The Koala and its Retroviruses:
Implications for Sustainability and Survival

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Koala Retrovirus (KoRV): Are Humans at Risk of Infection?

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Abstract. This manuscript summarizes the break-out session held on koala retrovirus (KoRV): Any risks of human infection? 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 break-out session were to discuss the zoonotic risk of koala retroviruses, the necessity to test human populations for exposure, and precautions to be taken to protect humans who transport or handle koalas *(Phascolarctos cinereus)*. Currently there is no evidence to support the zoonotic potential of KoRV, and the necessity to test humans for KoRV infection needs to be further justified. We recommend strict compliance with standard precautions when handling animals.

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Zoonotic potential of retroviruses

Based on their genomic structure, retroviruses are generally classified as either simple or complex. A number of complex retroviruses from nonhuman primates such as simian immunodeficiency virus, simian leukemic virus, and foamy virus have the capacity to jump species and infect humans. Koala retrovirus (KoRV), by contrast, is a simple retrovirus of the gammaretrovirus genus, whose members do not contain the accessory proteins required to counteract human cell restriction factors. Replication of a gammaretrovirus in human cells is therefore largely inhibited by human restriction factors, such as APOBEC 3 enzymes and tripartite motif (TRIM) proteins.

Viruses made in non-primate cells will also be inactivated by the complement system following binding of naturally occurring antibodies to the alpha gal epitope.

In the extensive studies and discussions that followed the discovery of a putative human retrovirus, xenotropic murine leukemia virus-related virus (XMRV), which was first isolated from human prostate cancer tissues but later shown to be a laboratory contaminant, the likelihood of a gammaretrovirus jumping species and replicating efficiently in humans was proposed and dismissed. Some gammaretroviruses have been shown to have the ability to infect human cells efficiently in culture yet show no evidence of transmission to humans, for example, feline leukemia virus (specifically FeLV subgroup B) and porcine endogenous retrovirus (PERV), both of which are related to KoRV. While 2–3% of U.S. domestic cats are infected with FeLV, which causes cat leukemia and lymphoma, FeLV infection of humans has not been detected (Levy *et al*., 2008; Hartmann, 2012). Neither has PERV been demonstrated to be zoonotic, PERV has not been detected in patients who

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have received clinical xenotransplantation of pig materials. In fact, when immunosuppressed small animals or nonhuman primates are inoculated with PERV, no infection occurs.

Transmission of KoRV

The gibbon ape leukemia virus (GALV), a highly oncogenic gammaretrovirus capable of inducing myeloid leukemia in juvenile gibbons, is conspecific with KoRV. Infectious GALV viruses have been detected in the blood, urine, and feces of infected gibbons and are known to be transmitted *in utero*, postnatally, or through contact of virus-free gibbons with infected gibbons. The koala retrovirus KoRV-A (subtype A) is an endogenous infectious retrovirus and transferred both horizontally and vertically in the germline, while the newly discovered KoRV-B (subtype B) appears to be exogenous (Xu *et al.,* 2013). KoRV-B is presumably transmitted either *in utero* or through the dam's milk, as evidenced by the detection KoRV-B from eight offspring of KoRV-B positive dams including two joeys ejected from the pouch of KoRV-B positive dams while the offspring of KoRV-B positive sires and KoRV-B negative dams are KoRV-B negative. Whether KoRV-B can be transmitted through contact of uninfected koalas *(Phascolarctos cinereus)* with infected koalas is as yet unknown. KoRV has been detected in blood and feces, but the assessment of virus in saliva and urine has not yet been performed.

Discussion

What we currently know about the risk of human infection by KoRV

The zoonotic potential of KoRV is generally compared to that of FeLV, a related virus that infects domestic cats, which has not been found to infect caregivers or to be associated with any disease in humans. To date, there is no evidence that any gammaretrovirus can jump species to infect humans, and both KoRV-A and KoRV-B are associated with diseases in koalas only. Establishing actual zoonotic risk from KoRV, however, requires more data on:

- *Routes of transmission:* whether KoRV-B can be transmitted from infected adult koala to uninfected adult koala through contact.
- *Mutation rates:* whether KoRV-A or B can mutate at rates high enough to circumvent innate human immune and antiretroviral response.
- *Epizoonotic transmission:* whether KoRV can be transmitted to predators of koalas.
- *Pathogenic potential:* The precise role of KoRV-A and B in koala disease remains to be firmly established.
- *Animal models for KoRV:* whether KoRV-A and B can replicate in non-human primates and cause leukemia or lymphoma.
- *Replication of virus in humans*: whether KoRV can replicate efficiently in primary human cells.
- *Mechanisms of human KoRV inhibition:* how different human restriction factors inhibit KoRV replication.

Testing humans for KoRV infection

Although the evidence currently suggests no zoonotic potential for KoRV, including exogenous KoRV-B, prudence suggests the need to exclude even the remote possibility of KoRV infection by testing the small population that comes into close contact with koalas and animal viruses (animal keepers, veterinarians, registered veterinary technicians, researchers, and possibly tourists with koala contact). PCR assays to detect KoRV specific sequences in blood and tissue samples are available, and ELISA and western blot assays can also be developed to test for KoRV antibody levels and viral protein expression. Any program to test human samples for KoRV infection, however, will depend on the following considerations:

- The justifiability of using resources to test humans for KoRV when there is currently no evidence of zoonotic transmission.
- How to interpret any positive results, given that laboratory contamination (as with XMRV), the sensitivity of modern assays, and cross-reactions can all result in false positives.
- Particularly in the absence of any documented transmission events even a low frequency of false positives is potentially controversial.

Guidelines for animal caregivers

Since zoonotic diseases can threaten the health of animal caregivers, zoos generally institute rigorous precautions, especially for the handling of non-human primates and other species at high risk for the transmission of serious zoonoses. Guidelines for handling nonhuman primates are available to prevent highly infectious zoonotic diseases.

Until now, koalas have not been considered in this category and are therefore handled using standard hygiene procedures. However, with the discovery of exogenous retrovirus KoRV-B, new questions have arisen about the potential risk to humans. While it is not yet clear whether KoRV-B presents a zoonotic threat to humans, contact with animals always carries some risk of disease transmission. At present there are no specific guidelines for handling koalas. To reduce the possibility of zoonotic diseases, we recommend strict adherence to standard precautions when handling animals especially when performing necropsies, and that every new koala should be presumed as KoRV-B positive before tests.

To decide whether it is necessary to establish more stringent guidelines for handling koalas, researchers will need to collect more data on:

- Viral load in koala urine, feces, and saliva.
- Effect of human complement on koala-derived viruses.
- Replication of KoRV in primary human cells.
- Inhibitory effects of different human restriction factors on the replication of KoRV.
- Reservoir for KoRV in animals.

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