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A Novel Exogenous Retrovirus Isolated from Koalas *(Phascolarctos cinereus)* with Malignant Neoplasias in a United States Zoo

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Abstract. Koalas in US zoos were screened for koala retroviruses in an effort to determine the viral mechanism for koala retrovirus induced malignant neoplasias. Although the previously characterized koala retrovirus (KoRV-A) was present in all US koalas, some koalas were also infected by a novel koala retrovirus, termed KoRV-B. The genome of KoRV-B is highly related to KoRV-A; however, certain regions within the viral genome, including the envelope gene, displayed diversity. These differences are sufficient to allow KoRV-B to employ a receptor (a thiamine transporter) that differs from that used by KoRV-A (a phosphate transporter). Of great interest was the strong correlation between the presence of KoRV-B and malignant disease (lymphomas) in koalas. All koalas that died from lymphoma were KoRV-B positive as were the dead joeys ejected from the pouch of KoRV-B positive dams. We found no evidence of KoRV-B transmission from sires to offspring but did from dam to offspring through de novo infection, rather than via genetic inheritance like KoRV-A. Detection of KoRV-B in native Australian koalas should provide a history, and a mode for remediation, of leukemia/lymphoma currently endemic in this population.

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Endogenous retroviruses (ERVs) have played an integral role in mammalian evolution. Elements derived from these genetically inherited ERVs comprise as much as 8% of the human genome (Bromham, 2002) and are known to regulate the expression of highly conserved gene clusters (van de Lagemaat *et al*., 2003). The majority of ERVs are defective remnants of exogenously transmitted retroviruses that likely integrated into the germline of mammalian progenitors millions of years ago. The discovery of koala retrovirus (KoRV) (Hanger *et al*., 2000) described the first endogenous retrovirus that is still actively producing infectious particles capable of transspecies transmission while being retained as an inherited part of the host genome. KoRV isolates described to date in Australia, Germany, and Japan have shown very limited genetic diversity (>99% sequence identity), characteristic of an endogenous virus. However, considering the likelihood that koala genomes also contain newly integrated forms of KoRV, we screened cohorts of 13 koalas from the Los Angeles Zoo (LAZ) and 28 koalas from the San Diego Zoo (SDZ) to detect more diverse KoRV isolates (Xu *et al*., 2013).

PCR amplification of viral sequences from koala specimens obtained from the LAZ was performed using genomic DNA prepared from blood or tissue and from viral RNA present in plasma, with primers specific to KoRV. Additionally, a viral marker rescue assay was developed using human cells containing an integrated replication incompetent retroviral genome that expresses GFP (green fluorescent protein). The GFP genome can be rescued and assembled into virus if KoRV is present in the koala peripheral blood mononuclear cells (PBMCs) co-cultured with the human-GFP cells. If KoRV rescues the GFP genome then supernatant containing KoRV-GFP vectors can infect naïve target cells that will