The Koala and its Retroviruses:
Implications for Sustainability and Survival

edited by

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Technical Reports of the Australian Museum, Online (2014) No. 24, pp. 103–105.
ISSN 1835-4211 (online)
http://dx.doi.org/10.3853/j.1835-4211.24.2014.1628

Koala Retrovirus Workshop Conclusion. The Future of KoRV Research—Foundational and Applied

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Abstract. This manuscript summarizes the conclusion session of the *Koala Conservation Workshop: The koala and its retroviruses: implications for sustainability and survival* held at San Diego Zoo, April 17–18, 2013. The main goals of the workshop were to determine the current state of foundational research of koala retrovirus (KoRV), the future foundational research needed, to initiate the need for applied research, and to create a collaborative international effort on KoRV that would directly help the sustainability and survival of both captive and free-ranging koalas *(Phascolarctos cinereus)*. The seven areas of future collaborative research of the workshop were determined to be: *(1)* Does KoRV cause disease in koalas? *(2)* Does KoRV cause population declines? *(3)* Are there KoRV-free koalas? *(4)* What is the importance of the variants of KoRV? *(5)* Is KoRV or its variants horizontally transmitted? *(6)* Do koalas develop an immune response to KoRV? *(7)* What is the role of prevention and therapy in free-ranging and captive koalas?

Pye, Geoffrey W., Rebecca N. Johnson, and Alex D. Greenwood. 2014. Koala Retrovirus Workshop conclusion. The future of KoRV Research—foundational and applied. In *The Koala and its Retroviruses: Implications for Sustainability and Survival*, ed. Geoffrey W. Pye, Rebecca N. Johnson and Alex D. Greenwood. *Technical Reports of the Australian Museum, Online* 24: 103–105.

Does KoRV cause disease in koalas?

There is plenty of supportive evidence to suggest that koala retrovirus (KoRV) causes lymphoid neoplasia in koalas *(Phascolarctos cinereus)* (Canfield *et al*., 1987, Canfield *et al*., 1988; Worley *et al*. 1993, Hanger *et al.,* 2000; Tarlinton *et al*., 2005), but, at this time, no-one has definitive proof of this. A missing resource impeding progress is the lack of an annotated koala genome. However, as presented by Rebecca Johnson, this situation is changing rapidly with the sequencing of a koala genome and transcriptome which is now in the annotation stage (Johnson *et al*., 2014, this volume). Important evidence for a causal role in disease by KoRV that is currently lacking is integration site differences of KoRV in diseased versus healthy tissues. It was agreed that this is crucial information that should be determined as soon as possible. It has been suggested that KoRV may cause disease by immunosuppression (Fiebig et al., 2006; Denner, 2014, this volume). However, KoRV positive koalas mount a strong immune response to antigens derived from *Chlamydia* (Timms, 2014, this volume). The consequence of KoRV on immune response thus requires further investigation to determine if KoRV has a broad, specific, or no effect on koala immune function. It was identified that there was a need to standardize both the collection of tissue samples from suspected KoRV-related diseased koalas and the epidemiological survey methods used to examine the data. In addition, studies looking at the potential immune suppressive effects of KoRV were identified as an important need.

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Are there KoRV-free koalas?

The studies by Tarlinton *et al.*, 2006 and Simmons *et al.*, 2012 suggest that potential KoRV-free free-ranging populations may be diminishing. It was identified that that the definition of KoRV-free koalas should be based on genomic information that shows that the individual koala is free of the potential to give rise to the virus. This will require an improvement in testing sensitivity from currently used methods. More sampling surveys are needed, particularly in southern Australia. If KoRV-free koalas are identified, then isolation should be considered, if sufficient numbers exist for a sustainable population.

Does KoRV cause population decline?

While KoRV likely has a significant effect on captive populations (Gillett, 2014, this volume; Hanger *et al.*, 2000, Miyazawa, 2014, this volume; Mulot, 2014, this volume; Pye *et al.*, 2014, this volume), there is little data on the prevalence of KoRV-associated disease in free-ranging populations (Hanger & Loader, 2014, this volume). Anecdotally there appears to be quite a difference in prevalence of expression of KoRV-associated disease across Australia, particularly between northern and southern areas. Even in Queensland where there is 100% prevalence of KoRV-A (Tarlinton *et al.*, 2006; Simmons *et al.*, 2012), there appears to a great deal of variation in the apparent prevalence of KoRV-associated disease (e.g., south-eastern Queensland versus central Queensland; Drs Amber Gillett and Sean FitzGibbon, pers. comm. 2013).

Much more data needs to be collected using standardized methods across the whole range of koalas. Current data on koala population declines is generally incomplete in Australia and typically does not include disease status. A considerable collaborative effort is needed to ensure sufficient standardized data from across the range to make a study of population or species decline meaningful as opposed to anecdotal. Comparison with data from a KoRV-free populations would be ideal if such populations truly exist.

What is the importance of the variants of KoRV?

With the recognition and recent sequencing of variants of KoRV, a standardized system is required to classify known and future variants. The system could be based on biological assay to determine the functional significance of identified variation, molecular clone development for comparative analysis and potential vaccine and serological resource development, serology where possible to determine prevalence in both captive and wild populations rapidly, samples from diverse populations to examine both epidemiology and among variant variation, uniform standardization and definitions to prevent nomenclature confusion in the literature, and through the sharing of reagents. Efforts should be made to determine how the viruses were generated e.g., by point mutation or recombination.

Currently the significance of KoRV variants on disease expression is unknown. It has been suggested that KoRV-B may be more pathogenic (Xu *et al.*, 2013). Examination of correlation of variant presence and mortality data over large populations in Australia may help determine the significance of the individual variants of KoRV. Until this is done, caution should be taken with mixing koalas of known differing variant status (e.g., KoRV-B positive koalas with KoRV-B negative koalas).

Is KoRV or its variants horizontally transmitted?

While it is well understood that endogenous KoRV is passed from koala to koala via the germ line, it is not known how exogenous forms of KoRV are transmitted or whether any KoRVs are currently being transmitted horizontally. It is possible that exogenous KoRV may be transmitted during copulation, in utero, via milk, via pap (the soft feces from the mother that the joey feeds on prior to beginning to eat *Eucalyptus*), or by direct contact (e.g., males fighting). Findings of KoRV-B positive tissue from 3-month-old joeys are suggestive that either in utero or via milk transmission occurs (Maribeth Eiden, pers. comm. 2013).

Opportunities may exist in the captive situations in Australia, Japan, and North America where KoRV-B positive and KoRV-B negative koalas have comingled to look at prevalence, integration- site distributions, pedigrees, and social interactions in order to model possible routes of transmission. In addition, longitudinal studies on populations where exogenous transmission appears to be occurring (e.g., Kangaroo Island) may be helpful in determining routes of horizontal transmission.

Do koalas develop an immune response to KoRV?

We need to determine if KoRV-infected koalas are tolerant to the virus, is there evidence of clearing of natural infection, are there resistant populations, and are the perceived cycles of infection real. Further development and standardization of assays in koalas is an important part of the process.

What is the role of prevention and therapy in free-ranging and captive koalas?

There are a large number of antiretroviral drug therapies available for HIV+ humans. Many of these drugs act to prevent replication of the virus and therefore prevent repeated cycles of infection. In koalas, we don't know whether cycles of infection occur or if they do, their role in the expression of clinical disease. Consequently we don't know if treatment would be beneficial in koalas infected with endogenous KoRV-A. Prophylactic treatment of female koalas negative for exogenous variants of KoRV (KoRV-B) may prove beneficial at times of breeding (e.g., if KoRV-B positive washed semen is used for artificial insemination).

Vaccination holds more promise due to the success of developing vaccines for FeLV. Uniform standards and definitions for challenge and protection will be required.

Progress in these seven areas will hopefully enhance our understanding of KoRV biology and lead to practical applications that can benefit captive and free-ranging koala health.

Acknowledgments. This workshop was kindly sponsored by San Diego Zoo Global, Los Angeles Zoo, Dallas Zoo, Albuquerque BioPark, and the Australian Museum.

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