Wildlife Health Australia

Koala retrovirus (KoRV)

Fact sheet

Introductory statement

The term koala retrovirus (KoRV) refers to a group of viruses specific to koalas. The viruses display a range of transmission behaviours (inherited vs horizontally transmitted), depending on the viral subtype and geographical region. The role of KoRV in koala disease is unclear. There is some evidence for association of KoRV infection with immune changes, ill thrift and disease in the koala host. Definite causative relationships between KoRV and disease in koalas have not been established. Further research is needed to determine the clinical and management implications of these viruses, as well as indicators of value in risk assessment and management.

Aetiology

Koala retrovirus (KoRV) belongs to the family *Retroviridae*, genus *Gammaretrovirus.* In other mammalian hosts, retroviruses may be linked to the development of cancer or immunosuppressive diseases. Gammaretroviruses include murine leukaemia virus (MuLV) and feline leukaemia virus (FeLV). The best known immunosuppressive retrovirus, human immunodeficiency virus (HIV), causes AIDS and belongs to the Lentivirus genus of *Retroviridae*.

KoRV comprises a family of related retroviruses that fall into three main groups, or clades (Chappell et al. 2017) each comprising one or more subtypes:

- KoRV A
- KoRV B/J
- a highly diverse clade comprising a polyphyletic subtype KoRV D, two previously described subtypes named KoRV C and E, and four novel subtypes KoRV F, G, H, I.

There is no strict definition for endogenous retroviruses (ERVs) however integration into the germ cells and inheritance from one generation of host to the next are considered to be essential. ERVs have been found in all vertebrates studied as well as some invertebrates. They are often millions of years old and the evolution of ERVs frequently mirror the evolution of the species involved. Mutations generally accumulate over time and many ERVs degrade into ‘junk DNA’; approximately 8% of the human genome is made up of ERVs. In contrast, exogenous retroviruses are spread horizontally and are integrated into the somatic cells but not the germ cells. These viruses are more frequently pathogenic.

In Qld, and in NSW down to the far south coast, KoRV A appears endogenous, displaying limited diversity consistent with a relatively inactive endogenous virus. KoRV A in Vic and SA, and all other subtypes of KoRV, are believed to be exogenous or to arise by recombination within hosts (Higgins and Maher 2019). This is reflected by a greater diversity, consistent with the dynamic evolution expected in actively infecting exogenous viruses (Chappell et al. 2017). Given the small and geographically restricted sample sets included in studies to date, it seems reasonable to expect that additional subtypes exist.

Natural hosts

KoRV has only been detected in the koala. It appears most likely that KoRV originated in a native Australian rodent, the grassland melomys (*Melomys burtoni*), in which an apparently ancestral virus (MbRV) was detected in all animals tested; no other candidate has been detected in the 42 rodent, bat, feral vertebrate and marsupial species screened to date (Simmons et al. 2014). KoRV's closest relative, the exogenous, pathogenic gibbon ape leukaemia virus (GALV) appears to have spilled over from rodents to primates in Southeast Asia in the mid-late 1960s, although there have been no new reports of infection with GALV in primates since 1978 (Hanger et al. 2000; Brown and Tarlinton 2017). There is no evidence for human infection with KoRV.

Occurrences in Australia

The prevalence of KoRV varies across the range of koalas in Australia. KoRV A is endogenized at a prevalence of 100% in Qld and in NSW populations at least to the far south coast (Higgins and Maher 2019). KoRV A is present at approximately 30 – 40% prevalence in Vic and SA (Legione et al. 2017). KoRV B occurs in Qld and NSW but has not yet been detected in surveys in Vic and SA. Subtypes other than KoRV A and B have only been detected in koalas in Qld but koalas in other states have not yet been tested.

World distribution

KoRV has been reported in captive koalas overseas. KoRV B/J was discovered in koalas in Japanese zoos (Shojima et al. 2013) and reported in koalas in Los Angeles Zoo (Xu et al. 2013); it has since been reported in two European Zoos [koalas from San Diego Zoo, of Qld origin] (Fiebig et al. 2015; Fiebig et al. 2016). KoRV C and D were reported in a koala in Japan [Qld origin] (Shimode et al. 2014). KoRV E and F were discovered more recently, in USA zoo koala populations [Qld origin] (Xu et al. 2015). All koalas of Qld and NSW provenance are expected to be endogenously infected by KoRV A.

Epidemiology

In Qld and NSW, where it has endogenized, KoRV A is inherited in the genome. It is believed other subtypes are exogenous but no route of transmission has been identified. Horizontal spread via exchange of body fluids (sexual contact and to a lesser extent fighting) is expected to be the main route of transmission (as with other infectious retroviruses). A longitudinal transmission study for KoRV B in a free-ranging Qld population estimated a 100% vertical transmission rate from mother to young (Quigley et al. 2018). There is some debate over the potential for arthropods to play a role in transmission (Higgins and Maher 2019). It is likely that multiple subtypes are co-circulating within koala populations, and that multiple subtypes can be formed within individuals as a result of mutation or recombination (Chappell et al. 2017)

To what extent, or if, KoRV A has endogenized in Vic and SA koalas has not been examined in full, but data to date suggests that KoRV A is not endogenous in southern koalas but is an ongoing exogenous infection (Simmons et al. 2012; Wedrowicz et al. 2016).

Originally, suspicion of a role for KoRV in disease was based on the fact that northern koalas, in particular, frequently suffer from a spectrum of diseases sometimes referred to as koala immune deficiency syndrome (KIDS). Some syndromes have been labelled presumptively as 'KoRV', based on the similarity of these diseases to those seen in humans with HIV/AIDS and cats with FeLV (Denner and Young 2013). Diseases in koalas putatively linked to KoRV include chlamydial infertility and mortality; a range of opportunistic infectious diseases; blood and bone marrow disorders (myelodysplasia); and neoplasia (e.g. lymphoma and leukaemia, mesothelial and craniofacial tumours) (Hanger et al. 2003; Tarlinton et al. 2005). Although lymphoma and severe chlamydial disease are more common in northern states, where KoRV prevalence is greater (Gillett 2014), differences in other potential drivers also exist between the populations, particularly with respect to genetics (Lau et al. 2014), chlamydial strains (Legione et al. 2017) and environmental pressures.

A recent study detected anti-KoRV antibodies in serum of koalas from Qld and SA, which suggests that both endogenously and exogenously infected koalas are able to mount an immune response against KoRV A (Waugh et al. 2017).

Although the role of KoRV in causing disease remains unproven, evidence is emerging incrementally for association of infection by exogenous subtypes with immune changes or disease. Suggestive evidence for association of KoRV B infection with lymphoid neoplasia in small captive koala populations in the USA and Europe (Xu et al. 2013; Fiebig et al. 2016), has been followed by association of KoRV B infection with changes in immune profiles of captive koalas of NSW origin (Maher and Higgins 2016). An association of KoRV B infection with chlamydial disease has been found in a small but well-characterised, longitudinally sampled, free-ranging group from south-east Qld (Waugh et al. 2017; Quigley et al. 2018). KoRV A positive koalas in Vic had immune changes consistent with those seen in retroviral immune suppression in other species (Higgins and Maher 2019). These koalas were also more likely to be in poor condition and show evidence of urinary incontinence than KoRV-negative koalas (Legione et al. 2017).

Causative relationships will be more difficult to establish and will likely require a combination of *in vitro* mechanistic studies and detailed longitudinal surveys. The widely supposed but unproven role of KoRV in development of cancer, impairment of immune function and disease is a key knowledge gap for koala management.

Clinical signs and pathology

Not applicable, as there is no proven link between KoRV infection and disease expression.

Diagnosis and sampling

Diagnostic assays for KoRV are not currently available outside of the research setting, although this may change as more evidence emerges to support their clinical application.

Diagnosis of infection is based on PCR of DNA from blood, faeces or tissues to detect provirus (retroviral insertions in the host DNA). In the case of exogenous viruses, it should be borne in mind that tissue tropism has not been defined so the virus may not be detected equally in all tissues.

Detection of circulating virus is by reverse transcription PCR of plasma.

For generic detection or quantification of all KoRV subtypes, *pol* gene primers can be used, but detection of specific subtypes requires the use of specific *env* gene primers. For screening of all known and unknown KoRV subtypes, deep sequencing of the *env* gene would be the appropriate approach.

The ability to detect anti-KoRV antibodies in koala serum (Waugh et al. 2016) is expected to have limited diagnostic value outside of research.

Laboratory diagnostic specimens

- For detection of KoRV provirus: whole blood in EDTA (fresh or frozen)
- For detection of KoRV viral RNA (circulating virus) and viral load determination: plasma in RNA-later (Qiagen)
- For detection of KoRV provirus in tissue: fresh, frozen or ethanol fixed tissue.

Treatment

No treatment is currently available. The difficulties associated with possible antiviral therapy are discussed in Kinney and Pye (2016).

Prevention and control

There is no vaccine available for KoRV and very little is known about ways in which this virus may be transmitted. Preliminary work on development of a vaccine is occurring (Waugh et al. 2016; Olagoke et al. 2018).

It is difficult to make recommendations on management of koalas based on KoRV status. In the absence of further information, it may be prudent to consider keeping KoRV-free and KoRV-infected individuals and populations separated. This may be an especially important consideration in koala translocation programs. Ongoing research is underway to identify which koala populations are infected or free of KoRV subtypes, to characterise modes of transmission, and significance to disease.

Surveillance and management

Wildlife disease surveillance in Australia is coordinated by Wildlife Health Australia. The National Wildlife Health Information System (eWHIS) captures information from a variety of sources including Australian government agencies, zoo and wildlife parks, wildlife carers, universities and members of the public. Coordinators in each of Australia's States and Territories report monthly on significant wildlife cases identified in their jurisdictions. NOTE: access to information contained within the National Wildlife Health Information System dataset is by application. Please contact admin@wildlifehealthaustralia.com.au.

Research is currently being conducted by several groups into the prevalence of KoRV across the Australian koala population.

Statistics

Data on prevalence of subtypes B to J varies with method and study population. *env* gene deep-sequencing of free-ranging koalas from south-east Qld found moderate to high prevalence of KoRV B/J, KoRV D and KoRV F, and low prevalence of KoRV G, KoRV H and KoRV I (Chappell et al. 2017). Based on *env* gene qPCR, however,

the prevalence of KoRV B/J in a south-east Qld population was only 25% (Waugh et al. 2017; Quigley et al. 2018)). KoRV B/J was not detected in surveys of Vic koalas (Legione et al. 2017) and, as in NSW, other subtypes have not been assessed. Consistent with these data, in Japanese zoos, KoRV J was detected in 67% of Queensland origin koalas, but in none of the Victorian origin koalas tested (Shojima et al. 2013).

Research

Key research questions

- Does KoRV cause disease, or does ill thrift predispose to KoRV infection?
- If KoRV causes disease, what are the driving factors?
    - Subtype, or interactions between subtypes?
    - Where the virus has inserted within the host DNA?
    - Co-infection with other non-retroviral agents (for example, herpesviruses)?
- What is the distribution and prevalence of KoRV subtypes in captive and free-ranging koala populations?
- How is KoRV transmitted?
- Should we be selecting animals for translocation or breeding programs based on their KoRV status?
- What is the need and feasibility of vaccination or anti-retroviral therapy? Will this be effective clinically and an efficient use of resources?

Human health implications

There have been no reported incidences of human infection with KoRV. *In vitro* studies have indicated that KoRV can enter the cells of many species. Based on studies with other retroviruses, the risk of human infection with KoRV is likely to be very low.

Conclusions

Given the complexity and uncertainty around the role of KoRV in disease, it would be premature to make clinical decisions based on detection of KoRV by PCR. Knowledge is changing rapidly, and accumulation of clinical, infection and immunological data is likely to assist the development and evaluation of prognostic tests on which clinical or population management decisions can be based.

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To provide feedback on this fact sheet

We are interested in hearing from anyone with information on this condition in Australia, including laboratory reports, historical datasets or survey results that could be added to the National Wildlife Health Information System. If you can help, please contact us at admin@wildlifehealthaustralia.com.au.

Wildlife Health Australia would be very grateful for any feedback on this fact sheet. Please provide detailed comments or suggestions to admin@wildlifehealthaustralia.com.au. We would also like to hear from you if you have a particular area of expertise and would like to produce a fact sheet (or sheets) for the network (or update current sheets). A small amount of funding is available to facilitate this.

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