# The Koala and its Retroviruses:
## Implications for Sustainability and Survival

edited by

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# Koala Retrovirus (KoRV) and its Variants

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Abstract. The recent, independent identification by several research groups of koala retrovirus (KoRV) variants was the focus of one of the break-out sessions at the *Koala Conservation Workshop: The koala and its retroviruses: implications for sustainability and survival* held at San Diego Zoo, April 17–18, 2013. The goals of this session were to discuss the differences and similarities between variants identified, to determine approaches to their nomenclature, the prevalence of these variants in wild and captive koalas, the relative pathogenicity of the variants, and the significance of the variants in managing koala populations.

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The nucleotide sequence of a koala retrovirus thought to be associated with lymphoma was first reported by Hanger in 2000 (Hanger *et al*., 2000) and named KoRV. More recently, separate research groups in Australia, Japan, and the United States have independently identified a number of KoRV variants (Miyazawa *et al*., 2011; Xu *et al*., 2013; Shojima *et al*., 2013; Shimode *et al*., 2014; own unpublished observations). At the time of this meeting in April, 2013 the only sequence analysis that had been publicly presented on KoRV variants was by the Miyazawa group at the 21st International Workshop on Retroviral Pathogenesis in Italy, September 2009. They showed that a variant, isolated from a captive koala at the Kobe Municipal Oji Zoo (KMOZ) was characterized by a significant sequence modification in the *env* gene, within the receptor binding domain (RBD), specifically in the variable region A (VRA) motif that is known to be involved in defining host cell receptor specificity. They referred to this variant as KoRV-B with the original Hanger strain designated KoRV-A and published the isolation of these viruses in the following year (Miyazawa *et al*., 2011). Recognition by other groups of variants with differing sequence stretches in this same region of the RBD VRA has led to the adoption of the Miyazawa labeling convention. Discussions during this break-out session at the 2013 San Diego meeting didn't reach consensus on KoRV nomenclature nor on a number of other issues raised, primarily because none of the sequences had at that stage been published and so direct comparative analyses could not be made. However, subsequent publications have helped to clarify the situation and the discussion below is intended to summarize the current state-of-play.

## KoRV variant nomenclature

The natural extension of the above naming convention has resulted in the publication so far of five KoRV variants with the original sequenced virus being designated KoRV-A and the remaining four being named KoRV-B, KoRV-C, KoRV-D, and KoRV-J (summarized in Denner & Young, 2013). The original Miyazawa KoRV-B had to be re-named KoRV-J as an isolate from the Los Angeles Zoo (LAZ) was given the KoRV-B designation in the first published sequence analysis of a KoRV variant (Xu *et al*., 2013). Ironically, subsequent sequence comparisons indicate that the LAZ KoRV-B VRA sequence is strikingly similar to KoRV-J placing these two viruses in the same phylogenetic grouping (Shimode *et al*., 2014). Furthermore, both of these viruses were shown to utilize the same receptor, the thiamin transport protein 1 (THTR1) for cell entry, a different receptor to that used by KoRV-A, the sodium-dependent phosphate transporter, Pit1 (Shojima *et al*., 2013; Xu *et al*., 2013). Full genome sequencing of these isolates has identified additional sequence variation from the prototype KoRV-A with both KoRV-B and KoRV-J showing additional, but distinct tandem repeats in the U3 region of the LTR (Shimode *et al*., 2014). Interestingly, the KoRV-J LTR was shown to display a significantly higher promoter activity than the KoRV-A LTR in selected cell populations hinting at a possible role in up-regulating the expression of host cell genes adjacent to proviral insertions (Shimode *et al*., 2014). Given the striking similarities between KoRV-B and KoRV-J it would be appropriate for both to be referred to as KoRV-B but each with a strain designation to separately identify them

(e.g., KoRV-B strains LAZ and KMOZ). Of particular note is the fact that all variants so far identified have only been found in animals that are also carrying KoRV-A. An obvious conclusion is that the deletions/insertions found in the same *env* location (RBD VRA) for all the variants are the products of a recombination hot spot.

Another convention that has been adopted was discussed at the meeting, that of referring to these isolates as "subtypes". As we probe further into both the koala genome and the KoRV variants that arise in individual animals, we are likely to detect many more of these variants. However as each new isolate is given a subtype listing we may generate unintentional nomenclature conflicts. Sequencing based approaches to virus taxonomy usually define genotypes as the higher order of classification with individual subtypes falling within these. The phylogenetic analysis provided by Shimode *et al*. (2014) of the currently available published sequences suggests three genotypes comprising multiple subtypes; KoRV-A, KoRV-B (containing both the LAZ and KMOZ viruses) and KoRV-C (clustering both KoRV-C and KoRV-D). The close genetic relationship between the KoRV-C and KoRV-D sequences suggests that they could be included in the same KoRV genotype but as separate subtypes. The field needs to have the discussion on selecting the appropriate consensus criteria for classification soon, so that the nomenclature does not become too messy.

An additional issue that needs to be considered, one that is unique to retroviruses, is the notion of virus isolation as a necessary criterion for defining genotypes/subtypes. This is not a scenario that needs to be addressed for most viruses where modern PCR based genotyping does not require virus isolation. Indeed, modern pathology laboratory diagnostics often rely almost solely on molecular detection, resulting in few viruses that have been reported in the literature ever having been isolated or cultured in a laboratory. However with retroviruses it is likely that many variant sequences will be identified following PCR of endogenized elements that may have arisen through recombination *in situ*. These may, or may not give rise to viable replicating viruses. As it happens, all of the reported KoRV sequences noted above have been derived from cultured viruses and so represent true genotypes/subtypes. Perhaps any new variant sequences derived only by PCR of extracted nucleic acid from koala tissue and/or blood should simply be referred to as variants, pending association with a replicating virus.

## KoRV variant prevalence in wild and captive populations

The vast majority of wild type sequences that have been generated and deposited in online databases are KoRV-A. All of the published literature reporting variant sequences to date has been generated from viruses isolated from koalas in captivity. Consequently, there is little information available on the spread of these and other variants in the wild Australian koala population. However it is certainly interesting that the KoRV-B and KoRV-J sequences are so similar, given their isolation from geographically separated koalas, suggesting a common infectious ancestry rather than *de novo* generation in their respective individual hosts. Ongoing studies in our laboratory are examining the presence of such variants in wild populations. Intriguingly, a variant we identified and sequenced in 2007 from a koala sampled on Kangaroo Island, off the south coast of Australia, is remarkably similar to KoRV-C, isolated from a koala at the Kobe Zoo and sourced from Queensland. This suggests a broader distribution of these variants in the wild than originally suspected, unless koalas from widely geographically distinct origins were brought together in a captive setting allowing horizontal transmission. Further testing of samples collected in the field will be required to answer this very important question.

## KoRV variant pathogenicity

A key question that lifts the discussion of these variants from an interesting taxonomic and evolutionary debate to one of critical importance to koala management is whether particular variants/subtypes are linked to more severe disease outcome. The study by Xu *et al.* (2013) directly examined two captive koala populations in the USA, one highly inbred colony where disease was uncommon and one where new animals were regularly introduced from Australia but where malignant neoplasias were noted. KoRV-B was isolated from animals from the latter but not former colonies. Furthermore, in this small data set, half of the animals from which KoRV-B was isolated (3/6) developed malignant lymphoma. The authors concluded that KoRV-B is associated with lymphoma development and that it may be more pathogenic. Given the nature of the likely generation of these particular variants, it is also possible that the isolation of KoRV-B may simply be a surrogate marker for increased recombination activity, which in turn could drive the increased pathogenic outcome.

Regardless of the answer to this mechanistic question, it is imperative that further studies are performed to validate this proposed pathogenic role for KoRV-B. Any correlation between the presence of a particular KoRV subtype and malignant disease in koalas will have clear implications for breeding programs that maintain stable koala populations in captivity. In wild populations, determining KoRV subtype prevalence and geographic spread should provide valuable insight into the spread of disease and perhaps offer clues to intervention strategies.

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