control Points by Disease Hazard* shows the CCPs identified across all hazards selected for detailed risk assessment.

2.3.2 Acceptable risk

Acceptable risk statements

Disease is a natural process and there will always be a level of illness and death in any population. This is recognized in disease risk analyses through a statement of ‘acceptable risk’ which provides guidance for decisions on how to use available resources to gain the maximum benefit for koala conservation.

Workshop Stakeholders debated the level of risk directly attributable to a disease they would accept on behalf of Australia’s koalas. When undertaking the risk assessments for each hazard these statements provided a threshold, above which risk management was recommended (see Table 10).

The acceptable risks defined for this disease risk analysis are:

For koalas:

- For any given wild population of koalas, the number of koalas in the population does not decrease, *as a result of disease*.
- Koala distribution in the wild does not decline from the current geographic distribution, *as a result of disease*.
- Less than 10% of koalas suffer from measurable ill-health at any one time, or as measured over 12 months, in any given free-ranging population, *as a result of disease*.
- Less than 5% of free-ranging koalas experience prolonged or severe negative animal welfare, as measured against recognised scientific criteria, *as a result of disease*.

For other animal species:

- A disease hazard associated with koalas does not present a previously unidentified risk of morbidity or mortality in another animal species.

For humans:

- A disease hazard associated with koalas does not pose a risk of an increase in morbidity or mortality in humans.

Assumptions and limitations of acceptable risk statements

In developing the acceptable risk statements, the following assumptions and limitations were recognised:

- Measuring the absolute number of free-living koalas is difficult, if not impossible, so acceptable risk should focus on trends within populations rather than overall numbers.
- Measuring certain aspects of ill health, such as infertility, can be difficult and may result in under-identification of populations suffering unacceptable risk as a result of disease.
- There are currently no recognised scientific criteria for the measurement of koala welfare in the wild.
- Although koala genetic diversity was recognised as an important facet of defining acceptable risk, there is currently insufficient knowledge of koala genetics to establish a meaningful acceptable risk statement. The sequencing of the koala genome is likely to result in a significant expansion of our understanding of the role of genetics in disease risks [6]. It will be critical to incorporate this understanding into an acceptable risk framework as this knowledge comes to hand.

2.3.3 Likelihood assessment

The following process was used to assess likelihood for each of the hazards taken forward for risk assessment.

The likelihood of a hazard occurring was determined from expert opinion supported by literature and database information.

The likelihood of a *non-infectious hazard* occurring was evaluated in a one step process.

The likelihood of an *infectious hazard* occurring was evaluated in a two-step process. First, evaluations were made of the *likelihood of entry* (also called “likelihood of release” [7]; refers to the likelihood of a hazard entering, or being present, in a specified environment or ecosystem) and *likelihood of exposure* (the likelihood of an individual encountering the hazard in the specified environment or ecosystem). The combined likelihood was determined to be the lowest of the two likelihood ratings for entry and exposure, recognising that the least likely step in a process will be the limiting factor for hazard likelihood. For example, if the entry and exposure likelihoods for a hazard were determined to be **HIGH** and **MODERATE** respectively, the combined likelihood for that hazard would be **MODERATE**.

Likelihood of entry of a hazard into a koala population was considered for the following pathways:

- koala population to koala population
- environment to koala population

- human population to koala population
- other animal population to koala population.

Likelihood of exposure of individual koalas to a hazard was considered for the following pathways:

- koala to koala
- environment to koala
- human to koala
- other animal to koala.

In addition, the *likelihood of an infected koala causing a hazard* to humans or other animals (via their direct or indirect exposure to koalas) was evaluated for contagious hazards.

Likelihood was evaluated as one of four categories as shown in Table 7.

Table 7 Categories and definitions of likelihoods [8]

Likelihood	Descriptive Definition
High	The event is highly likely to occur
Moderate	The event is moderately likely to occur
Low	The event is unlikely to occur but has been known to occur or may possibly occur
Negligible	The event will almost certainly not occur

If the likelihood for a hazard was evaluated as negligible, then the risk estimate for that hazard was classified as negligible and the risk analysis for the hazard was concluded at this point [7].

2.3.4 Consequence assessment

The consequences for a hazard were evaluated as one of four possible outcomes as shown in Table 8. If any one part of a more serious consequence definition was met, the more serious category was applied to the risk assessment. Measures of consequence were also evaluated against acceptable risk statements to determine the need for risk mitigation as noted above and shown in Table 8.

Hazard consequence was evaluated for all pathways where a non-negligible likelihood was identified. If a consequence was evaluated as negligible, then the risk estimate for that population was also negligible and was not considered further in the overall risk estimates for the hazard. If all consequences of a hazard were evaluated as negligible, then the risk analysis for the hazard was concluded at this point [7].

Table 8 Matrix for evaluating consequence, adapted from Cox-Witton et al. 2021 [9]

	Acceptable Risk Statements	CONSEQUENCE			
		Negligible	Minor	Moderate	Major
Koala population resilience & viability	<p>For any given wild population of koalas, the number of koalas in the population does not decrease as a result of disease.</p> <p>Koala distribution in the wild does not decline from the current geographic distribution as a result of disease.</p>	<p>No measurable decline in total population numbers. Isolated impacts in a single population.</p>	<p>Small declines in population numbers. Local short-term population loss. Low prevalence of koala illness or deaths in one or more populations.</p>	<p>Small to moderate population level effects with possible population extinctions. Illness or deaths in multiple populations.</p>	<p>Widespread, long-term overall population declines or high likelihood of population extinctions.</p>
Koala individual health & welfare	<p>Less than 10% of koalas suffer from measurable ill-health at any one time, or as measured over 12 months, in any given free-ranging population, as a result of disease.</p> <p>Less than 5% of free-ranging koalas experience prolonged or severe negative animal welfare, as measured against recognised scientific criteria, as a result of disease.</p>	<p>No detectable health and welfare impacts.</p>	<p>Koala illness with low health and welfare impacts.</p>	<p>Koala illness with moderate health and welfare impacts.</p>	<p>Significant koala illness with severe welfare impacts.</p>
Health & welfare of other species	<p>A disease hazard <u>associated with koalas</u> does not present a previously unidentified risk of morbidity or mortality in another animal species.</p>	<p>No detectable consequences.</p>	<p>Sporadic disease or welfare impacts; no population impacts.</p>	<p>Illness & death that may have population impacts on affected species.</p>	<p>Illness & death with significant population or welfare impacts on affected species.</p>
Human health & welfare	<p>A disease hazard <u>associated with koalas</u> does not pose a risk of an increase in morbidity or mortality in humans.</p>	<p>No detectable consequences in humans.</p>	<p>Rare, isolated cases (<1 per year).</p>	<p>Low number of cases (1-3 per year.)</p>	<p>Multiple cases (>3 per year).</p>

2.3.5 Overall risk estimate

The matrix in Table 9 was used to establish the overall risk estimate by combining the likelihood and consequence for each hazard.

Table 9 Matrix for combining likelihood and consequence to determine overall risk estimate. Adapted from Australian Government Department of Agriculture and Water Resources 2016 [10]

		CONSEQUENCE		
		Minor	Moderate	Major
LIKELIHOOD	High	Moderate	High	High
	Moderate	Low	Moderate	High
	Low	Low	Low	Moderate

A recommendation for risk management took into account the acceptable risk statements developed by stakeholders as shown in Table 10.

Table 10 Thresholds for determining the need for risk management based on stakeholders' agreed acceptable risk statements

	Acceptable Risk Statements	Threshold for Risk Management
Koala population resilience & viability	For any given wild population of koalas, the number of koalas in the population does not decrease as a result of disease. Koala distribution in the wild does not decline from the current geographic distribution as a result of disease.	Low (or greater) likelihood of: Small declines in population numbers. Local short-term population loss. Low prevalence of koala illness or deaths in one or more populations.
Koala individual health & welfare	Less than 10% of koalas suffer from measurable ill-health at any one time, or as measured over 12 months, in any given free-ranging population, as a result of disease. Less than 5% of free-ranging koalas experience prolonged or severe negative animal welfare, as measured against recognised scientific criteria, as a result of disease.	Low (or greater) likelihood of: Koala illness with low health and welfare impacts, based on an evaluation of current knowledge, including disease prevalence, distribution of clinical cases, severity of clinical signs, welfare impacts and mortality rates.
Health & welfare of other species	A disease hazard <u>associated with koalas</u> does not present a previously unidentified risk of morbidity or mortality in another animal species.	Sporadic disease or welfare impacts; no population impacts.
Human health & welfare	A disease hazard <u>associated with koalas</u> does not pose a risk of an increase in morbidity or mortality in humans.	Low number of cases (1-3 per year.)

2.3.6 Level of confidence in the risk assessment

The level of confidence in each aspect of the risk assessment (likelihood, consequence and overall risk) was evaluated for each hazard according to Table 11. The overall level of

confidence in the risk assessment for a hazard was taken as an average of all confidence assessments for that hazard.

Table 11 Rating scale for level of confidence. Adapted from Cox-Witton et al. 2021 [9]

DESCRIPTION	DEFINITION
High	Strong level of confidence in the assessment based on peer-reviewed scientific evidence which may be supported by unpublished reports provided by relevant experts.
Medium	Moderate level of confidence in the assessment. Limited peer-reviewed scientific evidence or unpublished reports provided by relevant experts. Some key knowledge gaps.
Low	Limited level of confidence in the assessment. Peer-reviewed scientific evidence and previous experience by experts is lacking. High degree of variation across the scenarios considered; high potential for variability in the outcomes. Significant knowledge gaps.

2.4 References

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Appendix 3 Hazard Refinement Outcomes and Rationale

Table 12 shows the detailed information collected for each disease hazard and the rationale for prioritising hazards for risk assessment (see decision tree in Figure 21, *Appendix 2*).

Table 12 Detailed information on each hazard and rationale for prioritisation

Hazard	Occurs in koalas in Australia	Causes disease in koalas	Significant population level disease in koalas?	Epidemiological knowledge gaps?	Retained for further risk assessment?	Notes	Ref.
INFECTIOUS DISEASE HAZARDS							
VIRUSES							
Barmah Forest virus	Yes	No	No	Yes	No – clinical disease not reported in koalas.	Some evidence koalas may be a wild reservoir. Human disease reports increasing but human disease not severe.	[1-5]
Encephalomyocarditis virus	No	No	No	Yes	No - not reported in koalas.	Non-listed WOAH wildlife disease [6].	[7-9]
Koala retrovirus (KoRV)	Yes	Yes	Yes	Yes	Yes – significant epidemiological knowledge gaps; likely role in co-morbidities.		[10-12]
Papillomaviruses	Yes	Yes	No	No	No – sporadic disease only.		[4, 13-15]
Phascolarctid herpesviruses	Yes	Possibly	No	Yes	Yes – significant epidemiological knowledge gaps; likely role in co-morbidities.	May have a synergistic role in the pathogenesis of clinical disease in koalas. Significant associations with concurrent <i>Chlamydia pecorum</i> infection.	[16-18]
Ross River virus	Yes	No	No	No	No – clinical disease not reported in koalas.		[4, 19-22]
BACTERIA							
<i>Acinetobacter lwoffii</i>	Yes	Yes	No	No	No - reports of disease are rare.	Mastitis and pouch young death reported in captive animals.	[4, 23, 24]
<i>Aeromonas hydrophila</i>	Yes	Yes	No	No	No - disease is sporadic and opportunistic.	Myositis in a captive koala; septicaemia in mixed infections.	[4, 25]
<i>Bacteroides</i> spp.	Yes	Yes	No	No	No - sporadic disease only.	Pyometra and vaginitis (n=4).	[4, 26]
<i>Bordetella bronchiseptica</i>	Yes	Yes	No	No	No - sporadic occurrence in the wild, occasional outbreaks in captive koalas. Further discussion in <i>Other Disease Hazards</i> in KDRA report.	Respiratory tract disease.	[4, 27]
<i>Burkholderia pseudomallei</i>	Yes	Yes	No	No	No – only one reported case in a wild koala.		[28, 29]
<i>Chlamydia</i> spp. (<i>C. pecorum</i> ; <i>C. pneumoniae</i> ; novel <i>Chlamydiales</i>)	Yes	Yes	Yes	Yes	Yes – primary pathogen in the wild, possible co-morbidities with other key hazards (e.g. KoRV, PhaHV).	WOAH animal disease listings include <i>Chlamydia</i> spp. but not <i>C. pneumoniae</i> and <i>C. pecorum</i> [6].	[4, 30, 31]

Hazard	Occurs in koalas in Australia	Causes disease in koalas	Significant population level disease in koalas?	Epidemiological knowledge gaps?	Retained for further risk assessment?	Notes	Ref.
<i>Chromobacterium violaceum</i>	Yes	Yes	No	No	No – sporadic disease only. Further discussion in <i>Other Disease Hazards</i> in KDRA report.	Opportunistic infections associated with flooding events.	[32]
<i>Clostridium piliforme</i>	Yes	Yes	No	No	No - only one reported case in koalas.	formerly <i>Bacillus piliformis</i>	[33]
<i>Clostridium septicum</i>	Yes	Yes	No	No	No – only one reported case in koalas.	Acute generalised enteritis.	[34, 35]
<i>Corynebacterium</i> spp.	Yes	Yes	No	No	No - normal flora, may cause infection secondary to <i>Chlamydia</i> .	Pneumonia; mucopurulent rhinitis due to <i>Rhodococcus</i> (formerly <i>Corynebacterium</i>) <i>equi</i> - probably secondary to chlamydiosis; vaginitis secondary to chlamydiosis; normal flora of semen and prepuce; otitis media.	[4, 34, 36]
<i>Coxiella burnetti</i>	Yes	No	No	No	No - koalas not known to be a reservoir.	WOAH listed disease [6].	[37]
<i>Enterococcus faecalis</i>	Yes	Yes	No	No	No - occasional opportunistic pathogen only.	Mastitis and pouch young death.	[4, 38]
<i>Escherichia coli</i>	Yes	Yes	No	No	No - ubiquitous opportunistic pathogen.	Nephropathy; typhlitis; pyometritis, septicaemia; pouch dermatitis, otitis, cystitis.	[4, 14, 31, 34, 39]
<i>Helicobacter</i> spp.	Yes	No	No	No	No - no clear link to overt disease in koalas.	Detected by PCR in koalas in the absence of clinical disease.	[40, 41]
<i>Klebsiella</i> spp. (including <i>K. pneumoniae</i> , <i>K. oxytoca</i>)	Yes	Yes	No	No	No - ubiquitous opportunistic pathogen and co-infections.	Isolated cases in pouch young. Septicaemia, mastitis, stomatitis, keratoconjunctivitis, pouch infections.	[4, 42]
<i>Leptospira</i> spp. (<i>L. interrogans</i> serovars)	Yes	No	No	No	No – clinical disease not reported in koalas.	1.5% seroprevalence in koalas in one study. Non-listed WOAH wildlife disease [6]	[4, 34, 43]
<i>Morganella morganii</i>	Yes	Yes	No	No	No – only one reported case in koalas.	Opportunistic pathogen isolated from a septicaemia case.	[4, 44, 45]
<i>Mycobacteria</i> spp. (including <i>M. ulcerans</i> , <i>M. scrofulaceum</i>)	Yes	Yes	No	No	No - sporadic disease only. Further discussion in <i>Other Disease Hazards</i> in KDRA report.	Historic reports of sporadic outbreaks of disease in isolated populations e.g. Raymond Island (Vic). Likely environmental transmission via wounds (e.g. fighting, mating).	[4, 46-50]
<i>Mycoplasma</i> spp.	Yes	Yes	No	No	No – low prevalence, unlikely population-level impacts.		[51]
<i>Nocardia asteroides</i>	Yes	Yes	No	No	No - only one reported case in a free-living koala.	Pneumonia, likely secondary invader.	[52, 53]

Hazard	Occurs in koalas in Australia	Causes disease in koalas	Significant population level disease in koalas?	Epidemiological knowledge gaps?	Retained for further risk assessment?	Notes	Ref.
Novel <i>Actinomyces</i> sp.	Yes	Yes	No	Yes	Yes - recently emerged, novel bacterial species.	Noted in SA since 2016; possible association with dental disease.	[54, 55]
<i>Proteus</i> spp.	Yes	Yes	No	No	No - opportunistic infection only.	Normal intestinal flora. Mixed pouch infections with <i>Klebsiella</i> spp. & <i>Pseudomonas aeruginosa</i> .	[4, 34]
<i>Pseudomonas aeruginosa</i>	Yes	Yes	No	No	No - opportunistic infection only. Further discussion in <i>Other Disease Hazards</i> in KDRA report.	Epidemic mortalities reported in pouch young in captivity. Usually fatal in rehabilitation animals. Wide range of clinical manifestations.	[4, 34, 56]
<i>Salmonella</i> spp. (incl <i>S. typhimurium</i> , <i>S. sachsenwald</i> , <i>S. bovismorbificans</i>)	Yes	Yes	No	No	No – sporadic disease only. Further discussion in <i>Other Disease Hazards</i> in KDRA report.	Mostly associated with captivity – septicaemia and peritonitis.	[4, 34]
<i>Serratia marcescens</i>	Yes	Yes	No	No	No – sporadic disease only.	Mastitis and death of pouch young.	[4, 57]
<i>Staphylococcus</i> spp. (incl <i>S. epidermidis</i>)	Yes	Yes	No	No	No –opportunistic infections only.	A variety of clinical manifestations in koalas. Secondary invader in <i>Chlamydia</i> infections.	[4, 58]
<i>Streptobacillus moniliformis</i>	Yes	Yes	No	No	No - only one reported case in koalas.	Pleuritis. Causes tendon sheath arthritis in turkeys.	[59, 60]
<i>Streptococcus</i> spp. (including α - and β -haemolytic <i>Streptococcus</i> spp.)	Yes	Yes	No	No	No –opportunistic infections only.	Suppurative osteomyelitis secondary to trauma (n=1), mastitis (n=1), stomatitis, pharyngitis.	[4, 34, 61]
<i>Ureaplasma</i> spp.	Yes	Yes	No	No	No – low prevalence, unlikely population-level impacts.		[51]
<i>Yokenella regensburgei</i>	Yes	Yes	No	No	No - only one reported case in koalas.	Otitis in one wild koala.	[31, 62]
FUNGI							
<i>Aspergillus</i> spp.	Yes	Yes	No	No	No - opportunistic disease only.	Cystitis. Captive koalas.	[31, 63, 64]
<i>Candida</i> spp. (including <i>C. catenulata</i>)	Yes	Yes	No	No	No - opportunistic disease only. Further discussion in <i>Other Disease Hazards</i> in KDRA report.	Stomatitis, colitis; cystitis. Significant clinical problem in koalas in rehabilitation.	[31, 63, 64]
<i>Coccidioides</i> spp.	Yes	Yes	No	No	No - only one reported case in koalas.		[31, 63, 65]
<i>Cryptococcus</i> spp. (<i>Cryptococcus gattii</i> , <i>C. neoformans</i>)	Yes	Yes	Yes	Yes	Yes – significant disease in captive populations.	Rhinitis, pneumonia; systemic infection.	[31, 63, 64, 66]
<i>Encephalitozoon intestinalis</i>	Yes	Yes	No	No	No - sporadic disease only.	Enteritis in captive joeys (n=2).	[67]

Hazard	Occurs in koalas in Australia	Causes disease in koalas	Significant population level disease in koalas?	Epidemiological knowledge gaps?	Retained for further risk assessment?	Notes	Ref.
Ringworm fungi (<i>Trichophyton mentagrophytes</i> ; <i>Microsporum gypseum</i>)	Yes	Yes	No	No	No - no significant population impacts.	Face, ears, dorsal feet and lateral limbs most often affected.	[14, 31, 63, 64]
PROTOZOA							
<i>Cryptosporidium</i> spp.	Yes	Yes	No	Yes	No - sporadic disease only.	Transient infection and mortality (n=4) in captive koalas.	[4, 34, 68]
<i>Giardia</i> spp.	Yes	Yes	No	Yes	No - clinical disease not reported in koalas.	Identified by PCR in koalas from Kangaroo Island.	[68, 69]
<i>Toxoplasma gondii</i>	Yes	Yes	No	No	No - sporadic disease only.	Pulmonary congestion and oedema (n=3). Peracute illness and death in captive animals. Non-listed WOAH wildlife disease list [6].	[4, 34, 70]
<i>Trypanosoma</i> spp. (including <i>T. copemani</i> , <i>T. gilletti</i> , <i>T. irwini</i> , <i>T. vegrandis</i> , <i>T. noyesi</i>)	Yes	Possibly	No	Yes	Yes – possible cause of disease, significant knowledge gaps.	Infected koalas generally healthy. Disease may be more likely with co-infection or immunosuppression [31].	[71-73]
INTERNAL MACROPARASITES							
<i>Bertiella obesa</i>	Yes	Yes	No	No	No - generally non-pathogenic.	Disease more common in debilitated koalas; may contribute to wasting.	[4, 34]
<i>Durikainema phascolarcti</i>	Yes	Yes	No	No	No - generally an incidental finding.	Heavy burdens might cause vascular and respiratory compromise.	[4, 34]
Nematodes (including <i>Breinlia mundayi</i> ; <i>Marsupostrongylus</i> , <i>Johnstonema</i> & <i>Ophidascaris</i> spp.)	Yes	Yes	No	No	No - clinical disease is rare.	Most signs of infection are microscopic; mild interstitial pneumonia reported due to <i>Marsupostrongylus</i> spp. Larval <i>Ophidascaris</i> obstructed hepatic blood vessels in one case.	[4, 34, 74]
EXTERNAL MACROPARASITES							
<i>Ctenocephalides</i> spp.	Yes	Yes	No	No	No - sporadic disease only.	Incidental cat flea cases reported.	[4]
<i>Demodex</i> spp.	Yes	Yes	No	No	No - sporadic disease only.	Periocular dermatitis.	[75]
Fly strike (<i>Lucilia cuprina</i>)	Yes	Yes	No	No	No - sporadic disease only.		[4]
<i>Koalachirus perkinsi</i>	Yes	Yes	No	No	No - not usually associated with clinical disease.	Outbreaks have been reported, causing pruritus and partial alopecia.	[4, 14]
Paralysis ticks (<i>Ixodes holocyclus</i> , <i>I. cornuatus</i> , <i>I. hirsti</i>)	Yes	Yes	No	No	No - sporadic disease only.	Koalas with no prior exposure may be susceptible to paralysis.	[4]
Sarcoptic mange (<i>Sarcoptes scabiei</i>)	Yes	Yes	Yes	Yes	Yes – increasing cases in wild koalas, significant clinical disease.	Outbreaks in free-ranging koalas in SA and Vic.	[4, 76]
Ticks other than paralysis ticks	Yes	Yes	No	No	No – secondary to other illness or as a	Changes that force koalas to travel on the ground may	[4]

Hazard	Occurs in koalas in Australia	Causes disease in koalas	Significant population level disease in koalas?	Epidemiological knowledge gaps?	Retained for further risk assessment?	Notes	Ref.
(including <i>Haemaphysalis</i> spp., <i>Ixodes tasmani</i>)					consequence of non-disease threats.	predispose to heavy infestations. Associated blood loss may be a cause of morbidity and mortality in individual koalas.	
NON-INFECTIOUS DISEASES							
DEGENERATIVE							
Degenerative joint disease	Yes	Yes	No	No	No - sporadic disease in aged animals.	Degenerative disease of the hips is associated with chronic injury in aging, free-living koalas. Generally not associated with mobility issues.	[77]
Degenerative ocular lesions	Yes	Yes	No	No	No - sporadic disease only.	Includes non-chlamydial keratitis and cataracts. Long-standing keratitis (n=22) in <i>Chlamydia</i> negative koalas from Magnetic Island. <i>Chlamydia</i> status not confirmed with molecular testing. Bilateral cataracts reported in some koalas entering rehabilitation.	[78, 79]
Hip and shoulder dysplasia	Yes	Yes	No	No	No - sporadic disease only.	Metabolic bone disease due to insufficient exposure to natural UV light postulated as pathogenic mechanism.	[31]
Periodontal disease	Yes	Yes	No	No	No - present in a high percentage of wild and captive koalas, but does not appear to have significant impacts.	Includes calculus, gingivitis, periodontal pockets, gingival recession and loss of gingival attachment. Associated with malocclusion (possibly stemming from low genetic diversity) in southern populations.	[80]
Tooth wear	Yes	Yes	No	No	No - a natural consequence of aging.	Associated with impaction of leaf material, periodontitis and phytobezoars. Can be a limiting factor in older koala's recovery from illness and injury.	[4]
NEOPLASTIC							
Neoplasia (includes lymphoid and non-lymphoid neoplasia)	Yes	Yes	Yes	Yes	Yes - high incidence of neoplasia in koalas. KoRV can be a causative factor in neoplasia in koalas.		[4, 12, 81, 82]
TOXICOSIS							
Aluminium toxicosis	Yes	Yes	No	No	No – isolated cases in specific geographic areas only.	Role as an inciting cause vs a consequence of renal failure is unclear.	[4]
Envenomation - snake bite	Yes	Yes	No	No	No - sporadic disease only.	Suspected tiger snake envenomation (n=1) euthanased after lack of response to treatment.	[4]

Hazard	Occurs in koalas in Australia	Causes disease in koalas	Significant population level disease in koalas?	Epidemiological knowledge gaps?	Retained for further risk assessment?	Notes	Ref.
Fluorosis	Yes	Yes	No	No	No – isolated cases in specific geographic areas only.	May predispose to periodontal disease and skeletal abnormalities (mandibular hyperostosis). Cases associated with increased environmental fluoride emissions.	[31, 83]
DEVELOPMENTAL							
Developmental cardiac disease	Yes	Yes	No	No	No - sporadic disease only.	Includes atrial septal defect & patent foramen ovale.	[34]
Developmental urogenital disease	Yes	Yes	No	No	No - sporadic disease only.	Includes intersex, testicular aplasia or hypoplasia, cryptorchidism and ureteral aplasia. Genetic associations for some of these diseases.	[4, 84, 85]
Hydrocephalus	Yes	Yes	No	No	No - only one reported case in koalas.	Neurological signs in a wild juvenile koala.	[4]
Iris cysts	Yes	Yes	No	No	No - only one reported case in koalas.		[4]
Malocclusion	Yes	Yes	No	No	No - not associated with significant morbidity or mortality.	High incidence in both free range (22%) and captive (30.5%) animals. Predisposes to periodontal disease; may be a genetic link.	[80]
Scoliosis and kyphosis	Yes	Yes	No	No	No - sporadic disease only.	Free-ranging koalas. Mobility generally unaffected but some reports of severe disease leading to emaciation and inability to climb.	[31, 86]
ENVIRONMENTAL							
Ballistics trauma	Yes	Yes	No	No	No - sporadic cases only.	Gunshot and other ballistics (e.g. bow and arrow)	[31]
Entanglement trauma	Yes	Yes	No	No	No - sporadic disease only.	Koalas become entangled in fences, wires, vines etc	[87]
Heat stress	Yes	Yes	Yes	No	Yes – large numbers of koalas potentially at risk.	Climate change and habitat loss likely to increase risk.	[31]
Ocular disease secondary to trauma	Yes	Yes	No	No	No - sporadic disease only.	Clinical manifestations include glaucoma, anterior chamber collapse syndrome and keratomycosis.	[4, 31, 34]
Motor vehicle trauma	Yes	Yes	Yes	No	Yes - common cause of injury in wild koalas.		[31]
Predator attack trauma	Yes	Yes	Yes	No	Yes – commonly reported hazard.		[31]
Reproductive disease secondary to trauma	Yes	Yes	No	No	No - sporadic disease only.	Clinical manifestations include spermatic granuloma and varicocele.	[31]
Thermal burn trauma	Yes	Yes	Yes	No	Yes – burns sustained in bushfires kill or injure large numbers of wild koalas.		[31]

Hazard	Occurs in koalas in Australia	Causes disease in koalas	Significant population level disease in koalas?	Epidemiological knowledge gaps?	Retained for further risk assessment?	Notes	Ref.
Trauma through falling from trees	Yes	Yes	No	No	No – sporadic cases only.	Population level effects where falls occur during harvesting or clear-felling but this is geographically isolated.	[31]
Trauma from intraspecific aggression	Yes	Yes	No	No	No – not known to have population-level effects; stems from natural koala behaviours.	Injuries inflicted during fighting and mating. Can result in falls from trees in wild koalas. Cause of trauma in captive koalas.	[31, 88]
OTHER NON-INFECTIOUS DISEASES							
Colloid goitre	Yes	Yes	No	No	No - sporadic disease only.	Incidental finding in one study (n=4).	[34, 89]
Diabetes mellitus	Yes	Yes	No	No	No - sporadic disease only.	Disease possibly related to transient stress-related insulin resistance.	[31]
Gastrointestinal torsion, intussusception or entrapment	Yes	Yes	No	No	No - sporadic disease only.	Aetiology unclear; may be related to stress or sudden diet changes.	[34]
Lithiasis (including struvite; calcium oxalate; uric acid)	Yes	Yes	No	No	No - sporadic disease only.	Gout reported in a single case. Urate crystals may occur with calcium oxalate in oxalate nephrosis.	[4, 90]
Microchip transponder reactions	Yes	Yes	No	No	No - sporadic disease only.	Fibrovascular proliferation at the site of implantation.	[34]
Oxalate nephrosis	Yes	Yes	Yes	Yes	Yes - frequent reports in free-ranging and captive koalas.		[31]
Phytobezoars	Yes	Yes	No	No	No - sporadic disease only.	May be a consequence of tooth wear, malocclusion or inappropriate captive diet.	[34]
OTHER CLINICAL SYNDROMES							
Gut dysbiosis (caecocolic dysbiosis/typhlocolitis syndrome)	Yes	Yes	No	Yes	No – lacks a clear single aetiology and case definition. Examined in more detail in <i>Other Disease Hazards</i> section of KDRA report.	Unique hindgut with specialised microbiome is highly susceptible to homeostatic disturbance. May have significant impact on rehabilitation populations.	[31]
Putative KoRV-associated disease syndromes	Yes	Yes	Unknown	Yes	No - lacks clear causality and case definition. Examined in more detail in <i>Other Disease Hazards</i> section of KDRA report.	Animals with multiple opportunistic infections possibly associated with KoRV status. Syndromes of multiple disease presentations or multisystemic combinations of disease.	[91]
Wasting syndromes	Yes	Yes	Yes	Yes	No - lacks a clear single aetiology and case definition. Examined in more detail in <i>Other Disease Hazards</i> section of KDRA report.	May stem from other diseases or from non-disease pressures.	[34]

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Appendix 4 Non-disease Threat Descriptions

This Appendix describes non-disease threats that may act as drivers of disease in koalas. These threats were identified during a generic threat analysis process undertaken with KDRA stakeholders during workshop sessions. The causal flow diagram in Section 3 *Problem Description* (Figure 4) in KDRA report was developed with stakeholder input based on the following information derived from the literature and stakeholders' expert opinion.

4.1 Habitat Loss, Degradation and Fragmentation

Australia has lost almost 40% of its forests since European settlement, with disproportionate loss on the fertile coastal soils which represent the best quality koala habitat [1-3]. In Vic, about 66% of native vegetation has been cleared since European settlement; the areas with the most fertile soils in Qld retain less than 10% of their original vegetation; and in the Sydney region of NSW, less than 1% of Sydney blue gum forests persist [1]. In addition to the absolute loss of large areas of habitat, clearing activities lead to alteration of floristic composition within habitats, thinning of tree/understory density, disruption of tree health and reduction in patch size and connectivity, such that remaining forests tend to be highly fragmented, disturbed and ecologically compromised [1, 4, 5].

As a species, the koala now primarily occurs in human-modified landscapes [6]. For the most part, habitat threats are related to direct and indirect anthropogenic pressures, reflecting the marked conflict between human land use and koala habitat requirements [7]. Specific causes include urban expansion (for housing, new roadways, industrial sites), land clearing for production purposes (forestry, agriculture, mining), introduction of pests, weeds and plant pathogens, and the habitat-altering effects of climate change (see *Appendix 4.2 Climate Impacts and Environmental Disasters* below). In areas of localised overcrowding (which can arise as a result of habitat fragmentation in certain cases), koalas may cause habitat destruction by defoliating remaining trees (see *Appendix 4.4 Localised Overcrowding*) [5].

Koalas are particularly sensitive to habitat loss, degradation and fragmentation due to their highly specific habitat requirements, centred around their reliance on particular tree species for food, shelter and thermoregulation, and their limited capability to move safely between disconnected habitats [8, 9]. Absolute loss of habitat, and the disproportionate loss of prime habitat, result in loss of food and shelter for koalas, limiting both the distribution and long-term viability of koala populations in a given area [5, 7, 8]. Degradation in habitat quality can increase physiological stress for resident koalas making populations more susceptible to disease [10-12] and less resilient to other stressors [13].

Koalas in degraded and fragmented habitats spend more time on the ground moving between trees [14], resulting in increased metabolic cost, increased likelihood of encountering misadventure (see *Appendix 4.3 Misadventure*) [9], and increased acquisition of ectoparasites [15]. Fragmentation of habitat also disrupts genetic flow between

populations, isolating groups of koalas and reducing corridors for dispersal of animals, with resultant reduction in opportunities for genetic mixing [16]. Decreased total area and increased fragmentation of habitats may cause koalas to reach unsustainable densities in the remaining habitat which, in turn, may threaten the viability of that habitat (see *Appendix 4.4 Localised Overcrowding*) [5].

Despite the importance of habitat loss, degradation and fragmentation to koalas, this threat is underrepresented in terms of scientific literature, with only 4% of threat-related publications concerning koalas focused on habitat loss and fragmentation [17]. This is particularly the case in the southern states where understanding of the ecology of koalas has received relatively little attention, possibly due to the perception that koala populations in these areas are stable or increasing [17].

4.2 Climate Impacts and Environmental Disasters

Modelling predicts a shift to a hotter and more variable climate in Australia due to climate change [18, 19], along with more frequent and widespread ecological catastrophes such as storms, droughts, fires and extreme rainfall events [20-23]. The widespread and catastrophic Australian bushfires of 2019-2020 had profound impacts on koalas and koala habitat [20, 24, 25], with more than 10% of total koala habitat estimated to have been affected [24]. This impact was cumulative to the effects of sustained drought and temperature extremes in many areas [13, 22, 24, 26, 27]. More recently, extensive flooding across large tracts of koala habitat in NSW and Qld in 2022 demonstrated the vulnerability of koalas to extreme rainfall events, particularly in marginal riparian habitats [28].

Koalas experience direct mortality in fire and heat events through smoke inhalation, burn trauma, dehydration and heat stress [25], and in flooding events through drowning. Extreme weather events also cause indirect mortality through the effects of habitat loss (see *Appendix 4.1 Habitat Loss, Degradation and Fragmentation*), compromised immunity due to physiological stress [8, 29], and an increased tendency to go to ground which exposes them to misadventure and associated disease risks [28, 30]. Fire events can have the potential to cause local extinctions, and fire is a primary contributor to koala population declines in certain regions [23]. Data emerging from the autumn 2022 floods in Qld suggests that significant population decline may also be linked to flooding, with population declines of 5-10% observed in two populations [28].

Climate change, extreme weather events and fire management are drivers of habitat degradation, due to their negative impact on the viability, configuration and abundance of trees [9, 31]. The change in the distribution and availability of preferred tree species directly affects the distribution, carrying capacity and viability of koala populations, with tree selection being mediated by climate [18, 32].

Sustained increases in temperature associated with climate change cause reductions in the moisture content of leaves, a critical consideration for koalas given leaf moisture is an

important factor in eucalyptus choice [33]. Climate-driven increases in both temperature and atmospheric CO₂ also negatively impact the nutritional quality of leaves [21].

There is evidence that extreme weather events can be associated with an alteration of disease prevalence in koalas. An increase in clinical chlamydia and chlamydial infection was noted in one population after heatwaves following a decade of drought [13]. A water-borne pathogen, *Chromobacterium violaceum*, was implicated in acute septicaemic deaths of koalas following the 2022 floods in Qld (see Section 7.2 *Opportunistic Infections* in KDRA report) [28].

The cumulative outcome of climate change and extreme weather events is that koalas and some of their preferred food trees are likely to experience significant range contractions, particularly in the northern parts of their range in south-east Qld, eastern NSW and eastern Vic [9, 17, 18, 21, 32, 34, 35]. Conservative climate projections predict a likely contraction of koala populations eastwards and southwards, with climate-driven contraction compounded by the impacts of other threats such as habitat loss and disease [12, 21, 34, 36].

4.3 Misadventure

Misadventure refers to poor health and welfare outcomes which befall koalas as a result of encounters with external (often anthropogenic) influences. Within the natural koala habitat, misadventure includes predator attack, vehicle strike, orphaning, falls from (or with) trees, gunshot and other ballistic injury, attack by non-predator species (e.g. deer, cattle), and natural disasters such as bushfire [37-39]. Koalas may experience misadventure by straying into areas which are not their natural habitat, with potential consequences including attack by domestic pets, trampling by livestock, accidental entrapment, drowning and entanglements [37, 38]. Misadventure also includes unnecessary rescues undertaken by community members who misinterpret the circumstances of their encounters with wild koalas [37].

Misadventure frequently results in traumatic disease and mortality in both healthy koalas and those affected by other disease. Misadventure can impact population viability through loss of breeding animals (particularly young males of breeding age that are dispersing), reduced fecundity, lower resilience and increased mortality [37-40].

Urbanisation and other land management practices which cause habitat fragmentation bring koalas increasingly into contact with human environments which can increase the likelihood of anthropogenic misadventure [9]. Disturbance of koalas in their natural habitat brought about by clearing and logging activities also increases misadventure risk [11, 38].

The estimation of the impact of various causes of misadventure is limited by the capacity to immediately investigate koala deaths. This is particularly the case for predation, which is probably underestimated as a cause of misadventure as carcasses are undetectable after consumption or burial [40].

4.4 Localised Overcrowding

Localised overcrowding occurs when koalas are present in an area at a density where their feeding results in defoliation of preferred food trees [5]. It is associated with koala densities of at least two koalas per ha, but population densities can be much higher, with 23 koalas per ha recorded in one study [41]. Localised overcrowding has been documented in 16 koala populations, almost all of which are southern koala populations in SA and Vic. Most (n=14) have occurred in areas where koalas have been reintroduced after local extinction. Seven populations are currently experiencing overcrowding issues [5].

The problem of localised overcrowding of koalas is commonly referred to as “overabundance” in scientific literature (e.g. [5]), reflecting the fact that koalas in these populations may have been thriving and breeding well, but may have had limited dispersal opportunity due to poor habitat connectivity [5, 41-43]. It was clear during discussions with workshop participants that this terminology creates significant confusion in the general community as to the threatened status of koalas, as well as detracting from the fundamental deficiencies in habitat management that are at the root of this problem. Consequently, for the purposes of this report, the term “overcrowding” is used in preference to “overabundance”.

Overcrowding has negative consequences for the ecosystem, koalas and other species. Widespread canopy defoliation depletes koala food resources, resulting in nutritional stress (and mass starvation in severe cases), with significant negative welfare implications for affected koalas [41]. Nutritional stress causes clinical abnormalities such as anaemia and poor growth rates (3), lowers immunity and resistance to disease [29] and is a contributing factor in susceptibility to many of the major disease hazards of koalas (see hazard flow charts in Section 5 *Risk Assessments for Selected Hazards* in KDRA report). Increased koala density provides more opportunity for infectious disease transmission and promotes intraspecific aggression leading to trauma. Defoliation, with or without additive habitat degradation by invasive plants and fungi [44], can lead to the death of individual trees which may have widespread impacts on its capacity to support both koalas and other species [5].

Localised overcrowding can give a false impression that koalas are less vulnerable than they really are, with negative impacts on community and political recognition of the overall threats facing koalas. For example, the relative abundance of southern koalas hindered legislative change to recognise northern koala populations as vulnerable under Commonwealth legislation [45].

Most cases of localised overcrowding are characterised by three factors: i) the presence of *Eucalyptus viminalis* (manna gum) or *E. ovata* (swamp gum), two highly palatable and nutritious species of eucalypt which are associated with small koala home ranges, high site fidelity and a reluctance of koalas to disperse even when trees become completely defoliated; ii) koala population densities of at least 2 per ha; iii) location on islands, or in areas where dispersal opportunities are limited by poor habitat connectivity [5, 41-43].

The gastrointestinal microbiome of koalas has been shown to differ depending on the eucalypt species they are eating [46]. If their diet is predominated by a restricted range of highly digestible species, this may limit their ability to switch to another food tree species, further limiting dispersal tendency when defoliation or increased koala density occur [5].

The management of overabundant koalas raises a debate as to the acceptability of certain response strategies, particularly culling, which often meet with public opposition [47, 48]. Response to localised overcrowding may be characterised by reactive management of koala welfare in response to public outcry, as opposed to more integrated and evidence-based long-term management strategies such as habitat restoration and annual monitoring to enable early recognition and prompt response [5, 43].

4.5 Low Genetic Diversity

Low genetic diversity refers to the loss of heterozygosity and allelic diversity within the koala genome which results from inbreeding.

In southern koala populations, loss of genetic diversity is associated with the low number of founding animals used in reintroduction efforts in the early 1900s [42, 49-51]. By the 1930s, koalas were presumed extinct in SA, and were undergoing dramatic decline in Vic [9]. In response, Vic koala populations were re-established through reintroduction programs, and koalas, sourced mainly from Vic, were introduced to a number of SA sites [9]. The originating sources for these translocations were island populations founded by very few individuals in the late 1800s and early 1900s [52]; the French Island population, which was the source for establishing koalas on Kangaroo Island (SA), is thought to have originated from as few as two or three founder individuals [52]. The interventions to re-establish koalas in Vic and SA resulted in severe genetic bottleneck effects [52] and genetic swamping of the few remnant Vic populations by the restricted gene pool of translocated animals [42]. The threshold of inbreeding which is associated with an increased probability of population extinction is exceeded by all southern koala populations studied to date [53].

Ongoing habitat loss and fragmentation are associated with increasing genetic erosion throughout the koala's distribution [49, 50, 54]. Successful dispersal of juvenile koalas of both sexes is impeded in fragmented habitat due to increased rates of juvenile loss to dog attacks and motor vehicle collisions [50, 55]. Koalas do not appear to exhibit active inbreeding avoidance behaviour (avoiding mating with closely related animals) [56]. Thus, declines in the heterozygosity of koala populations in fragmented habitat may be driven by both impeded gene flow and an increase in inbreeding [49-51, 54].

Inbreeding increases the probability of accumulation of deleterious recessive alleles and inbreeding depression, which in turn can reduce the viability of a population [42]. Although animal populations with low genetic variation are, as a general rule, considered to have lower survivorship, this does not appear to be the case with southern koala populations [50]. However, there is suggestion that loss of genetic diversity may express in other ways among southern koalas, including as elevated juvenile mortality [57], biased sex ratios in

offspring [58] and the occurrence of developmental abnormalities which are not present in listed koala populations [53, 59].

Genetic diversity is an important measure of a population's resilience, as it is a determinant of adaptive capacity to emerging environmental pressures, such as disease or climate change [53, 54]. Evaluation of whole genome sequencing data to date has identified a number of immune genes which are likely to be important to the immune response to diseases such as chlamydiosis, and in the future will likely help to quantify the adaptive potential of a population for coping with disease events [51, 60].

4.6 Community Perceptions and Engagement

The koala is a much-loved cultural icon in Australia [61] and a valuable component of the country's international tourism brand [62, 63]. Community actions and perceptions can have a significant impact on koala conservation, both directly (through community action and engagement) and indirectly (through advocacy and voter pressure). Public goodwill towards koalas is generally high, and many community members are closely involved in supporting koala conservation, through volunteer activities such as rehabilitation, local community advocacy, support of conservation organisations, or engagement in personal behaviours to benefit koalas (e.g. [64]). This support reached new heights during the Australian bushfire season of 2019-20, when national and international media attention highlighted the plight of koalas and the need to pro-actively protect them from anthropogenic impacts [61]. However, despite the good intentions of the general public, there are aspects of community perceptions and engagement that can pose threats to koalas.

If community members are not well informed of the risks to koalas they are less inclined to engage in personal actions, including advocacy to their government representatives, to support koala conservation [61]. There may be uncertainty in parts of the general community as to whether koalas are truly threatened with extinction. This uncertainty may stem from a range of factors, including the relative inconspicuousness of koalas in their natural environment, a lack of clarity regarding the number of koalas remaining in the wild [9], the regional disparities in koala population density and conservation status [6, 61] and the use of terms such as 'overabundance' which imply that koalas are thriving in some localities rather than competing for limited habitat remnants (See *Appendix 4.4 Localised Overcrowding*). The complexities of koala population fragmentation, and how habitat pressures and local ecology can result in dramatic population declines in some populations and localised overcrowding in others, may not be well understood by the community [5].

Koalas are increasingly located in areas where there are competing priorities for land use and land management. Some community members may be less inclined to support actions to safeguard koalas and their habitat if those actions compete with other land use priorities of greater personal importance to them [6, 61, 65-67]. Increasing urbanisation, with resultant disconnection of people from wildlife and wilderness, may result in a lack of the necessary public support for policies which promote koala conservation over land

development [66]. In the absence of public awareness of the true threats to koala survival, risk mitigation actions that require changes in personal behaviour may not be widely implemented if they are perceived as too difficult or inconvenient (e.g. [67, 68]).

There are tensions between ‘scientific’ management of koalas (which is perceived to isolate facts from values) and their ‘cuddly’ persona in popular culture, particularly where population control strategies are concerned [43]. Public outcry, guided by emotion-charged media portrayals of koalas and fuelled by the immediacy of social media, may stoke sentiment about koalas in ways that do not benefit their conservation or welfare [5]. Community response has resulted in the reversal of management decisions to cull overabundant koalas in southern Australia [43, 65] and the dismissal by state governments of culling as a management option for koalas over the last 25 years [47]. While many consider the elimination of culling a better ethical outcome, localised overcrowding issues have not yet been solved by alternative population reduction strategies, resulting in ongoing negative ecological and koala welfare impacts in these areas [65, 69].

A general public with limited understanding of animal welfare principles - particularly as they apply to population welfare - may not be equipped with the knowledge to recognise and support positive welfare decisions for wildlife [70, 71]. Members of the public who encounter koalas in the wild may also be unable to reliably distinguish between koalas in need of intervention and those that should be left undisturbed, and may “rescue” individuals unnecessarily [37]. Such well-intentioned, but misguided, efforts can be detrimental to koala health and welfare, and may lead to the unnecessary removal of animals from the wild [37, 72, 73].

4.7 Political Factors

The management of koalas in the wild is influenced by a range of policies, legislation, regulations and programs which are managed through all tiers of government (local, state and national), as well as landholders, communities, traditional owners, the private sector and non-government organisations [9]. The active engagement of many stakeholders allows the integration of diverse perspectives into decision-making processes. However, divergent approaches at different jurisdictional levels can present challenges to consistent, integrated and adaptive management of threats to koalas [6, 74], limiting the effective implementation of legislation or policy.

As described in *Appendix 4.1 Habitat loss, fragmentation and degradation*, the viability of koala populations is intimately connected to habitat viability, due to the koala’s reliance on particular tree species for food, shelter and thermoregulation [29, 75]. If environmental and land use regulation and legislation is insufficiently robust or inconsistent across jurisdictions, there may not be sufficient protection of koalas or their habitat which puts further pressure on remaining populations [76-78].

As has been recognised in the *National Recovery Plan for the Koala* [9], policy and management actions for koala management and protection must have a solid foundation in

evidence-based science in order to be effective. Where short-term political or economic concerns have prevailed over the needs of koalas, there has been a tendency toward reactive or high visibility koala management decisions, that address a limited aspects of the overall threat without taking into consideration the long-term viability of koala populations [6, 17, 65, 67].

Funding for conservation action in Australia, in the context of the rate of biodiversity loss, is low [17]. When available funding is tied to the prominent or “media-friendly” issues rather than long-term species and habitat viability goals, the overall benefit of the funding dollar to koalas may be short-lived [61]. Plans for koala conservation that lack the necessary processes and funding for implementation are unlikely to deliver tangible, long-term conservation outcomes [6].

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Appendix 5 Hazards for Detailed Risk Assessment – Literature Reviews



A wild koala sitting at the base of a tree (credit: Tamsyn Stephenson)

5.1 *Chlamydia* spp. in Koalas – Literature Review

5.1.1 Technical Information

Aetiological agent

Chlamydiae are Gram negative, obligate intracellular bacteria. The species that most commonly infects koalas is *Chlamydia pecorum*. *Chlamydia pneumoniae* is detected rarely and both species are classified in the taxonomic family *Chlamydiaceae* [1-3]. Several other *Chlamydia*-like bacteria outside the *Chlamydiaceae* family have also been identified in koalas using molecular techniques [4, 5] but their significance is unknown.

The taxonomy and nomenclature of the *Chlamydiaceae* has undergone many changes since the 1970s when the organism first isolated from koalas was identified as *Chlamydia psittaci*. For a comprehensive summary of the history of *Chlamydia* taxonomy with respect to koalas see Polkinghorne et al. 2013 [6].

Chlamydia pecorum can be classified into genotypes or sequence types based on genotyping methods that target the major outer membrane protein gene (*ompA*) [7] or multiple genetic loci [8-10]. Multi-locus sequence typing (MLST) identifies diverse sequence types of *C. pecorum* in koalas [11], which are mostly distinct from known livestock strains [12].

Listing

Chlamydiosis in koalas is not a WOA listed disease [13].

Chlamydiosis in koalas is not a nationally notifiable animal disease [14].

Chlamydiosis is not identified as a key threatening process under the Environment Protection and Biodiversity Act [15].

5.1.2 Epidemiology

Chlamydial infertility in both male and female koalas can have a profound effect on free ranging koala population viability. Introduction of *Chlamydia* to a naïve population has resulted in rapid population decline, reducing fecundity to zero in as little as 25 years [16]. Modelling studies indicate that active management of chlamydial infection can be a critical component in reversing koala population declines [17, 18].

The relative importance of chlamydiosis as a driver of population dynamics, compared with non-disease threats, is difficult to measure and probably varies across the koala's distribution [19, 20]. Interpretation is hampered by a scarcity of longitudinal population investigations, an inconsistent approach to disease classification, and the interrelatedness of chlamydial disease to other threats to koala populations [21-23]. A clear understanding of the role of chlamydiosis in population declines across the range of the koala is an important knowledge gap.

Host range

Chlamydia pecorum has a very wide host range, which includes domestic livestock (cattle, sheep, goats, pigs, water buffalo, reindeer) and birds [24-26]. It has also been found in other native marsupials including gliders, possums, bandicoots and quolls [24, 27, 28], though chlamydial disease occurs less commonly in these species [28].

The origins of koala *C. pecorum* are unclear. Several sequence types of *C. pecorum* found in koalas appear so far to be unique to the species [12, 29]. However, few Australian *C. pecorum* strains from other species have been genotyped and some koala sequence types may be more genetically similar to livestock strains than to other koala strains [9, 12]. Genotypes identical or very similar to those found in koalas have also been found in other Australian marsupials [28] and in Australian native birds [25].

Due to similarity of *C. pecorum* sequences between koalas and these other hosts, particularly livestock, the potential for cross-species transmission has been raised [28, 29]. However, more studies are needed to clarify whether postulated spill-over events have significant impact on *Chlamydia* epidemiology in koalas or instead represent rare evolutionary events [22, 30].

Chlamydia pneumoniae has been found in many other vertebrate species including amphibians, reptiles, horses and humans. Among Australian native wildlife it has been isolated from koalas, Shark Bay bandicoots, and Gilbert's potoroo [31-33].

Zoonotic potential

Chlamydia pecorum and koala *C. pneumoniae* are not known to be zoonotic [24, 34]. Human *C. pneumoniae* is believed to have evolved separately from koala *C. pneumoniae* [32, 35, 36].

There is strong circumstantial evidence that transmission of *Chlamydia* from koalas to humans does not occur. Although many thousands of free-ranging koalas with overt clinical signs of chlamydial disease have been captured, examined or brought into care over the last 80 years, there has been no report of transmission or seroconversion in humans in contact with these animals [37, 38].

Geographic distribution

Chlamydial infection has been recorded in virtually all wild koala populations throughout their range [39]. There are regional differences in the prevalence of both chlamydial infection and disease (chlamydiosis) in free-ranging koala populations.

Prevalence

Prevalence of chlamydial infection

Table 13 summarises the prevalence of *C. pecorum* and *C. pneumoniae* infection in koala populations in studies undertaken from 2013-2020. Confidence intervals are rarely reported

and are likely to be wide due to sample size limitations. Although the prevalence of *C. pecorum* can vary, *C. pecorum* is consistently more prevalent than *C. pneumoniae* in koalas. Reports of infection with *C. pneumoniae* in koalas have declined significantly in the last 20 years in all regions [39]. A 2021 study of koalas in south-east Qld failed to detect *C. pneumoniae* in over 2500 samples (nasal, conjunctival, urogenital and semen), whereas *C. pecorum* was present in over 60% of these samples [40]. Similar results have been found for koalas sampled in NSW from 2014-2022 [41].

Table 13 Prevalence of Chlamydia infection in Australian koala populations 2013-2020

*Chlamydia diagnosis was based on PCR in all cases. Where the same data were used in more than one study, the data are only tabulated once. NR = not reported; * F = free ranging; R = rehabilitation; U = unspecified; n = number of koalas unless otherwise specified.*

Location	Population status*	n	Prevalence		Reference
			<i>C. pecorum</i> n (%)	<i>C. pneumoniae</i> n (%)	
Queensland					
Brendale	F	22	11 (50%)	NR	Kollipara et al. 2013 [42]
Brisbane	R	677 samples	509 samples (71%)	0 (0%)	Palmieri et al. 2019 [43]
East Coomera	F	132	38 (29%)	NR	Kollipara et al. 2013 [42]
Elanora	F	31	19 (61%)	NR	Kollipara et al. 2013 [42]
Hidden Vale	F	24	14 (58%)	NR	Robbins et al. 2020 [10]
Koala Coast	U	23	20 (87%)	1 (4%)	Polkinghorne et al. 2013 [6]
Lower Beechmont	F	33	17 (52%)	NR	Kollipara et al. 2013 [42]
Moreton Bay	F	254	89 (35%)	NR	Robbins et al. 2020 [10]
Moreton Bay	F	160	49 (31%)	NR	Nyari et al. 2017 [44]
Narangba	F	16	8 (50%)	NR	Kollipara et al. 2013 [42]
North Stradbroke Island	F	10	3 (30%)	NR	Kollipara et al. 2013 [42]
SE Qld	F, R	250	155 (62%)	0 (0%)	Hulse 2021 [40]
SE Qld	F	13	5 (38%)	NR	Wedrowicz et al. 2018 [45]
St Bees Island	F	36	10 (28%)	NR	Kollipara et al. 2013 [42]
NSW					
Byron Bay	F	5	1 (20%)	NR	Kollipara et al. 2013 [42]
Gunnedah	F	140	93 (66%)	NR	Fernandez et al. 2019 [8]
Koala Beach	U	29	19 (67%)	3 (10%)	Polkinghorne et al. 2013 [6]
Pilliga State Forest	U	26	2 (8%)	2 (8%)	Polkinghorne et al. 2013 [6]
Port Macquarie	R	73	46 (63%)	NR	Kollipara et al. 2013 [42]
SE NSW	F	11	3 (27%)	NR	Wedrowicz et al. 2018 [45]
Tanilba Bay	R	41	24 (59%)	NR	Kollipara et al. 2013 [42]

Table 13 (continued)

Location	Population status*	n	Prevalence		Reference
			<i>C. pecorum</i> n (%)	<i>C. pneumoniae</i> n (%)	
Victoria					
Ballarat	U	10	9 (90%)	2 (20%)	Polkinghorne et al. 2013 [6]
Cape Otway	F	41	2 (5%)	NR	Wedrowicz et al. 2018 [45]
Framlingham	U	10	0 (0%)	0 (0%)	Polkinghorne et al. 2013 [6]
French Island	F	237	2 (1%)	0 (0%)	Patterson et al. 2015 [3], Legione et al. 2016 [9], Legione et al. 2016 [46]
French Island	U	5	0 (0%)	0 (0%)	Polkinghorne et al. 2013 [6]
Greater Gippsland	F	30	11 (37%)	0 (0%)	Legione et al. 2016 [46]
Mallacoota	F	5	0 (0%)	NR	Wedrowicz et al. 2018 [45]
Mornington Peninsula	F	13	6 (46%)	0 (0%)	Legione et al. 2016 [46]
Mt Eccles	F	168	36 (21%)	0 (0%)	Patterson et al. 2015 [3], Legione et al. 2016 [46]
Raymond Island	F	153	50 (33%)	0 (0%)	Patterson et al. 2015 [3], Legione et al. 2016 [46]
Raymond Island	F	26	21 (81%)	NR	Wedrowicz et al. 2018 [45]
South Gippsland	F	176	107 (61%)	NR	Wedrowicz et al. 2018 [45]
South Gippsland	R	22	10 (45%)	NR	Wedrowicz et al. 2018 [45]
South West Coast	F	210	15 (7%)	1 (0.5%)	Legione et al. 2016 [46]
South Australia					
Eyre Peninsula	R	3	2 (67%)	NR	Speight et al. 2016 [47]
Kangaroo Island	U	10	0 (0%)	0 (0%)	Polkinghorne et al. 2013 [6]
Kangaroo Island	F	170	0 (0%)	NR	Fabijan et al. 2019 [48]
Kangaroo Island	F	81	0 (0%)	NR	Speight and Funnell 2020 [49]
Mt Lofty Ranges	F	17	57 (88%)	9 (53%)	Polkinghorne et al. 2013 [6]
Mt Lofty Ranges	R	62	55 (89%)	NR	Speight et al. 2016 [47]
Mt Lofty Ranges	F	75	35 (47%)	NR	Fabijan et al. 2019 [48]
Mt Lofty Ranges	F	188	95 (50.5%)	NR	Stephenson 2021 [50]

The prevalence of *Chlamydia* in SA koala populations was historically thought to be low because of the lack of reports of chlamydial disease. However, with the exception of the

Kangaroo Island population, infection prevalence in the SA populations appears comparable to populations in other states [6, 47, 48].

Assumptions of *Chlamydia*-free status in any koala population should be viewed with caution given the otherwise ubiquitous distribution of chlamydial infection, its high transmissibility and the logistical limitations of screening free-ranging populations. Some island populations of koalas, created by the historic translocation of clinically healthy, ostensibly *Chlamydia*-free animals, were postulated to be free of chlamydial infection based on serological assays and absence of observed disease. This included populations on St Bees Island and Magnetic Island in Qld, French Island in Vic and Kangaroo Island in SA [6, 22, 51]. Molecular techniques (PCR) have since detected *C. pecorum* in clinically healthy koalas on St Bees Island and French Island [9, 12]. Only Kangaroo Island has demonstrated recent absence of *Chlamydia* in koalas using PCR [48].

There are some populations of mainland koalas where scat analysis and absence of clinical disease suggest that *Chlamydia* is present at very low prevalence; examples include Campbelltown, Kanangra and Mumballa in NSW [41, 52]. These populations are sometimes referred to as being “*Chlamydia*-free”. The circumstances that lead to pockets of low *Chlamydia* prevalence in wild koalas are not well understood. It is likely to reflect a complex combination of factors including geographic isolation, extirpation of infected koalas followed by repopulation with uninfected animals, or a preponderance of *Chlamydia* strains with low pathogenicity.

Chlamydial genotypes (as defined by *ompA*) and sequence types (as defined by MLST) are reasonably distinct in northern and southern koala populations, although a few genotypes occur in both [22]. There appears to be a lower diversity of *C. pecorum* genotypes and sequence types in southern koala populations compared with northern populations [10, 42, 45] which may reflect the restricted genetic provenance of these populations [7, 46]. Differences in sequence types can exist between neighbouring populations within the same region [8], and multiple sequence types can be detected within the same koala population, and also within the same individual from different anatomical sites [12].

Gender bias to the presence of chlamydial infection has not been identified in most studies [1, 3, 47] although one study found that male koalas were 2.7 times more likely to be positive for *C. pecorum* [46]. Although *C. pecorum* infection rates increase with age class in some studies [1, 3], other studies have not detected statistically significant associations between age and infection status [3, 47].

Prevalence of chlamydial disease

The prevalence of chlamydial disease is lower than the prevalence of chlamydial infection as chlamydial infection is commonly detected in apparently healthy koalas [1, 47, 53-57]. However, not all signs of disease can be determined by visual examination. A proportion of koalas which have ‘inapparent’ disease, that is only identifiable with specialised equipment or via internal or microscopic examination, may be misidentified as ‘subclinical’ (i.e. no signs

of illness) [3, 6, 44, 47, 58]. In addition, a proportion of animals which are healthy but infected at the time of examination will go on to develop disease at a later date; longitudinal data from south-east Qld found that 66% of koalas diagnosed with chlamydial infection via PCR progressed to overt disease within 3-4 years [57].

Chlamydial disease had a prevalence of 28% in one study of free-ranging koalas in Qld [44]. In a Vic study, prevalence of 'wet bottom' ranged from 38-44% in studies of three koala populations and internal sonographic abnormalities were noted in 11-43% of animals [3]. Chlamydial disease appears to be less prevalent in SA populations than in other states, with clinical disease observed in only 4% (3/75) of koalas in the Mount Lofty Ranges of free-ranging koalas [59, 60].

Chlamydial disease accounted for 52% of admissions [61] and 59% of post mortem diagnoses [62] in south-east Qld rehabilitation facilities, and 20.4% and 46% of admissions to two NSW rehabilitation facilities [23, 63]. Hospital admissions data are not available for Vic and SA populations but chlamydial disease was present in 63% [47] and 12% [64] of post mortem cases in two SA studies.

Both urogenital and ocular disease are reported throughout the koala's range, with urogenital disease more commonly reported in most cases [3, 46, 48, 61-63]. There are recent anecdotal reports that ocular disease is occurring with increasing frequency in some populations in Vic [65]. Although chlamydial disease is generally considered to be less severe in southern populations [3, 46-48], cases of severe disease can still occur [48].

Mode of transmission

It is generally accepted that urogenital chlamydial infection is transmitted sexually between koalas [6, 63] as the organism has been found in both the semen of infected males [66, 67] and the urogenital tract of infected females [68]. However, chlamydial disease has been reported in koalas below expected breeding age [1], suggesting that early transmission from infected dams to juveniles may be important. This could occur during pap feeding [6, 44, 69, 70], or through direct contact with the genital epithelium of the infected dam during parturition [71].

Other potential sources of transmission have been proposed but are likely of lesser importance to the epidemiology of the disease in koalas. These include direct transfer of infected discharges from the eyes or urogenital tract, [63, 71], aerosol inhalation [72, 73], and mechanical transfer by arthropod vectors [71, 74].

Incubation period

The incubation period for natural chlamydial infections in koalas is not known but *in vitro* studies using koala isolates have determined a cell cycle of approximately 46 hours for *C. pecorum* [75]. A report of experimental infection undertaken in koalas showed an incubation period of 7-19 days for the onset of ocular disease (conjunctivitis) and 25-27 days for development of cystitis [71, 76].

Persistence of agent

Chlamydia are capable of survival outside of the host under appropriate conditions, in spite of their obligate intracellular life cycle, which may have implications for potential transmission via fomites. A study undertaken *in vitro* showed that the extracellular elementary bodies could survive drying for two days and remained viable at 18-23°C for up to 28 days [77]. The same study showed *Chlamydia* maintained infectivity for cell culture after three days' exposure on forest red gum (*Eucalyptus tereticornis*) leaves.

Chlamydia pneumoniae survived in aerosols at 15 to 25°C in conditions of high relative humidity for at least five minutes, on various manufactured surfaces for 1-4 hours, and on human skin for up to 30 minutes [78]. *Chlamydia pneumoniae* could also be recovered from hands that had touched contaminated surfaces for up to three minutes [79].

5.1.3 Pathogenesis

Current understanding of the pathogenesis of chlamydiosis in koalas is incomplete, although extrapolation from other species is considered appropriate given the similarities in disease manifestation between koala chlamydiosis and disease in other species [80, 81].

All known members of the *Chlamydiales* share a similar and distinct biphasic life cycle, alternating between the extracellular, infectious elementary body (EB) and the metabolically active, intracellular non-infectious reticulate body (RB) [34]. Following infection, the EB penetrates the target cell, transforms into an RB and undergoes replication within the host cell while enclosed in a host-derived vacuole, or 'inclusion' [36]. The newly-replicated RB transforms back into an EB which is released from the host cell by exocytosis or lysis and is then infectious to other host cells [82].

Both RBs and EBs produce a range of virulence factors which act in a variety of ways to exploit the host cell and evade its defences [82-84]. Infected cells produce inflammatory mediators, and chronic hypersensitivity to chlamydial antigens leads to further tissue damage [39].

Chlamydiae can remain in a non-replicative, "persistent" intracellular state within host cells, evading host immune responses while preserving host cell activities [85]. In this state, *Chlamydiae* can elicit a profound host inflammatory response with immunoglobulin production and fibrosis [80, 86].

A complex interplay of host, pathogen, environmental and immune factors appear to determine the disease outcomes following chlamydial infection [10]. These factors are summarised below.

Pathogen factors

Pathogen species

Chlamydia pecorum is consistently the most pathogenic species of *Chlamydia* that infects koalas and is the primary cause of chlamydial disease in this species [1, 6, 46, 61, 63].

Disease due to *C. pneumoniae* in the absence of *C. pecorum* is rarely reported [72], although PCR detection of *C. pneumoniae* is not always undertaken, so it may have a role in disease where signs of chlamydiosis are found in animals which are PCR-negative for *C. pecorum* [47].

Several novel *Chlamydia*-like bacteria outside the *Chlamydiaceae* family have been identified in koalas in Qld, NSW and Vic [4, 5, 87]. The potential of these bacteria to cause disease is unclear; they have been found predominantly, but not exclusively, as co-infections with either *C. pecorum* or *C. pneumoniae* and have also been associated with several cases of plasmacytic enteritis where neither *C. pecorum* nor *C. pneumoniae* was detected [5].

Pathogen load

Chlamydial load is a strong predictor of urogenital disease, but not ocular disease [88]. The urogenital load of *C. pecorum* is significantly higher when koalas acquire a new infection, and declines in chronic (>3 months) infections [57, 80], although there may still be a high level of shedding from apparently healthy animals [68, 89]. Progression of urogenital tract disease is significantly associated with an increased chlamydial infection load [57].

Pathogen type

Genetic diversity in *C. pecorum* can exist between neighbouring koala populations within the same region [7, 8] and molecular typing is therefore of potential value in understanding multiple disease emergence events and identifying the risks of pathogen transfer between populations. A lack of prior exposure to *C. pecorum* (or to a novel *C. pecorum* type) may be associated with increased disease expression [48] although more information is needed to inform what degree of strain difference constitutes a 'novel' type, and whether non-exposed individuals from chlamydia-infected populations have inherited or acquired protection or are as naïve as those from chlamydia-negative populations.

Although studies have identified associations between sequence types or genotypes of *C. pecorum* and disease expression in koalas [8, 10, 44, 46, 57, 88], there is no clear causative evidence that particular strains are more pathogenic than others. The potential for differing pathogenicity exists, given that *Chlamydia pecorum* genotypes or sequence types vary in the expression of known virulence-associated factors, including chlamydial cytotoxin genes, effector proteins and chlamydial virulence plasmid [83, 90, 91]. There is some evidence for variation in pathogenesis for *C. pecorum* in cattle [29, 30] and *C. trachomatis* in humans [92].

Host factors

Host signalment

The effect of koala age and gender on chlamydial disease expression is equivocal [23].

A longitudinal study of 38 free-ranging, *Chlamydia* positive koalas in Qld demonstrated no age or sex influence on progression to disease [57], and neither age nor sex were predictors

of clinical disease in a modelling study involving 204 free-ranging Qld koalas [88]. Female koalas and aged koalas are overrepresented in post mortem and hospital admission studies of chlamydial disease [23, 47, 63], but this probably reflects other biases in data (such as the relative ease of detection of reproductive disease in female koalas compared with males [93, 94]) rather than an intrinsic influence of age or gender in the tendency to develop disease [63].

Low body condition is more commonly encountered in rehabilitation cases admitted for chlamydiosis than other causes such as trauma [23]. It is unclear whether this indicates an increased susceptibility to chlamydial disease in animals with a low body condition, or whether urogenital chlamydial disease is an inciting cause for loss of condition.

Host immune response

An effective anti-chlamydial host response involves a combination of cell-mediated immunity, humoral immunity and cellular inflammatory responses [10, 95]. The balance of immune responses may be a mechanism through which environmental and genetic factors drive chlamydial pathogenicity in koalas [80] but the nature (and relevance) of this balance is not well understood. Koalas with more severe chlamydial disease show increased expression of certain cytokines [96] and high titres of anti hsp60 IgG are associated with chronic infection and fibrosis [80].

Immunogenetics

Studies have identified associations between particular immune genes and susceptibility to chlamydial disease. Chlamydial antigen is detected and presented to the immune system via major histocompatibility complex (MHC) molecules, and some association of MHC genotype with disease expression has been observed [10, 97, 98]. Koalas capable of resolving a *C. pecorum* infection without medical intervention contained variants in a number of immune genes (MHC, toll-like receptor and gamma interferon) when compared to koalas which were unable to resolve infection [99]. The role of immunogenetics in chlamydial disease progression and resolution is likely to be influenced by the interaction between particular host and chlamydial genotypes (and potentially other host traits) rather than genetic diversity alone [10].

Site of infection

The ability of a koala to resolve a chlamydial infection may vary with anatomical site of infection. Urogenital tract infections had a 13% likelihood and ocular infections an 85% likelihood of resolution in one longitudinal study [57].

Other urogenital microflora

Koalas with high loads of *C. pecorum* had a lower microbiota diversity at ocular and urogenital sites, dominated by different bacterial species than those seen in koalas with a low or negative chlamydial infectious load [100], though it is not clear whether the changes caused or resulted from infection and inflammation.

Co-infections

The role of host co-infections in *Chlamydia* pathogenesis is discussed below in *Associations with other disease hazards of koalas*.

Environmental factors

The effect of external environment on the development of chlamydial disease is complex and is likely to reflect a range of co-factors operating over different scales of time [88, 101]. In one study, habitat reduction and increasing urbanisation were associated with an increase in the prevalence of chlamydial infection, following a 3 to 4 year time lag, possibly reflecting associated increases in physiological stress, contact rates, sexual encounters and territorial behaviour [101]. However, koalas in high quality habitat may also demonstrate a high prevalence of chlamydial infection [101]. The relative probability of admission of koalas with clinical signs of chlamydiosis did not change over 30 years in one study, in spite of local urbanisation [63]. Increased level and proximity of human disturbance is postulated to increase chlamydial disease expression [53, 54], but this is not a consistent finding [88].

The role of stress in chlamydial disease expression in koalas is difficult to quantify, and direct studies are lacking [21, 22], but stress and malnutrition have been implicated in exacerbating chlamydial disease in several other species [102-104]. A variety of environmental stressors could affect the prevalence of overt chlamydial disease in individual koala populations, including overcrowding, declining food resources and extremes of weather [54, 105-107].

5.1.4 Associations with other disease hazards of koalas

Many studies have identified associations (but not causation) between aspects of chlamydial infection or disease and a range of co-infections and disease states [50, 55, 88, 107-113].

Currently there is no clear causative evidence of KoRV inducing more severe chlamydial disease in koalas, although associations between chlamydial disease severity and increased KoRV proviral or viral load have been identified in a number of studies [60, 114-117]. Detection of certain KoRV variants has been associated with more severe chlamydial disease in Qld koalas [55, 88, 108, 117]. Presence of replication-competent KoRV in Vic koalas was associated with “wet bottom” [116] and a decrease in gamma interferon IFN γ and interleukin production, which could render koalas more susceptible to developing chlamydiosis [109]. It may be that chlamydial disease leads to increases in KoRV load, rather than the other way round, since koala lymphocytes that are stimulated by inflammation are likely to increase circulating KoRV viral and proviral loads [118].

The presence of koala gammaherpesviruses (PhaHV-1 and PhaHV-2) has been associated with infection with *C. pecorum* in both male and female koalas in Victoria [110, 111]. An association between reproductive disease caused by *C. pecorum* and PhaHV co-infection has

been detected in SA koalas (Stephenson, 2021). See *Appendix 5.9 Phascolarctid Herpesviruses – Literature Review* for further discussion.

Chlamydial disease is likely to be exacerbated by opportunistic infections from a range of microbes, including Gram-positive cocci, rods and curved rods; Gram-negative rods, filamentous bacteria and yeasts [37, 89, 107, 112].

5.1.5 Diagnosis

Chlamydial infection may be non-pathogenic, or it may cause disease which can be further classified as ‘overt’ or ‘inapparent’ [6]. In non-pathogenic infection there are no signs of disease [1, 48]. Overt disease denotes the presence of obvious external clinical signs, while inapparent disease can only be detected using specialist equipment or internal examination [22]. Studies suggest that in koalas inapparent chlamydial disease is commonly misdiagnosed as non-pathogenic infection [44, 47].

Clinical signs

The three main clinical presentations of chlamydiosis are ocular disease, urinary tract disease and reproductive disease, although other manifestations are occasionally seen.

Ocular disease [6, 39, 68, 119] manifests as serous ocular discharge, blepharospasm and conjunctival/scleral hyperaemia in the acute phase, progressing to mucopurulent discharge, conjunctival lymphoid hyperplasia and hyperaemia, sometimes with keratitis and corneal scarring in the chronic-active phase. The chronic inactive phase signifies end-stage ocular disease and is characterised by extensive conjunctival hyperplasia, minimal erythema and mature scarring in the absence of exudate (unless deformity of the eye or nasolacrimal duct occlusion predispose to bacterial infection). Blindness may ensue as a result of chronic corneal damage or conjunctival hyperplasia; severe ophthalmitis and globe rupture have been reported. Disease may be unilateral or bilateral. Figure 22 shows severe ocular disease in a koala with chlamydiosis.



Figure 22 Image of a koala with severe conjunctivitis as a result of chlamydiosis (credit: Amber Gillet)

Urinary tract disease [58, 68, 81, 89, 120, 121] manifests as frequent and occasionally painful urination and haematuria and pyuria associated with cystitis. Affected animals develop “wet bottom”, a common (but not pathognomonic) finding of urogenital chlamydiosis which refers to the brownish staining and wet fur around the cloaca and rump due to constant wetting with urine.

Reproductive tract disease may be inapparent, with reduced fertility of both male and female koalas as the only indicator [5, 6, 47, 48], although granulomatous orchitis in males may present with palpable changes to the testis or epididymis [122]. In females, fibrotic occlusion commonly leads to the development of unilateral or bilateral, fluid-filled para-ovarian (bursal) cysts in the caudal abdomen or pelvic inlet [89, 120], which may be palpable or detectable by ultrasound [58] dorsal to the epipubic bones in anaesthetised females [37]. Endometritis, pyometra, and vaginitis may also occur [60].

Occasional atypical presentations of chlamydiosis occur. Two outbreaks of rhinitis characterised by dyspnoea, coughing, sneezing and a serous to purulent nasal discharge have been attributed to chlamydial infection [37, 72]; in one of these episodes, *C. pneumoniae* infection was present without concurrent *C. pecorum* infection [72]. Some of these cases also demonstrated acute shifting lameness, epidermal ulceration at pressure points and swelling of the hands and feet, possibly associated with polyarthritis [37, 72]. A joey which developed *C. pecorum*- associated pneumonia presented with a cough and audible respiratory noises [73]. Arthritis associated with *C. pecorum* has also been described [123].

There is only circumstantial evidence linking novel *Chlamydia*-like infections to clinical disease, although post-mortem pathology has been reported [4, 5].

Clinical pathology

There are no consistent haematological or biochemical abnormalities associated with chlamydiosis. Leukocyte counts are often within normal range, although neutrophilia is also common [124]. Debilitated koalas may exhibit a non-regenerative anaemia, hypoproteinaemia and evidence of dehydration. If renal disease is present then urine specific gravity is often lowered and blood urea nitrogen and creatinine values may be elevated [125, 126]. If cystitis is present, urinalysis may demonstrate a range of abnormalities which may occur individually or together, including presence of erythrocytes, leukocytes, bacteria, yeasts, epithelial cells, and occasional renal tubular casts [37].

Pathology

The pathology of chlamydiosis in koalas is characterised by inflammation of mucosal tissues, progressing to fibrosis and scarring with chronicity [89, 120].

Ocular pathology presents histologically as acute active conjunctivitis, chronic active conjunctivitis, or chronic inactive keratoconjunctivitis. Affected koalas may have an

accumulation of predominantly plasma cells and neutrophils along with villous hypertrophy and hyperplasia of the conjunctival epithelium, and corneal vascularisation [81, 127].

Urinary tract pathology involves the kidneys, bladder and urethra in both sexes. Cystitis is characterised by mucosal hyperplasia, degeneration and hydropic change of the epithelium with mixed inflammatory cells and fibrosis of the lamina propria and submucosa, which can lead to hydronephrosis and hydroureter. Severely affected bladders display polypoid hyperplasia and hypertrophy [81, 89, 127]. Renal changes include tubular dilatation and degeneration, protein cast formation and focal to widespread pyelitis or pyelonephritis with mixed inflammatory cell infiltrates and fibrosis [89]. Chronic cases may develop an inactive cystitis with marked bladder wall thickening in the absence of pyuria [81, 89].

Male koalas with reproductive pathology commonly develop prostatitis with degeneration of the glandular epithelium, large accumulations of necrotic debris, inflammatory cells in the ducts and gland parenchyma and fibrosis of the lamina propria and submucosa of the prostatic urethra [127]. Granulomatous orchitis and epididymitis with interstitial fibrosis have been described [121]; involvement of the bulbourethral glands, testes, vas deferens and epididymis is less common [43]. Affected koalas have an increased incidence of sperm fragmentation and abnormal sperm morphology [128, 129].

Female koalas have evidence of vaginitis, cervicitis, metritis, salpingitis and ovarian diverticulitis, all characterised by a mixed inflammatory infiltrate. More chronic cases demonstrate squamous metaplasia of the uterine tubes, cystic dilation and destruction of uterine glands, submucosal fibrosis, and thickening and distortion of the walls of the genitalia. There may be fibrous adhesions between the ovaries and bursal walls. Para-ovarian cyst formation in the ovarian bursae is commonly encountered and may be unilateral or bilateral. Chlamydial inclusions may be present in epithelial cells in all parts of the reproductive tract as well as in submucosal macrophages [89, 120].

Infection with novel uncultured *Chlamydiales* has been seen in association with plasmacytic enteritis at necropsy, but their aetiological role remains speculative [5].

Differential diagnosis

Signs of ocular disease such as blepharospasm, discharge, hyperaemia and conjunctival swelling can be associated with trauma, exposure to environmental irritants, or other bacterial or fungal infections [37]. However, chronic chlamydial conjunctivitis is characterised by proliferative hyperplasia of the conjunctiva and nictitating membrane which is not commonly seen in ocular disease due to other aetiologies. Keratitis has been observed in *Chlamydia* seronegative koalas on Magnetic Island [37] and St Bees Island [124] which was speculated to be related to exposure to external environmental agents such as insect bites. [47].

While koalas with ‘wet bottom’ are frequently assumed to have chlamydiosis, the syndrome has been seen in the absence of *Chlamydia* [3, 130], although it should also be noted that it may be difficult to detect chlamydial inclusions in chronic chlamydial disease cases [81, 89].

Nephrosis and nephritis of non-chlamydial origin may be associated with oxalate deposition [131]. *Mycoplasma* spp. and *Ureaplasma* spp. can elicit ocular, urogenital and respiratory disease similar in presentation to chlamydiosis [132]. Recently, PhaHV has been implicated in reproductive disease in female SA koalas [50].

Diagnostic testing

Definitive diagnosis of a chlamydial aetiology for disease is complicated because diseased hosts do not always shed *Chlamydia* organisms, and organisms may be shed by infected hosts in the absence of disease [6]. Reasonable certainty of diagnosis is best achieved by combining sensitive PCR-based detection techniques with a thorough veterinary examination, which includes diagnostic aids such as urinalysis and ultrasound evaluation to detect inapparent disease [6, 39, 47, 58].

Detection of *Chlamydia*

Molecular detection of *Chlamydia* in koalas is important for the detection of subclinical cases (which may be the most amenable to treatment) and for confirming presumptive cases when clinical signs are present [133].

The preferred chlamydial detection method is PCR testing of ocular and urogenital swabs [39]. PCR testing is highly specific and sensitive, and is capable of detecting subclinical infection. A range of PCR methodologies are used by different diagnostic laboratories [132]. However, there are practical limitations to the use of PCR, as it must be conducted in a laboratory and may be considered cost prohibitive. Using the correct swabbing technique and correct swab types is also important to consistency and accuracy of PCR testing [134].

The most widely used technology currently in use for point-of-care detection of *C. pecorum* is loop mediated isothermal amplification (“LAMP”) DNA amplification. LAMP assays, which are rapid and sufficiently robust for field use [135-137], are now widely used in koala rehabilitation and hospital facilities in QLD and NSW for detection of *C. pecorum* [124]. However, commercially-available LAMP assays do not allow re-use of samples, testing can be expensive, and false negative results are reasonably common, based on anecdotal reports [138].

Detection of chlamydial shedding via PCR-testing of scats is being employed as a non-invasive means of evaluating chlamydia status of free-ranging koalas [45, 139], but further validation of these methods is required to improve and quantify sensitivity.

Diagnostic ultrasound

Sonography is an effective method of detecting and grading the severity of chlamydial urogenital disease in both sexes, and consequently ultrasound evaluation is becoming part

of the routine clinical assessment in many koala hospitals [37]. Sonographic changes commonly encountered with chlamydiosis include measurable bladder wall thickening (reflecting cystitis), and the presence of unilateral or bilateral fluid-filled paraovarian cysts in the caudal abdomen or pelvic inlet in females [37, 39, 58]. Sonographic changes in the prostate of males may be seen but are less commonly encountered [39].

Serological testing

Serology is no longer used as a diagnostic screening tool for chlamydia in koalas. A koala-specific ELISA was in use for many years in Australia but is no longer available [37]. Complement fixation tests used in the past were shown to have a sensitivity of only 7%, in addition to which detectable CF antibodies took at least 3-4 months to develop in *Chlamydiae*-naïve koalas [37].

Immunohistochemistry

Immunohistochemistry for chlamydial antigens can be used to assist in localising *Chlamydiaceae* within formalin-fixed material [89] but its sensitivity as a diagnostic tool is impacted by the condition of the mucosal epithelial cells and the variable impacts of formalin fixation.

Surveillance and monitoring

There is no targeted national surveillance or monitoring program in place for *Chlamydia* in koalas, although there is capacity to utilise the Wildlife Health Australia national wildlife health information system database (eWHIS) as a place for collating these data as part of national general wildlife surveillance activities.

Local population surveillance programs are undertaken for *Chlamydia* in koalas throughout their range, at various times, and generally in association with other koala monitoring activities. For example, the SA Department for Environment and Water (DEW) koala program undertook surveillance for ocular and urogenital disease as part of a regular sterilisation program to control koala numbers on Kangaroo Island for several years [48]. However, such programs are reliant on engagement by particular research groups and screening is not necessarily part of project planning from the outset, which can limit the effectiveness of the surveillance effort.

5.1.6 Treatment

The decision to treat koalas with chlamydiosis is based on considerations of animal welfare and prognosis for eliminating the infection. For wild animals, the potential welfare impacts of prolonged hospitalisation must be balanced against the prospects for release. Animals with co-morbidities, such as other chronic diseases, advanced tooth wear or poor body condition are unlikely to be suitable treatment candidates [39]. It may also be necessary to consider the breeding prospects of koalas which have had reproductive tract infections when evaluating suitability for release [63, 140]. The welfare considerations for captive animals are similar to wild animals, although they may be better habituated to human

intervention. Biosecurity risks for conspecifics must also be considered in the captive situation [141].

Treatment for chlamydiosis involves antibiotic therapy in combination with various adjunctive treatments as outlined below.

Systemic antibiotic therapy

Antibiotic treatment of chlamydiosis is complicated by i) the chronicity of disease in many presenting cases, which may make them refractory to antibiotic treatment; ii) the risk of antibiotic-induced gut dysbiosis leading to opportunistic infections (particularly candidiasis) and potentially fatal effects on the koala's digestive physiology; iii) koalas' efficient metabolic pathways for excretion, which may increase the rate of elimination of drugs; and iv) risk of antibiotic resistance development [142-144].

The ideal antibiotic for treating koalas not only requires a favourable microbial cure rate with minimal adverse effects, but must also be practical for use in wild animals in the rehabilitation environment, i.e. readily available and inexpensive, not cause injection discomfort, be deliverable in small volumes by either subcutaneous or intramuscular injection, and require infrequent dosing [142].

Chloramphenicol has been the drug of choice for chlamydiosis in koalas in recent years, administered as an injectable agent to treat systemic chlamydiosis and also as a topical preparation to treat ocular cases [39]. The recent withdrawal of chloramphenicol from commercial sales in Australia [145], along with some treatment failures [146] and the potential for serious side effects [147] prompted the search for alternatives. Florfenicol, a synthetic analogue of chloramphenicol, demonstrated equivocal efficacy, plasma concentrations below the minimum inhibitory concentration (MIC) for *C. pecorum*, and adverse dysbiosis effects [148]. Fluoroquinolones (enrofloxacin and marbofloxacin) are often used to treat chlamydiosis due to their perceived safety and anecdotal effectiveness [149]. However, re-emergence of infection after treatment is common [150], and based on pharmacokinetic studies fluoroquinolones are unlikely to reach therapeutic levels via oral or subcutaneous administration at the doses generally given [151], which increases the risk of antimicrobial resistance development.

In a recent clinical comparison of five antibiotics in the treatment of koala chlamydiosis, doxycycline had the best treatment success, with a 97% cure rate compared with 81% for chloramphenicol, 75% for enrofloxacin, 66% for florfenicol and 25% for azithromycin. Doxycycline had the advantage of requiring weekly, rather than daily, administration, although it caused irritation at the site of injection, which was reduced by dilution of the drug prior to administration [142]. Anecdotally, doxycycline therapy appears more likely to cause serious gut dysbiosis, and less likely to resolve serious disease, than chloramphenicol, consequently chloramphenicol remains the drug of choice for treating chlamydiosis in koalas [39, 124].

Controlled trials have yet to be conducted to determine the optimum duration of treatment to ensure microbial cure. As a general rule, treatment for 28 days is undertaken for koalas with overt clinical disease [39], but trials in rehabilitation facilities are ongoing to establish the efficacy of shorter (14 day) treatment periods [124]. There is a need to balance the benefits of shorter treatment durations in reducing time in rehabilitation against the risks of encouraging the development of antibiotic resistance by insufficient treatment [152].

The perceived efficacy of antibiotic treatment in curing chlamydial infection may depend on the extent of post-treatment testing used to confirm treatment success, as well as the *Chlamydia* status of the population the koala is released to. If koalas are released back into an area with high *Chlamydia* prevalence, they may be likely to become re-infected [140] which may be perceived as a failure of treatment.

Treatment of ocular disease

Where ocular disease is present, topical ophthalmic antibiotics may be used in combination with systemic treatment. There is little evidence-based data for the relative efficacy of topical treatments for ocular chlamydiosis [133], although including an anti-inflammatory component anecdotally results in the greatest clinical benefit to affected animals [124]. Therapeutic approach varies between rehabilitation facilities and topical preparations which have been used include dexamethasone, chloramphenicol plus hydrocortisone, oxytetracycline plus polymyxin B sulphate, ofloxacin, oxytetracycline HCl plus oleandomycin phosphate plus neomycin sulphate [37, 124].

Many koalas with ocular disease develop persistent exudative build-up, and benefit from flushing of the nasolacrimal ducts [39]. Surgical intervention to remove proliferative conjunctiva is only warranted if the tissue impairs vision at the completion of medical treatment.

Adjunctive treatments

Specialised nursing care, excellent nutritional support and prevention, early detection and treatment of iatrogenic conditions (related to the stress of treatment and antibiotic therapy), are important for positive long-term clinical outcomes for koalas being treated for chlamydiosis [133, 143].

Oral analgesics and anti-inflammatories have been used to treat pain, inflammation and fibrosis associated with chlamydial disease and are essential to treatment success in chronic cases [124].

Koalas surviving antibiotic treatment were shown to have a greater diversity of faecal bacteria than those that died, and a greater abundance of the tannin-degrading *Lonepinella koalarum* [153]. The administration of koala faecal matter or caecal contents to mitigate the risk of dysbiosis is commonly suggested [133, 142, 143] but controlled studies evaluating the efficacy of this treatment are lacking. A study in which faecal microbiome is administered orally to koalas using enteric-coated tablets (to bypass gastric acids) is in progress [154].

Vaccination is showing promise as an adjunctive therapy for reducing the severity of clinical signs [155] – see *Prevention and Control*.

Bilateral chronic structural change in the female reproductive tract is invariably associated with permanent infertility, persistence and progression of inflammation, fibrous adhesions and pain. Ovariohysterectomy is an effective and beneficial treatment for these cases from a welfare perspective [143]. However, not all animals are suitable candidates for surgery: individuals of advanced age, or with associated chronic disease or poor body condition are not suitable candidates for surgery. Furthermore, the future release and holding options for a surgically sterilised animal should be explored within the jurisdiction before surgery is entered into [39].

A plethora of treatments which are not supported by evidence or have no known role in treating chlamydiosis in any species have been incorporated into treatment regimens in the past [133]. All untested ancillary treatments have the potential to do harm, both by increasing stress and by exposing animals to products of unknown safety [133]. Consequently, unsystematic, uncontrolled experimentation with treatments should be avoided.

5.1.7 Prevention and control

Free-ranging populations

Determining the best approach to control and prevention of *Chlamydia* in free-ranging populations is challenging due to the multitude of factors which affect the pathogenesis of chlamydial infection in koalas. There are no standard, nationally-accepted biosecurity and testing requirements for *Chlamydia* in koalas; consequently, organised programs for prevention or control of *Chlamydia* in free-ranging koalas are largely experimental and tend to be localised in approach.

The control of *Chlamydia* in free-ranging populations where clinical disease is prevalent may involve capture of clinically affected animals and transfer to rehabilitation facilities for assessment, treatment or euthanasia, depending on the severity of clinical signs. This can be a resource-intensive but effective tool for reversing population decline in defined populations [17, 143]. Modelling suggests that treatment of individual free-ranging koalas with chlamydiosis can have a significant impact in reversing population declines over a number of years [17]. While antibiotics provide no protection against reinfection [57], treatment of individual koalas may be sufficient, within a coordinated program, to re-establish population growth if other threats are also controlled [17]. A safe and effective vaccine may also be a useful tool in reducing population declines in certain circumstances (see *Vaccination* below).

Given the likely impacts of other stressors, including land management practices, on chlamydial pathogenesis (see *Pathogenesis*), support of good general health of free-ranging populations is an important consideration for controlling the impact of *Chlamydia*.

Retention of connected, protected habitat which provides for the nutritional and shelter needs of koalas and reduces their exposure to other threatening processes is likely to promote the capacity of populations to resist the detrimental effects of *Chlamydia*.

Managers of populations that are thought to be *Chlamydia*-free, or which have a very low prevalence of *Chlamydia*, may restrict koala movements to prevent the introduction of diseased individuals into the population [59]. Restrictions should be based on robust evidence of the current *Chlamydia* status of the population. Protocols should consider all entry pathways for disease (including potentially contaminated equipment) and use robust and systematic *Chlamydia* testing methods to screen incoming animals [156].

Translocation might also increase the risk of expression of chlamydial disease through stress-related changes to immune function [101]. In populations where *Chlamydia* is present, avoiding the introduction of novel *Chlamydia* genotypes may be a consideration [3], although there is currently insufficient knowledge and diagnostic technology to inform decision-making (see *Pathogenesis*).

Rehabilitation populations

The limited availability of reliable and cost-effective point-of-care diagnostic testing and the lack of standardised biosecurity and testing requirements for *Chlamydia* pose challenges for management of risk in the rehabilitation setting.

Both general and specific biosecurity principles are important to preventing the spread of *Chlamydia* within rehabilitation facilities [141]. These include excellent barrier hygiene between animals of different disease status, undertaking health evaluation (preferably with diagnostic testing to detect *Chlamydia*) to identify animals with chlamydiosis on admission, and a rigorous, standardised treatment protocol for chlamydiosis if treatment is indicated.

Important practices for preventing transmission of *Chlamydia* (and other pathogens) from rehabilitation facilities to free-ranging koalas include releasing rehabilitated individuals at their location of origin, isolating animals from different locations while in care, and preventing cross-contamination through appropriate disinfection, personal protective equipment and equipment use practices [141]. These practices will also reduce the risk of the dissemination of novel *Chlamydia* genotypes.

Captive populations

Koala management in captive populations is generally oriented towards individual animals. Many Australian zoos and wildlife parks aim to maintain *Chlamydia*-negative captive populations of koalas. The objective is to prevent new acquisitions bringing *Chlamydia* into their collections through stringent biosecurity practices, quarantine and diagnostic testing during isolation. Routine testing of existing captive koala populations may also be undertaken.

Although nationally-accepted biosecurity and testing requirements for *Chlamydia* in koalas are lacking, many institutions will have their own (often comprehensive) protocols. A

quarantine period of 45 days is commonly used for koalas entering a *Chlamydia*-free captive institution; this duration appears to be derived from quarantine regimes used for *C. psittaci* in birds. Incoming animals will commonly be sampled for PCR testing on one or two occasions during the quarantine period. Koalas being moved to other *Chlamydia*-free captive institutions will commonly receive a physical examination and PCR testing prior to shipment. Australian regulations stipulate that koalas intended for export from Australia are tested twice via PCR, with negative results, no less than 21 days apart within a 45 day pre-export isolation period [157].

The response to the detection of *Chlamydia* infection in captive koalas will vary from institution to institution, but may include isolation, systemic antibiotic treatment and follow-up diagnostic testing [37]. Environmental decontamination and disinfection may also be considered [71].

Vaccination

Development of a vaccine for *C. pecorum* in koalas has been a prominent focus of chlamydial research, with many vaccination trials having been undertaken over the last 15 years [155, 158-168]. Critical comparison of vaccine trials is limited by the absence of placebo groups in many studies and the diversity of *C. pecorum* antigens, adjuvants and dose regimens which have been used. The development of a safe, effective, widely-available vaccine has the potential to deliver positive impacts to koala health. Possible benefits of vaccination that have been suggested by research include:

- reducing chlamydial load or clearing infection in infected koalas [161, 165].
- reducing severity or prevalence of disease in infected koalas, which may in turn reduce need for antibiotic therapy [155, 165].
- reducing the number and proportion of healthy infected koalas that progress to disease [165].
- conferring a level of ongoing immunity to joeys of vaccinated dams [70].
- enhancing the koala's immune function and improving ability to fight off other infections [169].

Modelling of south-east Qld populations indicated that a vaccine with 75% protective efficacy could be a useful tool in reversing current population declines in that region if a vaccination program covering approximately 10% of the population per year, and targeting young females was used [170].

5.1.8 References

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5.2 *Cryptococcus* spp. in Koalas – Literature Review

5.2.1 Technical information

Aetiological agent

Cryptococcosis is caused by fungal agents of the *Cryptococcus* genus, particularly *C. gattii* and *C. neoformans* species complexes [1]. Other species in the genus may cause disease in mammals but have not been reported in Australian wildlife [2].

The nomenclature of the pathogenic members of the genus has a complex history which is comprehensively summarised in Krockenberger et al. 2019 [2] and Danesi et al. 2021 [3]. In this chapter, the nomenclature of two species complexes (*C. gattii* and *C. neoformans* species complex), and their respective molecular types (*C. gattii* VGI-V; VGIV/VGIIIc; *C. neoformans* VNI-IV; VNB) will be used [3].

Listing

Cryptococcosis is not a WOAHL listed disease [4].

Cryptococcosis is not a notifiable animal disease in Australia [5].

Cryptococcal disease is not identified as a key threatening process under the Environment Protection and Biodiversity Act [6].

5.2.2 Epidemiology

Cryptococcus species complex organisms are widely distributed in the environment [7]. The *C. gattii* complex is more important as a pathogen of Australian wildlife than the *C. neoformans* complex. *C. gattii* VGI is the most widespread and commonly documented cause of cryptococcal disease in koalas [8], probably because of its strong association with *Eucalyptus* spp. trees [9], particularly *E. camaldulensis* and *E. tereticornis*, both of which are consistent environmental sources of the organism and common feed species for koalas across their range [9-11].

Both *C. gattii* and *C. neoformans* can cause disease in immunocompetent animals, including humans, although in humans *C. neoformans* occurs more commonly in immunosuppressed individuals [12]. There is little evidence that *C. neoformans* infection is associated with immune status in other species, including koalas [13].

Given the vast majority of cases of cryptococcus infection in koalas are caused by *C. gattii*, the remainder of this chapter will focus on the *C. gattii* species complex unless explicitly stated.

Host range

Cryptococcus gattii infection and disease has been documented in a wide range of domestic and wild species, including dogs, cats, goats, horses, ferrets, cheetah, squirrels, porpoises, dolphins, parrots, cockatoos and kiwi [14]. Among Australian wildlife, in addition to koalas,

C. gattii infection has been reported in phascogales, bandicoots, gliders, wallabies, potoroos and native rodents [2, 8, 15-19]. *Cryptococcus gattii* is both a primary and an opportunistic pathogen in humans [20].

Zoonotic potential

Direct zoonotic transmission of *Cryptococcus* spp. does not occur [21], although koalas can amplify the organisms in the environment [22], thus increasing environmental load.

Geographic distribution

Cryptococcus gattii has a global distribution in soil, trees and tree hollows [14], although different molecular types predominate in different regions of Australia. *Cryptococcus gattii* VGII is considered endemic to south-western WA and NT [23], while VGI is more widespread and has strong associations with trees in the *Eucalyptus* genus throughout Australia, thus presenting an important source of infection to koalas [23]. VGI has been detected in association with eucalypts (*Eucalyptus*, *Angophora* and *Corymbia* spp.) and other native trees (*Melaleuca*, *Myoporum*, and *Syncarpia* spp.) in eastern, inland, southern and western parts of Australia [2]. The various molecular types of *C. gattii* have been isolated from the soil and hollows of over 50 species of tree worldwide, and it has been suggested that the species of tree is less important than the capacity of the tree to form decayed hollows, thus providing an effective substrate and environmental niche for the organism [3, 24, 25].

Most reported cases of cryptococcosis in koalas occur in NSW, with cases in free ranging and captive koalas mostly occurring in the Sydney region and on the north coast [22, 26, 27]. However, cryptococcosis is more extensively studied in NSW koala populations than in other areas of Australia, so the occurrence of disease in other regions is probably under-represented [28]. Clinical disease occurs sporadically in free-ranging and captive koalas in northern and south-east Qld [29, 30]. Recent retrospective necropsy studies of koalas in SA (n=85) did not detect any cases of cryptococcosis [31] and there are no reports of cryptococcosis in free-ranging koalas in SA or Vic.

Cryptococcal disease has been reported in captive populations in Qld, NSW and WA [23, 32]. An unusual cluster of cryptococcal disease, nasal colonisation and subclinical infection due to *C. gattii* (predominantly VGII) that occurred in three captive facilities in northern Qld was associated with movement of infected koalas between the facilities, and may have involved transmission from an infected animal imported from WA [23]. Isolated cases of *C. gattii* VGII have occurred in captive koalas in south-western WA, where this molecular type is endemic [3, 23, 26].

Prevalence

There are three phases of cryptococcus infection in koalas: nasal colonisation, subclinical infection, and cryptococcal disease (see *Pathogenesis*). Subclinical infection is characterised by the presence of antigenaemia (cryptococcal antigen detectable in the blood of the host) in the absence of disease.

In captive environments, high koala density may be important to maintaining a high environmental presence of *C. gattii* [13], but this is not the case in natural habitat, where heavy environmental loads of *C. gattii* may occur in the absence of koalas [22]. The density of Eucalyptus trees with hollows, and the presence of hollows colonised by *C. gattii* within a koala's home range, were significant predictors of nasal colonisation in free-ranging koalas in the Liverpool Plains of NSW, but the cryptococcal load in tree hollows was not associated with nasal colonisation [25, 33]. These findings suggest that frequency of exposure is more important than the environmental load per se in driving nasal colonisation.

In considering the prevalence of nasal colonisation in koalas, it should be noted that the sensitivity of nasal swabbing as a means of determining and quantifying cryptococcal colonisation is unknown [34]. The prevalence of cryptococcal nasal colonisation (6%) and antigenemia (7%) in free-ranging koalas in NSW is much lower than in captive populations, where nasal colonisation has been reported at close to 100% prevalence, with subclinical antigenemia prevalence of >50% in some facilities [22, 27, 35]. The high prevalence figures for captive populations in NSW support the hypothesis that koalas amplify *C. gattii* VGI in their immediate environment [22, 34]. The mechanisms of amplification are not clear. In free-ranging koalas, no clear epidemiological pattern to the occurrence of nasal colonisation or subclinical infection has been identified, although a possible link between high relative abundance of *E. camaldulensis* in a koala's habitat and the development of antigenemia merits further study [34].

No seasonal or gender variations have been detected in the prevalence of nasal colonization or antigenaemia in free-ranging koalas, or antigenaemia in captive koalas [22, 34]. One study of captive NSW koalas indicated a significant bias of nasal colonisation in males, which was suggested to reflect the greater tendency of males to use smell to explore territory [36]. There are no published data on prevalence of colonisation and antigenemia in koalas outside of NSW.

Cryptococcal disease (cryptococcosis) is well recognised in koalas, but is much less prevalent than nasal colonisation or subclinical infection. In retrospective post mortem studies, only 3–4% of koalas (mostly from NSW) had cryptococcal lesions [34]. The comparative rarity of clinical disease, given the ubiquity of *Cryptococcus* in the environment, suggests that, in the majority of cases of infection, the host response is sufficient to contain or eliminate the pathogen [37].

In a survey of Australian institutions housing koalas in captivity (n=16 institutions in Qld, NSW, SA, WA and Vic), cryptococcosis was noted in 11% of 263 diseased koalas (from any cause) [32]. Although cryptococcal disease is predominantly observed and documented in captive koalas, case clusters also occur in free-ranging populations [26, 34], and represent 39% of all reported cases of cryptococcal disease in koalas [3]. There does not appear to be a gender bias to cases of clinical disease [3].

Mode of transmission

Cryptococcus organisms exist in the yeast form within the host, and likely as a filamentous fungus in the environment [7]. Transmission occurs when the host makes direct contact with environmental cryptococcal organisms. In koalas, inhalation of airborne cryptococcal organisms into the nasal passages, with subsequent colonisation of the sino-nasal mucosa, is the most common route of entry [2, 36].

Solitary cutaneous cryptococcal lesions have been identified in numerous koalas [26], suggesting that direct inoculation is also a possible route of infection, possibly via minor grooming injuries [38]. In one case, spinal osteomyelitis occurred in the absence of lesions in other tissues. Overlying rake marks suggested that direct inoculation may have occurred via fighting injuries [39].

Seemingly healthy koalas are able to carry *C. gattii* VGI and VGII with them when translocated within Australia or internationally, either through nasal colonisation or within constrained foci of infection [23, 40, 41].

Incubation period

As with many fungal diseases, the incubation period for *Cryptococcus* remains poorly defined because the time of exposure is often unknown [42]. Incubation period for *C. gattii* infections in people during outbreaks in USA and Canada ranged from 2-13 months [24, 42], with a median incubation of 6-7 months in the Canadian outbreak [42].

In koalas the incubation period is thought to be variable and extended [26]. In a study of 28 healthy koalas with subclinical cryptococcal infection, one animal developed cryptococcal pneumonia within 6 months, while another developed cryptococcal meningoencephalitis two years after the start of the study [27].

Determination of incubation period is complicated by the fact that transmission does not always result in disease. Progression from one phase to the next may occur rapidly or slowly, but is not inevitable; nasal colonisation may occur in the absence of the development of clinical disease or subclinical infection (see *Pathogenesis*), and both colonisation and subclinical infection may spontaneously resolve over time with effective adaptive immune response [3].

Environmental exposure may significantly pre-date disease development: a cluster of *C. gattii* VGII cryptococcosis cases in captive koalas in eastern Qld was attributed to environmental seeding by an infected koala imported from WA approximately 10 years prior, which introduced *C. gattii* VGII into environments previously free of *Cryptococcus*, and also into environments where *C. gattii* VGI was endemic [23].

Persistence of agent

Cryptococcus gattii can colonize the environment in tropical, subtropical, temperate and dry climates. Environmental isolates tolerate temperatures as high as 37°C [20] and are adapted for survival in dry, nutrient-deprived soils [20].

There is some evidence that *C. gattii* may persist longer in the environment in the presence of plant tissue, reflecting the organism's strong environmental association with plants [43]. The bark of eucalypts is high in dihydroxyphenylalanine, which is metabolised by *Cryptococcus* species to produce melanin, and may contribute to their environmental survival by helping them to resist UV irradiation [20].

In studies of *C. gattii* in Mediterranean Europe, the organism was unable to tolerate low temperatures during winter and did not survive when the minimum temperature dropped below 0°C. Survival also dropped rapidly if rainfall in the driest month increased beyond 100 mm [44]. These conditions correspond with the climatic requirements for olive trees, an important ecological niche for *C. gattii* in the region. It is likely that *C. gattii* in Australia is subject to similar environmental constraints, which also favour the presence of the organism's preferred Australian tree species.

The virulence factors which promote the organism's survival in mammalian hosts (see *Pathogenesis*) also promote environmental persistence, including survival within soil amoebae [3].

5.2.3 Pathogenesis

As noted, there are three phases of cryptococcus infection in koalas: i) nasal colonisation; ii) subclinical infection; and iii) clinical disease.

Colonisation of the sino-nasal mucosa occurs very commonly in koalas, particularly in captivity (see *Prevalence*), and probably requires a threshold environmental exposure of infectious agent in order to occur [25]. Once colonisation is established, infection may either spontaneously resolve (a common occurrence in young koalas soon after independence), or may progress to subclinical infection or clinical cryptococcal disease [22, 36].

Subclinical infection probably reflects early, limited invasion of the respiratory mucosa. It is defined as cryptococcal capsular antigenaemia (as determined by a positive serological titre) in the absence of clinical signs or identifiable lesions. Subclinical infections can either i) resolve in an immunocompetent host, ii) persist as a localised infection, or iii) progress to clinical disease [22, 27, 36]. The presence of concurrent adverse factors such as stress and poor nutrition may play a role in the progression of subclinical infection to clinical disease [2, 34].

Key virulence factors for *Cryptococcus* pathogens include [3, 45, 46]:

- the organism's thick polysaccharide capsule, which enables avoidance and suppression of host immune response. The capsule undergoes "phenotypic switching" during infection, developing its mucoid properties which enables longer survival in macrophages, and avoids antibodies and complement-mediated phagocytosis. The mucoid forms are associated with a low inflammatory response and the formation of granulomas.
- the ability to grow at mammalian body temperature.
- the ability to produce melanin to resist host and environmental free radicals.
- production of binding proteins and sugars which facilitate tissue invasion and help evade host immune response.
- development of "titan cells" which further resist phagocytosis.

The relative importance of these virulence factors to pathogenesis is not known.

Different cryptococcal antigen proteins are produced during different phases of the infectious process in koalas, possibly reflecting shifts in virulence pathways as disease progresses [37]. Some antigenic proteins are unique to particular molecular types of *C. gattii*, possibly indicating differences in the virulence mechanisms which might explain differences in the epidemiology and pathogenesis of *C. gattii* molecular types in koalas [37]. Additionally, it is possible that genetic variation exists between different strains within molecular types which may affect the virulence of the organism in a given situation [25].

5.2.4 Associations with other disease hazards of koalas

The role of immunosuppression or association with co-infection in the pathogenesis of cryptococcosis in koalas remains unclear. Given cryptococcal disease in koalas is commonly caused by *C. gattii*, which generally infects immunocompetent hosts in other species, an association with the potential immunosuppressive effects of KoRV is perhaps less likely [2]. Investigations into this association are ongoing [34].

A 9 year old koala which was diagnosed with a novel alphaherpesvirus infection was also found to have cryptococcal organisms in the lung, cultured as *C. gattii*. Cryptococcal organisms were not cultured or detected histologically in any other tissues. It was postulated that cryptococcal disease may have contributed to physiological stress which activated a latent herpesvirus infection in this individual [30].

5.2.5 Diagnosis

Nasal colonisation and subclinical cryptococcal infection can be diagnosed by serological detection of cryptococcal antigen (see *Diagnostic testing*). Serology alone is insufficient to diagnose cryptococcal disease, which generally requires a combination of clinical signs presenting in conjunction with indicative serological titres, culture or cytology [2]. Diagnostic imaging techniques such as radiography, computed tomography and magnetic

resonance imaging are useful complementary tools where discrete masses or bony involvement occur [2, 38, 47].

Identifying cryptococcal infection at the colonisation or subclinical stage/s may enable interventions which prevent progression to clinical disease, an important consideration given the poor prognosis once clinical signs are apparent (see *Treatment and Prevention and control*).

Clinical signs

Most koalas with clinically apparent cryptococcosis appear to be systemically unwell, and may demonstrate general signs of illness including inappetence, lethargy, depression and weight loss [2, 48]. However, some may only exhibit minor signs of ill health such as coughing, sneezing and nasal discharge, and only become systemically unwell when the lesion becomes obstructive or progressive [49]. Occasionally, sudden death may be the only presenting sign [48].

The typical presentation in the koala are signs localising to the respiratory tract [2, 26]. Most common is upper airway disease with sino-nasal disease characterised by sneezing, difficult or noisy breathing, nasal discharge, nose bleed and facial distortion. Nasopharyngeal involvement may result in dyspnoea, swallowing air and gastric dilatation. Involvement of the lower respiratory tract, including trachea, lungs or pleural space, is likely to be more subtle in clinical presentation, but may be associated with dyspnoea and coughing [2, 26].

Signs of neurological disease typically reflect meningoencephalitis and include blindness, nystagmus, limb paresis, opisthotonos and seizures [2, 26, 48, 50].

A case of cryptococcal osteomyelitis of the distal tibia and proximal tarsus due to *C. gattii* VGI was associated with lameness, an extensive ulcerative mass, diffuse soft tissue swelling and local lymph node enlargement. There was radiographic evidence of severe osteolysis in the affected bones [38].

Unilateral exophthalmos was the only presenting sign in a case of *C. neoformans* cryptococcosis in a captive koala in a European zoo [47].

Clinical pathology

There are no haematological or serum biochemistry findings which are diagnostic for cryptococcal disease in koalas. An inflammatory leukogram indicative of a general stress response may be seen [38]. Cytological methods may be used to detect the characteristic gram positive yeasts with a wide, poorly staining extracellular capsule in infected or colonised tissues [2].

Pathology

A positive culture from a normally sterile site such as lung, lymph node or cerebrospinal fluid is diagnostic for cryptococcosis. In contrast, a positive culture from a nasal swab in the

absence of a positive cryptococcal antigen test is not evidence of disease and should be considered colonisation until proven otherwise [2].

Cryptococcal organisms have a classic microscopic appearance. They are 5-30 µm, round or ovoid, gram positive staining yeasts with a wide, poorly staining or negatively staining extracellular capsule. Organisms are easily detected by cytology of fine needle aspirates of masses, or from smears of nasal exudate, nasal washes or bronchoalveolar lavage, and are readily identified on histopathology [2]. Molecular detection of cryptococcal DNA using panfungal PCR and sequence analysis may be attempted, although yields may be low from formalin-fixed tissue [2].

Grossly, cryptococcal lesions tend to appear as masses, but may be ulcerative if mucosal surfaces are involved. Masses may range in appearance from classic gelatinous multinodular lesions to solid granulomatous lesions. The host response may vary from minimal tissue response to a florid granulomatous response. Early inflammatory exudates can be neutrophilic or eosinophilic in nature. Abscessation and pus formation is seen rarely [2, 28].

The most common site of pathology in koalas is the respiratory tract (78% of cases). 73% of cases with respiratory tract involvement have pneumonia and 48% have upper respiratory tract pathology [2]. The organism is neurotropic in koalas with 32% of cases showing central nervous system involvement, although relatively few cases (14%) have neurological disease without involvement of other organ systems [3]. Oral cavity lesions have been observed in several individuals [49].

Disseminated disease is relatively common (34% of cases). Both upper and lower respiratory tract disease can lead to haematogenous dissemination. Disseminated disease can localise in the skin, bone, gastrointestinal tract, urogenital tract, spleen or lymph nodes [26, 38].

Skin involvement may be seen as a primary focus [26] or following haematogenous dissemination from a respiratory infection [38].

Spread of disease to regional lymph nodes is commonly present (20% of cases) but is rarely found in isolation of other tissue involvement [3, 26].

Differential diagnosis

In Australia there are few diagnostic alternatives for large encapsulated budding yeasts, and identification of such organisms in diseased tissue is diagnostic for *Cryptococcus* [2]. Culture and serology are useful ways of eliminating rare fungal diagnostic alternatives. Major differential diagnoses for upper and lower respiratory tract disease in koalas include chlamydiosis, *Bordetella* rhinitis and neoplasia. Neurological signs in koalas may be associated with trauma, meningoencephalitis due to other infectious agents, or neoplasia. Palpable or visible tissue masses may be indicative of neoplasia, trauma, or lymph node enlargement due to other pathogens. Ulcerative skin lesions may be consistent with trauma, burns, bacterial dermatitis or other fungal infections such as ringworm.

Diagnostic testing

There are two major antigen detection methods for cryptococcosis in koalas: the latex cryptococcal antigen test (LCAT) and the lateral flow immunochromatography assay (LFA). Both detect cryptococcal capsular antigen and are highly sensitive and specific. However, serological testing should always be interpreted in the light of clinical findings [2].

The LFA may be run on blood, plasma, serum or urine, is inexpensive, and is simple to use in a field situation. LFA has a high sensitivity and excellent negative predictive value for the diagnosis of cryptococcosis in koalas, and is therefore an excellent screening test for the disease, although positive results should be confirmed via LCAT [2].

LCAT is useful for determining serological end-point titres for monitoring subclinical and clinical disease [2, 27]. Low level positive LCAT titres (1:2 to 1:8) are generally typical of self-limiting subclinical infection, although they may also indicate early clinical disease. Titres less than 1:64 often resolve spontaneously in captive koalas, particularly in the absence of concurrent stressors such as poor nutrition and transport [27]. Titres \geq 1:64 are strongly suggestive of clinical disease and warrant further investigation [2].

Surveillance and monitoring

There is no targeted surveillance program for cryptococcosis in koalas. However, the finding of cryptococcal disease in samples from free-ranging koalas would be considered interesting and unusual and would therefore be logged in the Wildlife Health Australia national wildlife health information system (eWHIS) as part of national general wildlife surveillance activities.

Cryptococcosis is a substantial management concern in captive koala facilities, particularly due to the potential role of stressors in promoting the progression of nasal colonisation and subclinical infection to disease. Serological monitoring via LFA or LCAT is generally recommended as a component of a standard health check at least once or twice a year, and prior to transporting koalas between facilities [3]. Nasal cytology or mycological culture may assist in understanding the prevalence of subclinical infection in a captive group, which may provide indication for environmental decontamination strategies such as substrate change and furniture replacement [2, 3].

5.2.6 Treatment

Successful treatment of cryptococcal disease in koalas is extremely challenging once clinical signs are present, and is almost exclusively attempted in captive koalas. Aggressive antifungal drug therapy, alone or in combination with surgical debulking of lesions, is required [2, 35, 41, 47, 51]. Signs of neurological involvement are a very poor prognostic indicator [48].

The drug regimens used for koalas are based largely on those used with success in domestic cats [26]. Amphotericin B is a fungicidal formulation which is an essential component of the treatment regimen in cases of extensive or disseminated disease, but care must be taken to avoid its nephrotoxic effects by concurrent administration of fluids, and renal function

should be monitored throughout treatment [48]. The liposomal formulation of amphotericin B is less toxic, but is very expensive and requires IV administration [2].

Amphotericin B may be used alone or in combination with oral triazole antifungals, most commonly fluconazole [35, 41, 51], but also itraconazole [35, 41, 47]. Second generation triazole formulations (e.g. voriconazole, posaconazole, isavuconazole) are very expensive and untested in diseased koalas, and the gastrointestinal absorption of posaconazole in koalas is highly variable [52].

Triazole drugs are relatively less toxic than amphotericin B but are fungistatic rather than fungicidal and are therefore less effective in treating symptomatic disease. The elimination half-life of fluconazole in healthy koalas is much shorter than in many species, and oral bioavailability is low, with MIC values failing to reach the levels required to inhibit *C. gattii* when used as the sole therapeutic agent [51, 53]. There appears to be substantial variability in bioavailability of fluconazole between individual koalas, which may be related to differences in disease status, drug formulation, gastrointestinal absorption and the nephrotoxic effects of concurrent amphotericin B administration [51]. Monitoring of drug plasma concentrations is therefore essential to effective triazole therapy [2, 41, 51]. Specific antifungal sensitivity testing is also recommended, as certain strains of *C. gattii* show intrinsic resistance to fluconazole *in vitro* [51]. In spite of these limitations, triazoles have been used successfully as the sole therapy in koalas to manage early, localised or subclinical infections [38, 48, 49].

Where cryptococcal disease presents as accessible localised lesions, surgical excision or debulking of lesions may be a useful adjunct to successful treatment [41].

Serial monitoring of cryptococcal antigen titres via LCAT is an essential component of determining effectiveness of treatment regimens [41]. Antifungal drug therapy should be continued until antigen titres are continuously negative for at least one month [48]. In one case of a captive koala in the USA where antigen titres persisted in spite of clinical resolution after surgical debulking and combined antifungal therapy, treatment with itraconazole was continued indefinitely [41].

There is a single report of treatment of a free-ranging koala, in which a joey whose mother was euthanased due to disseminated cryptococcosis was treated with oral fluconazole twice daily for 15 months, with response to therapy based on resolution of cryptococcal antigenemia. This animal was released approximately 21 months after admission [38].

5.2.7 Prevention and control

Early diagnosis of subclinical infection is the key to successful outcomes [2]. In captive colonies, a screening program should be established to monitor for cryptococcal colonisation through monitoring of antigenic titres, and possibly also nasal cytology and culture (see *Surveillance and monitoring*). Management options for low positive antigen titres might include monthly monitoring, with or without triazole medication [2].

In captivity, re-use of eucalypt browse from koala enclosures appears to be associated with the development of cryptococcosis in other captive species [2]. Environmental decontamination strategies such as regular changes of substrate and disinfection or replacement of enclosure furnishings may be recommended in colonies where disease outbreaks or subclinical infection are present. It may also be prudent to avoid collecting browse from locations where cryptococcal disease outbreaks have been reported in free-ranging koalas.

The stress of transport appears to be a key cause of progression to cryptococcal disease [2, 23]. Preshipment screening for subclinical infection should include serological testing and nasal cytology. Animals with evidence of subclinical infection should not be subjected to the stress of transport due to the risk of precipitating clinical disease [2, 23].

Given the predilection of *C. gattii* for environments and plant species also favoured by koalas, there is little capacity for preventing exposure of free-ranging koalas to the organism. However, translocation of free-ranging animals from regions where *C. gattii* is environmentally abundant is likely to present similar risks as shipment of captive animals. Every effort should be made to minimise stress during translocation. Serological testing via LFA should be used to avoid translocating animals with subclinical infection. Given the inherent stress in the rehabilitation process, serological testing of koalas entering rehabilitation from areas where *C. gattii* is commonly present is also likely to be an important tool.

5.2.8 References

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5.3 Heat Stress in Koalas - Literature Review

5.3.1 Technical information

Description of hazard

Heat stress is a physiologic event that occurs when an animal has extended exposure to ambient temperatures above their thermoneutral zone (TNZ) [1]. In heat stress, animals are unable to dissipate heat and their body temperature (T_b) rises above the normal range (hyperthermia) [1, 2]. Heat related illness in mammals can range from mild hyperthermia with panting and sweating, through to severe inflammatory changes, multiple organ damage and disseminated intravascular coagulation [3-5].

5.3.2 Epidemiology

Causes of hazard

Extended periods where the daily maximum ambient temperature remains above the upper limit of the koala TNZ (24.5°C), or where ambient heat dissipation at night is reduced, can lead to heat stress in koalas. This is most apparent when the ambient temperature exceeds 35°C [6].

Since the 1950s, the average ambient temperature in Australia has been increasing and heatwaves have become more frequent, longer, and hotter across the geographical range of koalas [7]. This situation is likely to get worse due to climate change [8].

Periods of prolonged heat and drought have resulted in high mortality in koalas [9-14]. Ambient temperatures and water availability have been shown to impact survivability of koalas in their northern and western range [15, 16].

Geographic distribution

Heat stress has been reported as a cause of morbidity and mortality in koalas across their geographic range [17-20].

Mapping of the likely distribution of koala heat stress events has not yet been attempted. Heat stress mortality in Australian flying-fox colonies can be predicted based on weather forecasting with a high degree of accuracy (>70% within 24-48 hours of a forecast maximum of $\geq 42^\circ\text{C}$). Extrapolation of such techniques to koala populations should be possible, but would require detailed knowledge of the species' thermal tolerance [21].

Prevalence

There have been few studies to investigate the prevalence of heat stress in koalas. One study from SA reported that 12% (n=225) of presentations to a wildlife centre (2013-2014) showed signs of heat stress [18]. The majority of cases presented on the day of, or the day following, ambient temperatures exceeding 40°C [18]. Retrospective studies of post mortem data for koalas in south-east Qld identified heat stress as a cause of death in some

individuals, but figures were included within the 13% of cases attributed to “other” causes [20]. In 2019-20, drought or extreme heat (as distinct from bushfire) were recorded as reasons for admission in 36 out of 1613 koala rescues (2.2%) reported to the NSW Government [22]. The lack of consistent reporting may reflect the seasonal and sporadic nature of heat stress events [23-25].

The impacts of heatwaves and droughts on koala population viability have been described. In Mungallala Creek, Qld it was estimated that over 60% of the koala population died in the summer of 1979-1980 due to a combination of drought and heat [10]. An estimated 25% of the Gunnedah koala population (NSW) succumbed to heat in 2009 [11]. Monitoring of populations in south-west Qld over the 2000-2009 drought showed an approximate decline of 80% [12].

5.3.3 Pathogenesis

Koalas generally maintain their T_b within the normal range if ambient temperatures do not exceed the upper limit of their TNZ (24.5°C). When the ambient temperature is above 24.5°C, T_b is less well regulated and hyperthermia may occur. Increasing T_b correlates strongly with ambient temperature when the latter exceeds 30°C [6].

Koalas use convection, evaporation and behavioural responses to dissipate excess heat. Convective heat loss via peripheral vasodilation and air currents becomes less effective as ambient temperature increases [6, 26]. Evaporative cooling mechanisms include sweating from hand and foot pads, panting and hypersalivation [26]. These mechanisms become less effective in hotter and more humid conditions, and result in increased water losses for the animal [3, 9, 10, 15]. Koala behavioural responses to increased ambient temperature include seeking cooler microclimates (e.g. lower tree branches, understory, tree hollows), tree hugging, spreading their body out on cool branches and increased free water drinking [13, 15, 27, 28]. These behaviours increase the efficacy of evaporative and convective heat loss. Koalas in severe heat stress will eventually become prone on the ground in any available shade [29].

Koalas obtain about 75% of their water intake from foliage [30]. The remainder of their water needs are met by drinking free water [31]. Koalas need to ingest fluid more frequently in summer to compensate for reduced leaf moisture content [11] and increased water turnover associated with convection and evaporative cooling [15, 28]. Prolonged heat can cause koalas to become inappetent [29], further reducing their water intake and reducing their ability to dissipate heat through evaporative means.

Body heat is generally acquired during the day and dissipated at night. The body temperature of koalas has been shown to peak late in the afternoon, approximately two hours after the peak in ambient temperature. It has been postulated that during hot weather, the koala's peak in T_b shifts to later in the evening or into the night, reducing the night time window of opportunity to efficiently dissipate heat, and further predisposing them to hyperthermia and heat stress [6].

Dehydration may cause increased water re-absorption from the very large fluid volume of the koala caecum. Koalas that develop heat stress may have impaired gut function as a result of dehydration of the caecum and its contents [32].

Prolonged hyperthermia in animals has been demonstrated to cause a significant inflammatory response, widespread endothelial damage and eventually organ damage in dogs and primates [3, 4, 33]. These changes have also been seen in koalas with heat stress [32].

5.3.4 Associations with other disease hazards of koalas

No studies have demonstrated associations of heat stress with other diseases in koalas. However, any disease process that inhibits convection, evaporative or behavioural means of thermoregulation would likely decrease the koala's ability to cope with elevated ambient temperatures. Such diseases could include oxalate nephrosis, chlamydiosis and other renal diseases (due to increased hydration stress), pneumonia or other respiratory disease, and cardiac disease, that might affect panting behaviour and circulation.

5.3.5 Diagnosis

Clinical signs

The normal rectal temperature range for koalas is reported to be 35.5–36.5°C [34], which is similar to the long term diurnal temperature range of 35.4–36.9°C measured in free-ranging koalas using surgically-implanted data loggers [6]. Maximum and minimum core body temperatures for koalas as measured by data loggers were 34.2°C and 39.0°C. The rectal temperatures of heat-stressed koalas are likely to be over 37°C [32], although some affected koalas may have “normal” rectal temperatures [35].

Koalas which are heat stressed may exhibit evaporative cooling behaviours including panting (respiration rates >60), sweating (from palmar and plantar aspects of paws) and hypersalivation [18]. Heat stressed koalas are likely to show signs of dehydration including prolonged capillary refill times, “muddy-coloured” mucous membranes, skin tenting, sunken eyes and weak pulse [36]. They may drink profusely and have small, shrunken faecal pellets if heat stress has led to chronic dehydration [32]. Severely affected koalas may show signs of depression, inappetence, inability to climb and collapse. Heat stressed koalas may be found inactive at the base of a tree [35]. They may be in poor body condition and have increased ectoparasite burdens if they have been trying to survive in a drought-affected environment for prolonged periods [11]. Heat stressed koalas are often too debilitated to eat or drink, leading to secondary complications of chronic anorexia and gut dysbiosis [29]. Figure 23 shows a debilitated, heat-stressed koala at the base of a tree.



Figure 23 A debilitated wild koala sitting at the base of a tree during an extreme heat event (credit: Ian Hough)

While there are no koala-specific criteria for determining the severity of heat-related illness, systems established for domestic dogs are probably appropriate for extrapolation.

Table 14 shows a system developed for dogs which uses updated terminology ('mild', 'moderate' and 'severe' heat-related illness) in place of the terms 'heat stress', 'heat exhaustion' and 'heat stroke', and outlines clinical signs and recommended management [5]. Although not all of the signs in

Table 14 have been reported in koalas, it is likely that severely affected koalas could suffer similar metabolic and physiologic disturbances as those described in dogs, including hypovolaemic shock, endotoxaemia, metabolic acidosis and DIC leading to reduced organ perfusion and tissue necrosis [5, 33, 37].

Table 14 The VetCompass “Clinical Grading Tool for Heat-Related Illness in Dogs”, which could be applied to koalas (adapted from Hall et al. 2021 [5])

Stage	Previous Terminology	Clinical Signs	Suggested Treatment
Mild	Heat stress	Continuous panting or respiratory effort unresolved following cessation of exercise or removal from hot environment. Lethargy, stiffness or unwilling to move.	Active cooling if hyperthermia present. Rehydration (may need oral only). Supportive care for organ systems affected (e.g. oxygen for dyspnoea). Monitor for progression of clinical signs.
Moderate	Heat exhaustion	Progression of mild stage: – no response to cooling and/or fluids. Hypersalivation, diarrhoea and/or vomiting (no blood present). A single seizure. Episodic collapse with spontaneous recovery (no impaired consciousness).	Active cooling if hyperthermia present. Rehydration - may require intravenous fluids. Supportive care for organ systems affected (e.g. gastrointestinal support). Consider hospitalisation to monitor progression of clinical signs.
Severe	Heat stroke	Progression of moderate stage. Any of: <ul style="list-style-type: none"> • Central nervous system impairment (ataxia, two or more seizures, profound depression, unresponsive, coma). • Liver or kidney dysfunction. • Gastrointestinal haemorrhage. • Petechiae/purpurae. 	Requires hospital care. Active cooling if hyperthermia present. Coagulation assessment required. Supportive care for organ systems: <ul style="list-style-type: none"> • neurological support (e.g. osmotic agents, seizure management) • Intravenous fluid therapy, blood glucose and electrolyte management • Respiratory support (e.g. oxygen, intubation) • Circulatory support (e.g. vasopressors) • Gastrointestinal support (e.g. antiemetics, gastrointestinal protectants) • Transfusion products.

Clinical pathology

Clinical pathology changes are dependent on the severity of heat-related illness and the organs affected.

Heat stressed dogs will show clinical pathology indicative of dehydration including increased total protein and increased symmetric dimethylarginine (SDMA). Renal, liver and muscle parameters may be elevated due to organ damage. Prolonged prothrombin time and activated partial thromboplastin time have been observed in dogs affected by heatstroke-induced disseminated intravascular coagulation [3, 33].

Pathology

There are no detailed reports of koala pathology associated with heat stress. Pathology associated with heat-related mortality in dogs includes generalised congestion, oedema, haemorrhage and thrombosis [38]. Multiple organs are affected, with changes often seen in brain, kidney, liver, heart, intestine and spleen [38]. Hyperthermia can cause inflammatory changes, endothelial damage and damage to multiple organs [3, 4, 33]. Koalas succumbing to heat stress often have noticeably dehydrated caecal contents [32].

Differential diagnosis

Presentations of renal failure may show similar signs of dehydration, depression and water seeking behaviours, however koalas in renal failure are not hyperthermic. Trauma with subsequent unwitnessed seizure activity may present in a similar fashion to heat stress, and koalas may also be hyperthermic. Debilitating infectious disease of the respiratory system that increases respiratory rate, and causes pyrexia, lethargy and depression may mimic heat stress. Figure 24 shows a wild, dehydrated koala drinking from a bowl of water.



Figure 24 A dehydrated koala in rehabilitation care drinking water from a bowl (credit: Adelaide Koala and Wildlife Centre)

Surveillance and monitoring

Currently, there is no monitoring or standardised reporting of heat stress in koalas. There are likely to be records and reports in wildlife hospitals and rehabilitation facilities of heat stress admission and treatment, but currently there are no central records and few published reports.

5.3.6 Treatment

Treatment of heat stress in animals includes provision of a cool environment with good air flow and low humidity, to help restore evaporative and convection heat dissipation mechanisms [5]. Fluid therapy is used to help restore circulating blood volume and to manage dehydration. Supportive care for affected organs should also be provided. As with all cases where koalas require hospitalisation, specialised nursing care and excellent nutritional support are essential. A variety of species of high-quality browse with a high moisture content should be provided, along with additional misting of leaves or sprinkler systems to maintain foliage hydration [32]. Misting of browse may also help to increase the koala's fluid intake if the animal is eating voluntarily. If anorexic, regular syringe feeding of leaf paste provides koalas with fluid and nutrients [35].

Prognosis may be grave if there is progression to severe stages of heat-related illness. Dogs with signs of mild heat-related illness (Table 14) have a much greater chance of survival (85-90%) than dogs with severe signs (7-54%) [5], and this would be expected to be the same for koalas. It is very difficult to restore function to a dehydrated caecum and many koalas with severe dehydration will eventually succumb to caecal dysfunction [32].

5.3.7 Prevention and control

Climate change is likely to play a significant role in the frequency and severity of heat stress events of free-living koalas. Prolonged high temperatures have a profound effect on the quality of koala habitat, reducing both the moisture content of browse, the suitability of trees for shelter, and free water available in the environment [11, 31]. Koalas living in drought-affected landscapes will be more prone to heat stress, due to reduced dietary and free water intake, increased evaporative water turnover and reduced options for behavioural cooling strategies [9, 16]. Understory and midstory habitats provide refugia from heat events [39] and the loss of these are likely to highly disadvantage koalas during times of high ambient temperatures. Mitigation of all aspects of these processes is not possible, but supportive actions could help koalas cope with an increase in ambient temperatures. Actions include ecological assessments, protection of refugia, and mitigation of the cumulative effects of low water availability, low quality browse and fragmented habitat [40, 41].

Koalas will drink free water, especially during times of prolonged heat [31], so the provision of accessible, strategically-placed and well-maintained watering stations would help free-ranging koalas to meet increased water demands [28]. Awareness and education programs

which give clear instructions on how to safely provide water for heat-stressed koalas would ensure that the correct assistance is given by the community and emergency workers [42, 43].

For captive or rehabilitating koalas, misting or spraying browse with water will help to preserve the moisture content of the leaf, as well as incidentally increasing the koala's water intake, both of which offset increased water turnover [34, 44]. Spraying the koala's natural evaporative surfaces (e.g. undersides of feet, oral mucous membranes) may assist with evaporative cooling. Feeding captive koalas in the evening rather than in the morning during warmer months appears to reduce their peak body temperature, and may assist in avoiding heat stress effects [45, 46]. Careful storage of cut browse branches (refrigeration; standing the cut end in water which is replenished daily) will help to maintain leaf moisture [46, 47].

Koalas make use of cooler microclimates to help manage their heat, including lower tree trunks, trees with high foliage cover and trees in cooler gullies [27]. Consequently, ensuring best quality habitat and diversity of tree species and protection of refugia including midstory and non-fodder trees with high foliage cover, will support koalas to employ these behaviours [16, 28, 48]. Actions include ensuring sufficient availability of a diverse range of trees and ensuring effective corridors to connect scattered habitat. Maximising the potential microclimates within the koala's geographical range will mitigate the impacts of increased ambient temperatures. Such actions require planning well in advance of extreme heat events.

Establishing systems for forecasting heat events, similar to the monitoring and interventions recommended for flying-fox colonies [21], may help to target the supply of free water stations, encourage monitoring of populations and prepare resources for care of heat-stressed koalas.

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5.4 Koala Retrovirus – Literature Review

5.4.1 Technical information

Aetiological agent

Family: *Retroviridae*; genus: *Gammaretrovirus*; species: koala retrovirus (KoRV).

Retroviruses are RNA viruses [1] which replicate by reverse transcribing their RNA genomes to make DNA copies of themselves (proviruses) which integrate into the host cell's genome. If these proviruses are incorporated into germ cell lines (i.e. egg and sperm cells), they become a permanent part of the host's germ line DNA, passed on to the next generation of the host through Mendelian inheritance [2]. A retrovirus inherited in this way is known as an "endogenous" retrovirus. A retrovirus that integrates into the genome of somatic (non-reproductive) cells, rather than germ cells, and is not inherited by the next generation, is termed "exogenous". Koala retrovirus (KoRV) is a relatively young retrovirus which exists in both endogenous and exogenous forms [3, 4].

The complete KoRV genome consists of three genes: *gag*, *pro-pol* (often referred to as *pol*) and *env*. There are twelve identified variants or subtypes of KoRV denoted as KoRV-A to KoRV-M (KoRV-J has been reclassified as KoRV-B), based around phylogenetic groupings of the *env* gene. KoRV-A has the full KoRV gene complement, exists as both an endogenous and exogenous virus, and is thought to be the endogenous virus from which other variants have arisen [5, 6]. Variants other than KoRV-A are thought to be only exogenous. Some exogenous variants lack the full KoRV gene complement and most are incapable of replication competent [5, 7-10].

In addition to the variants of KoRV based on the *env* gene, defective (presumably endogenous) retroviral elements, known as recKoRV (recombinant KoRV) occur in koalas throughout their range. RecKoRV elements lack the complete KoRV gene complement and are non-replicating [5, 7, 11].

Listing

KoRV is not a WOAHL listed disease [12].

KoRV is not a notifiable animal disease in Australia [13].

KoRV-associated disease has not been identified as a key threatening process under the Environment Protection and Biodiversity Act [14].

5.4.2 Epidemiology

To understand the complexities of KoRV epidemiology, it is necessary to have some understanding of the endogenisation processes of retroviruses in general, and KoRV in particular. New discoveries are constantly refining this understanding and the reader is encouraged to refer to current publications for the most up-to-date information.

Most endogenous retroviruses within mammalian DNA derive from infections that occurred millions of years ago; these retroviruses have since become “fixed” in their integration sites and consequently are found in most individuals in a population in the same location within the host genome. Many of these ancient retroviruses degrade into “junk DNA”; 8% of the human genome consists of such retrovirus-like elements [15]. In contrast, KoRV-A is a relatively young endogenous retrovirus, which probably initially became incorporated into the koala genome less than 50,000 years ago [3, 4], and is still undergoing transition from an active, newly endogenised retrovirus into a genomically-fixed entity. Consequently, endogenous KoRV-A retains many of the pathogenic features more commonly associated with exogenous retroviruses, including the ability to replicate and produce infectious virus [16, 17]. The endogenisation process is an ongoing conflict between the virus and the host, with provirus repeatedly integrating into the host genome, and causing further host genetic mutations in the process [15]. Research continues into the epidemiological and pathological implications of the ongoing KoRV endogenisation process, with new information continually coming to light.

KoRV-A is present in all individuals which test positive for KoRV and exists in both exogenous and endogenous forms [11]. Other KoRV variants have only been found in koalas that also harbour KoRV-A [6, 9, 18, 19] and they appear to be exogenous only, based on their high genetic diversity, inconsistent detection within koala family groups and relatively low numbers of proviral copies per cell in infected animals [20-22].

Based on *pol* PCR, proviral loads of competent KoRV (which possesses *pol*) are much higher in northern than southern koala populations, with proviral integrations of 165 copies per cell reported in Qld koalas compared with 1 copy per 10,000 cells in some Vic populations [17]. This indicates that KoRV-A endogenisation is more advanced in northern populations than southern populations since copy numbers above 1 per cell suggest that the infection is being inherited rather than transmitted [23].

Testing of koalas for the presence of KoRV has predominantly focused on a conserved region of the *pol* gene [24]. However, studies have identified *pol*-negative, non-replicative, presumably endogenous recKoRV in Vic and SA koalas [11, 25] which are distinct from similar recKoRV versions in northern populations [5, 7], and would have been identified as KoRV negative using *pol* gene PCR. Most notably, recKoRV was found in koala from the population on French Island, from which virtually all southern koala populations are derived. Consequently, it is possible that all southern koalas have recKoRV in their genome. If this is the case, it may be misleading to label these koalas as “KoRV-free” or “KoRV-negative”. In recognition of this distinction, for the purposes of this report, the term “*pol*-negative” will be used to refer to koalas which carry recKoRV elements only, “*pol*-positive” will be used for koalas whose genome contains replication-competent KoRV containing the full genome complement, and “*pol* KoRV” will be used to denote replication competent, complete KoRV. The presence of recKoRV, in the absence of endogenized *pol* KoRV, has led to speculation on the endogenisation history of KoRV in southern koalas [11]. RecKoRV elements are not

replication-competent and are unlikely to have integrated into the genome of southern koalas by themselves. Their presence suggests that southern koalas have historically been exposed to a replication-competent form of KoRV which enabled recKoRV to be “carried” into their genome. It is possible that the host alleles which historically contained replication-competent KoRV never integrated endogenously in southern koalas, or that they were lost from the host genome due to the well-documented genetic bottlenecks which occurred in southern populations [11]. It is also possible that other endogenous viral elements were involved in recKoRV becoming ubiquitous in southern koalas in the absence of endogenised KoRV [25]. Further investigations of southern koalas are required to test these hypotheses.

Host range

Koala retrovirus is host specific to koalas and has not been shown to transmit to other species *in vivo* [26]. However, several KoRV variants are capable of *in vitro* infection of cells of many different host species, including humans [20, 27-32]. This probably reflects the ubiquitous nature of the host cell transporters used for viral entry [20, 27-31].

Retroviruses related to KoRV are found in flying-foxes, *Melomys* rodents and gibbons [33-36]. It appears most likely that KoRV originated via cross-species transmission from a melomid Australo-Papuan rodent [37]. Spillover of KoRV’s closest relative, the exogenous gibbon ape leukaemia virus, to endogenised forms in *Melomys* rodents, has occurred [34, 38]. These findings suggest the possibility for trans-species infections with KoRV, although KoRV appears to be substantially less infectious than gibbon ape leukaemia virus based on *in vitro* studies [20, 39].

Zoonotic potential

The low zoonotic potential of other simple gammaretroviruses, such as feline leukaemia virus (FeLV) [26], suggests KoRV is unlikely to be zoonotic. However, further assessment of routes of transmission, mutation rates and replication ability in human cells as well as interaction with human viral restriction factors are needed to fully assess the zoonotic potential of KoRV [26, 40].

Geographic distribution

KoRV (or recKoRV elements) are present in all free-ranging koala populations that have been tested in Australia [11, 41-44]. Studies of KoRV in museum koala skins confirm that it was ubiquitous in northern Australia by the late 19th century [3, 22]. The variants (KoRV-A through -M) differ in distribution throughout the geographical range of koalas [6, 16, 24, 25, 29, 30, 41, 45-47]. KoRV and most of the variants are also present in many captive populations throughout the world, where they have been the subject of extensive study [29, 30, 32, 45, 48-51].

Prevalence

Based on presence of full viral sequence (including *pol*) in both proviral and viral forms, koalas from northern populations exhibit 100% prevalence of replication-competent KoRV

[31]. Replication competent KoRV (*pol* KoRV) is much less prevalent in southern populations and only appears to exist in exogenous form, however endogenous *pol*-negative recKoRV variants have been discovered recently and appear to be widespread [9, 16, 41, 43, 44, 46, 52], with all koalas in southern populations hypothesised to possess recKoRV [11, 31], and with similar, but distinct, versions existing in northern populations.

Table 15 summarises KoRV prevalence reports, based on *pol* detection, in Australia since 2012. KoRV was first confirmed at 100% prevalence in Qld in 2006 [16], and in NSW in 2012 [41]. All subsequent studies of northern populations have detected 100% KoRV prevalence [6, 9, 18, 19, 23]. Prevalence, based on *pol* detection, in southern populations varies from 0% to 82% over time and between populations.

Table 15 KoRV *pol* prevalence reported between 2012 and 2021 in koalas in Australia. Results use *pol* PCR/qPCR primers developed in Tarlinton et al. 2005 [24]

Region	Number of koalas	Number (%) <i>pol</i> positive	Reference
Queensland			
Blair Athol	4	4 (100%)	Tarlinton et al. 2006 [16]
Blair Athol	27	27 (100%)	Simmons et al. 2012 [17]
St Bee's Island	4	4 (100%)	Tarlinton et al. 2006 [16]
South-east Qld	90	90 (100%)	Tarlinton et al. 2006 [16]
South-east Qld	250	250 (100%)	Simmons et al. 2012 [17]
South-east Qld	71	71 (100%)	Sarker et al. 2020 [46]
New South Wales			
Pilliga	57	57 (100%)	Simmons et al. 2012 [17]
Port Macquarie	43	43 (100%)	Simmons et al. 2012 [17]
Victoria			
Raymond Island	17	5 (29%)	Tarlinton et al. 2006 [16]
Raymond Island	29	10 (35%)	Simmons et al. 2012 [17]
Raymond Island	136	38 (28%)	Legione et al. 2017 [43]
Gippsland	20	11 (55%)	Simmons et al. 2012 [17]
Greater Gippsland	33	6 (18%)	Legione et al. 2017 [43]
Strzelecki Ranges	26	18 (69%)	Simmons et al. 2012 [17]
Snake Island	12	6 (50%)	Simmons et al. 2012 [17]
Far North Vic	15	6 (40%)	Legione et al. 2017 [43]
General mainland	43	36 (82%)	Simmons et al. 2012 [17]
Mornington Peninsula	15	4 (27%)	Legione et al. 2017 [43]
French Island	28	6 (21%)	Simmons et al. 2012 [17]
French Island	94	23 (24%)	Legione et al. 2017 [43]
Phillip Island	11	0 (0%)	Simmons et al. 2012 [17]
Southwest Coast	178	30 (17%)	Legione et al. 2017 [43]
Ballarat	5	3 (60%)	Tarlinton et al. 2006 [16]
Far West	167	52 (31%)	Legione et al. 2017 [43]
South Australia			
Kangaroo Island	26	0 (0%)	Tarlinton et al. 2006 [16]
Kangaroo Island	162	24 (15%)	Simmons et al. 2012 [17]
Kangaroo Island	170	72 (42%)	Fabijan et al. 2019 [44]
Mt Lofty Ranges	75	49 (65%)	Fabijan et al. 2019 [44]
Mt Lofty Ranges	97	37 (38%)	Sarker et al. 2020 [46]
Mt Lofty Ranges	216	89 (41%)	Stephenson et al. 2021 [53]

The prevalence of KoRV variants other than KoRV-A is difficult to determine due to small sample sizes and non-standardised diagnostic techniques with widely differing sensitivities (see *Diagnostic testing*). Although some studies indicate that southern koalas possess

multiple KoRV variants [9, 11, 31, 54], a recent investigation argued these findings are likely to be artifacts [25]. Table 16 summarises reports of KoRV variant prevalence in Australia since 2012.

Table 16 Prevalence of KoRV variants reported in koalas in Australia 2012-2021

^a Results use *pol* PCR/qPCR primers as developed in Tarlinton et al. 2005 [24]; ^b KoRV-A and KoRV-B results using specific variant PCRs; ^c deep amplicon sequencing technology used for variant detection; ^d D/F intermediate variants; ^e other intermediate variants accounted for <1% of reads. Four new hypervariable region variants were detected in a small number of koalas (<6%).

Region	No. of koalas	KoRV Variant									Ref.	
		A	B	C	D	F	G	H	I	K		
Queensland												
Northern (1891-1980s)	16	15 (94%)										[3] ^b
South-east Qld	12	12 (100%)										[55] ^b
South-east Qld	290	290 (100%)	83 (29%)									[18] ^b
South-east Qld	16	16 (100%)	4 (25%)		14 (88%)	4 (25%)	-	-	-			[19] ^c
South-east Qld	33	33 (100%)	33 (100%)		33 (100%)	-	11 (33%)	-	32 (97%)			[9] ^c
South-east Qld	18	18 (100%)	14 (78%)		17 (94%)	8 (44%)	2 (11%)	1 (6%)	1 (6%)			[6] ^c
South-east Qld	71	71 (100%)										[46] ^b
South-east Qld	151	151 (100%)	83 (55%)		138 (91%)	132 ^d (87%)		9 (6%)				[56] ^{c, e}
South-east Qld (captive koalas)	45	45 (100%)	39 (87%)	1 (2%)	43 (96%)	5 (11%)	3 (7%)	18 (40%)	30 (67%)	5 (11%)		[47] ^c
South-east Qld (captive koalas)	64	64 (100%)	53 (77%)	0 (0%)	61 (95%)	3 (5%)	7 (11%)	23 (36%)	23 (36%)	30 (47%)		[47] ^c
New South Wales												
North-east NSW	12	12 (100%)										[55] ^b
Port Macquarie	15	15 (100%)										[55] ^b
Victoria												
Southern (1891-1980s)	3	1 (33%)										[3] ^b
Mallacoota	3	0 (0%)										[55] ^b
Raymond Island	18	4 (22%)										[55] ^b
South Gippsland	203	64 (32%)										[55] ^b
Sth Gippsland, Raymond Island	19	9 (47%)										[55] ^b
Central Gippsland	17	13 (76%)										[55] ^b
Phillip Island	6	0 (0%)										[55] ^b
Cape Otway	11	2 (18%)										[55] ^b
South Australia												
Kangaroo Island	170	72 (42%)	0 (0%)									[44] ^{a, b}
Mt Lofty Ranges	28	28 (100%)	28 (100%)		28 (100%)		12 (43%)		18 (64%)			[9] ^c
Mt Lofty Ranges	75	49 (65%)	0 (0%)									[44] ^{a, b}
Mt Lofty Ranges	97	88 (91%)										[46] ^b

Within captive Australian populations which hold northern koalas, KoRV prevalence is assumed to be 100%, due to the endogenous nature of KoRV-A in northern koalas. This has been confirmed in two captive institutions in Qld [47] but data on KoRV prevalence in a wider range of Australian zoos is not readily available. Japanese zoos have reported prevalence of 100% for KoRV-A, 50-64% for KoRV-B and 50% for KoRV-C [29, 45, 50, 57]. In a study of nine Japanese zoos holding koalas originating from Vic, 36% (4/11) were positive for KoRV-A and 0% for KoRV-B [29].

Mode of transmission

Transmission of retroviruses occurs through endogenous or exogenous means. In endogenous transmission, the provirus within the DNA of the host's germ cell line (oocytes and spermatozoa) is inherited by the offspring, through Mendelian dominant inheritance. Consequently, all the cells of the offspring have retroviral DNA within them and proviral loads are greater than one proviral copy per cell [2, 17].

Based on general retroviral behaviour and the defective nature of many KoRV variant sequences, it is possible that transmission of exogenous variants between koalas does not occur *in vivo*, and that the exogenous variants arise solely through recombination or mutation with endogenous KoRV-A elements in the genome within a single individual [22]. However, studies have identified patterns of KoRV variants in koala family groups, consistent with maternally transmitted exogenous KoRV infection [47, 52]. In addition, many exogenous variants are geographically restricted, but locally abundant, which is consistent with an initial host recombination with endogenous KoRV, followed by transmission to other individuals by exogenous means [25, 47, 52].

If exogenous transmission does occur, it is likely that close contact between animals would be required [58], as most retroviruses do not survive well in the environment [2, 59]. Dam-to-joey transmission is considered the most likely means of exogenous transmission and could occur through infected milk, pouch contact or *in utero* [18, 47, 52, 60, 61]. Sire-to-joey transmission has also been postulated, given that some joeys have exogenous proviral profiles closer to that of the sire than their dam [32, 52]. Sexual transmission, or contact during fighting, have also been suggested but are less likely [18]. Other transmission methods considered theoretically possible include vectors [60] and iatrogenic transmission via blood transfusions and trans-faunation, since KoRV virus and provirus may be present in blood and gut contents [47, 62-64].

Transmission of KoRV via fomites or environmental contamination is considered unlikely, due to the limited survival of gammaretroviruses in the environment [65]. In infection studies of FeLV, environmental transmission was not recognised [66, 67]. However, the fact that koala retrovirus is likely to have transferred at some point from rodents to koalas suggests that the possibility of fomite or environmental transmission should not be entirely discounted.

Incubation period

The incubation period of KoRV, from acquisition of the virus to demonstration of related disease, is currently unknown. Most koalas are found to be positive for KoRV without clinical signs of disease [24, 43, 46, 68, 69].

Persistence of agent

Environmental survival of KoRV has not been examined, but survival of other gammaretroviruses (such as FeLV) is short, and the viral particles are readily inactivated by disinfectants, heat and desiccation. Survival of retroviruses in general is increased in moist, room-temperature settings [65, 70].

5.4.3 Role of KoRV in expression of disease

KoRV provirus and virus are readily detected in koalas both with and without signs of disease [24, 43, 46, 68, 69]. The completeness of the KoRV genome determines the capacity of KoRV to replicate and therefore potentially cause disease. In contrast to northern koalas, which have 100% prevalence of the intact KoRV genome [17, 24, 46], KoRV detected in southern koalas often lacks some proviral gene segments, rendering it replication-defective [5, 11, 46, 56]. This may be an underlying basis for the lower prevalence of clinical disease in southern koalas.

Based on association between disease and presence of some exogenous KoRV variants, in particular KoRV-B/J, it has been proposed that some exogenous variants are more pathogenic than endogenous KoRV-A [30, 69]. However, it is appearing likely that these findings actually reflect an association between disease and proviral load (with higher loads appearing above detection limits of less sensitive assays) rather than differing variant pathogenicity. Studies finding associations between the presence of multiple KoRV variants and disease states in koalas [19, 29, 30, 32, 43, 71, 72] suggest that disease may be most associated with escape and proliferation of the virus in any competent form; whether this association is a cause or result of disease is uncertain [46].

In broad terms KoRV is thought to potentially influence disease expression via two mechanisms: firstly, the active integration of KoRV into the koala genome increases the mutagenic load experienced by the koala; secondly, the replication of KoRV in the koala's white blood cells (WBC) is associated with a range of negative impacts on immune cell function, which for the purposes of this document will be referred to collectively as "immune modulation" (as it is recognised that a variety of different mechanisms, both stimulatory and suppressive, may be involved). The mutagenic and immunomodulatory properties of KoRV may be expressed in a variety of ways, leading to a highly complex and diverse range of disease associations which are the subject of ongoing investigation. It is likely that a combination of longitudinal and mechanistic *in vitro* studies will be needed to prove or disprove causation [73].

KoRV and neoplasia

KoRV proviral integration into koala DNA is a mutagenic event which is thought to be a key initiating process for many koala neoplasms [15], and which is likely to be a contributing factor to the high incidence of neoplasia in koalas (see *Appendix 5.6 Neoplasia in Koalas – Literature Review*). Several mechanisms have been identified for the oncogenic potential of KoRV integration into the koala genome. Neoplastic tissues from koalas have been found to contain new KoRV integrations in the vicinity of oncogenes, suggesting that upregulated expression of these genes may have occurred, increasing the likelihood of cell transformation or oncogenesis [15, 74]. KoRV integrations in or near oncogenes could lead to an inherited predisposition for specific cancers [15]. KoRV has also been found to acquire oncogenes from the host, re-integrate (transduce) them into its genome, and express the transduced genes as viral oncogenes [15]. “Candidate” genes, that could be associated with the induction of neoplasia by KoRV, have been identified in several recent studies in koalas, but the exact mechanisms of action are not yet confirmed [15, 74, 75].

Further information on the associations between KoRV and neoplasia is in *Appendix 5.6 Neoplasia – Literature Review*.

KoRV and immune modulation

There are several identified mechanisms by which KoRV may cause immune modulation. By replicating within WBC, KoRV causes these cells to be targeted for elimination by the host’s immune system. The KoRV provirus integration causes disruption of the WBC genome and as a result, it is unable to function properly [76]. In studies conducted *in vitro*, KoRV is shown to significantly upregulate various cytokines, interleukins and interleukin receptors in koala cells [31], which is likely to disrupt function of infected cells; its impact on other koala immune cells is not confirmed. In southern populations, KoRV *pol*-positive koalas have been shown to have a more limited range of T lymphocyte ratios than *pol*-negative koalas, which might suggest a reduced capacity to regulate immune responses [77]. KoRV is also known to carry an ‘immunosuppression domain’ (*isu*) which may dysregulate the host’s immune response, as has been reported in other retroviruses with this domain [68], though the impact of this domain is contentious.

5.4.4 Associations with other disease hazards of koalas

Many studies have identified statistical associations between various KoRV traits (such as viral load, proviral load, variant prevalence and *pol* prevalence) and a range of disease states and co-infections in koalas [19, 24, 29, 30, 32, 43, 56, 69, 71, 72, 74, 78-81]. While such studies are important for identifying avenues for future research, they should not be taken to imply a causative link between KoRV and the associated variable.

Increased severity of chlamydial disease is associated with high KoRV proviral and viral loads in koalas throughout their range [24, 43, 46, 56]. Presence of certain exogenous variants has been associated with more severe chlamydiosis in some studies of northern koalas [29, 30,

56, 69]. In southern populations, KoRV associations with increased incidence of urogenital chlamydiosis and periodontal disease have been identified [43, 74, 78-80]. There are fewer reports of associations between KoRV and other disease states in southern koalas, and associated diseases are often not as severe as those reported in northern populations.

Other infections in koalas, such as koala herpesviruses, trypanosomiasis and pulmonary actinomycosis have not been directly associated with infection by KoRV [74, 82, 83], but studies are in their infancy and further work is needed to develop understanding of these interactions.

The variety of associations between KoRV traits and disease states may suggest that disease is most associated with escape and proliferation of KoRV in any competent form [25]; whether this association is a cause or result of disease is uncertain, and it may be that the causation runs in the opposite direction, with severe disease providing opportunity for KoRV proliferation and escape from host immune control [46].

5.4.5 Diagnosis

Clinical signs

There are no clinical signs associated with the presence of KoRV infection *per se*. Disease states associated with KoRV infection (i.e. neoplasia, chlamydiosis etc.) are described in the relevant chapters of this report.

“Ill thrift”, and other poorly-defined clinical syndromes of koalas, are often presumptively attributed to KoRV infection (see Section 7.1 *Clinical Syndromes with Undefined or Multiple Aetiologies* in KDRA report for further discussion). The term “KoRV koala” has been commonly used by those who care for sick koalas in NSW and Qld to summarise a wide range of signs linked to poor general health, high incidence of opportunistic infections and poor response to treatment. There is an identified need to develop a more definitive and evidence-based syndrome description to capture the range of clinical conditions that might represent KoRV-associated immunosuppressive disease [84].

Clinical pathology

KoRV infection *per se* does not cause any consistently recognised clinical pathological changes. However, KoRV viral loads (KoRV RNA copies per μL of plasma) and proviral loads (KoRV DNA/ 10^3 beta-actin copies) were associated with increased immature red blood cell (metarubricyte) and lymphocyte counts, and decreased erythrocyte and neutrophil counts, in free-ranging Qld and SA koalas [78]. A study from healthy koalas in SA showed that *pol*-positive koalas had significantly lower haematocrits than KoRV negative koalas but the difference was not clinically significant [85]. Clinical pathology changes seen in associated diseases (e.g. neoplasia, chlamydiosis) are described in the relevant chapters.

Pathology

KoRV presence *per se* is not indicated by any typical pathological changes. Pathology changes seen in associated diseases (e.g. neoplasia, chlamydiosis) are described in the relevant chapters.

Diagnostic testing

Diagnostic testing for KoRV detection is based on molecular technology. Each technique currently available has both diagnostic and logistical limitations, and the selection of the most appropriate approach is dependent on the aim of testing.

PCR can be used to detect presence or absence of particular KoRV genes, as targeted by the selected primers [37]. Design of PCR tests to detect KoRV variants is challenging so this technique is better suited to “presence/absence” studies, as might be indicated for screening southern koalas for the presence of *pol* KoRV.

Quantitative PCR (qPCR) is approximately ten times more sensitive than PCR [73]. Design of qPCR tests to detect KoRV variants is challenging. It is best used for determining the load of replication-competent provirus (if DNA is used) or the viral load (if RNA is used). Quantification of *pol* might be indicated in both northern and southern koalas to detect associations between KoRV viral or proviral load and disease processes [56].

Deep amplicon sequencing is performed on the *env* gene. It is used for detection and quantification of KoRV variants and is approximately ten times more sensitive than qPCR [73]. This method is best suited to testing large numbers of samples in batches when rapid turnaround time is not required. Use of deep amplicon sequencing might be indicated for screening of koalas to identify and prevent introduction of novel variants to a naïve population; to gather data on distribution and diversity of KoRV variants; and to identify animals with a high diversity of KoRV variants, which may indicate undesirable traits that allow proliferation of KoRV.

Rehabilitation facilities are not currently resourced to undertake KoRV testing routinely. Subtype analysis is expensive, has long turnaround times and is difficult to arrange for small numbers of samples, making it impossible to apply in the clinical context. Cost and availability of *pol* testing is similar to *Chlamydia* PCR [73].

Diagnostic testing for KoRV is currently only available through research laboratories. These diagnostic tests can be performed on any samples containing DNA (including blood, skin and faecal material), but replication of gammaretroviruses occurs most commonly in immune cells, so the highest proviral and viral loads are likely to be found in blood, lymph nodes and spleen [86].

Surveillance and monitoring

There is no co-ordinated surveillance for KoRV status of either wild or captive koalas in Australia. Captive koalas in Australia are tested for KoRV on an *ad hoc* basis and there is

currently no central repository for this data. Koalas entering rehabilitation facilities are not routinely tested for KoRV status due to the cost and logistic challenges to accessing diagnostic testing.

There are numerous projects in free-ranging koalas which continue to collect data on KoRV status (using various molecular methods) however there is no nationally coordinated research program for KoRV in koalas.

5.4.6 Treatment

Currently, there are no established regimens for treatment of KoRV by eliminating replicating virus. Many anti-retroviral medications approved for treatment of human immunodeficiency virus have broad antiviral activity and might theoretically be of use in treating KoRV, but koala-specific protocols for their use have not been established. The efficacy of such medications in koalas is unknown, and caution is required as the pharmacodynamics of many drugs in koalas are known to be significantly different to other eutherian mammals [87-89]. There is a report of a koala infected with KoRV-A, KoRV-B and KoRV-F being treated with anti-retroviral medications marketed for humans (raltegravir and tenofovir), but there was no effect on viral load or disease progression and the animal was euthanased after 33 days of treatment [90]. In addition to questions of drug safety, efficacy and treatment logistics, fundamental questions regarding the indications and goals of anti-retroviral treatment for KoRV remain; for example, anti-retroviral medications do not remove virus from already infected cells and therefore treatment of the dam would need to be started prior to birth if the aim was to minimise the risk of exogenous dam-to-joeey transmission [91].

KoRV vaccination has been proposed as a means of controlling the viraemic state in koalas with endogenous KoRV, thereby reducing the likelihood of associated disease [92, 93]. However, no KoRV vaccines are commercially available at present. There are safety concerns that vaccination may lead to auto-immunity in endogenously infected koalas [94], and there is debate as to whether immunotolerance may exist which would render vaccination ineffective [95, 96]; these fundamental immunological questions must be resolved before vaccination can be considered as a treatment tool for KoRV (see further discussion of vaccination in *Prevention and control*).

5.4.7 Prevention and control

There are currently no consistent national or regional approaches to prevention and control of KoRV and KoRV-associated disease states in koalas. Maximising adaptive potential by reducing mortality from other causes, maintaining large population sizes and minimising inbreeding are key considerations in active management of koala populations and these strategies are also likely to maximise the opportunities for resilient koala populations to “co-adapt” to the presence of KoRV.

Some captive and zoo-based koala populations undertake screening for KoRV using various molecular techniques. In most cases this data is not published and may not be shared between koala holders. Increased testing, and improved data sharing between captive koala populations, as well as an evidence based, scientifically coordinated approach to managing KoRV status of Australia's captive breeding koala population is recommended.

A collection of koalas (n=30) with no detectable KoRV based on *pol* and *env* PCR has been established at Cleland Wildlife Park (owned and operated by the SA Department of Environment and Water) [97]. There may be other captive collections in Australia where replication-competent KoRV is absent, although no such collections are reported.

Approaches to prevention and control are necessarily drawn from the currently limited knowledge base. Prevention and control strategies should be revised regularly, given the rapid rate of development of knowledge of KoRV. A range of methods for prevention and control are discussed below.

Identifying and maintaining “*pol*-negative” koala populations

It is assumed that all northern koalas have endogenous replication competent KoRV-A in their genome. Recent studies suggest that some southern koalas are likely to be “*pol*-negative” rather than entirely “KoRV free”, although recKoRV elements are incapable of replication and therefore are unlikely to be infectious [11]. Protecting *pol*-negative populations of koalas from the entry of replication-competent KoRV would involve identification and managed isolation of these populations to prevent breeding with *pol*-positive koalas. The creation of KoRV *pol*-negative populations would protect the future offspring from the disease risks apparently associated with replication-competent KoRV, and act as valuable controls for longitudinal studies to examine the impact of freedom from replicative endogenous KoRV-A on koala clinical health. However, such populations are unlikely to be of benefit to supplementing wild populations due to their narrow genetic base, and the benefits of maintaining *pol*-negative populations must be balanced against other considerations for population genetic management.

Managing risks associated with high KoRV load in individual koalas

Animals with high proviral and viral loads of KoRV, or high diversity of KoRV variants, may have transmissible or heritable traits that have allowed KoRV to “escape” containment. Conversely, *pol*-negative koalas may have some genetic alleles that restrict infectious viral replication and prevent endogenisation of infectious KoRV [11]. Selecting animals with low viral loads for breeding and translocation and avoiding breeding and translocation of those with high viral loads, would encourage the retention of the most robust genetic profiles for avoiding disease consequences of KoRV. There is currently no consistent and clearly articulated protocol to enable selection based on KoRV profile, nor are there defined thresholds or consistent methodologies for quantifying proviral and viral load.

Minimising the spread of exogenous KoRV variants

Prevention and control strategies to minimise the spread of exogenous KoRV variants remain precautionary at present, pending further understanding of KoRV transmission dynamics and pathogenicity. Given that close-contact transfer from dam (or sire) to joey early in life appears to be the most likely route of exogenous transmission, *env* PCR screening of adults to identify their exogenous KoRV variant profiles would enable preferential breeding from adults with the lowest number of variants, or with variants known to be incapable of replication. Screening for KoRV variants prior to relocation would help to avoid introducing novel exogenous variants to a naïve population.

Managing risks of individual koalas with a history of KoRV-associated disease

The possibility of a KoRV-mediated inherited predisposition to neoplasia should be considered in koala populations or genetic lines with a high prevalence of neoplasia. Minimising of breeding from such lines, and investigation of KoRV profile as outlined above, may be indicated (see also *Appendix 5.6 Neoplasia in Koalas – Literature Review*).

Individual koalas from populations or pedigrees that have a history of diseases recognised to be potentially KoRV-associated (e.g., severe chlamydiosis, neoplasia, ill thrift, persistent or multiple “opportunistic” infections) may have heritable deleterious KoRV integrations, or other traits that have allowed KoRV to “escape” containment and then create deleterious integrations. Clinical syndromes of this nature require better definition (see *Identified gaps in knowledge* in Section 5.4 *Koala Retrovirus - Risk Assessment* in KDR report) and the presence of these syndromes could flag the need for closer incorporation of KoRV status (as described above) into breeding and translocation decisions.

Vaccination

An effective KoRV vaccine could be an important tool in developing and maintaining populations free from replication-competent endogenous KoRV. Vaccination for KoRV is in the early stages of research and there are no commercially available vaccines. Some studies indicate that endogenous KoRV-A may be protected from host immunological attack because it is incorporated into the inherited genome and is therefore recognised as “self”, limiting the likely effectiveness of vaccination [95, 96]. Other studies suggest that neutralising antibodies may be produced in response to vaccination [98, 99], raising the converse concern that vaccination of endogenously-infected animals might trigger an autoimmune reaction in the host [94]. These risks must be explored carefully if vaccination is to become a useful tool for KoRV treatment, prevention and control.

Anti-retroviral drug therapy

Although some researchers have suggested the theoretical strategies for incorporating antiviral drug therapy into KoRV prevention and control [47], there is insufficient knowledge to warrant their use at this time (see *Treatment*). Theoretically, individual koalas with persistently high viral and proviral loads of KoRV might benefit from anti-retroviral

medication, as this may reduce the likelihood of immunosuppression, development of neoplasia or worsening of other disease states. As dam-to-joey transmission is thought to be a significant mechanism for exogenous transfer, focusing anti-retroviral treatment on the period of time when the dam is raising the joey might reduce the risk of exogenous KoRV transfer to the joey [47].

5.4.8 References

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5.5 Motor Vehicle Trauma in Koalas – Literature Review

5.5.1 Technical information

Description of hazard

In the context of this report, “motor vehicle trauma” refers specifically to road vehicle trauma, because collisions between koalas and road vehicles (cars, trucks etc.) are by far the most common type of motor vehicle trauma [1]. Much of the information on hazard diagnosis, treatment, prevention and control will also apply to train collisions.

5.5.2 Epidemiology

Causes of hazard

Koalas encounter motor vehicles when moving across open ground [2]. Their movements across a landscape reflect their search for trees which meet their nutritional and shelter needs [3, 4], their drive to establish solitary home ranges [5], and the seasonal mate-seeking behaviour of males [6]. These movements bring them into contact with roads and the vehicles which use them.

The tendency for individual koalas to cross roads reflects the roles of breeding activity and juvenile dispersal as key drivers of koala movement along the ground. Males are three times more likely to cross roads than females. Females with joeys are particularly unlikely to exhibit road crossing behaviour [7-9]. Younger koalas (<5 years old) are four times more likely to cross roads than older animals [8].

Few studies have examined individual koala road-crossing behaviour but there are indications of significant individual variability. In one study of 51 koalas in south-east Qld, only 18 koalas (35%) ever exhibited road-crossing behaviour over the 30 month period of study, and two individuals accounted for 50% of observed road crossings [8]. In a study of koalas in north-east NSW, all koalas crossed roads at some stage over a two year tracking period, but frequency of crossing ranged from 5 to 53 times, with one individual koala crossing a busy highway at least 32 times [10].

Human population growth, with associated urbanisation of landscapes, leads to a corresponding increase in road density and traffic volumes [11], as well as an increase in habitat loss and fragmentation [12]. These landscape changes increase the risk of motor vehicle accidents (MVA) for koalas by increasing the likelihood and frequency of attempted road crossings [2, 13-15]. This is particularly the case where development coincides with high (>1.0/ha) koala population density [14] or abundance [16, 17]. A study in north-east NSW demonstrated that koala home ranges in that area contained, on average, four times more “non-habitat” than habitat with primary feed trees [10].

The vast majority of koala road crossings occur at night. Although koalas are usually encountering roads at the time of lowest traffic volume, it also coincides with lowest visibility, and potentially higher levels of driver fatigue [18, 19].

The fatality rate from MVA is very high in koalas, with reports ranging from 50-83% [1, 2, 10, 14, 20-22]. Motor vehicle accidents may cause sufficient mortality to contribute to koala population decline, and may render vulnerable or small populations intrinsically unviable or reliant on immigration from other source populations in order to persist [23]. Motor vehicle accidents tend to involve otherwise healthy animals [24, 25], thus potentially affecting population viability by reducing healthy breeding animals.

Geographic distribution

Mortality and trauma to koalas from MVA occurs throughout their natural distribution although the relative impact varies with the particulars of the landscape (see *Prevalence*) [2, 13, 15, 24-28]. The hazard is not restricted to urban areas, as major roads also cross through relatively intact koala habitat [28].

Prevalence

Trauma from MVA accounts for a variable proportion of wild koala admissions to veterinary clinics (Table 17), but is the most common cause of trauma admissions across the koala's distribution [13, 15, 21, 22, 24-26, 28, 29]. Trauma caused by MVA was the most common cause of death among rehabilitation admissions in studies conducted in Vic [2, 15], NSW [30, 31] and Qld [14], although other causes such as disease and predation may dominate in certain populations [22, 26, 28]. The adverse effect of motor vehicle trauma is likely to be underestimated by wildlife admissions; a recent study identified that, in addition to 596 hospital admissions due to MVA over 31 years, an additional 544 koalas were sighted as roadkill during the same reporting period [22].

Table 17 Prevalence of MVA trauma in koalas

^a includes sightings as well as rehabilitation admissions; ^b longitudinal population study.

Location	Total Cases/ Admissions	MVA Trauma Cases	Reference
Rehabilitation admissions			
Qld	3590	1307 (36%)	Taylor-Brown et al. 2019 [21]
Qld	10139	2030 (20%)	Burton and Tribe 2016 [29]
NSW	5051	596 (12%)	Lunney et al. 2022 [22]
NSW	12543 ^a	1223 (10%)	Charalambous and Narayan 2020 [26]
NSW	3781	802 (21%)	Griffith et al. 2013 [13]
Vic	2584	1423 (55%)	Schlagloth et al. 2021 [2]
Post mortem studies			
Qld	519	109 (21%)	Gonzalez-Astudillo et al. 2019 [24]
Qld	291 ^b	10 (3%)	Beyer et al. 2018 [28]
NSW	374	71 (19%)	Hopkins and Phillips 2012 [31]
NSW	127	38 (30%)	Canfield 1987 [30]
Vic	55	12 (22%)	Obendorf 1983 [15]
SA	240	40 (17%)	Stephenson 2021 [25]
SA	85	35 (41%)	Speight et al. 2018 [27]

Male koalas appear to be more commonly affected by MVA trauma based on rehabilitation admissions [2, 6, 13, 14, 22, 32] although this is not a consistent finding [27, 33]. Male koalas tend to travel more frequently and broadly than females due to sexual activity, particularly during the breeding season [6]. Seasonal variation and peaks in MVA mortality have identified greater prevalence of admissions and fatalities occurring between August and December [2, 6, 9, 13, 14]. This coincides with the peak mobility of breeding males and the dispersing season for juveniles, although it may also reflect a seasonal influx of tourists and traffic in certain areas [13]. There is also evidence for an age predisposition to MVA trauma, with young animals significantly more likely to be involved [14, 27]. The dispersal age for koalas is around 20-36 months [34], and animals of this age are likely to range more frequently and more broadly seeking home range territory [14].

Estimations of the prevalence of trauma in koalas demonstrate a sampling bias, as reports are generally a subset of rehabilitation or veterinary admissions, or necropsy studies. Where there is concerted effort to sample koalas which have not come to ground for other reasons, or koalas are monitored longitudinally, different prevalence patterns may emerge (e.g. Beyer et al. 2018 [28]).

Once koalas have established a home range, they may be less at risk of MVA trauma. A two year study of 14 radio-collared koalas in northern NSW recorded no cases of MVA in the six mortalities during the study although two animals died of MVA shortly after the collars were removed [9].

Although MVA trauma can occur concurrently with disease [6], it more commonly affects healthy animals [30]. Most MVA admissions are in good to excellent body condition, suggesting that these animals are active, robust individuals which suffered vehicle strike as a result of increased mobility [24, 25].

5.5.3 Pathogenesis

Motor vehicle collisions cause physical impact injuries to koalas which may be immediately fatal, or may cause damage to bones, muscles and internal organs (see *Clinical signs*).

5.5.4 Association with other disease hazards of koalas

Post mortem studies of koalas in NSW [6] and SA [25] suggest that healthy koalas are more likely to suffer MVA trauma than those with pre-existing disease conditions.

Treatment for motor vehicle trauma may require prolonged hospitalisation and rehabilitation, and this may be associated with the development of other diseases related to long-term medical management such as gut dysbiosis, candidiasis and secondary wound infection [35].

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5.5.5 Diagnosis

Clinical signs

Koalas struck by vehicles have clinical signs consistent with trauma. External signs include skin abrasions, broken claws, deformities associated with fractures and external haemorrhage.

Internal traumatic injuries caused by MVA may include limb fractures, haemothorax, haemopericardium, liver rupture and diaphragmatic hernia [27, 30]. Head injuries are common, and often involve fractures of the skull and mandibles, as well as intracranial haemorrhage [1, 30]. It has been suggested that the koala's skull height in relation to the ground clearance of a car predisposes them to head trauma during MVA [6].

Koala MVAs have a high fatality rate due to the debilitating effects of massive haemorrhage and shock and the high incidence of head injuries and irreparable fractures [1, 24]. Figure 25 shows a live wild koala recovering from motor vehicle trauma.



Figure 25 A wild koala recovering in a rehabilitation facility after significant motor vehicle trauma injuries to the head (credit: Yasmine Muir)

Cellulitis, abscessation and cutaneous myiasis (fly-strike) are also reported, and may reflect a prolonged period between injury and examination [15].

Pathology

Gross pathology of MVA trauma is associated with blunt trauma impact, and includes head and limb fractures, haemoperitoneum, haemothorax and spinal injuries [6, 27]. Injuries of the front half of the body, particularly the head, are commonly encountered [27, 30].

Diagnostic testing

Diagnostic testing for koala trauma cases is similar regardless of the nature of the trauma and should include full body radiographs, complete blood count and biochemistry, in addition to any screening for pre-existing disease [35]. Abdominocentesis and ultrasound are indicated in all cases of trauma as internal bleeding and gastrointestinal tract rupture are common. Thoracocentesis may be indicated based on radiography [36, 37].

Koalas with haemorrhage may demonstrate red blood cell indices consistent with haemorrhage, including regenerative anaemia and hypoproteinaemia. Packed cell volume (PCV) and total protein (TP) should be monitored daily for at least three days post trauma [35]. Repeat ultrasound, abdominal and thoracocentesis may be performed on a daily basis, along with analysis of fluids obtained, to monitor for haemorrhage, peritonitis or organ rupture [38].

Differential diagnosis

Other causes of trauma, particularly animal attacks, may cause similar injuries to MVA trauma, including fractures, internal haemorrhage and head injuries [35, 39]. Puncture marks and dried saliva on the fur can distinguish predator attacks from MVA [35].

Koalas struck by vehicles are likely to be found on or near a road, which may assist in differentiating them from other causes of trauma.

Surveillance and monitoring

There is no targeted national surveillance or monitoring program in place for trauma of any kind in koalas. However, rehabilitation facilities and veterinary hospitals maintain good records of trauma admissions and post mortem examinations, enabling retrospective longitudinal studies [2, 13, 24, 25, 27]. Online databases hosted by the state governments of Qld [40] and NSW [41] collate koala rehabilitation admission data for their respective states, including information on the number of admissions attributed to vehicle trauma.

5.5.6 Treatment

Koalas with motor vehicle trauma should be triaged and treated for life-threatening injuries. Once the animal is stabilised, further investigation and diagnosis can direct prognosis and inform the decision to euthanase or continue with treatment and rehabilitation [38]. The

prognosis for koalas with MVA trauma is generally poor [1]. There is a high case fatality rate, and many animals will be euthanased on prognostic or welfare grounds.

Treatment of MVA trauma in koalas is symptomatic and may include fluid therapy, analgesia, wound management and surgical repair. As with all hospitalised koalas, reducing stress and providing excellent nutrition are critical to positive outcomes [35]. Antibiotics are generally not required and given the risks of dysbiosis and candidiasis, should be avoided unless there is a clear requirement for their use [35, 42].

Based on a retrospective study of admissions to wildlife hospitals in Qld over a period of 13 years [1], traumatic injuries associated with a decision to euthanase included:

jaw fractures - associated with cranial trauma, and may lead to misalignment of molar teeth which affects the koala's ability to chew leaves properly.

damage to the gingival junction of the jaw– commonly associated with intractable impaction of vegetation and secondary infection.

fractures in close association with vital organs (skull, spine and pelvis).

However, it is difficult to generalise about the prognosis of traumatic injuries, and many injuries involving the jaw, skull and pelvis may be suitable for treatment [36, 37].

5.5.7 Prevention and control

Prevention of MVA trauma is vitally important given the high fatality rate and poor prognosis for injured koalas. Koalas which die as a result of MVA trauma are often the healthiest individuals, and strategies to reduce trauma are likely to increase the number of healthy koalas in a given population [25].

Understanding koala behaviour patterns in relation to encounters with roads is an important aspect of successful risk mitigation. Breeding and dispersal are inevitable and predictable activities of wild koala populations, so the provision of viable, connected habitat is an important (and under-utilised) strategy for reducing koala encounters with vehicles [10, 13, 43, 44].

In many cases, road routes which present a risk of MVA trauma to koalas are long-established, so it is necessary to explore mitigation methods for prevention and control [17]. Over 40 types of road mitigation measures that aim to reduce wildlife mortality on roads have been identified, but there is little information about the relative effectiveness of the different measures in reducing risks to wildlife, including koalas [11, 18, 43, 45]. In many cases, selection of wildlife road mitigation strategies is based on considerations of cost and opinion rather than evidence of effectiveness [43].

Strategies that have been used in the context of koalas are outlined below.

Road planning: There is growing recognition that the design of public infrastructure, including roads, must acknowledge and better address the environmental cost of their establishment [18, 44]. Modelling suggests that modifying existing roads to carry more

traffic is generally more effective in mitigating MVA risk to koalas than building more roads to accommodate increased traffic [18]. If new roads are necessary, locations close to existing high value habitat should be avoided [11]. Road architecture which deters the approach of koalas may also be a strategy for exploration; there is some indication that koalas may be less inclined to approach roads across a long and steeply inclined cutting [17].

Traffic management: Traffic-calming efforts are commonly proposed as a method to reduce MVA risks for koalas [15, 46]. However, introducing warning signs and reducing the speed limit from 80 km/h to 60 km/h did not result in a significant reduction in koala mortalities in one study in south-east Qld [14], suggesting that much lower speed limits would be necessary to effectively mitigate the risk of koala MVA trauma. While dynamic road signs (particularly those displaying a koala) have been shown to be effective in causing motorists to reduce their speed [46], it is not clear whether this translates to a reduction in MVA trauma and fatalities for koalas. A longitudinal study of radio-collared koalas monitored one individual which successfully crossed a road at least 32 times over a two year period (although several near-misses were reported to researchers). The authors speculated that the presence of a roundabout in the vicinity which slowed traffic to 40 km/h may have been a factor in this koala's ability to cross unharmed [10].

Studies of the frequency and timing of daily road crossings by koalas indicate that switching to daylight saving time in Qld could reduce koala vehicle collisions by 8% on weekdays and 11% on weekends, by changing the timing of peak commuter traffic relative to the peak ground travel times of koalas [47].

Wildlife road crossing structures: Koalas show variable use of wildlife road crossing structures and their impact in reducing MVA hazard is not widely studied. Koalas have been shown to use underpasses, but not overpasses [7, 11, 48]. "Escape ramps" embedded in roadside exclusion fences to enable wildlife to escape if they find themselves on the wrong side, appear poorly used by koalas [48]. The effectiveness of road crossing structures depends on their size and location; in the case of koalas, such structures would only be of use if located within their home range [44]. Some studies suggest that wildlife crossing structures are not effective unless fences are also present [45], but this is not a consistent finding [49].

Physical barriers: Construction of exclusion fencing, either on its own or as an adjunct to fauna crossings, has been successful in reducing koala road-kill in several instances [9, 49]. Barriers and road-crossing structures require good maintenance and careful attention to location and design in order to be effective [43, 44].

It is likely that the ideal prevention and control plan will incorporate both habitat connectivity and road mitigation strategies, and will consider the specific characteristics of a particular landscape, and the behaviours of koalas, in determining which combination of strategies is likely to be most effective.

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5.6 Neoplasia in Koalas – Literature Review

5.6.1 Technical information

Description of hazard

Neoplasia is the uncontrolled, abnormal growth of cells and is a common cause of disease and mortality in both free-ranging and captive koalas [1].

5.6.2 Epidemiology

Causes of hazard

In general terms, neoplasia arises as a result of the transformation and subsequent damage to cellular DNA, resulting in uncontrolled cell growth and division, as the neoplastic cells no longer respond to normal growth-controlling mechanisms [2].

The majority of the diagnoses of neoplasia in both captive and wild koalas are lymphoid tumours, followed by craniofacial tumours (osteochondroma) and mesothelioma [1, 3-5]. A large number of other types of neoplasia have been reported as isolated cases.

Lymphoid tumours include lymphoma/lymphosarcoma and lymphoid leukaemia. Generally, lymphoid leukaemia is defined as neoplasia arising from progenitor cells in the bone marrow, whereas lymphoma and lymphosarcoma arise from other lymphoid tissues. Lymphoma and lymphosarcoma may also have a leukaemic phase, and conversely lymphoid leukaemia can infiltrate the tissues and give rise to solid tumours, so the three are not necessarily separate clinical entities [6, 7].

Osteochondroma is a benign neoplasia of cartilage and bone. In koalas, the bones of the skull are most commonly affected, but similar proliferations in other locations have been recorded, including the pelvis, ribs, clavicles and long bones [6].

Mesothelioma is a distinct serosal proliferative neoplasm of koalas which was historically classified as nodular or granulomatous peritonitis, fibrosarcoma or myxofibrosarcoma [8]. It is usually a diffuse, malignant nodular tumour of the abdominal surfaces (e.g. peritoneum, mesentery, and gastro-splenic ligament) or the pleura or pericardium [6].

Koala retrovirus (KoRV) status is likely to play a key role in the high incidence of neoplasia in koalas [9]. Viral particles associated with cases of lymphoid neoplasia were recognised as far back as 1988 [10]. The identification and sequencing of KoRV followed in 2000 using koala blood samples and lymphoma tissue [11]. The development of neoplasia in koalas shows strong epidemiological links to *pol*-positive KoRV status [12-19]. KoRV viral loads have been shown to be increased in koalas suffering from leukaemia or lymphoma when compared with healthy animals [12], and there is a significant association between KoRV proviral load (the form of the virus which is initially integrated into the host genome) and koala neoplasia [20]. *Pol*-positive KoRV-A is present in all northern and some southern koalas. Other KoRV variants are also present in northern and southern koalas. The complex nature of KoRV

infection in koalas does not support simplistic associations between KoRV prevalence and the occurrence of neoplasia [9].

The wide variety of non-lymphoid neoplasms recorded in koalas are listed in Table 18.

Table 18 Neoplasia types (other than lymphoid neoplasia, osteochondroma and mesothelioma) described in koalas

Type of neoplasm	Sites	References
Adenocarcinoma/ cystadenocarcinoma	Bile ducts; genitourinary tract; liver; lung; intestine; kidney; mammary gland; pancreatic duct; urinary bladder	Ladds 2009 [1], Hanger and Loader 2014 [6], Blanshard and Bodley 2008 [8], Gillett 2014 [18], Canfield et al. 1987 [21], Canfield et al. 1990 [22], Higgins and Canfield 2009 [23], Stephenson 2021 [24], Giovannini et al. 2015 [25]
Adenoma/ cystadenoma	Bile ducts; oviduct; pituitary; skin (sebaceous gland)	Tong 2019 [5], Blanshard and Bodley 2008 [8], Canfield et al. 1990 [22], Giovannini et al. 2015 [25], Antonsson and McMillan 2006 [26]
Adenomatosis	Subcutaneous	Hanger and Loader 2014 [6]
Fibroadenoma	Skin	Tong 2019 [5]
Fibroleiomyoma	Uterus	Tong 2019 [5]
Fibrosarcoma	Oral; peritoneal; pleural; spleen; sternum; skin (associated with vaccination site)	Ladds 2009 [1], Tong 2019 [5], Hanger and Loader 2014 [6], Blanshard and Bodley 2008 [8], Canfield et al. 1990 [22]
Giant cell tumour	Periosteum	Gillett 2014 [18]
Granulosa-cell tumour	Ovary	Tong 2019 [5]
Haemangiosarcoma	Spleen; unknown primary	Blanshard and Bodley 2008 [8], Gillett 2014 [18]
Leiomyoma	Intestine; urinary bladder; uterus	Ladds 2009 [1], Tong 2019 [5]
Leiomyosarcoma	Intestine; liver; spleen	Tong 2019 [5], Canfield et al. 1990 [22]
Lipoma	Pouch	Giovannini et al. 2015 [25]
Liposarcoma	Ovary	Tong 2019 [5]
Myeloid leukaemia	Bone marrow; brain and spinal cord	Hanger and Loader 2014 [6]
Myxofibroma	Subcutaneous (transponder- associated)	Vogelnest et al. 1997 [27]
Myxosarcoma	Liver; periocular; stomach	Ladds 2009 [1], Gillett 2014 [18], Stephenson 2021 [24]
Nephroblastoma		Gillett 2014 [18]
Osteosarcoma	Long bone	Gillett 2014 [18], Worley et al. 1993 [28]
Papilloma	Oral cavity	Ladds 2009 [1], Tong 2019 [5], Gillett 2014 [18]
Phaeochromocytoma		Gillett 2014 [18]
Rhabdomyoma	Skeletal muscle (site not specified)	Connolly 1999 [29]
Rhabdomyosarcoma	Diaphragm	Ladds 2009 [1]
Spindle-cell tumour	Nasal bones	Bercier et al. 2012 [30]
Squamous cell carcinoma	Digit; lung; nictitating membrane; skin; tongue	Tong 2019 [5], Blanshard and Bodley 2008 [8], Gillett 2014 [18], Kobayashi et al. 2021 [31]
Teratoma	Testis	Canfield et al. 1990 [22]

Geographic distribution

Neoplasia of various types have been reported throughout the koala's wild distribution [4, 5, 18, 20-22, 32, 33]. Neoplasia is also widely reported in captive collections of koalas in Australia [3, 18, 21, 22, 33, 34]. A survey of koalas in Australian captive institutions found that neoplasia accounted for approximately 55% of disease cases [18]. There were 30 primary cases of neoplasia in koalas in the national wildlife disease database [35].

Prevalence

Prevalence of neoplasia varies geographically throughout the wild koala's range.

McEwen (2021) reports that the prevalence of lymphoma in koalas is "at least an order of magnitude higher than in humans [9]. Other publications report a high incidence of neoplasia in koalas, relative to most other species [21, 22, 36-39].

In studies of both post mortem and clinical admission records, neoplasia is more prevalent in northern populations of koalas than in southern populations [4, 18, 20, 24, 32, 33]. One study of admissions to wildlife hospitals in Qld found that 1% of koalas presented with neoplasia [40]. A second retrospective study of Qld koala rehabilitation admissions found a prevalence of 3.2% [6]. It is likely that these figures under-represent neoplasia prevalence as rehabilitation data may not include cases where neoplasia was diagnosed incidentally, or where neoplasia was found as a co-morbidity to other disease processes, and will not capture data on cases that occurred in wild koalas which did not enter the care or rehabilitation system [41].

There is growing evidence that regional differences in the prevalence of neoplasia are associated with differences in the nature of KoRV infection in northern and southern koala populations [9, 19, 42, 43]; see *Appendix 5.4 Koala Retrovirus - Literature Review* for further discussion. Other unidentified factors may also be at play. Table 19 summarises reports of post mortem detection of neoplasia in koalas admitted to rehabilitation.

Table 19 Post mortem detection of neoplasia in koalas admitted to rehabilitation

Lymphoid tumours include lymphoma, lymphoid leukaemia and lymphosarcoma. Mesothelioma includes serosal neoplasia diagnosed as myxosarcoma.

Location	Neoplasia cases n/total (%)	% of most commonly diagnosed neoplasia types			Reference
		Lymphoid tumour	Osteochondroma	Mesothelioma	
Northern populations					
Qld	9/67 (13%)	56%	33%	11%	Fabijan et al. 2020 [4]
Qld	41/519 (8%)	49%	17%	5%	Gonzalez-Astudillo et al. 2019 [32]
Qld	3/26 (12%)	33%	66%	0%	McKenzie 1981 [44]
NSW	6/127 (5%)	83%	17%	0%	Canfield 1987 [38]
Southern populations					
SA	9/240 (4%)	67%	11%	0%	Stephenson 2021 [24]
SA	5/92 (5%)	80%	20%	0%	Fabijan et al. 2020 [4]
Vic	0/44 (0%)	-	-	-	Obendorf 1983 [45]

Lymphoid neoplasia is by far the most commonly reported neoplasia of koalas in both northern and southern populations [4]. Reports of higher than expected prevalence of lymphoid tumours in koalas date back to the 1970's [46]. Lymphoid neoplastic disease comprised 16.1% of all disease diagnosed in koalas submitted to the Australian Registry of Wildlife Health from 1998-2018 [5]. Koalas affected by lymphoid neoplasia are usually middle-aged (3-7 years), but may be as young as one year or as old as 10 years [1]. No sex predilection to lymphoid neoplasia has been noted in koalas [21].

Lymphoid neoplasia in koalas has four morphologic types: leukaemic (i.e. extends to the circulation); multicentric; alimentary; and focal [37]. Of 45 cases of lymphosarcoma in koalas in the Australian Registry of Wildlife Health database from 1998–2017, 51% were leukaemic and 42% were multicentric [5].

Cases of osteochondroma have been reported in both captive and free-ranging koalas in both northern and southern populations but appear more common in NSW and Qld [4, 6]. Osteochondroma is seen most commonly in mature age koalas [39]. One report suggests that males may be overrepresented in osteochondroma cases [39], but this sex bias has not been explored in recent studies.

Koalas affected by mesothelioma are most commonly 3-8 years of age [1]. No sex predilection has been noted. Other neoplasms of koalas (Table 18) are reported only as isolated events and no prevalence data are available.

Neoplasia is a common cause of disease in captive koalas. A survey of 15 captive facilities found that neoplasia was overwhelmingly the most prevalent condition of koalas, affecting

almost 56% of 263 cases [18]. The relative prevalence of neoplastic types reflected the trends in wild populations, with lymphoma the most prevalent tumour (70% of neoplasia cases), followed by osteochondroma and mesothelioma (equal prevalence at 4.8% of neoplastic cases).

5.6.3 Pathogenesis

Oncogenesis is a multi-stage process of initiation, progression and maintenance of abnormal cell growth and division. This sequence is almost certainly a consequence of gene changes within the host, either by over-expression through gene amplification, inappropriate expression of normal genes, or mutations in a critical region of a gene [2]. This sequence is commonly initiated by exposure to a cancer-producing agent (carcinogen).

There is a growing understanding of the means by which KoRV integration into the koala genome (a mutagenic event) may promote development of neoplasia in koalas. Retroviral oncogenesis is a phenomenon recorded in many species, and molecular research in koalas suggests that the insertion of KoRV within the koala genome could be a key initiating process for many neoplasms [9]. Every cell of the body of a northern koala contains at least one KoRV copy because endogenised, replication-competent KoRV is inherited within the DNA of northern koalas [9, 20]. In any individual koala, further integrations can occur in somatic cells through reintegration of endogenous KoRV, and also by the integration of exogenous KoRV variants. The presence of exogenous KoRV variants in southern koalas is disputed [42, 47-50], and endogenised replication-competent KoRV appears absent from southern populations [19, 49-51]. Consequently, KoRV has more opportunities to exert a mutational effect in northern koalas, and the chances of a KoRV integration occurring near a site where it could alter gene expression and lead to neoplasia is correspondingly higher in northern koalas. Regional differences in the nature of KoRV infection in northern and southern koalas is described further in *Appendix 5.4 Koala Retrovirus - Literature Review*.

There are integration 'hot spots' within the koala genome where KoRV insertions are more likely to occur. These hot spots are often in close proximity to genes involved in cell growth and proliferation [9]. Neoplastic tissues of koalas have also been found to contain new KoRV insertions in the vicinity of oncogenes. The insertion of KoRV into the genome can induce gene dysregulation, potentially compromising the removal of early neoplastic cells, and promoting tumour growth and malignant transformation [52]. *In vitro* studies of the effect of KoRV on naïve human cell lines also demonstrates significant upregulation of several oncogenes, including those associated with lymphocytic leukaemia [47].

Two captive koala facilities in Qld noted a potential hereditary pattern of lymphoma and leukaemia in koalas, with at least three successive generations of animals succumbing to these neoplastic conditions at similar ages. This may reflect the inheritance of KoRV proviral copies within the genome [18], but may also indicate unidentified inherited predispositions which are independent of KoRV status.

5.6.4 Association with other disease hazards of koalas

The associations between KoRV and development of neoplasia have been discussed earlier in this chapter.

Neoplasia is commonly reported concurrently with chlamydiosis, with 39% (16/41) of koalas with neoplasia also diagnosed with chlamydiosis in one post mortem study [32]. This association may reflect the high prevalence of *Chlamydia* as a co-morbidity, or possibly co-infection with KoRV, rather than a direct association between chlamydiosis and the development of neoplasia. Associations between KoRV and *Chlamydia* infection are discussed in the respective chapters.

A wide range of other associations and causal factors for neoplasia have been identified in other species, particularly humans [2]. Osteochondroma in humans, horses and dogs can be inherited as an autosomal dominant trait [53]. The development of mesothelioma in humans and other animals has been associated with exposure to asbestos, the SV40 virus and chemicals such as ethylene oxide, nitrofurazone, nitrotoluene and potassium bromate [54]. Gammaherpesvirus infections of humans are responsible for thousands of new cases of neoplasia annually [55]. Other than KoRV, no such predisposing factors or associations have been investigated for the development of neoplasia in koalas.

5.6.5 Diagnosis

Clinical signs

Clinical signs of lymphoid neoplasia in koalas are variable. Body condition may be good or poor, and illness may be protracted or result in sudden death [1, 29]. Signs of lymphosarcoma reflect the location and severity of body system involvement, although lethargy, depression, listlessness and weakness are commonly encountered [1, 5, 29].

Alimentary lymphosarcoma in koalas commonly results in non-specific signs of gastrointestinal dysfunction including diarrhoea, abdominal pain which manifests as grunting or moaning, and soil pica [1]. Pale mucous membranes may be seen if anaemia is present. Lymphomatous infiltration of the reproductive tract can be associated with vaginal prolapse [38] or loss of pouch young [1]. Other signs of lymphoma may manifest depending on the location of lesions, including polydipsia [1], ocular discharge and swollen conjunctivae [21, 38], lameness [14], posterior paralysis [44], nystagmus and head tremors [37], and terminal convulsions [37]. Figure 26 shows a wild koala with a large facial swelling due to lymphoma.



Figure 26 A wild koala in care with a large facial lymphoma (credit: Amber Gillet)

Enlargement of superficial lymph nodes is a common finding, reported in 35% of lymphosarcoma cases [37]. The spleen and thymus may also be palpably enlarged and thymic enlargement may be accompanied by dysphagia [1].

Clinical signs of osteochondroma are associated with obstruction and compression of adjacent structures. Facial distortion is commonly seen but is not a consistent presentation. Disruption of the temporomandibular joint can cause malocclusion and degenerative joint disease. Obstruction and expansion into the nasal cavities can cause difficulty breathing and eating, a chronic nasal or oculo-nasal discharge and bleeding. Neurological signs may be seen if the tumour compresses the brain [5, 8].

Koalas with mesothelioma may present with few clinical signs until disease is advanced. Abdominal distension due to ascites may be seen if the neoplasia is localised in the abdomen, or dyspnoea if localised in the pleura. Affected individuals are often in fair to poor body condition. Abdominocentesis or thoracocentesis usually yields viscous, blood-tinged fluid [5, 6].

Clinical pathology

Approximately 50% of koalas affected by lymphoid neoplasia have circulating neoplastic cells (i.e. leukaemic lymphosarcoma), resulting in markedly elevated white cell counts in

excess of 100×10^9 cells/L. If the white cell count is normal, a relative lymphocytosis or lymphoblastosis may be present. Affected koalas may be neutrophilic or neutropaenic, and thrombocytopaenia is common. Mild to severe anaemia, which is usually non-regenerative, often accompanies lymphoid neoplasia. Common biochemical findings include elevated lactate dehydrogenase, elevated blood urea and hypoalbuminaemia [1, 5, 8, 37].

Neoplastic changes in mesothelial cells may be observed during cytological examination of abdominal fluid from koalas with mesothelioma. Dysplastic cell types are also seen on bone marrow examination.

Pathology

The major gross finding in lymphoid neoplasia is often enlargement of superficial, mesenteric or thoracic lymph nodes, and commonly splenic enlargement. The enlargements seen grossly are due predominantly to infiltrates of neoplastic lymphocytes [1, 21, 37, 56]. Pale nodules or streaks may be visible at gross necropsy although it is possible for significant infiltration of the tumour to occur without any grossly visible signs in the affected organ [1, 29]. Immunohistochemical studies showed that approximately half of lymphoid neoplasia cases were of T cell origin, one quarter of B cell origin and one quarter failed to stain with the markers used [3].

In a post mortem study of 51 koalas with lymphoid neoplasia, 54.8% of cases involved abdominal organs, especially the liver (13.8%) and spleen (12.1%). Solitary or multiple lymph nodes were frequently involved (12.9%). Infiltrates were also observed in a range of atypical sites such as lung, brain and conjunctiva (collectively 13.3%), and 5.8% in the cervico-mediastinal area including thymus [3].

Craniofacial osteochondromas present as firm masses of the facial bones, including the orbit, buccal cavity, nasal cavity, maxilla and hard palate. Masses are white to cream, smooth, shiny, nodular and with a lobular appearance on cut surface. Microscopically they consist of neoplastic nodules divided by connective tissue septae. These nodules contain dysplastic cartilage and bone with cartilage matrix and hypertrophied chondrocytes. Tumours are benign and do not metastasize. Necrosis and inflammation may be associated with ulceration of compressed and displaced tissues [1, 5, 8, 39].

Mesotheliomas are located in the abdominal or thoracic serosal layers. Grossly there is serosal thickening with multiple glistening nodules distributed diffusely across serosal surfaces. Lesions are most common in the abdominal cavity, associated with ascites, but may also occur in the pleura, pericardium, pelvic cavity and scrotum. Fluid accumulating in cavities is notably high in volume and of a viscous consistency. Histologically, tumours demonstrate various proportions of fibrous, myxoid and mesothelial tissues [1, 5, 6, 8, 57].

Differential diagnosis

As many neoplasms present with non-specific clinical signs, there are a range of differential diagnoses, including infectious disease, malnutrition, exposure to toxins and trauma.

Craniofacial facial swelling associated with cryptococcosis may mimic osteochondroma.

Diagnostic testing

Diagnosis of neoplasia is made based on cytological or histological examination of affected tissues. In the live animal, cytological examination of aspirates from enlarged lymph nodes or masses, bone marrow or free thoracic or abdominal fluid may demonstrate neoplastic cells [5, 8, 37, 56]. Lymphoid neoplasia may manifest as leukaemia, which is diagnosed by examination of blood smears for circulating neoplastic cells [8].

Diagnosis of mesothelioma can be confirmed by cytological assessment of abdominal fluid and histopathology using immunohistochemistry [58].

Surveillance and monitoring

There is no active surveillance and monitoring program for koala neoplasia. However, cases of neoplasia are commonly captured by the Australian Registry of Wildlife Health and the surveillance database of Wildlife Health Australia (eWHIS), and wildlife hospitals and rehabilitation facilities may keep records of cases of neoplasia.

5.6.6 Treatment

The prognosis for the commonly encountered neoplasms in koalas (lymphoid, osteochondroma and mesothelioma) is very poor, and treatment is rarely attempted [5]. Surgical resection of focal masses has been successful in a very small number of cases [8, 59], but success probably depends on early diagnosis [5]. Craniofacial osteochondromas treated by surgical resection have recurred [8].

Most cases of neoplasia in koalas are diagnosed during end-stage disease or at post mortem examination. In one review of 41 cases of lymphoid neoplasia, 49% were diagnosed at necropsy and 29% were euthanased at first clinical presentation based on a diagnosis or a strong suspicion of neoplasia. Five of the koalas were given supportive care but died, on average, within two weeks [5].

High doses of prednisolone may provide temporary symptomatic relief in a koalas with lymphoid neoplasia, but are unlikely to cause clinical remission [8]. Chemotherapy using other agents has been attempted on very rare occasions without success [29].

5.6.7 Prevention and control

Options for prevention and control of neoplasia are limited due to the lack of information on the causal factors of neoplasia in koalas. The association between KoRV status and neoplasia indicates that prevention of some cases of neoplasia in koalas may be addressed through prevention and control measures for KoRV. Proposed prevention and control

measures for KoRV are outlined in Section 5.4 *Koala Retrovirus - Risk Assessment* and Appendix 5.4 *Koala Retrovirus - Literature Review*.

The possibility of inherited predisposition to neoplasia should be considered in koala populations or genetic lines with a high prevalence of neoplasia. Prevention of breeding from such lines may be indicated. As a general principle, maintaining genetic diversity in koala populations will encourage retention of the most robust koala genetic profiles to minimise the impact of neoplasia on the species.

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5.7 Novel *Actinomyces* sp. in Koalas – Literature Review

5.7.1 Technical information

Aetiological agent

Actinomyces is a genus of anaerobic or facultative aerobic, Gram-positive, filamentous bacteria [1]. A novel species of *Actinomyces* (termed here *Actinomyces* sp. nov.) has been found, associated with pneumonia in a number of free-ranging South Australian koalas [2]. The *Actinomyces* sp. found in koalas was sequenced from multiple isolates and showed less than 95.1% similarity to the closest *Actinomyces* species, *A. timonensis* [2].

Listing

Novel *Actinomyces* sp. is not a WOA listed disease [3].

Actinomycotic pneumonia is not a notifiable animal disease in Australia [4].

Actinomycotic pneumonia is not identified as a key threatening process under the Environment Protection and Biodiversity Act [5].

5.7.2 Epidemiology

Actinomyces spp. can be found in the healthy microbiota of the human oropharynx, gastrointestinal tract and urogenital tract [6-9] and on nasal, oral or oropharyngeal mucosal surfaces and within the urogenital tract of other animals [1, 8-11].

Species of *Actinomyces* can cause sporadic or rare disease in humans and animals [1, 6, 7, 12-14]. Disease from *Actinomyces* spp. are most often associated with opportunistic infections where there has been a breakdown in the normal immunological defence of the animal, which has allowed opportunistic entry of the bacteria [1, 9]. Lumpy jaw (caused by *A. bovis*), a disease of cattle, occurs when there is a loss of integrity of the oral mucosa (generally caused by a penetrating wound). Bacteria then gain entry to the submucosal tissues and cause disease [15]. *Actinomyces* spp. have also been found in cases of progressive periodontal disease in macropods, as a part of multi-organism infections [16-18]. In humans, dental disease has been associated with pulmonary actinomycosis [6, 12, 19].

Novel *Actinomyces* sp. has been found as the predominant bacteria isolated from 17 cases of pneumonia in wild South Australian koalas since 2016. The disease in koalas has been termed pulmonary actinomycosis [2].

Host range

The host range of the novel *Actinomyces* sp. found in koalas is not known, however it has not been identified in host species other than the koala.

A small number of other wildlife species have been reported with pulmonary actinomycosis, including two free-ranging chamois [20], one free-ranging black-tufted marmoset [21], a

captive red kangaroo [22] and a captive bare-nosed wombat [23]. Most studies did not differentiate the *Actinomyces* involved to the species level but *A. hyovaginalis* was identified in the bare-nosed wombat [23].

Zoonotic potential

There is no evidence of animal-to-human transmission of any *Actinomyces* spp. Human-to-human transmission of *Actinomyces* spp., via contaminated bite wounds, has been reported [8].

The zoonotic potential of the novel *Actinomyces* described in koalas has not been determined, but due to the nature of the non-transmissible, opportunistic autologous infections seen in other animal species, the zoonotic potential is considered very low.

Geographic distribution

Species of *Actinomyces*, and resulting disease, have been reported globally [1, 6, 7, 12-14]. The novel *Actinomyces* sp. causing pneumonia in koalas has only been reported in SA [2]. Investigations in Qld have detected *Actinomyces* spp. in a case of aspiration pneumonia in a wild koala (eWHIS case 14690) but the species is not known [24].

Prevalence

A mortality study of SA koalas (2016-2019) reported 17 cases of pulmonary actinomycosis, of a total of 240 cases [25]. Subsequently, there have been at least four more cases of pulmonary actinomycosis reported in SA koalas [26].

Mode of transmission

Actinomyces spp. infections are classified as endogenous. These bacteria are not considered contagious and are generally not transmitted directly between individuals [1, 9, 12]. The pathway by which koalas acquire novel *Actinomyces* infection is currently not known. It is hypothesised that *Actinomyces* sp. nov. is a normal part of the koala's gastrointestinal microbiome, as *Actinomyces* spp. have been found in both healthy and diseased oral microbiomes of other mammals [1, 6, 10, 18]. Opportunistic infections may arise when there is a disturbance in immunological defence mechanisms in the individual koala.

Incubation period

The incubation period for *Actinomyces* spp. in general is not known, however the development of disease as a result of *Actinomyces* infection is generally considered to be slow and chronic in nature. Due to the prolonged timeline, the inciting cause often cannot be identified. Cases of pulmonary actinomycosis in humans have shown chronic progression over months [6, 12, 19]. The incubation period in koalas is not known.

Persistence of agent

Assuming *Actinomyces* sp. nov. is a commensal organism, persistence in the environment is unlikely to be important in the epidemiology of the disease. However, this remains unverified.

5.7.3 Pathogenesis

There is limited understanding of the pathogenesis of pulmonary actinomycosis in the koala. It is assumed that the non-contagious, commensal bacteria, forming part of the healthy gastrointestinal microbiome of the koala, opportunistically invade the lower respiratory system, as a result of unidentified initiating factors [2].

In humans, aspiration is a leading initiator of *Actinomyces* pneumonia with parenchymal or lobar pneumonia affecting the lower lung lobes [6, 7, 27]. A similar pattern of pneumonia has been seen in affected koalas. Other types of aspiration in koalas are generally associated with bottle-feeding orphaned joeys, and have a more cranial lung lobe distribution [28, 29]. Histological plant fragments, indicative of aspiration, were found in microscopic examination of lung tissues in two of the cases of pulmonary actinomycosis in koalas [2]. Culture of lung tissue from cases of pulmonary actinomycosis in koalas also isolated other anaerobic bacteria species which are commonly found in the oral microbiome of the koala [2, 30].

Because actinomycotic pneumonia has only been diagnosed in SA koalas, one hypothesis is that the disease may have a genetic component [31]. Koalas that develop pulmonary actinomycosis may be genetically predisposed to the disease because they lack key immune alleles that would enable them to mount an effective immune response. Koalas may also be genetically predisposed to carry novel *Actinomyces* sp. as part of their alimentary microbiome, rendering them more likely to develop pulmonary actinomycosis if inhalation of alimentary tract content occurs. Alternatively, affected koalas may have genetically-based anatomical differences that predispose them to aspiration, such as an elongated soft palate, a straighter trachea which encourages laminar flow to deep within the lungs, or dental malocclusions which lead to a dental disease [32]. These factors are speculative but may underpin a predisposition to actinomycotic pneumonia in the SA population [31].

5.7.4 Associations with other pathogens

No associations have been found in affected koalas between pulmonary actinomycosis and infection with koala retrovirus (KoRV) or *Chlamydia pecorum* [2, 21], although only a few cases have been investigated. No other disease associations have been investigated or hypothesised at this stage.

5.7.5 Diagnosis

Clinical signs

Koalas presenting with pulmonary actinomycosis may show signs of respiratory distress and may also have a reduced body condition score (emaciated, poor or fair). Radiographic investigations may show extensive or lobar pneumonia, but this is not specific for pulmonary actinomycosis [2].

Secondary hypertrophic osteopathy was found in four cases of pulmonary actinomycosis in koalas, and was diagnosed radiographically [33].

Clinical pathology

There are no reported clinical pathology findings diagnostic of pulmonary actinomycosis [2].

Pathology

Pulmonary actinomycosis in koalas is characterised by marked pyogranulomatous lobar pneumonia, most commonly affecting the left caudal lobe. *Splendore-Hoeppli* phenomenon and intralesional, variably Gram-staining, non-acid fast filamentous bacteria, consistent with *Actinomyces* sp., were present in all investigated cases. These findings support the hypothesis that a significant pathogen in all reported pneumonia cases is a novel *Actinomyces* sp. The observed lesions in koalas were consistent with actinomycotic lesions observed in other species [20, 21]. Disseminated actinomycosis has been reported in one free-ranging koala from SA, with lesions in the kidney and skeletal muscle, as well as lung [34].

Differential diagnosis

Pathogens reported to cause pneumonia in koalas include *Bordetella bronchiseptica* [35, 36], *Chlamydia* spp. [29, 37, 38] and *Cryptococcus gattii* [35, 39]. *Bordetella bronchiseptica* has been identified in outbreaks of pneumonia in captive koala colonies in Qld [36] and NSW [35, 40], either as a primary pathogen, or secondary to other diseases such as cryptococcosis or chlamydiosis [35]. Cryptococcal infections have not been reported in koalas in SA, therefore this is considered less likely as a differential in SA koala populations. Other bacterial infections have been reported to be associated with pneumonia in koalas [40-44].

Secondary hypertrophic osteopathy has not been reported in koalas in the absence of pulmonary actinomycosis [33].

The histopathological changes seen in actinomycosis (*Splendore-Hoeppli* phenomenon) may also be seen in nocardiosis or botryomycosis [20]. Differentiation of the pathogen occurs through special staining and histopathological examination. *Nocardia* are acid-fast, but *Actinomyces* are not, and in botryomycosis, the organisms implicated are generally non-filamentous [20].

Diagnostic testing

Broncho-alveolar lavage or tracheal wash can be used to help diagnose the presence of pneumonia and differentiate the cause. Ultrasound-guided fine needle aspirate of lung lesions has been used to aid diagnosis in a macropod [22]. Diagnosis of novel actinomycosis in koalas was via histopathology of lung tissue [2].

Surveillance and monitoring

Novel *Actinomyces* of koalas is an emerging disease, and no formal surveillance or monitoring is currently being conducted.

5.7.6 Treatment

No treatment protocols have been trialled or established for novel *Actinomyces* infection in koalas. Choice of antibiotic should be based on culture and sensitivity testing of samples obtained via bronchioalveolar lavage. Chloramphenicol has been used to treat similar cases in other animals and humans.

Cattle with lumpy jaw are treated with intravenous sodium iodide with concurrent broad-spectrum antibiotic therapy, with a guarded prognosis if deformity and malalignment of the jaw is present [45]. Cats and dogs with actinomycosis have been treated with drainage of abscesses or effusions and prolonged antibiotic therapy, inclusive of penicillin, amoxicillin and chloramphenicol [46]. In humans, prolonged high dose antibiotic treatment (6-12 months) with penicillin or amoxicillin is needed and surgical resection may help reduce the longevity of treatment [6].

5.7.7 Prevention and control

Currently, no prevention or control methods have been identified for actinomycotic pneumonia in koalas. Novel *Actinomyces* is not considered transmissible between koalas. It is considered unlikely that a vaccine would be developed for this pathogen. As dental disease has been associated with pulmonary actinomycosis in humans, and dental disease is often seen in koalas [47, 48], it is thought that this may contribute to the incitement of pulmonary actinomycosis [2]. A greater understanding of the likely initiating factors, incubation period and treatment options will be required before prevention and control strategies for pulmonary actinomycosis in koalas can be developed.

5.7.8 References

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5.8 Oxalate Nephrosis in Koalas – Literature Review

5.8.1 Technical information

Description of hazard

In the context of this document, nephrosis is defined as a degenerative or inflammatory disease primarily affecting the tubules of the kidney [1]. Nephrosis is commonly associated with acute and chronic renal failure in koalas.

This chapter considers only oxalate nephrosis (ON) i.e. nephrosis associated with renal oxalate crystal deposition.

Listing

Oxalate nephrosis is not a WOAHA listed disease [2] or a notifiable animal disease in Australia [3].

It is not identified as a key threatening process under the Environment Protection and Biodiversity Act [4].

5.8.2 Epidemiology

Causes of hazard

The causes of ON in koalas are poorly understood. It is likely that the disease involves a combination of genetic, dietary, gastrointestinal and environmental factors [5, 6].

A genetic predisposition to abnormal oxalate metabolism is suspected in koalas, due to the marked regional bias in the prevalence of this condition, with the majority of cases recorded in the Mount Lofty Ranges (MLR) population in SA [7, 8]. SA koala populations are principally derived from a small number of founder animals and demonstrate high inbreeding coefficients, which have been associated with testicular abnormalities [9]. Some Vic koala populations which are similarly derived from a very small number of founder animals from French Island have also been shown to have a higher prevalence of ON than more genetically diverse Vic koalas [10].

In humans, a well described disease called ‘primary hyperoxaluria’ occurs (hyperoxaluria = elevated oxalate levels in the urine). Affected humans have gene mutations that cause liver enzyme dysfunction and endogenous oxalate overproduction, causing increased oxalate excretion and deposition within the kidneys [11]. It is not known if this specific syndrome exists in koalas from populations with a high prevalence of ON [12]. Primary hyperoxaluria has been recently identified in another marsupial, the Gilbert’s potoroo [13]. Another gene candidate for ON has been recently identified in a KoRV-based study which analysed the differential expression of genes in lymph node tissue between koalas in Qld, where ON is less prevalent, and koalas in MLR. This study revealed statistically significant upregulation of a mutated gene in MLR koalas that codes for an anion transporter involved in oxalate transport in the kidney and intestine [14]. In experimental studies with mice and rats this

gene is linked to increased urinary oxalate levels and renal oxalate deposition [15-17]. The gene has also been investigated in a limited number of preliminary studies in humans [18, 19]. In koalas the upregulation of this mutated gene may reflect a dysfunctional transporter that results in elevated oxalate levels, or conversely, a compensatory response to high oxalate due to another cause. As not all koalas demonstrating this genetic upregulation develop ON, the involvement of other genes and other factors in disease manifestation is likely [14].

In humans with inherited predisposition to abnormal oxalate metabolism, increased dietary oxalate exacerbates disease [20]. The leaves of eucalypt species eaten by koalas have a relatively low total oxalate content (<1% dry weight) when compared with plant species such as soursob, which are known to cause acute oxalate toxicity in other herbivore species (>10% dry weight), suggesting that excessive dietary oxalate intake is unlikely to be a primary cause of ON in koalas [12]. However, SA eucalypt species favoured by koalas have significantly higher (although still generally low) levels of oxalate than Qld species, and oxalate content of leaves may increase in times of drought, so there is potential for koala oxalate intake to increase depending on the types and quality of leaf being ingested [12]. An increase in dietary oxalate intake might in turn have an influence on the occurrence of ON in koalas already predisposed to the condition.

Gastrointestinal oxalate-degrading bacteria play an important role in ON manifestation in humans [21]. The role of these bacteria, particularly *Oxalobacter formigenes*, in the development of ON has received some attention in koalas. A study of faecal and caecal samples from MLR koalas (n=22) found that *O. formigenes* was present in all subjects, irrespective of ON status, and there was no significant difference in the relative abundance of *O. formigenes* or other known oxalate-degrading bacteria between affected and unaffected koalas [5]. Koalas from the MLR population had much lower levels of *Oxalobacter* spp. than previously detected in two Qld koalas [22], but this may reflect differences in methodology rather than true differences in overall abundance. It has been suggested that antibacterial treatment may be a factor in the depletion of koalas' oxalate-degrading gastrointestinal bacteria, thereby predisposing to the development of ON [23, 24]. Further investigation is required to clearly elicit the role of *O. formigenes* or other oxalate-degrading bacteria in oxalate metabolism and development of ON in koalas.

Dehydration can be a risk factor for ON in the koala. Koalas ingest water through the moisture content of the browse they consume or by drinking free water [25]. In hot weather, they experience increased water loss through convection and evaporative cooling [26, 27], and have reduced water intake due to lower leaf moisture content and lower availability of free water [25, 28], making them vulnerable to dehydration and renal stress. Food and water intake are closely associated in koalas, which obtain about 75% of their water intake from the foliage they feed on [29]. A reduction in food (and therefore water) intake due to ill health, injury, physiological stress, loss of habitat or inappropriate husbandry may similarly increase the risk of dehydration, thereby increasing the risks of ON.

Mount Lofty Ranges koalas with ON were more likely to die or be euthanased in the months following high ambient temperatures and in the months following low rainfall, although seasonal measures of eucalypt leaf water content were not associated with deaths [6]. These findings suggest that evaporative water loss and lack of availability of free water exacerbate ON in koalas in SA, which typically has long hot and dry summers. The relationship between hydration and ON has also been seen in captive koalas. In one captive institutions in SA, which has a prevalence of ON over 50%, based on presence of oxalate in urine, maintaining free water availability and hydration of browse are important to preventing exacerbation of clinical signs [30].

Marsupial young are born at a very early stage of development, and consequently are heavily dependent on their mothers' milk during organogenesis [31]. Maternal nutritional stress during lactation can have negative impacts on renal development for the joey, including nephron numbers. This may reduce the functional capacity of the growing kidneys, thereby making the joeys of nutritionally compromised dams more vulnerable to developing ON if they are otherwise predisposed [31]. This possibility warrants further investigation given the high incidence of ON reported in young koalas [32].

Geographic distribution

The majority of reported cases of ON in free-ranging koalas are associated with the MLR population in SA [7, 8, 10], with sporadic cases reported elsewhere in both captive and free-ranging koalas in Qld, NSW and Vic, and in captive WA koalas [32-36]. Oxalate nephrosis is a leading cause of disease in the MLR population in SA [7]. Other populations with increased prevalence are emerging outside of SA as targeted investigations are undertaken [10].

Prevalence

Reported cases of ON are presented in Table 20. Oxalate nephrosis is reported in both free-ranging and captive koalas in SA. In the MLR population, there was a reported prevalence of 25-54.9% in wild koalas at post mortem examination [7, 20, 37]. The Kangaroo Island population of koalas is less well studied in the context of ON but appears to have a lower prevalence of disease based on presence of oxalate crystals on urinalysis of wild koalas [32]. Several clinical cases were diagnosed in wild koalas rescued from the 2019-20 bushfires [38]. Urinary oxalate levels in SA koalas (both Kangaroo Island and MLR populations) were reported to be 5-20 times higher than those of Qld koalas [32], supporting the likelihood of higher oxalate intake or impaired endogenous oxalate metabolism in SA koalas [12, 32].

Table 20 Reported cases of oxalate deposition or nephrosis in koalas

Location	Cohort	Total	ON cases	Reference
Queensland				
SE Qld	Wild	519	5 (1%)	Gonzalez-Astudillo et al. 2019 [39]
SE Qld	Wild	10139	0 (0%)	Burton and Tribe 2016 [34]
SE Qld	Wild	17	2 (12%)	Speight et al. 2014 [32]
New South Wales				
Central and northern NSW	Wild	12543	0 (0%)	Charalambous and Narayan 2020 [40]
Central and northern NSW	Wild & captive	235	4 (1.7%)	Canfield 1989 [35]
Port Macquarie	Wild	3781	0 (0%)	Griffith et al. 2013 [41]
Port Macquarie	Wild	127	2 (1.6%)	Canfield 1987 [36]
Various locations	Wild & captive	110	3 (2.7%)	Connolly 1999 [42]
Victoria				
Cape Otway	Wild	13	1 (8%)	Speight et al. 2020 [10]
Central region	Wild	2	0 (0%)	Speight et al. 2020 [10]
French Island	Wild	5	2 (40%)	Speight et al. 2020 [10]
Raymond Island	Wild	11	2 (18%)	Speight et al. 2020 [10]
Somers	Wild	6	0 (0%)	Speight et al. 2020 [10]
Strzelecki region	Wild	12	0 (0%)	Speight et al. 2020 [10]
Western region	Wild	14	4 (29%)	Speight et al. 2020 [10]
unknown	Wild	44	0 (0%)	Obendorf 1983 [43]
South Australia				
Kangaroo Island	Wild	25	1 (4%)	Speight et al. 2014 [32]
Mt Lofty Ranges	Wild	85	27 (31.8%)	Speight et al. 2018 [7]
Mt Lofty Ranges	Wild & captive	51	28 (54.9%)	Speight et al. 2013 [20]
Mt Lofty Ranges	Wild	240	60 (25.0%)	Stephenson 2021 [37]

Oxalate nephrosis prevalence in free-ranging koalas in Qld and NSW ranges from 0 to 12% [32, 34-36, 39-42]. In these populations, disease is more commonly seen in koalas admitted to care for other reasons, that develop ON subsequent to admission [44]. In Qld, ON is only reported in free-ranging koalas. Facilities housing both captive and rehabilitation koalas, receiving the same range and species of browse, report that only individuals from the wild develop the disease [23]. The reason for this is not clear, but a difference in the prevalence of oxalate-degrading bacteria in the microbiome has been hypothesised [44].

Oxalate nephrosis is reported in both free-ranging and captive koalas in Vic. In Vic, ON has been recorded in wild koalas on French Is, and in several other Victorian populations which received significant numbers of koalas from French Island during reintroduction programs in

the 1930s [10, 45]. In contrast, the Strzelecki Ranges population in Vic, which is believed to be a remnant original population rather than reintroduced, had no evidence of ON based on histological examination of a small number of kidney samples [10]. The association between provenance and ON prevalence in Vic is based on low numbers of animals and is not conclusive, given ON was absent from two Vic populations (central region and Somers) which were derived from French Island founders. However, the finding lends support to the likelihood that inherited abnormalities of oxalate metabolism underlie regional patterns in the prevalence of ON [10, 14].

5.8.3 Pathogenesis

The primary pathogenic mechanism for ON in koalas is physical damage and associated inflammation caused by the deposition of calcium oxalate crystals in renal tissue [23]. The blockage of nephron tubules by crystals is also a common feature and can lead to widespread tubule dilation, tubule rupture and glomerular atrophy [20]. The progressive damage to the renal tissue compromises the kidney's ability to concentrate urine, leading to renal insufficiency and eventually renal failure [32]. It has been suggested that oxalate may also cause renal damage prior to precipitation of crystals, and that this may explain the finding of renal dysfunction in the absence of renal calcium oxalate deposition in some MLR koalas, which were shown to be hyperoxaluric [32]. In chronic cases of oxalate nephrosis, fibrosis replaces functional renal parenchyma, contributing to further deterioration in renal function [20].

Elevated levels of oxalate in the urine of affected koalas support the premise that ON is a primary cause of renal disease, since ON which occurs secondary to renal failure is generally associated with a decrease in urinary oxalate [32]. The fact that many koalas succumbing to ON are less than 2 years old also supports a primary pathogenesis [7, 32], as renal failure is not otherwise a common cause of disease in young animals.

The factors which lead to hyperoxaluria and the triggers for crystal formation within renal tissue are poorly understood [23], although a number of causes have been suggested (see *Epidemiology*).

5.8.4 Associations with other disease hazards of koalas

Any disease hazard which predisposes koalas to dehydration (or inappetence leading to dehydration) might also be expected to increase the risk of development of ON in susceptible individuals.

Prolonged antibacterial therapy, as is commonly used for the treatment of chlamydial infection, can markedly alter the koala microbiome [46]. Antibacterial therapy would be expected to deplete oxalate degrading bacteria in the koala digestive tract, although whether this could precipitate ON is yet to be demonstrated. At one wildlife hospital in Qld, over 70% of koala ON cases were associated with animals in rehabilitation receiving antibacterial treatment [23].

A post mortem study of 240 koalas from the MLR population found that koalas with ON were less likely to be infected with replication-competent KoRV than koalas without ON. It was speculated that the insertion of the KoRV provirus into host DNA might disrupt genes associated with ON development, but further understanding of KoRV pathogenesis, and the role of genetics in ON, is required to test this hypothesis [37].

5.8.5 Diagnosis

Clinical signs

Clinical signs associated with ON reflect a loss of renal function. Affected koalas may demonstrate acute, dramatic weight loss (400-1000g within less than 5 days), polyuria and polydipsia, reduced appetite, dehydration and high rates of mortality [23, 32]. Free-ranging koalas affected by ON in SA are usually rescued after being found on the ground in poor body condition or with signs of polydipsia and polyuria [20, 23, 47].

While ON is commonly associated with signs of ill health, mild ON has been detected as an incidental finding in otherwise healthy free-ranging koalas and as an incidental post mortem finding [23, 36]. Whether this reflects a preclinical phase of disease in which renal function remains adequate is unclear.

Clinical pathology

Clinical pathology findings are consistent with renal dysfunction. Serum biochemistry is consistent with renal insufficiency and is often characterized by azotaemia in conjunction with poorly concentrated urine [23, 32]. Symmetric dimethylarginine (SDMA) levels in affected koalas correlate well with other renal function parameters and are elevated above reference intervals in most cases of ON [48]. Other biochemistry parameters are generally unremarkable.

Urinary oxalate to creatinine ratio becomes elevated in animals with hyperoxaluria, although hyperoxaluria is seen in SA koalas both with and without ON, so it may not be a good indicator of clinical disease [32].

Total blood calcium is anecdotally reported to be low in affected individuals and has been used as a prognostic indicator [23]. However, this finding is not supported by systematic studies of calcium levels in affected koalas in SA [32].

Urine sediment examination is characterised by the presence of oxalate crystals, although not all affected animals will show crystalluria [32]. “Wheat-sheaf” or “bow tie” crystal formations (consistent with calcium oxalate monohydrate) are commonly seen in SA cases, whereas crystals in koalas from Qld are typically rectangular or oval, consistent with calcium oxalate dihydrate [23, 32]. Crystalline urinary casts may be visible in fresh urine samples, suggesting that in SA koala urine sediment the crystals are renal in origin [32]. In severe cases, a macroscopic pale yellow sediment is visible in the urine without the need for sedimentation techniques. Infrared spectroscopic analysis of the urine crystal sediment, and

renal precipitate, have shown a consistent pattern of calcium oxalate with some uric acid and phosphate components [20, 32].

Pathology

Pathological changes mainly involve the renal system. Kidneys may show surface pitting, mottling or streaking in the cortex, cortical pallor and white foci in the medulla [10]. Severe cases may show streaks of heavy precipitation in the papilla and medulla and a gritty yellow precipitate in the renal pelvis [20].

Renal histopathology includes tubular loss and dilation, cortical fibrosis and glomerular atrophy [20]. Yellow or clear birefringent crystals and crystal 'ghosts' (where crystals are lost during processing) may be visible, arranged in rosettes or fan shapes and varying in morphology as previously described [10, 20, 37]. A mixed intratubular inflammation may be associated with the crystals or a multifocal mononuclear interstitial inflammation [10].

Differential diagnosis

The most common cause of urinary tract disease in koalas is chlamydiosis, which can be distinguished from ON by the presence of bladder pathology and pyuria, pathology of other organ systems and the absence of crystalluria.

Clinical signs of dehydration and weight loss seen with ON are non-specific and may be associated with most koala diseases. Polydipsia may be seen in animals that are dehydrated or heat stressed, but this is not accompanied by polyuria unless there is renal compromise.

Diagnostic testing

Definitive diagnosis of ON is based on identification of the characteristic crystal morphology in sediments of voided urine or on renal histopathology [20, 32]. Renal ultrasound is an important diagnostic tool in live animals [23]. Renal function testing through serum biochemistry (urea, creatinine and SDMA) and urinalysis should be part of the diagnostic process where ON is suspected [48].

Surveillance and monitoring

There is no targeted national surveillance or monitoring program for ON in koalas, although there is capacity to utilise the Wildlife Health Australia national wildlife health information system database (eWHIS) as a place for collating these data as part of national general wildlife surveillance activities.

5.8.6 Treatment

Treatment of ON is challenging and generally unsuccessful in advanced cases. Prevention, early detection and early intervention are important to successful management. Most aspects of treatment are supportive in nature, including fluid therapy, optimising nutrition and minimising stress [23].

The provision of a high diversity of good quality eucalypt and non-eucalypt browse species is always important in the supportive care of koalas. In cases of ON it may be important to specifically supply low oxalate browse to the koala, and to manage dehydration by leaf spraying and provision of drinking water, particularly on hot days [6, 12, 23]. Increasing fibre and fluid intake through syringe feeding of leaf or pumpkin puree has been recommended by clinicians [30].

Maintaining a healthy gut biome is considered critical in the clinical management of ON [30]. As well as providing high quality nutrition, oral administration of koala caecal contents may be beneficial in restoring oxalate-degrading bacteria to the gut. In humans with abnormal oxalate metabolism, the administration of probiotics containing *O. formigenes* has a supportive role in reducing urinary and plasma oxalate levels, but this treatment has not been tested in koalas with ON [5, 23].

Some therapeutics used in the treatment of humans with renal disease and ON, including benazepril [49], stiripentol [50] and vitamin B6 [51], have been incorporated into the treatment of koala cases. No pharmacokinetic studies of these treatments have been undertaken in koalas, although benazepril anecdotally appears to reduce ON disease severity and likelihood of recurrence [30]. Propentofylline (Vivitonin®) has been used by some clinicians as an adjunctive therapy [30] as this family of drugs has been shown in some species to improve renal blood flow [52, 53], although its effects on the koala kidney function have not been investigated. Such ongoing medical treatments are likely to be most suitable for managing ON cases in captive koalas.

Oral treatment with calcium carbonate theoretically assists in binding dietary oxalate in the gastrointestinal tract and reducing absorption of unbound oxalate in cases of ON [51]. Anecdotally, this has been beneficial in preventing advancement of disease in rehabilitation koalas in Qld, allowing them to maintain sufficient renal function to enable eventual release [44], but no such therapeutic benefit has been seen in SA koalas [30]. Calcium carbonate therapy may induce temporary hypercalcaemia, causing diarrhoea and inappetence [23].

Response to rehydration and return of urine specific gravity to normal levels within 3 days of treatment are considered good prognostic indicators in koalas with ON that experience episodic illness [30].

5.8.7 Prevention and control

Prevention of ON in free-ranging koalas focuses on strategies to prevent dehydration. Maintaining habitat quality, quantity, diversity and connectivity is likely to be important in this context. Strategies which reduce hydration stress on trees, such as improving catchment and slowing water outflow, are important considerations for maintaining the moisture content of foliage on koala feed trees [54], and may also reduce the concentration of oxalate in leaves [12]. Habitat quality, quantity, diversity and connectivity are also essential to provide refugia from thermal stress for koalas [55].

Free water stations may be a practical management tool for offsetting water loss in free-ranging koalas and might slow or prevent development of ON in susceptible populations [6, 27].

Strategies to prevent dehydration of koalas and koala browse are also important to preventing the development of ON in captive and rehabilitation facilities. Misting or spraying browse with water will help to preserve the moisture content of the leaf, as well as incidentally increasing the koala's water intake [6, 12, 56]. Careful storage of cut browse branches (refrigeration; standing the cut end in water which is replenished daily) will help to maintain leaf moisture [23]. Supportive strategies to reduce stress, concurrent disease or debilitation are important for ensuring good nutrient and moisture intake in koalas in captivity and in rehabilitation. This is particularly important for lactating mothers to ensure healthy renal development in joeys.

There is currently insufficient information to determine the feasibility or benefit of diversifying the genetics of populations susceptible to ON. However, supporting genetic diversity in koala populations individually and as a whole will encourage retention of the most robust koala genetic profiles for adapting to a changing climate and coping with the impacts of high ambient temperatures and drought.

5.8.8 References

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5.9 Phascolarctid Herpesviruses – Literature Review

5.9.1 Technical information

Aetiological agent

Koalas are known to be hosts to three herpesviruses, designated phascolarctid herpesvirus 1 (PhaHV-1), phascolarctid herpesvirus 2 (PhaHV-2) and putatively phascolarctid herpesvirus 3 (PhaHV-3) [1, 2]. PhaHV-1 and PhaHV-2 are gammaherpesviruses [1-3]. A novel alphaherpesvirus (PhaHV-3) was identified in a sick captive female koala from Qld [4]. The animal had pneumonia, hepatitis and adrenalitis as well as concurrent lung disease due to *Cryptococcus gattii*. The novel alphaherpesvirus was shown to be most closely related to macropod alphaherpesvirus-1. No other cases have been identified of this novel herpesvirus and it is not known if the natural host of the novel virus is the koala, or if in this instance the virus spilled over from another host. This alphaherpesvirus is not considered further in this literature review.

All herpesviruses detected so far in marsupials belong to the *Alphaherpesvirinae* and *Gammaherpesvirinae* subfamilies [1, 2, 5].

Listing

Phascolarctid herpesviruses are not WOAHA listed animal diseases [6].

Herpesvirus infection due to phascolarctid herpesviruses is not a notifiable animal disease in Australia [7].

Disease due to phascolarctid herpesviruses is not identified as a key threatening process under the Environment Protection and Biodiversity Act [8].

5.9.2 Epidemiology

The study of koala herpesviruses is still in its infancy. Epidemiological data are only available for free-ranging populations in Vic [9, 10] and SA [11]. Only a few studies have attempted to evaluate the association between PhaHV infection and risk factors for disease [4, 9-11].

Phascolarctid herpesviruses 1 and 2, like other gammaherpesviruses, appear to be host specific and probably co-evolved with their host [5]. The detection of PhaHV-1 and -2 in the French Island koala population, which has been closed to introductions for over 100 years, supports the likelihood that these viruses have been present since before European habitation of Australia [10]. Data are required for koala populations in Qld and NSW, and for more geographically widespread captive populations, to support this theory.

Once infected with a herpesvirus, an animal remains infected for life. The virus generally lies dormant within host cells (“latent” infection), until infection is reactivated by factors such as stress or immune compromise. During the “lytic” (active) phase, the virus replicates within the host cell, and releases a new generation of viruses when the infected host cell lyses. The

viral infection can be transmitted to other individuals during the lytic phase (see *Pathogenesis*) [12].

Adult koalas in both SA and Vic free-ranging populations were found to be significantly more likely to be positive for PhaHV-1 (but not PhaHV-2) than juveniles [10, 11]. For PhaHV-2, juveniles were as likely to be positive as older animals. Increasing age was a significant risk factor for co-infection with PhaHV-1 and -2 in both female (Vic and SA) and male (Vic) koalas [10, 11]. The discrepancy in age associations between the two viruses might point to differences in transmission dynamics (see *Mode of transmission*).

No differences in sex prevalence have been identified for PhaHV-1 and -2 infections. However, female koalas without pouch young were more likely to be positive for PhaHV-1 or PhaHV-2 or coinfecting with PhaHV-1 and PhaHV-2, than females with pouch young, suggesting a possible association between herpesvirus infection and reduced fertility [10].

Where one gammaherpesvirus subtype is detected in an individual koala, there is a high likelihood that the other gammaherpesvirus will also be detected. Oropharyngeal detection of PhaHV-1 was 3.5 times more likely if PhaHV-2 was also present [11].

Host range

The only known host of PhaHV-1 and PhaHV-2 is the koala, consistent with the host specificity of gammaherpesviruses in general [5].

Zoonotic potential

The zoonotic risk of PhaHV is considered very low as gammaherpesviruses are highly host-specific [3, 12].

Geographic distribution

Published investigations of PhaHV-1 and -2 are currently only available for Vic and SA koalas, where both PhaHV-1 and -2 have been detected in all free-ranging and captive populations of koalas tested to date [10, 11]. A study to detect PhaHV-1 and -2 in NSW and Qld free-ranging populations is in progress and has so far identified PhaHV-1 in both Qld and NSW free-ranging koalas [13].

Prevalence

Prevalence studies of PhaHV in koalas have been undertaken using PCR assays. PCR tests cannot differentiate between the lytic and latent phases of PhaHV infections [10]. PhaHV occurs at moderate to high prevalence in studied populations. A survey of 810 koalas from widespread locations across Vic demonstrated significant variation in prevalence, ranging from 7.4-45.5% for PhaHV-1 and 0.9-54.6% for PhaHV-2 [10]. Significant longitudinal variations in prevalence have been reported. Over the course of three years, the prevalence in the Raymond Island population decreased from 45% to 17%, and that of the Cape Otway area population reduced from 39% to 22%. Conversely, PhaHV-2 prevalence was higher in the French Island population in 2013, compared to 2011 [10].

A study of wild koalas in the Mount Lofty Ranges of SA detected PhaHV in 73% (58/80) of individuals. The prevalence of PhaHV-1 and -2 was 57.5% (46/80) and 40.0% (32/80) respectively in wild caught koalas, and 46% (40/87) and 27.5% (24/87), respectively in necropsied koalas [11]. Positive oropharyngeal swabs were found in 74.6% of the infected cases (54.0% of the euthanased cohort). Co-infection with both PhaHV-1 and PhaHV-2 (as determined from post mortem sampling of spleen) was significantly correlated with presence of both virus subtypes in oral swabs.

Research is underway to investigate prevalence of PhaHV-1 and -2 in koala populations in Qld and NSW [13].

Mode of transmission

The means of transmission of herpesviruses in koalas has not been extensively studied, and what is known is mostly extrapolated from studies of related viruses in humans and other animal species. Gammaherpesviruses may be shed from various sites in the body during their infective lytic stage, which affects the possible means of transmission; for example, the viral load of ovine herpesvirus-2 is high in the lung and the vesicular gland, suggesting that both respiratory and venereal transmission might be possible [14].

The fact that PhaHV-1 prevalence increases with age while PhaHV-2 prevalence does not appear to do so, may suggest PhaHV-1 is more likely to be acquired through activity related to maturation, such as sexual contact or aggressive behaviour associated with breeding, whereas PhaHV-2 may be more likely to be acquired in infancy, through close contact with the mother while in the pouch [10].

Incubation period

There is no information available on the incubation period of PhaHV. Because initial infection with gammaherpesviruses can be asymptomatic, it is difficult to determine incubation period. The permanency of gammaherpesvirus infection, and the latency and reactivation behaviour of these viruses also means that incubation period is not likely to be of epidemiological relevance.

Persistence of agent

The persistence and stability of PhaHV in the environment has not been studied. Herpesviruses in general are fragile in the environment and have known sensitivity to several different chemical disinfectants, solvents and detergents [3].

The persistence of herpesviruses as a latent infection within the host is discussed in *Pathogenesis*.

5.9.3 Pathogenesis

Knowledge of the pathogenesis of PhaHV-1 and -2 is based on extrapolations from other herpesviruses. Features of pathogenesis common to all herpesviruses include destruction of infected cells during lytic infection; the occurrence of virus transcription, DNA synthesis and

nucleocapsid assembly in the nucleus of infected cells; and the reversion to latency within host cells, from which reactivation to the lytic stage can occur [12].

Gammaherpesviruses are lymphotropic by nature, infecting epithelial cells initially, then typically establishing latency within lymphocytes in the host spleen and lymph nodes [5, 12]. During latency, only a small subset of the herpesvirus genome is expressed, and the virus lays dormant. The virus prevents the death of the infected cell via the translation of effector proteins which interfere with natural cell pathways, thus evading the host's immune system. Reactivation from the latent state to the replicating, lytic state is typically associated with host immunosuppression, external or environmental stressors, or concurrent infection [14]. Reactivation is followed by rapid virus replication, lysis of the host cell, and shedding of active virus [12].

The viral and cellular mechanisms controlling latency and reactivation by gammaherpesviruses are not well understood, but it is thought that latency represents a “default” mechanism where failure of immediate gene expression leads to maintenance of the genome in a circular configuration [12].

It is likely that the typical cellular behaviour of gammaherpesviruses also occurs with PhaHV-1 and PhaHV-2, although the details have yet to be elucidated. Sites of latency and shedding for PhaHV-1 and -2 have not been confirmed [10]. In the SA study of euthanased koalas, all koalas which tested positive on oropharyngeal swab also had positive tests from splenic swabs, which was postulated to suggest that PhaHV forms latent infections in the spleen [11]. However, if lymphocytes are sites of latency for PhaHV, this finding might also reflect the high concentration of these cells within the spleen, rather than the spleen being a specific organ for viral latency [12].

The detection of both PhaHV-1 and -2 in oropharyngeal swabs suggests that virions may be shed from epithelial cells in koalas, but it is also possible that detection of PhaHV-1 and -2 in swabs of rostral and caudal epithelial sites may represent detection of latent virus at those sites. Severe lymphoid depletion in lymph nodes and spleen was reported in the index cases of PhaHV-1 and PhaHV-2 infection but this may have been related to other disease [1, 2].

In humans, gammaherpesviruses include the lymphotropic tumour viruses Epstein-Barr virus and Kaposi's sarcoma-associated herpesvirus, both important causes of lymphomas and other neoplasms in immunosuppressed populations, particularly in association with the lentivirus human immunodeficiency virus [15]. Given the high incidence of lymphomas and other neoplasia in koalas and the likely role of KoRV in increasing neoplasia risk (see *Appendix 5.6 Neoplasia in Koalas – Literature Review*), this association merits further exploration.

5.9.4 Associations with other disease hazards of koalas

The presence of both PhaHV-1 and PhaHV-2 has been strongly associated with concurrent infection with *Chlamydia pecorum* in koalas [9, 10], although in one SA study this

relationship was only identified in females [16]. The nature of the association between *Chlamydia* and herpesvirus infection in koalas remains unclear but possibilities include reactivation of latent PhaHV infection secondary to the immunological challenge of concurrent *Chlamydia* infection (or vice versa); concomitant transmission of both pathogens; increased susceptibility to PhaHV entry in *Chlamydia*-infected cells; or PhaHV-induced persistence of *Chlamydia* [9, 10]. *In vitro* studies of *C. trachomatis* and human herpesviruses have demonstrated synergistic effects of co-infection for pathogenicity of both agents. Herpesvirus can induce persistence of chlamydial infection within cells, while the entry of herpesvirus into cells is facilitated if cells are infected with *Chlamydia* [17, 18].

The link between PhaHV and KoRV status in koalas is unclear and associations have not been extensively studied. KoRV-positive female koalas in Vic were twice as likely to be positive for PhaHV-1 (but not PhaHV-2) than those where KoRV was not detected [10]. There was no identified relationship with KoRV status in males. Interactions between PhaHV-1 and KoRV could result in an increased risk of opportunistic infections causing disease [10].

In other species, including birds [19] and humans [20], retroviral oncogenesis is augmented in the presence of gammaherpesvirus infection. An association has been identified between the incidence of neoplasia and co-infection with KoRV and PhaHV-2 [16], although the data are insufficient to draw any conclusions regarding viral cooperation in oncogenesis.

Molecular testing of 137 koalas from the Mt Lofty Ranges in SA found co-infection with KoRV, *Chlamydia* and PhaHV in 16.1% of individuals, but the only significant co-infection association was between *C. pecorum* and PhaHV-1 [16].

5.9.5 Diagnosis

Clinical signs

There are no clinical signs that are pathognomonic (uniquely diagnostic) for PhaHV infection. However, associations have been described between PhaHV infection and certain clinical presentations.

Associations between herpesvirus infection and general markers of ill health, have been identified in a study of koalas (n=87) euthanased on welfare grounds in SA [11]. In this study, increased tooth wear was positively and significantly correlated with PhaHV-1 detection. In addition, koalas positive for PhaHV-2 (but not PhaHV-1) were 3.5 times more likely to have a low body condition score than those which were not positive for the virus. These findings may suggest a role for herpesvirus in contributing to overall debilitation in koalas or may be reflective of increased shedding of virus due to compromise in an animal's health status. The possibility of reactivation and subsequent detection of herpesvirus secondary to other disease processes, or due to general poor health of the host, should be considered when interpreting PhaHV results in koalas [10].

Clinical disease of the urogenital tract has been associated with herpesvirus infection. "Wet bottom", a clinical syndrome often attributed to chlamydiosis in koalas [21-23], was

identified as a significant risk factor for concurrent infection with both PhaHV-1 and -2 in koalas in Vic populations [10]. The presence of both PhaHV-1 and -2 are significantly associated with urinary incontinence and genital tract abnormalities (uterine and ovarian cysts; testicular malformation) in female and male koalas, as well as lowered fertility in females [10, 24]. The association with reproductive abnormalities was stronger with PhaHV-2, whereas the association with wet bottom was stronger for PhaHV-1 [10]. Because of the high incidence of co-infection between the herpesviruses and *Chlamydia*, it is difficult to separate the clinical variables associated with each individual agent. However, given that wet bottom is not pathognomonic for chlamydial infection (see *Appendix 5.1 Chlamydia spp. in Koalas - Literature Review*), the role of herpesviruses in this syndrome warrants further investigation.

A retrospective study of koala post mortem cases in SA found an association between reproductive disease and PhaHV infections. Of koalas with paraovarian cysts, 81% were infected with either PhaHV-1 or PhaHV-2, and *C. pecorum* was only ever identified in co-infection with PhaHV in koalas with paraovarian cysts, never as the sole infectious agent present [16]. This may suggest a role for PhaHV in exacerbating reproductive disease in koalas.

Conjunctivitis was associated with PhaHV-1 detection, and not with *Chlamydia* detection, in a study of SA koalas [16]. This may suggest a possible role for herpesvirus as a differential diagnosis for ocular disease in cases where *Chlamydia* is not detected.

The three koalas in which PhaHV-1 and -2 were first identified demonstrated clinical signs associated with various comorbidities, including dermatitis associated with sarcoptic mange, and chronic interstitial nephritis, cystitis and conjunctivitis consistent with chlamydiosis (although the *Chlamydia* status of these individuals was not reported). No signs were common to all three animals [2].

Clinical pathology

No clinical pathology signs have been typically associated with PhaHV-1 and -2 infection, although systematic studies are lacking.

Pathology

There has been no systematic study of pathology in koalas infected with PhaHV-1 and -2, with studies to date identifying correlations but not causations. Although intranuclear inclusion bodies have been identified on histopathology for other marsupial herpesviruses and for PhaHV-3 [4, 5], they have not been reported for PhaHV-1 and -2.

The three koalas in which PhaHV-1 and -2 were first identified demonstrated marked to severe lymphoid depletion in the spleen and various lymph nodes, consistent with chronic stress or chronic infectious disease [1, 2]. However, in the absence of detailed information on disease screening for other infectious aetiologies (particularly *Chlamydia*), it is not

possible to determine the extent to which this pathology was attributable to PhaHV infection.

Differential diagnosis

In the absence of a definitive clinical picture, there is no clear differential diagnosis for PhaHV-1 and -2 infection.

Diagnostic testing

Phascolarctid herpesvirus DNA has been detected from conjunctival, nasal, oropharyngeal, cloacal and prepuce swabs, and from the liver and spleen of koalas, using PCR techniques [1, 2, 4, 5, 9-11, 25]. Current molecular methods used to detect PhaHV-1 and -2 are sensitive but labour intensive. There is no commercially-available diagnostic molecular test for PhaHV-1 and -2 that would enable diagnosis in a clinical setting.

In Vic populations, PhaHV-1 and 2 were twice as likely to be detected by PCR from urogenital/cloacal swabs, rather than swabs collected from oral/nasal/conjunctival sites [10]. In SA populations, the oropharyngeal site was most likely to detect PhaHV in live animals, and the spleen was the most likely site of detection in necropsied koalas [11]. This may reflect the high concentration of lymphocytes within the spleen rather than the role of the spleen as a site of latency [26].

Koalas that test positive on PCR should be assumed to have lifelong infections, given the latency potential of herpesviruses [5]. Currently, latent and lytic infection cannot be differentiated by PCR test.

Tests to detect serum antibodies against PhaHV are not currently available. Serological tests would be a valuable means to detect herpesvirus exposure in animals in which PCR tests are negative at the time of sampling.

Surveillance and monitoring

There is no targeted surveillance or monitoring program in place for PhaHV in koalas. However, there is capacity to utilise the Wildlife Health Australia national wildlife health information system database (eWHIS) as a place for entering data as part of national general wildlife surveillance activities.

5.9.6 Treatment

There is no effective treatment for marsupial herpesvirus infections and there are no reports of the use of antiviral drugs for treating clinical cases in koalas. Further *in vivo* studies are required before recommending the clinical use of antiviral drugs in herpesvirus disease in koalas [5].

Treatment of herpesvirus infections in koalas is likely to include supportive care such as good nutrition, symptomatic treatment for clinical illness and management of concurrent diseases. Given the role of immunosuppression and stress in the pathogenesis of

gammaherpesviruses, reducing sources of stress and demands on the koala's immune system is also a priority [5].

5.9.7 Prevention and control

There are few prevention or control methods identified for PhaHV. PhaHV-1 and -2 have been detected in all free-ranging and captive populations of koalas tested to date, at high rates of prevalence [10], and it is highly likely that they have co-evolved with their host [5]. Therefore, the options for preventing exposure to the virus in wild koalas are likely to be limited. Testing koalas in rehabilitation for herpesvirus, and managing individuals while in care based on their herpesvirus status has been proposed as a possible risk mitigation option. Koalas undergoing rehabilitation are likely to be physiologically stressed, and this may increase their likelihood of shedding virus if latently infected, or increase the rate of viral expression [14], thereby increasing herpesvirus transmission risk.

Management of concurrent infections and minimising external environmental stressors are likely to be very important to control of herpesvirus-associated disease [5].

5.9.8 References

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5.10 Predator Attack Trauma in Koalas – Literature Review

5.10.1 Technical information

Description of hazard

This hazard refers to trauma and trauma-related mortality which occurs in koalas as a result of being attacked by a predator species. Predators may be carnivores (e.g. dogs, cats and foxes) or non-carnivore species (e.g. pythons and raptors).

In the context of this chapter and unless otherwise specified, the term “wild dogs” refers to dingoes, dingo-dog crosses and domestic dogs that are no longer living in a domesticated environment (as opposed to stray or unsecured pets).

Other species, including deer, cattle and horses, are implicated in attacks on koalas which can result in significant trauma [1-3]. These attacks are not considered predatory in nature and are not addressed further in this chapter.

5.10.2 Epidemiology

Causes of hazard

Domestic and wild dogs are both responsible for predator attacks on free-living koalas [4-7]. In protected forests or bushland habitat, wild carnivores (mostly wild dogs but also foxes) are primarily thought responsible for predator attacks on koalas [4, 7-12]. Feral and domestic cats are considered capable of attacking and killing an unattended koala joey [13]. A number of non-carnivore native species are also known to be predators of koalas, including carpet pythons, monitors and large raptors such as eagles and owls [9, 14, 15].

Koalas are at greatest risk of predator attacks when moving across open ground [6, 9], although non-carnivore predators such as raptors and carpet pythons may also attack juvenile koalas in the canopy during daylight hours [9, 14]. Factors which increase the likelihood of koalas going to ground will also increase their vulnerability to predation. Such factors include habitat loss and habitat fragmentation, illness, dispersal and breeding activity [4, 5, 16-18]. Figure 27 shows a wild koala crossing open ground, and vulnerable to predator attack.



Figure 27 A wild koala moving across open country is vulnerable to predation (credit: Tamsyn Stephenson)

Predator presence, abundance and behaviour may affect the likelihood of attacks on koalas. Koalas are more likely to encounter domestic pets, particularly dogs, in areas of higher dwelling density, where increased habitat fragmentation combines with higher numbers of predators [19, 20]. Both wild dogs and pythons may be present in degraded, rural and urbanised habitats, as well as being the predominant predators in more intact landscapes [9, 12]. Carnivore predators can readily attack and kill adult koalas, whereas non-carnivore predators disproportionately (but not exclusively) prey on juvenile koalas [9, 14].

Not all dogs will attack koalas. A Qld survey showed that domestic dogs over 10 kg in size were responsible for the vast majority (96%) of attacks, and attacks were more common if more than one dog was present [21]. Some breeds of dog are over-represented in data as the cause of koala attacks [13], but it is not clear whether this reflects breed propensity to attack koalas or other correlative factors such as breed preference of owners in certain areas. Individual wild dogs may be more inclined to target koalas, with a single individual thought to be responsible for as many as 75 fatalities in one study [12].

The impact of predation on koala populations is difficult to determine because there is limited data on predator attacks and carcasses are rarely discovered [6, 9, 11, 12]. Owners may also avoid reporting predation events if their dogs are involved [3]. Modelling suggests that predation by dogs may lead to koala population declines, particularly in fragmented landscapes [5, 16, 22, 23]. Predation by non-carnivore species is often difficult to quantify but may have significant impact in some populations, as has been demonstrated in the

eastern Moreton Bay region of Qld, where carpet pythons accounted for 7.2% of all koala mortalities, and 14% of confirmed predator attacks on koalas [9, 12].

Geographic distribution

Trauma due to predation occurs throughout the koala's distribution [4, 6, 24, 25].

Prevalence

There are few available prevalence figures for predation in koalas. Predation is very difficult to quantify, and may be an under-recognised threat, because koala population field monitoring rarely finds koalas immediately after death, many researchers are not trained to recognise the signs of predator attack and carcasses are rarely discovered by the public [3, 12, 26]. A small number of longitudinal studies of free-living koalas have identified predation as the predominant cause of mortality in the populations under investigation. One study of approximately 500 koalas (mostly fitted with tracking devices) over four years in the eastern Moreton Bay region of Qld found predation as the cause of 49.5% of all mortalities [12]. The majority (64.3%) of predator attacks were attributed to wild dogs, followed by carpet pythons (11.5%) and domestic dogs (3.3%). The remaining 20.9% of predation mortalities were presumptively attributed to wild dogs. In a study of 40 koalas south-east of Brisbane, dog attacks accounted for 30% of mortalities over four years of monitoring [27]. In the Port Stephens population of NSW, dog attacks were identified as the cause in 43% of mortalities (n=23) in a cohort of 50 radio-tracked animals [5].

Admission and post-mortem records from wildlife hospitals and rehabilitation facilities give an indication of the impact of predator trauma on koalas (Table 21). Data rarely distinguish between different predator species [6]; presumably in many cases the term “dog attack” is used to refer to any case suspected to be caused by a carnivore predator.

Table 21 Predator attacks as a percentage of koala rehabilitation facility and hospital admissions

* also includes livestock attacks

State	Overall number of cases	Number (percentage) of cases attributed to predation	Reference
Qld	519	40 (7.7%)*	Gonzalez-Astudillo et al. 2019 [4]
Qld	3590	495 (13.8%)	Taylor-Brown et al. 2019 [28]
Qld	10139	775 (7.6%)	Burton and Tribe 2016 [29]
Qld	8503	765 (9%)	Schlagloth et al. 2021 [6]
NSW	5051	398 (7.9%)*	Lunney et al. 2022 [30]
NSW	12543	534 (4.3%)	Charalambous and Narayan 2020 [24]
NSW	3781	749 (19.8%)	Griffith et al. 2013 [17]
NSW	374	17 (4.5%)	Hopkins and Phillips 2012 [20]
NSW	127	9 (7.1%)	Canfield et al. 1987 [31]
Vic	55	0 (0%)	Obendorf 1983 [32]
Vic	2584	466 (18%)	Schlagloth et al. 2021 [6]
SA	85	11 (12.9%)	Speight et al. 2018 [25]
SA	240	25 (10.4%)	Stephenson 2021 [33]

In koala hospital and rehabilitation admissions, mortality rate for predator trauma is high. In retrospective studies of admission data, mortality rate from dog attacks for koalas under treatment ranges from 52-69% [6, 28, 30]. Mortality rates for predation by species other than dogs are not widely reported. Predator attacks accounted for 10-29% of mortalities in hospitals in one study [6].

There are seasonal trends in admission data for koala predator attacks, with more admissions and fatalities occurring in spring and summer, coinciding with the peak mobility of breeding males and dispersing juveniles [6, 17, 30, 34, 35]. Male koalas were more commonly admitted as a result of predator attack than females in one study [30], but in most studies no sex or age trends are reported.

The prevalence of non-carnivore predation in koalas has not been extensively studied. In a review of NSW rehabilitation admissions, 2% of all cases were animal attacks which were not clearly identified as dog-related, and included attacks by monitor lizards [30]. In a population north of Brisbane in Qld, carpet python predation was an important cause of death, particularly for near-independent and back-rider offspring. Predation was highly seasonal, with 71% of deaths occurring in the summer months (December to February), coinciding with the peak activity of pythons. Python predation occurred on the ground or in the canopy, and was only recorded on days with maximum daytime temperature exceeding 24°C [9].

An increase in time that koalas spend on the ground, due to debilitation, may be a factor in increasing risk of predation. In one study, 57.5% of koalas which died due to predation were classified as “unhealthy” based on post mortem findings [4]. Koalas suffering animal attacks are also more likely to be in poor body condition compared with those involved in MVA trauma [4, 10].

5.10.3 Pathogenesis

Predator attacks result in trauma to koalas from biting, crushing, clawing and shaking [4, 25, 26]. Koalas attacked in the canopy may also suffer impact injuries in falling from trees [9]. Injuries may be immediately fatal, or may cause damage to bones, muscles and internal organs (see *Clinical signs*).

Surveys of dog owners in south-east Qld indicate that most domestic dog attacks on koalas occur within the dog’s own yard when koalas enter private property [21].

5.10.4 Association with other disease hazards of koalas

No associations between specific infectious disease hazards and predator trauma have been identified, although debilitation and poor health resulting from infectious disease may be a factor in koalas spending time on the ground, thus putting them at increased predation risk [4, 10].

Treatment of koalas with predator injury may require prolonged hospitalisation and rehabilitation, and this may be associated with the development of other diseases related to long-term medical management such as gut dysbiosis, candidiasis and secondary wound infection [26].

5.10.5 Diagnosis

Clinical signs

Koalas attacked by predators have clinical signs consistent with trauma. However, these signs can be subtle and easily missed by untrained observers. External signs may be confined to subtle abrasions, minor punctures or slicking of fur with saliva; internal damage may be severe and extensive in spite of the external appearance [26]. Figure 28 shows a post mortem examination of a wild koala killed by dog attack.



Figure 28 A post mortem examination of a wild koala killed by dog attack trauma, with extensive bruising, haemorrhage and deep punctures (credit: Veterinary Diagnostics Lab, Roseworthy Campus, University of Adelaide)

Injuries from carnivore attacks include canine tooth puncture wounds in the skin with subcutaneous soft tissue and organ damage, fractures and haemorrhage [4, 25]. Blood or saliva may be visible on the fur [25, 26].

Fractures are a common result of dog attacks. Analysis of data from 185 dog attack cases in wild koalas at a Qld rehabilitation facility found that fractures from dog attacks were more likely to involve the ribs and torso. This may be a result of the canine predisposition to maul these areas or the relative fragility of the ribs [35]. Skull and upper forelimb fractures are also commonly reported in rehabilitation admissions, perhaps because dogs tend to grab the head or the limbs if a koala endeavours to escape [3, 13].

It can be difficult to distinguish predation by different species on the basis of clinical signs. Hallmarks of python predation (not present in all cases) include a U-shaped primary bite site, and slicking of the fur around the koala's face with python saliva [9]. Due to the koala's thick fur, the fine puncture marks resulting from a python bite can often be difficult to detect unless the body is thoroughly shaved. There are no reports of koala injuries or pathologies which distinguish raptor predation although raptors have been reported to pluck joeys from their mother's back [13], so talon punctures on the dorsal aspect might be expected. Koalas suffering raptor attacks have talon marks on the face, belly and thorax, which are generally larger than the puncture marks associated with cat bite wounds [3, 36].

Pathology

Dog bites cause crush injuries that can result in fractured bones, significant internal haemorrhage and organ rupture. The most common region of injury is the abdomen, followed by chest, head and peripheral limbs. Haemabdomen is often present in abdominal injuries, and injuries to the chest include haemothorax and rib fractures. Head injuries may include skull fractures, hyphaema and ocular prolapse. In many cases, bites also cause bruising and haemorrhages over the surfaces of abdominal organs [1, 26, 35]. Full thickness puncture of the caecum has also been noted by clinicians, and may be present in the absence of obvious skin trauma [13, 37]. Damage to the caecum can also lead to caecal necrosis and peritonitis [38].

The constrictive attack methods of python predation may cause pulmonary congestion [9].

Differential diagnosis

Visible bite wounds, talon puncture wounds and saliva on the fur can distinguish predator attack from other causes of trauma such as MVA, attacks by other animals, and falls from trees, all of which may also cause wounds, haemorrhage, bruising and fractures [2, 4, 6, 9, 25].

Diagnostic testing

Diagnostic testing for predator trauma cases is similar to vehicle trauma, see *Appendix 5.5 Motor Vehicle Trauma – Literature Review*.

Surveillance and monitoring

There is no targeted national surveillance or monitoring program in place for predator trauma, or trauma of any kind in koalas. However, rehabilitation facilities and veterinary hospitals maintain records of trauma admissions and post mortem examinations, enabling retrospective studies [4, 6, 17, 25, 33].

5.10.6 Treatment

Treatment of predator trauma in koalas is symptomatic and may include fluid therapy, analgesia, wound management and surgical repair. Reducing stress and providing excellent nutrition are critical to positive outcomes. Systemic antibiotics are generally not required except for severe dog bites or where abdominal trauma and septic peritonitis have been identified. Given the risks of gut dysbiosis and candidiasis, antibiotics should be avoided in koalas unless there is a clear requirement for their use [26, 39]. Serial (every 3 day) abdominocentesis can be used to assess for abdominal haemorrhage or peritonitis [38].

5.10.7 Prevention and control

Viable, connected habitat is critical to allow koalas to minimise their time on the ground while travelling, thus reducing the risk of predator attacks [16, 17, 40, 41]. Even small

numbers of non-endemic trees in urban habitat, such as back yards, allow koalas to shelter from dogs and other predators [18].

Prevention of domestic dog attacks on koalas focuses on strategies to lower the likelihood of dog and koala interactions. As most domestic dog attacks on koalas occur in the dog's own yard [21], education and community engagement programs may increase dog owner awareness and provide information on strategies to prevent back yard attacks. Strategies include restricting dog movements to private land during dawn and dusk [4], securing a climbing pole against fences to enable koalas to escape, and fencing yards to prevent koala access [21, 42]. Other aspects of responsible pet ownership include encouraging the selection of smaller breeds of dogs which are less likely to attack koalas, and encouraging obedience and wildlife aversion training for dogs [21, 42]. Covenant restrictions on dog breed size by councils and land managers may help in reducing dog attacks on koalas [3]. The success of community engagement and education in reducing the incidence of dog attacks on koalas is not well studied, but behavioural changes and avoidance training of dogs require significant commitment and engagement of owners to the process [13, 42].

Reducing the number of free-ranging predators, particularly wild dogs, has been successful in reducing koala mortality in certain situations [12]. However, some studies suggest that identifying and removing individuals that are responsible for predation events may be more effective and ethical than reducing overall predator abundance [7, 12]. The fact that dingoes are a threatened species in some jurisdictions may be a complicating consideration, particularly because recent genetic testing suggests that the majority of 'wild dogs' have a high level of dingo ancestry [43]. Dingoes are considered to fill the ecological niche of the terrestrial apex predator in mainland Australia, and may mitigate the predatory impacts of mesopredators such as cats and foxes [44], so their reduction in the wild may have unintended negative ecological consequences.

Prevention or control of attacks on koalas by native predators such as pythons and raptors has generally not been recommended, as they are considered a natural predator in the ecosystem.

5.10.8 References

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5.11 Sarcoptic Mange in Koalas – Literature Review

5.11.1 Technical information

Aetiological agent

Sarcoptic mange is a highly contagious skin infection caused by the parasitic mite, *Sarcoptes scabiei* [1].

Listing

Sarcoptic mange is not a WOAHA listed disease [2].

Sarcoptic mange is not a notifiable animal disease in Australia [3].

Sarcoptic mange is not identified as a key threatening process under the Environment Protection and Biodiversity Act [4].

5.11.2 Epidemiology

Sarcoptic mange can have dramatic short-term effects on wildlife populations, with epidemics associated with very high morbidity and mortality [5]. In many populations, epidemics are self-limiting and population recovery occurs, but long-term effects may be much more serious in fragmented and isolated populations, leading to local extinctions [5, 6]. Sarcoptic mange is a disease of emerging significance in koalas, and its effect on koala population viability is currently unknown.

Host range

Sarcoptes scabiei has been documented to infect a broad host range of approximately 150 mammal species globally, including humans [7, 8]. Sarcoptic mange is considered to be an emerging infectious disease in Australia, based on its continued spread into new hosts [7, 9, 10]. It has been documented in many Australian native mammal species, including bare-nosed wombats, southern hairy-nosed wombats, koalas, bandicoots, dingoes, possums, potoroos and wallabies [1]. Mange is recorded in non-endemic mammalian species in Australia and non-endemic canids (wild dogs, domestic dogs and foxes) are likely to be significant in the origins and epidemiology of mange in Australian wildlife [7].

Historically, *S. scabiei* was thought to have a number of relatively host-specific varieties [5]. However, the taxonomy of sub-species varieties is no longer used and *S. scabiei* is now considered a single mite species which can develop local adaptations to enable sustained transmission in new host species [7, 11].

Zoonotic potential

Disease in humans caused by *Sarcoptes scabiei* is known as ‘scabies’, and is acquired from other infected humans or animals [12]. Humans have acquired scabies from infected Australian wildlife [13], and zoonotic transmission from koalas has been anecdotally reported [14].

Geographic distribution

Sarcoptes scabiei is believed to be widely distributed throughout Australia and was probably introduced via European settlers and their domesticated animals [7, 15]. It is primarily a public health and domestic dog issue in northern Australia [16, 17] and a wildlife and feral animal disease in southern areas of Australia. While there is little detailed information on the geographic distribution of *S. scabiei* across the country [1], it seems likely the mite is present in most locations where koalas populations are present, given that sarcoptic mange has been reported in bare-nosed wombats in NSW, Vic, SA and the south-eastern regions of Qld [18] and reports of mange outbreaks in wild canids occur sporadically throughout Australia [15, 18-21].

Sarcoptic mange as a disease of wildlife is most commonly reported in the southern regions of Australia [7, 19]. This also appears to be the case in koalas, with confirmed cases reported sporadically in Vic and SA [22-25], but rarely in NSW and Qld [26-30]. Victorian cases are widely distributed, with reports in koalas from northern, western, eastern and central Vic populations [22]. South Australian cases are less widespread, with all reports occurring within a 12 km radius of the index case [22].

Prevalence

There is little information on the prevalence of sarcoptic mange in koalas. Written reports of mange in koalas date back almost 50 years, but until recently, disease appeared to be restricted to isolated cases, usually occurring in koalas in rehabilitation or captivity [14, 24, 31]. Since 2011, localised outbreaks of sarcoptic mange in wild koalas have been reported from various sites in the north, west, east and central regions of Vic [22] and around the Mt Lofty Ranges (MLR) in SA [22, 23]. Prevalence of sarcoptic mange in the 2012-13 outbreak in MLR was 8% [23]. A recent post mortem study of 240 koalas from MLR identified sarcoptic mange in 4.2% of cases [32]. In a retrospective post mortem study from the mid-1970s in Vic, prevalence of sarcoptic mange was 3.6% [24].

Retrospective studies of rehabilitation facility admissions and post mortem records in NSW and south east Qld, spanning 17-30 years, did not identify sarcoptic mange or skin disease as a reason for admission, suggesting a low prevalence in these areas [26-29]. Sarcoptic mange was not reported as a cause of disease in a 2014 survey of 16 captive koala facilities across six Australian states [33].

In koalas, most reported cases occur in adults, although cases in juveniles [14] and subadults [22] have been reported. Male koalas appear over-represented and it has been suggested that they are more likely to become infested due to roaming and fighting behaviour [22]. In contrast to sarcoptic mange in wombats, mange in koalas appears least likely to occur in winter [22], but comprehensive epidemiological studies are lacking.

Mode of transmission

Transmission of *S. scabiei* mites occurs via contact, which may occur directly between hosts, indirectly via contaminated environments, or by both means [12, 15, 34]. The predominant means of transmission varies among host species, with direct transmission more likely to be important in social species with a high host density (e.g. herd species), and indirect transmission through shared environments more important in species with more solitary habits (e.g. bears, wombats) [34].

The source of the *S. scabiei* mite in koala cases has not been confirmed, and it is unclear whether sustained koala-to-koala transmission occurs, or if koala cases represent repeated transmission from other species or the environment [14, 35]. There is a high genetic similarity between mites infecting koalas and those infecting wombats, suggesting that transmission between these species may occur [15]. Genomic sequences detected in koalas in Vic and SA have also been identified in foxes in Vic and dogs in NT, supporting the hypothesis that canids may be an important reservoir for marsupial *S. scabiei* infestation [19]. Outbreaks in Vic koalas coincided with the observation of several foxes with clinical signs of advanced mange, possibly suggesting spillover from foxes [22]. The possibility of mite transmission to koalas via tree surfaces has been suggested, based on the fact that the distribution of lesions corresponds with the areas of the koala's body which are in contact with trees [22], however there is no evidence to support this hypothesis at this stage.

Incubation period

There is no information on the incubation period for *S. scabiei* in koalas. Experimentally infected wombats showed clinical signs of infection by day 14 [12, 36].

Persistence of agent

Sarcoptes mites are able to survive off the host for up to 19 days in an optimised microclimate of high relative humidity (97%) and low temperatures (10-25°C) [37, 38].

5.11.3 Pathogenesis

The pathogenesis of sarcoptic mange has not been studied in koalas. The following information is based on studies in other species, including wombats.

Sarcoptes mites burrow into the skin and consume host cells and secretions, creating tunnels and depositing irritating and allergenic material including mite excretions, dead mites, moulted exoskeletons, and egg casings [5]. The acute signs of sarcoptic mange (skin inflammation and itching) are caused by hypersensitivity reactions to this allergenic material [5]. A type I hypersensitivity response (dominated by mast cells and eosinophils) is associated with relatively mild disease, whereas the most severe forms of mange are characterised by a type IV hypersensitivity reaction (dominated by lymphocytes) [34, 39]. Hair loss, erosions and crusting are a result of self-trauma caused by pruritus; as pruritus increases, the number and severity of secondary lesions also increases [5].

There is a marked variation in severity of sarcoptic mange between different host species. The factors which lead to this variability probably include previous exposure, differing environmental conditions and host co-morbidities [40]. It is not known whether koalas can mount an effective immune response to clear *S. scabiei* infection, rather than developing sarcoptic mange.

Mange-infected animals suffer a cascade of physiological and behavioural effects that increase the pathogenic impacts of infestation with *S. scabiei* [41]. Individuals with mange lose more heat to the environment than healthy animals due to hair loss [41-43], and have higher metabolic rates due to inefficient thermoregulation and the inflammatory response [41]. They may also be less capable of meeting increased metabolic demand because foraging activities are less effective than in healthy animals [41, 43-45], They are also less able to rest, due to interruptions from mite-associated irritation [41, 45, 46].

Sarcoptic mange may also alter nutritional balance in detrimental ways. The fatty acid composition of mange-affected bare-nosed wombats has been shown to shift toward chronic inflammatory (omega 6) rather than anti-inflammatory (omega 3) fatty acids, which may promote disease progression and accelerate host mortality [41].

5.11.4 Associations with other disease hazards of koalas

No disease hazard associations have been postulated for koalas with mange. In wombats, sarcoptic mange appears to be more common in animals with co-morbidities, and it is possible that mange contributes to the development of co-morbidities in the host [1]. A link between new or reactivated herpesvirus infections and debilitation caused by sarcoptic mange has also been suggested in wombats [47]. The role of debilitation due to sarcoptic mange in exacerbating or activating other pathogenic processes has not been investigated in koalas.

Given the importance of immune response in the pathogenesis of sarcoptic mange in other species, co-infection with pathogens which might affect host immune function (e.g. KoRV) might have an impact on disease expression of *S. scabiei* infection.

5.11.5 Diagnosis

Clinical signs

Most reports of sarcoptic mange in koalas describe animals with severe clinical signs that are very debilitated by the time they are rescued [1, 48]. At that stage, disease is characterised by dry, pruritic, encrusted lesions which may occur over the entire body, but commonly affect the face and distal fore- and hind-limbs, particularly the interdigital areas [22]. Exudative fissures are commonly seen on digits and paws and may ooze serosanguinous fluid [22]. Enlarged lymph nodes in affected body regions were noted in a number of koalas [22]. Many affected animals are found dead, presumably due to the septicaemic effects of secondary bacterial infection of affected skin [22].

In other species, pathological manifestations of sarcoptic mange range from mild to severe depending on chronicity of infection; on first principles it is likely that the same occurs in koalas. Early signs of sarcoptic mange in one captive koala colony were reported as small, dry, raised, circumscribed lesions beneath the fur [31]. Figure 29 shows a wild koala with severe sarcoptic mange on the abdomen.



Figure 29 A wild koala with sarcoptic mange on the abdomen (credit: Pam Whiteley)

Clinical pathology

No specific clinicopathological changes are reported for sarcoptic mange in koalas. Wombats infected with mange demonstrate significant changes in haematology and serum biochemistry, including lower red cell parameters (haematocrit, mean cell volume, mean cell haemoglobin and total haemoglobin), creatinine and albumin, and elevations in white blood cell counts (particularly neutrophils and lymphocytes), globulins, liver enzymes and creatine kinase [49, 50]. Changes are consistent with anaemia, inflammation and starvation [49].

Pathology

Sarcoptes mites cause classic thickening and scale production in the skin of affected koalas associated with a hyperplastic and hyperkeratotic dermatitis [22]. Microscopic lesions are characterised by acanthosis, orthokeratotic and segmentally parakeratotic hyperkeratosis, and numerous intracorneal mites [22]. Chronic cases with secondary bacterial infection demonstrate intracorneal pustules and mixed perivascular and interstitial dermatitis [22].

Differential diagnosis

Chronic dermatophyte infection ('ringworm') may also present with dermatitis and skin thickening, although it is not generally pruritic in koalas [51]. Ringworm is distinguished from sarcoptic mange by the presence of fungal hyphae within hair follicles on skin biopsy, and the absence of mange mites on skin scraping.

Benign disorders of keratinization such as volar hyperkeratosis may cause crusting and skin thickening in koalas, but these conditions are not pruritic and are distinguishable from sarcoptic mange due to absence of mites and characteristic histopathology [52]. Mild and moderate clinical effects of sarcoptic mange in koalas are not described, but based on the manifestations in wombats, differential diagnoses for mildly affected animals could include trauma, or infections due to bacteria, fungi or other ectoparasites [53, 54].

Diagnostic testing

Classical diagnostic testing for sarcoptic mange involves skin scraping the epidermis for visualisation of the mite or its eggs by microscopy. In studies of wombats, the sensitivity of this method was variable, especially in the early stages of disease, when false negative diagnosis was commonly encountered [55]. PCR detection was more sensitive than microscopy in detection of mites in skin scrapings, and may also be of potential value for detecting mites in less invasive samples such as skin swabs [55]. The use of PCR techniques in the diagnosis of sarcoptic mange in koalas has not been investigated. Clinical cases in koalas are commonly at an advanced stage at the time of diagnosis. Microscopy of superficial skin scrapings generally identifies numerous mites [22].

Surveillance and monitoring

There is no formal surveillance or monitoring program for sarcoptic mange in koalas. However, there is capacity to utilise the Wildlife Health Australia national wildlife health information system database (eWHIS) as a place for entering cases of sarcoptic mange in koalas as part of national general wildlife surveillance activities.

5.11.6 Treatment

There is no accepted global standard treatment regimen for mange in wildlife, and there is species-specific variation as to what constitutes best-practice treatment in free-ranging wildlife [1].

The study of the treatment of mange in Australian wildlife is most advanced in wombats, as sarcoptic mange is the most frequently observed debilitating disease condition in bare-nosed wombats and also impacts southern hairy-nosed wombats [1]. However, extrapolation of treatment protocols to koalas should be undertaken with caution, as koalas have demonstrated idiosyncratic pharmacokinetics for a number of drug formulations [56-58].

General principles of treatment

In making decisions on treatment of sarcoptic mange in wildlife, general recommendations are as follows [53, 59]:

Confirmation of the diagnosis should be undertaken where possible prior to treatment, to rule out differential diagnoses, especially for mild or moderate skin disease.

Pre-treatment assessment should be carried out by a veterinarian with expertise in the assessment of sarcoptic mange. This should include evaluation of disease severity (preferably using a standardised approach as has been developed for wombats in some jurisdictions [60]), body condition, general health and presence of other disease. This evaluation ensures that treatment is only undertaken after assessing the costs and benefits for the individual from a welfare and prognosis perspective.

Suffering of affected animals should not be prolonged if the prognosis for recovery is poor. Skerratt et al (2021a) includes guidance on euthanasia criteria for mange-affected wombats, which could be modified for use in koalas.

Use of antiparasitic drugs

As is the case with other Australian wildlife species [1], the efficacy of many antiparasitic drug regimens in treating koalas with mange is anecdotal, with combinations of medications and variable dose regimens being used off-label with unknown animal health and welfare implications. Anecdotally, mortality of mange-affected koalas has been attributed to the use of certain anti-parasitological formulations [48, 61], but evidence-based investigation and clinical reporting is lacking. Antiparasitic medications which have been used in the treatment of sarcoptic mange in koalas include ivermectin (subcutaneous injection), topical selamectin (Revolution®), pour-on moxidectin (Cydectin®), topical fluralaner (Bravecto®) and topical benzyl benzoate [1, 48, 61].

Where humane and practical, the current recommended antiparasitic treatment options **for wombats** are:

- injectable ivermectin at recommended livestock doses given weekly over several months [35, 53].
- topical moxidectin or selamectin for repeated treatments (special permit required), particularly as a means of reducing handling when efficacy of injectable avermectins has been established [53, 62].
- topical fluralaner (special permit required) [63, 64].

Due to the need for multiple treatments and handling, injectable ivermectin is only considered feasible for wombats in captivity or in rehabilitation [53], and it is likely this would also be true for koalas. Oral ivermectin has been used safely in koalas at relatively high doses to treat demodectic mange [54], suggesting that it might also be appropriate to use for sarcoptic mange via the oral route.

Repeated applications of topical selamectin pour-on over prolonged periods has been used under special permit conditions for treatment of free-ranging wombats via medicated burrow flaps or a “pole and scoop” method of direct application to individual wombats [53]. Dose rates and regimens vary widely and the lack of controlled trials poses safety and efficacy risks to populations under treatment [65].

Tropical fluralaner has shown efficacy against mild to moderate mange in trials on bare-nosed wombats [63]. Anecdotally, this drug has also been used with success for treating mange in common brush-tailed possums [53]. Dose regimens remain experimental and use of this drug in koalas would only be possible under the direct supervision of a veterinarian, as the product is currently only approved for use in wombats under special permit conditions [35]. Pharmacokinetic studies of fluralaner in koalas are currently in progress [66].

Other antiparasitic treatments historically used to treat sarcoptic mange in koalas and other wildlife (such as malathion baths [14] and amitraz [31]) are considered obsolete.

Supportive therapy

Supportive treatment can greatly improve the success of sarcoptic mange treatment and the welfare of the individual. Supportive therapies which are recommended for wombats would also apply to koalas and may include pain relief, fluid therapy, antibiotics (if secondary infections are present), excellent nutrition and careful management of the thermal environment, as well as removal of crusts and organic debris from affected skin [53, 59]. Juvenile koalas may particularly benefit from supplementary feeding during treatment; iron and vitamin B supplementation have also been used [61].

Treatment of free-ranging koalas

Effective treatment of free-ranging individuals and populations for mange would benefit from good understanding of the parasite’s epidemiology, including transmission pathways and sources of infection [53]. This information is limited for koalas, and consequently individual and population-level treatment has not been attempted *in situ* in this species [1]. Treatment of entire populations of free-ranging mammals has been undertaken in several species worldwide, with varying success [43, 67-70]. In Australia, treatment of free-ranging wombat populations has been attempted using various antiparasitic medications and administration methods. The long-term feasibility and outcomes of treating wombats remains poorly understood. Considerations of feasibility, efficacy, ecologic impact, drug resistance and cost should all be part of the evaluation process in determining if and how

antiparasitic medications should be used for population level management of sarcoptic mange [71].

5.11.7 Prevention and control

It is presently challenging to eliminate or sustainably control sarcoptic mange in wildlife populations, and this challenge can be compounded when there is greater than one pathogen reservoir present (e.g. free-ranging canids and wombats)[1].

Biosecurity precautions may be an important consideration for preventing spread of mange within rehabilitation facilities [14].

Antiparasitic medications which have been used to treat sarcoptic mange in koalas include ivermectin, topical selamectin, pour-on moxidectin and topical fluralaner. There are no recommended treatment protocols for mange prevention or control in koalas, and all treatments used so far have been based on protocols developed for other host species.

Measures which reduce environmental stress to koalas, such as conserving habitat and minimising human disturbance, are likely to be of general benefit to reducing the impact of this disease, as appears to be the case for wombats [72].

Improving or at least maintaining genetic diversity in koala populations as a whole is likely to encourage the retention of the most robust koala genetic profiles for avoiding serious consequences of infection with *S. scabiei*.

5.11.8 References

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5.12 Thermal Burn Trauma in Koalas – Literature Review

5.12.1 Technical Information

Description of hazard

Koalas suffer burn injuries during bushfires, or subsequent to bushfires as they negotiate a burnt landscape. In the context of this chapter, the term “bushfire” includes wildfires which ignite spontaneously, as well as fires which are deliberately lit for vegetation control (“prescribed” burns) or with malicious intent.

The risk of thermal burns to koalas is not the same as the overall risk of fire to koalas. There are many additional implications of bushfire events to koala health, welfare and population viability that are only discussed in this chapter if they have clear implications for burn trauma, or have a flow-on effect to burn trauma.

Thermal burns sustained due to contact with other sources of heat are not considered in this review, although the principles of assessment and treatment would be similar.

5.12.2 Epidemiology

Causes of hazard

Bushfires are the predominant cause of thermal burns in koalas. Wildfires occur with varying intensity, extent and severity depending on a variety of environmental, seasonal and climatic factors. Prescribed burns may also place koalas at risk of thermal injury [1].

Bushfires are an inherent part of the Australian landscape [1]. Fire is considered an ecologically threatening process to koalas [2], and is recognised as a contributing factor in koala population declines [3-6], including population extirpations [5, 7-9]. Severe wildfire events are often preceded by significant drought and heat events [10-14], and the impacts of all three can have a cumulative impact on koala health and well-being [11, 15, 16]. The devastating 2019-2020 Australian bushfires resulted in an estimated loss of 80% of the koala population of Kangaroo Island [16] and 8.7% of the total NSW koala population [5]. However, koala populations may recover from fire events provided an adequate quality and quantity of unburnt adjacent habitat is present [17, 18].

The nature of the fire determines the likelihood of koalas being injured or killed. Koalas tend to climb higher when they perceive danger, so severe fires that burn the forest canopy, as opposed to the understorey and ground vegetation, are most likely to be associated with koala burns, mortalities and long-lasting population impacts [6, 7, 19]. However, koalas are also susceptible to burn injuries to the hands and feet if they travel along the ground during or after low-intensity fires [1], or when descending burning trunks of trees [20].

Geographic distribution

Fire is a common, but irregular, landscape event throughout Australia, with 55 out of a total of 134 million hectares (approximately 40%) of Australia's forest area burning on at least one occasion between 2011 and 2016 [11].

The 2019-2020 Australian bushfires were unprecedented in extent and severity, burning more than 12.6 million hectares; affecting more than 10% of the total wild koala habitat; and killing an estimated 60,000 koalas [7, 21]. It is anticipated that climate change will increase the frequency of such "mega-fires" [1, 7].

Prevalence

Records of koala admissions to rehabilitation facilities and hospitals during and after a bushfire give an indication of the prevalence of burns, although they underestimate the true number of burn victims, as many affected animals will succumb to their injuries or to secondary hazards (such as shock, predation or nutritional stress) before they can be rescued [16, 17, 22]. There is a high variability in koala admission statistics following bushfires because fires do not occur on a regular or predictable basis, and data are sometimes excluded from retrospective reviews to prevent skewing of data [23]. On Kangaroo Island during the 2019-20 bushfires, burns were observed in 67.4% of koala admissions, with a post-admission fatality rate (from death or euthanasia) of 45.6% [16].

Burns *per se* are not generally separated from general bushfire impacts in rehabilitation admission statistics, and data could include animals suffering displacement, shock, smoke inhalation and nutritional stress due to bushfires without necessarily having burn injuries [24]. Bushfires accounted for 0.7% and 4.2% of admissions in two NSW studies [24, 25] and 0.1% of admissions in a Qld study [26]. The prevalence of burns as a cause of death in koalas is variable, with retrospective studies of koala post mortem records revealing no cases of burns in some instances [27, 28], and a prevalence of 2% in one SA study [29].

Most koala burn admissions are adults, and males and females appear to be equally represented [9, 16].

5.12.3 Pathogenesis

There is no specific information on the pathogenesis of thermal burns in koalas, but human and domestic animal models appear to be appropriate to describe the process [16, 30-32]. Many koalas burnt in bushfires are already suffering dehydration, malnutrition and other disease as a result of preceding drought and heat events [10-14], rendering them more likely to come to ground, where they become burnt, and less capable of withstanding the detrimental effects of burns [16-18, 22, 32, 33].

Application of heat to skin results in three zones of injury: an inner *zone of coagulation* representing tissue destroyed at the time of injury; a surrounding *zone of stasis* characterised by inflammation and low perfusion, and an outer *zone of hyperaemia*, where perfusion is not impaired. The initial burn often expands in area and depth for the first 48

hours as tissue death occurs in the zone of stasis. The release of kinins, particularly bradykinins, cause vascular permeability, smooth muscle contraction and pain at the burn site [31].

In addition to the burn injury itself, severe burns induce responses that affect almost every organ system through a variety of processes, including inflammation, hypermetabolism, muscle wasting and insulin resistance. The initial 24-72 hours after a burn are associated with increased vascular permeability and fluid shifts which lead to oedema, protein loss, hypovolemia and shock. Thermal injury produces deleterious free radicals which induce an immunosuppressed state that predisposes patients to sepsis and organ failure [31].

5.12.4 Association with other disease hazards of koalas

The majority of koalas affected by thermal burns are presumed to be otherwise healthy. However, if treatment requires prolonged hospitalisation and rehabilitation, this may be associated with the development of other diseases related to long-term medical management such as gut dysbiosis and candidiasis [20, 34] – see Section 7.1 *Clinical Syndromes with Undefined or Multiple Aetiologies*. The immunosuppressive state induced by burns contributes to the risk of secondary disease and opportunistic infections, as well as increasing the likelihood and severity of disease expression for infection. For example, it is not uncommon for koalas admitted to care with burns to develop clinical chlamydia some weeks after arrival [21].

In the aftermath of a fire, the risk of predation trauma increases due to the loss of habitat which forces koalas to move on the ground in search of food and shelter, and because burn trauma impedes climbing ability and is associated with general debilitation, increasing the likelihood of koalas coming to ground [16-18, 22, 32, 33].

5.12.5 Diagnosis

Clinical signs

It is vitally important that experienced and highly-trained personnel undertake triage of all burnt koalas, as soon as possible after they are brought into care [16, 32]. Individuals with poor welfare or prognosis for release should be considered for euthanasia. Welfare, health and recovery should be re-assessed at regular intervals by experienced and highly trained personnel, with no assumptions that previous prognosis assessments will continue to be valid [20].

Koala fur is highly insulative and burn damage is generally most marked in less furred areas such as the face, ears, genitals and the palms and soles of the paws [9, 16, 19, 29]. Burnt animals may present with singed fur and whiskers and charred claws. Deeper burns present with oedema, blisters and burnt dead tissue (eschars) [32]. Figure 30 shows deep burns on the digits of a wild koala caught in a bushfire. This koala was euthanased due to the severity of the burns.

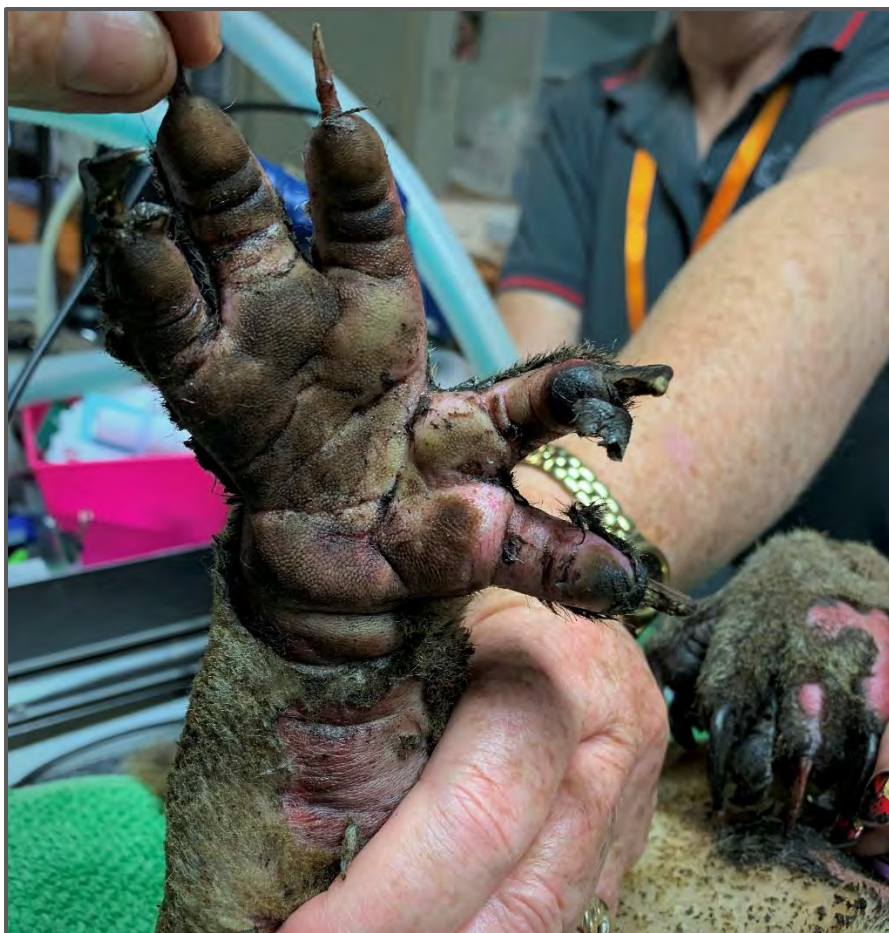


Figure 30 A wild koala with severe burns to extremities (credit: Cheyne Flanagan, Port Macquarie Koala Hospital)

In alignment with the human model, burns of koalas are classed as superficial, partial thickness or full thickness, depending on the extent of destruction of the various skin layers. Burn severity is further classified based on the percentage of the body surface, or the number of body regions, which are affected [16, 32]. In the 2019-20 fires on Kangaroo Island, the extent of burns was scored on a scale of 1-5 according to the number of burnt regions present; the mean for 72 koalas was 2.86 [16].

The most common body regions burnt during the Kangaroo Island fires were the limbs (68-78%), followed by the head and face (47%), with burns to the body and trunk rarely seen. Although the site of limb burns was not consistently recorded, 28/128 koalas (approximately 22%) were specifically reported as having burns to hands, feet and nailbeds. Most animals demonstrated superficial burns (61%) with approximately 11% demonstrating full thickness burns [16]. It is not unusual for burnt koalas to have minor burns on furred areas, and significant burns on extremities [17, 21].

Scarring associated with burns can lead to loss of function and may impact suitability for release, especially if affecting the eyelids, nose, genitals, feet and pouch. Progressive digital

necrosis is a very serious sequel of burns to the paws of koalas and may not become apparent until well after the thermal injury was sustained [35].

Poor body condition and dehydration are commonly seen in burnt koalas [11, 16, 32], and affected over 70% of animals in the 2019-20 fires on Kangaroo Island [16]. This may reflect the detrimental impacts of burn injuries on fluid, electrolyte and protein balance [31], as well as the deprivation of both food and moisture associated with the habitat destruction caused by fire. The impact of preceding drought conditions has also been implicated as a cause of poor body condition in some instances [10-14].

Burnt koalas may demonstrate systemic signs of illness including shock, and respiratory distress secondary to smoke inhalation [16, 19], although many koalas with significant smoke inhalation die before rescue can occur [20].

Clinical pathology

Clinicopathologic changes in burnt koalas are non-specific and may reflect shock, inflammation, dehydration and sepsis.

Pathology

The gross appearance of burned koalas includes charred claws, singed fur and whiskers, and typical burn injuries whose appearance will depend on their depth and chronicity [36]. Thermal burns of the upper respiratory tract are less obvious on external examination but may be associated with burns around the nose and blistering of the nasal mucosa [35].

There are no specific reports describing the histopathology of burns in koalas, but presumably it is similar to what is described for domestic animals.

Differential diagnosis

The case history and appearance of a burnt koala are pathognomonic.

Surveillance and monitoring

There is no targeted national surveillance or monitoring program in place for thermal burn trauma, or trauma of any kind in koalas. A study of koalas released to the wild following fires in Port Stephens, NSW, in 1994 found that the 12 month survival of burnt koalas (58%) was not significantly different to the survival of unburnt koalas (67%) [33]. Following the 2019-20 fires, fourteen koalas which were rehabilitated for burns in Vic are being monitored via GPS and radiotelemetry to inform rehabilitation protocols, release location selection criteria and post-release survival [37].

5.12.6 Treatment

Treatment of thermal burns in koalas is symptomatic and may include cooling of burnt areas, fluid therapy, analgesia and wound management [34]. Reducing stress and providing excellent nutrition are critical to positive outcomes [34]. Systemic antibiotic treatment is

generally not required and given the risks of dysbiosis and candidiasis, should be avoided unless burns are infected or there is evidence of sepsis [19, 34].

Deeper and more extensive burns, poor body condition and dehydration in burnt koalas carry a poor prognosis for return to the wild. Partial or full thickness burns to the hands, feet, claws and nailbeds carries a poor prognosis due to the importance of viable claws and footpads to the koala's ability to climb and forage [16, 20]. Of 304 koalas evacuated from fire grounds in the 2019-2020 fires on Kangaroo Island, 54.4% were released to the wild, with burnt koalas having a significantly lower likelihood of successful rehabilitation than unburnt animals [16]. A decision to euthanase should be made if burns cover >50% of the body, if extensive full-thickness burns are present, or if there are severe burns affecting the face, hands or feet (particularly pads, nail beds and claws) or genitals [19, 20, 34].

5.12.7 Prevention and control

Mitigation of the impact of fire on koala populations has been a focus of national consideration in the aftermath of the 2019-2020 mega-fires. The National Environmental Science Programme of the Threatened Species Recovery Hub has developed a decision support framework to help identify management actions that can be taken to support koalas in fire prone landscapes. Prevention strategies outlined in the framework include: recognition and management of co-existing threats to koala populations; long-term monitoring of koala populations; restoring habitat connectivity; understanding local habitat response to fire; maintaining appropriate ecosystem fire regimes which incorporate alternatives to prescribed burning [1].

Restoration and preservation of habitat is an important aspect of risk mitigation for thermal burns in koalas [17, 18, 22, 33]. Koalas will be less likely to be burnt if they are able to move through the landscape via the canopy, rather than having to move at ground level; this requires improved habitat quality as well as connectivity. Maintaining habitat connectivity provides means of escape and refugia options for koalas during fire and may also make habitats more resilient to fire by decreasing edge flammability [1, 38].

Where prescribed burning is used, a number of recommendations and actions have been identified which can be applied to reduce the risk of koala burn trauma and mortality. Strategies related to reducing the frequency, intensity, nature or extent of burning include strategic lighting patterns; test burning to ensure canopy preservation; incorporating alternatives to burning; and using knowledge of traditional owners on habitat-preserving fire management practices. Strategies which focus on specific koala risk mitigation include: use of koala detection dogs to assist in relocation of koalas; exclusion of areas known to be used by koalas; timing burns to avoid breeding and dispersal periods when koalas are more likely to be mobile; and wetting and reducing fuel load around the base of trees known to be used by koalas [1, 39].

Thermal burns are more likely to result in irreversible damage if there is a delay in treatment of koalas. Rapid post-fire response and assessment is needed to allow early

identification of koalas in need of intervention, and to facilitate rescue and evaluation by competent and experienced personnel [16, 37]. Timely access to firegrounds by experienced wildlife rescue teams would improve koala outcomes by enabling evacuation before koalas are burnt, or rescue before their burns are untreatable [16, 20]. Significant prior planning is required to incorporate wildlife workers and veterinarians into the incident response [37].

The care of burnt koalas takes a high emotional and psychological toll on responders, which can increase the burden of making difficult welfare-based decisions to euthanase animals. This can result in treatment decisions which may not be in the best welfare interests of koalas [40]. In recognition of this gap, programs to provide wildlife first aid awareness and training to fire fighters, and to provide training in wildlife triage and response to veterinary personnel and wildlife carers, are in development [37, 41] .

5.12.8 References

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5.13 *Trypanosoma* spp. in Koalas – Literature Review

5.13.1 Technical information

Aetiological agent

Trypanosomes are flagellated protozoa which have an indirect life cycle involving an arthropod vector and a vertebrate host [1, 2]. Trypanosomes found in Australian mammals appear to be endemic to the continent [3]. Among the introduced trypanosome species believed to be widely distributed in Australia (*Trypanosoma lewisi*, *T. melophagium* and *T. theileri*), only *T. lewisi* has been identified in an Australian mammal, specifically an endemic rodent [3]. The remainder of this chapter will therefore focus on endemic Australian trypanosomes.

Six trypanosome species have been identified to date in koalas: *T. irwini*, *T. gilletti*, *T. copemani*, *T. vegrandis*, *T. noyesi* and a novel species currently identified as *Trypanosoma* sp. AB-2017 [4-7].

Listing

Surra (*T. evansi*) and tsetse-transmitted trypanosomiasis (*T. congolense*, *T. vivax*, *T. brucei*, *T. uniforme*, *T. simiae*) are the only WOAHA listed trypanosome diseases [8].

Koala trypanosome infections are not notifiable animal diseases in Australia [9].

Disease due to trypanosome infection is not identified as a key threatening process under the Environment Protection and Biodiversity Act [10].

5.13.2 Epidemiology

Host range

With the development of sensitive molecular techniques and increasing surveys of vertebrates over a wider geographical range, a growing number of hosts are being identified for Australian trypanosome species [3]. Table 22 summarises current knowledge of host range and distribution in Australian mammals.

Table 22 Host range and geographic location for trypanosome species found in koalas [1]

Trypanosome species	Native Australian mammalian hosts	Geographic locations	References
<i>T. irwini</i>	Koala, eastern bettong, brush-tailed rock wallaby, yellow-footed rock wallaby, eastern quoll, swamp wallaby	Qld, NSW, Vic	Barbosa et al. 2019 [1], Krige 2022 [11], Barbosa et al. 2017 [12]
<i>T. gilletti</i>	Koala, brush-tailed bettong	Qld, NSW, WA	McInnes et al. 2011 [5], Barbosa et al. 2017 [7], Cooper et al. 2018 [13], McInnes et al. 2011 [14]
<i>T. copemani</i>	Koala, brush-tailed possum, bare-nosed wombat, Gilbert's potoroo, quokka, quenda, eastern quoll, spotted-tailed quoll, chuditch, brush-tailed bettong, brush-tailed rock wallaby, Tasmanian devil	Qld, NSW, Vic, Tas, WA	McInnes et al. 2011 [5], Barbosa et al. 2017 [7], Krige 2022 [11], Austen et al. 2011 [15], Austen et al. 2009 [16]
<i>T. vegrandis</i>	Koala, northern brown bandicoot, quenda, tammar wallaby, western grey kangaroo, chuditch, brush-tailed bettong, Gould's wattled bat, lesser long-eared bat, black flying-fox, little red flying-fox	Qld, NSW, WA, NT	Barbosa et al. 2019 [1], Barbosa et al. 2017 [7], Krige 2022 [11]
<i>T. noyesi</i>	Koala, eastern grey kangaroo, common brush-tailed possum, burrowing bettong, brush-tailed bettong, banded hare wallaby, swamp wallaby, bush rat, chuditch	Qld, NSW, ACT, Vic, WA, NT	Barbosa et al. 2019 [1], Krige 2022 [11], Botero et al. 2016 [17]
<i>Trypanosoma</i> sp. AB-2017	Koala	Qld	Barbosa et al. 2017 [7]

Zoonotic potential

While some trypanosome species are zoonotic, no Australian trypanosome species have been known to infect humans. However, *T. copemani* has been shown to grow in human serum *in vitro* [18] and further research is required to determine its zoonotic potential [7].

Geographic distribution

Trypanosome infection has been reported in koalas in NSW and Qld (Table 22) and studies still in progress have also detected trypanosomes in SA koalas [19]. There are only isolated studies (n <10 individuals) involving koalas located elsewhere in Australia [20, 21], none of which have detected trypanosomes. Several of the trypanosome species known to infect koalas have been detected in other Australian mammals elsewhere in Australia (Table 22), so a broader distribution of trypanosomal infection in koalas is likely.

Prevalence

An understanding of the true prevalence of trypanosome infection in koalas is hindered by the restricted geographic distribution of investigations to date [1]. A high proportion (almost 74%) of koalas in one study were infected with at least one trypanosome species, with *T. irwini* being the most commonly detected species (71.1%), followed by *T. gilletti* (21.5%) and *T. copemani* (4.4%) [14]. *T. vegrandis*, *T. noyesi* and the novel *Trypanosoma* sp. AB-2017 have been much less commonly reported to date, but this may reflect low levels of parasitaemia and the use of non-specific PCR primers rather than truly low prevalence [7].

It is difficult to determine geographic variations in trypanosome prevalence with confidence based on current data. *T. irwini* and *T. gilletti* infection were significantly more prevalent in koalas in NSW than in Qld in one study, but this result may have reflected sampling bias [14].

There is some evidence to suggest that prevalence of some *Trypanosoma* sp. infections increases with age, with koalas younger than dispersal age (2-4 years old) having a lower prevalence of *T. irwini* than adults. This may reflect an increased risk of contact with infected vectors which coincides with the increase in social behaviour, movement and activity patterns associated with dispersal and commencement of breeding [14].

Variation in prevalence by gender has not been extensively investigated. In one study, male koalas had a significantly higher prevalence of infection with *T. gilletti* and *T. copemani* (but not *T. irwini*) than females. The reasons for this difference are unclear, but could reflect different physiological status, or differential activities of the sexes that are associated with differences in vector exposure [14].

Polyparasitism with as many as five trypanosome species has been observed in koalas [5, 7], with the prevalence of multiple infections (27.4%) being significantly higher than the prevalence of single infections (4.8%) [7]. Co-infections composed of *T. irwini*, *T. gilletti* and *T. copemani* are most frequently observed [7].

Mode of transmission

The transmission dynamics of Australian trypanosomes are not well studied and there are significant knowledge gaps in regard to our basic understanding of host-parasite relationships, developmental biology and potential for cross-species transmission [22]. The information here is largely extrapolated from the behaviour of non-endemic trypanosome species.

The trypanosome life cycle generally involves a vertebrate definitive host and an intermediate host vector [3]. Within the vertebrate host, trypanosomes are deposited in dermal connective tissue by the infected vector, from which they enter draining lymphatics and the bloodstream. The “slender” trypomastigote stage multiplies by binary fission during this parasitaemia event, with the level of parasitaemia peaking, going into remission or recrudescing as host antibodies are produced to the variable antigen types (VSGs) presented

by successive generations of organisms. Infected blood is ingested by the intermediate host vector, where further development takes place, unless the vector is a strictly mechanical host [23].

There are two broad means of trypanosome transmission from vector to vertebrate host: “salivarian” trypanosomes develop into their infective stage after migrating to the mouthparts and salivary glands, and are transmitted when the vector obtains a blood meal from the vertebrate host [11]. “Stercorarian” trypanosomes transform into their infective form in the hindgut, and are transmitted to a vertebrate host via faecal contamination of the bite site during feeding [11]. Certain *Trypanosoma* species can also be transmitted mechanically, as an invertebrate vector passes infective blood forms from one vertebrate host to another [11].

Intact *T. copemani* life stages have been detected in the faeces of *Ixodes australiensis* ticks after 30 days of incubation, suggesting stercorarian transmission dynamics in this species [15]. It may be that some trypanosomes employ more than one strategy, as well as behaving differently in different vectors. For example, intact *T. noyesi* parasites have been demonstrated within the salivary glands and proboscis of tabanids, suggestive of salivarian and mechanical modes of transmission, but within the gut contents of questing ticks, suggesting the stercorarian route [11].

The vectors of the trypanosomes infecting koalas and other Australian wildlife remain unconfirmed, and there are significant logistic challenges to conclusively confirming vector candidates [22]. Trypanosomal DNA of each of the species infecting koalas has been identified in tick species that infect koalas [7, 11]. However, these studies have not conclusively demonstrated that DNA originated from viable trypanosomes, as opposed to being genetic remnants from an infected blood meal [24]. Recent detection of *T. noyesi* in “questing” ticks (i.e. ticks off the host) supports the likelihood that ticks are vectors rather than accidental dead-end ingesters of this parasite, since a tick which enters the questing state has metabolised its previous blood meal [11].

Other arthropods, including fleas and mites, have been suggested as possible vectors for koala trypanosomes on the basis of their close association with trypanosome-infected koalas [4], but to date none of the trypanosomes infecting koalas has been detected in arthropod species other than ticks. The detection of trypanosomal DNA in arthropods which parasitize Australian species also supports a role for tabanid flies [11, 17], sand flies [17] and leeches [25] as vectors for various Australian trypanosome species.

Incubation period

The incubation period of trypanosome infection in the koala is unknown. The incubation period of other trypanosome species is highly variable and can range from four days to two months [26], with incubation periods of years reported in some instances [27].

Persistence of agent

Trypanosome survival outside of a host is very limited. They may survive for a few hours in blood [26].

5.13.3 Pathogenesis

Many trypanosome infections are subclinical and the majority of wildlife trypanosomes have historically been considered benign to their vertebrate hosts [1]. The mechanisms of pathogenesis described here are generic to trypanosomes rather than representing a confirmed process in koalas.

Trypanosomes may cause extravascular erythrocyte destruction through the attachment of trypanosome antigen to host erythrocytes, increasing antibody mediated erythrophagocytosis. Trypanosomes can also physically damage erythrocytes, release cytotoxic and haemolytic factors and interfering with the coagulation cascade, potentially resulting in thrombocytopaenia and disseminated intravascular coagulation [2].

As well as erythrocyte effects, some trypanosome species can invade other host tissues. Among the species known to infect koalas, *T. copemani* has this capacity, although intracellular entry is not an obligate stage of its life cycle [28, 29]. *T. copemani* infections in brush-tailed bettongs were associated with inflammatory infiltrates in the muscles of the heart, oesophagus and tongue in three individuals, which may have reduced host fitness and increased susceptibility to predation [29].

Trypanosomes have been shown to suppress both cellular and humoral immunity, affecting the activity and function of B cells, T cells and macrophages [1, 30, 31]. The predominant surface antigens of trypanosomes are variant-specific glycoproteins (VSG) which promote chronic parasitemia and prevent lysis by inhibiting the complement pathway. By inducing host immunosuppression, trypanosomes can potentiate concurrent infections, and also reduce the capacity of the host to respond to immunisation against other pathogens [14].

Trypanosomes can demonstrate ‘condition dependent pathogenicity’, increasing in virulence when they encounter a new or naïve host species [3], or immunosuppressed individuals [14].

5.13.4 Associations with other disease hazards of koalas

Although koalas (and other vertebrates) demonstrate a high incidence of infection with multiple trypanosome species within a single host, the implications of trypanosome polyparasitism for levels of parasitaemia, individual parasite virulence or pathogenicity are not clear [7].

Because trypanosomes can compromise the immunity of their host (see *Pathogenesis*), they can potentiate the effects of concurrent infections [1]. Outside of Australia, infection with trypanosome species has been associated with severe, sometimes fatal, disease in the presence of co-infections [14]. It has been suggested that co-infection of *Trypanosoma* spp.

with pathogens such as *Chlamydia* and KoRV may be associated with poor health and decreased survival of koalas [7], but in the absence of studies to elucidate this relationship, these associations remain speculative.

5.13.5 Diagnosis

Clinical signs

The majority of koalas infected by trypanosomes are clinically healthy [4, 14], reflecting the general premise that most trypanosomal infections of wildlife are subclinical and benign [1]. Clinical manifestations may be most likely to arise where concomitant infections with other pathogens exist, or where immunosuppression occurs due to other stressors [32].

Disease caused by trypanosome infection (trypanosomiasis) in other species often manifests as haemolytic anaemia caused by extravascular erythrocyte destruction [1]. Signs consistent with such a process were observed in the index case for *T. irwini* in a koala, which demonstrated depression, pale mucous membranes and generalised weakness associated with a profound anaemia [4]. A few juvenile koalas in Qld have also been observed to have profound and highly regenerative anaemia in association with detection of circulating trypanosomes on blood films, and in the absence of other identifiable causes for the anaemia. It is possible that joeys transitioning to independence are more susceptible to the haemolytic effects of trypanosome infection [33].

Few studies have explored associations between clinical disease and trypanosome infection in koalas; where clinical signs are noted, it is often unclear if these signs can be attributed to the trypanosomes [1]. Clinical signs consistent with trypanosomiasis, including severe regenerative anaemia, neurological signs (nystagmus, tremors and seizures) and lethargy, have been observed in a small number of koalas with parasitaemia [1, 4, 33].

The most comprehensive study of the clinical impact of trypanosomes on koalas examined clinical records of koala admissions to a Qld rehabilitation facility over three years [14]. This study found that koalas infected with *T. gilletti* alone, or in co-infection with *T. irwini*, had significantly lower body condition scores than uninfected koalas [14]. This association was not seen for koalas infected with *T. irwini* or *T. copemani* alone. Koalas in the study originating from NSW were significantly more likely to die or be euthanased if infected with *T. gilletti* or a mixed trypanosome infection [14].

Given the limited understanding of the potential clinical effects of koala infection with trypanosomes, clinically abnormal koalas with trypanosomes identified should receive a full diagnostic work-up to identify any other possible causes of illness or mortality [34].

Clinical pathology

Trypanosome parasitemia is confirmed by examination of fresh or Giemsa stained blood smears under light microscopy. *Trypanosoma* spp. can be detected extracellularly among the red blood cells [3].

Although reductions in packed cell volume (PCV) were detected in trypanosome-infected koalas in the study by McInnes et al 2011 [14], a causative role for trypanosomes is difficult to establish. Mild anaemia is a common and non-specific clinical sign in diseased koalas which is associated with a range of clinical syndromes, including complicated cystitis, lymphosarcoma and heavy tick infestations [14]. In many cases, the observed reductions in PCV in trypanosome-infected koalas may not be biologically significant. While koalas infected with *T. gilletti* or *T. copemani* demonstrated significantly lower mean PCV than uninfected koalas, no such association was detected for *T. irwini*, and the PCV of *T. gilletti*-infected koalas were still within reference ranges for the species. The very small number of animals which were positive for *T. copemani* in this study (n=6) precludes further interpretation of findings [14].

Anecdotally, there have been a small number of cases where profound anaemia occurs in association with trypanosome parasitaemia. These cases exhibit PCV in the order of 9-12% (normal PCV is 30-40%) with a very strong regenerative response (marked anisocytosis and polychromasia) along with erythroid hyperplasia on bone marrow cytology [33]. These findings are similar to the clinical pathological findings reported for the sentinel koala diagnosed with *T. irwini*, which had a PCV of 9% and haemoglobin of 32g/l (normal range is 88–140g/l) as well as bone marrow erythroid hyperplasia [4].

Pathology

Published description of pathology associated with trypanosome infection in koalas is limited. A post mortem examination of the first koala diagnosed with *T. irwini* revealed pathology in a range of tissues, but it was not clear the extent to which pathology was attributable to trypanosome infection as opposed to co-morbidities [33]. Changes included benign osteochondromas of ribs, haemosiderosis and extramedullary haematopoiesis of the spleen, extreme atrophy of lymphoid tissues, bone marrow hyperplasia, severe necrosis of the liver, nephritis and oxalate nephrosis of the kidneys and pneumonia. Grossly there was marked subcutaneous oedema, large volumes of peritoneal fluid and markedly enlarged mesenteric lymph vessels [4, 35]. The KoRV status of this individual was not described, but he was PCR-positive for *Chlamydia* and had clinical signs of keratoconjunctivitis [33].

Trypanosome-infected koalas which developed neurological signs appeared to have trypanosome life stages present in the liver, central nervous system and choroidal vessels, as well as a lymphocytic/plasmacytic choroiditis [1].

Differential diagnosis

Other causes of poor body condition in koalas include advanced dental disease or tooth wear, poor nutrition, renal disease, chlamydiosis, neoplasia, untreated injuries and opportunistic bacterial and fungal infections. Potential causes of anaemia and low PCV in koalas include blood loss due to trauma, poor nutrition, chronic inflammatory disease, neoplasia, bone marrow dysplasia and ectoparasite infestation [36].

Diagnostic testing

Identification of trypanosomes on blood smears via light microscopy is a relatively insensitive detection method, particularly if parasitaemia levels are low. Molecular methods of detecting trypanosome DNA via PCR are sensitive and commonly utilised for studies of Australian trypanosomes [3-7, 13, 16, 20]. Next generation sequencing methods have been shown to be of greater accuracy than traditional PCR methods for detecting novel and rare trypanosomes in mixed infections in native species [7, 13].

Although most molecular testing is carried out on blood samples, PCR methods have also been used to detect trypanosome DNA in the tissues of Australian vertebrates, including brain, lung, liver and skin. Five out of seven healthy koalas tested positive for trypanosome infection on testing of liver samples using molecular techniques [37].

Surveillance and monitoring

There is no coordinated, targeted national program for surveillance of trypanosomes in Australian native wildlife. However, the findings of trypanosomes in samples from wildlife in Australia would be considered interesting and unusual and would therefore be logged in the Wildlife Health Australia national wildlife health information system (eWHIS) as part of national general wildlife surveillance activities [34].

5.13.6 Treatment

There are no specific treatment recommendations for trypanosome infection. Treatment of infected, symptomatic individuals has been attempted using melarsomine dichlorhydrate, but this failed to kill circulating trypanosomes. Imidocarb has also been used in an attempt to clear parasitaemia, but without success [33]. Anecdotal cases where trypanosomiasis has been suspected as the cause of profound, regenerative anaemia have generally been unresponsive to treatment, but a small number have survived with careful nursing, multiple blood transfusions and iron supplementation [33]. Symptomatic therapy based on clinical signs, reduction of stress, and specific treatment for co-infections are indicated [1].

5.13.7 Prevention and control

There are no identified methods of prevention or control of trypanosome infection in koalas. Prevention and control of trypanosome infection in general depends on breaking the cycle of transmission, which in turn requires knowledge of competent vectors [34]. Limiting exposure to vectors is unlikely to be feasible in wild koalas, but may be indicated as part of a general reduction of stressors and vectors for disease spread in captivity and rehabilitation.

5.13.8 References

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Appendix 6 Common and Taxonomic Names of Species

Taxonomic names for the species mentioned in this report are listed in Table 23.

Table 23 Taxonomic names of animal species mentioned in KDRA report

Common Name	Taxonomic Name
Banded hare-wallaby	<i>Lagostrophus fasciatus</i>
Bare-nosed wombat	<i>Vombatus ursinus</i>
Black flying-fox	<i>Pteropus alecto</i>
Black-tufted marmoset	<i>Callithrix penicillata</i>
Brush-tailed bettong	<i>Bettongia penicillata</i>
Brush-tailed rock-wallaby	<i>Petrogale penicillata</i>
Burrowing bettong	<i>Bettongia lesueur</i>
Bush rat	<i>Rattus fuscipes</i>
Carpet python	<i>Morelia spilota</i>
Chamois	<i>Rupicapra</i>
Chuditch	<i>Dasyurus geoffroii</i>
Common brush-tailed possum	<i>Trichosurus vulpecula</i>
Dingo	<i>Canis familiaris dingo</i>
Domestic cat	<i>Felis catus</i>
Domestic dog	<i>Canis familiaris</i>
Eastern bettong	<i>Bettongia gaimardi</i>
Eastern grey kangaroo	<i>Macropus giganteus</i>
Eastern quoll	<i>Dasyurus viverrinus</i>
Red fox	<i>Vulpes</i>
Gilbert's potoroo	<i>Potorous gilbertii</i>
Gould's wattled bat	<i>Chalinolobus gouldii</i>
Grassland melomys	<i>Melomys burtoni</i>
Koala	<i>Phascolarctos cinereus</i>
Lace monitor	<i>Varanus varius</i>
Lesser long-eared bat	<i>Nyctophilus geoffroyi</i>
Little red flying-fox	<i>Pteropus scapulatus</i>
Northern brown bandicoot	<i>Isoodon macrourus</i>
Quenda	<i>Isoodon fusciventer</i>
Quokka	<i>Setonix brachyurus</i>
Red kangaroo	<i>Osphranter rufus</i>
Shark Bay bandicoot	<i>Perameles bougainville</i>
Southern hairy-nosed wombat	<i>Lasiorhinus latifrons</i>
Spotted-tailed quoll	<i>Dasyurus maculatus</i>
Swamp wallaby	<i>Wallabia bicolor</i>
Tammar wallaby	<i>Macropus eugenii</i>
Tasmanian devil	<i>Sarcophilus harrisii</i>
Western grey kangaroo	<i>Macropus fuliginosus</i>
Yellow-footed rock-wallaby	<i>Petrogale xanthopus</i>



Photo: A healthy wild koala and joey in a tree (credit: Amber Gillet)