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Koala Retrovirus Genetic Diversity and Transmission: Advice for Breeders

Briony A. Joyce

The University of Queensland, St Lucia Queensland QLD 4072, Australia

Abstract. The rapid spread of koala retrovirus (KoRV) across Australia and international zoo populations has necessitated appropriate control measures. Along with pathogenicity, the genetic diversity of the virus and how it transmits between animals also needs to be considered when deciding the most suitable measures. Next generation sequencing has become the gold standard approach for KoRV diversity studies due to the high sensitivity, accuracy, and throughput. This approach has identified a large proportion of known KoRV diversity and has provided a broader understanding of KoRV prevalence and abundance within koala (*Phascolarctos cinereus*) populations, specifically identifying individuals with low diversity. Recent evidence has demonstrated that exogenous KoRV transmits from mother to joey, likely through the ingestion of milk and/or pap, and that koalas are not likely to acquire additional KoRV subtypes/sequences later in life. This finding strongly indicates that breeding with KoRV negative or endogenous KoRV-A positive only females is the best chance at alleviating exogenous KoRV from koala populations worldwide. Captive breeders are therefore urged to determine the KoRV profile of all animals included in their breeding program through deep sequencing methods (where feasible) and use this to inform their future breeding regimes.

Introduction

Koala retrovirus (KoRV) is a gammaretrovirus discovered in 2000, closely related to feline leukaemia virus (FeLV) and gibbon ape leukaemia virus (GaLV) (Hanger *et al*., 2000). Alike other retroviruses, KoRV is putatively associated with the onset of neoplasia and other associated cancers in koalas (*Phascolarctos cinereus*) (including leukaemia and lymphoma) and is suspected to cause immunodeficiency and opportunistic disease in this species (Tarlinton *et al*., 2005; Fabijan *et al*., 2020). Whilst habitat destruction and fragmentation, domestic dog attacks and vehicle collisions are among the greatest threats that wild koalas face, the putative KoRV-associated diseases are currently the major contributor towards captive koala mortality. Initially established from wild koala gene pools, captive koala breeding programs are now commonplace in zoos around Australia and internationally. These animals are often exchanged between institutions and, in some cases, exported overseas to increase genetic diversity within colonies. Occasionally, wild koalas are also incorporated into the captive setting and either used for display or as part of the breeding program. Animals approved for this integration are often hand raised and show no wild instincts or have sustained significant injuries, making them unfit to return to the wild. Understanding how to effectively manage these captive populations to reduce the impact from this virus is therefore crucial. The current advice based on recent publications will be addressed in this manuscript.

KoRV genetic diversity

KoRV was first discovered by Hanger *et al*. (2000) in koala genomic DNA through PCR with degenerate primers. This prototypic sequence was later classified as KoRV-A. Since its discovery, more than 10 additional subtypes (B-M) have been identified across multiple institutions around the world (Xu *et al*., 2013; Shojima *et al*., 2013; Xu *et al*., 2015; Chappell *et al*., 2017; Joyce *et al*., 2021; Blyton *et al*., 2021), each with a unique amino acid signature within the receptor binding

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ORCID iD: Briony A. Joyce https://orcid.org/0000-0001-7713-1648
Corresponding author: Briony A. Joyce b.joyce@uq.net.au
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