The Koala and its Retroviruses: Implications for Sustainability and Survival

edited by

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Retrovirus-related Disease in Zoo-based Koalas (Phascolarctos cinereus) in North America

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ABSTRACT. Koala retrovirus (KoRV)-related disease is a major suspected cause of death in koalas *(Phascolarctos cinereus)* in zoos in North America. There are currently eleven zoos exhibiting koalas in North America. A mortality survey of these institutions indicated that mortalities directly related to KoRV (e.g., lymphoma, leukemia, anemia, bone marrow hypoplasia, osteochondromatosis) and mortalities suspected to be KoRV-related (e.g., immunosuppression, unusual opportunistic infections [e.g., Coccidioidomycosis], potentially other neoplasia) account for 41% of deaths. Testing of the living North American koala population for a recently reported, exogenous koala retrovirus variant (KoRV-B) identified four KoRV-B-positive individuals in a population of 54 koalas (7.4%).

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Koalas (Phascolarctos cinereus) have been exhibited in North America since 1925 with the North American regional studbook tracking koalas since 1971. Koala retrovirus (KoRV) has been suspected to be a major cause of mortality in some zoo-based koala populations in southeastern Queensland, Australia, where it has been reported anecdotally to cause up to 80% of mortalities (Hanger et al., 2000). The incidence of mortality related to KoRV in US-based koalas has not been previously reported. In 2011, a novel variant of KoRV (KoRV-B) was reported following a number of malignant cancers and deaths related to KoRV in koalas at the Los Angeles Zoo (Xu et al., 2011; Xu et al., 2013). KoRV-B appears to be exclusively exogenous, unlike the originally sequenced endogenous KoRV-A which is both exogenous and endogenous (Xu et al., 2013). The prevalence of KoRV-B has not been previously reported in the US and is currently unknown in Australia.

Methods and materials

A KoRV mortality survey was emailed in February 2013 to veterinarians at nine of the eleven institutions in North America currently exhibiting northern koalas. Two zoos were not emailed surveys due to only recent acquisitions of northern koalas with no deaths. All nine emailed-institutions responded (Table 1).

Fresh-EDTA treated blood samples were collected from koalas currently living in ten zoos in the US. One zoo (Miami Metro Zoo) was excluded due to the geriatric, nonreproductive age of their two koalas. Samples were sent at room temperature overnight and tested for the presence of KoRV-B by PCR using plasma and peripheral blood mononuclear cell DNA (Table 2).

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institution	number of deaths ^a	direct KoRV ^b	suspect KoRV ^c	total %
San Diego Zoo ^f	124	36	13	40
Los Angeles Zoo	7	4	1	71
San Francisco Zoo	14	2	2	29
Riverbanks Zoo and Garden	5	2	0	40
Cleveland Metroparks Zoo ^d	8	2	4	75
Columbus Zoo and Aquarium ^d	7	1	0	14
Miami Metro Zoo ^d	1	1	0	100
Lowry Park Zoo ^{d,e}	3	0	1	33
Albuquerque Zoo ^e	0	n/a	n/a	n/a
Dallas Zoo ^d	0	n/a	n/a	n/a
Palm Beach Zoo ^e	0	n/a	n/a	n/a
total	169	48	21	41

Table 1. Deaths related directly or suspected to be related to KoRV in northern koalas (Phascolarctos cinereus) at US zoos.

a Deaths with necropsy and histopathology results.

b Deaths directly associated with KoRV (e.g., lymphoma, leukemia, anemia, bone marrow hypoplasia, osteochondromatosis).

c Deaths suspected to be associated with KoRV (immunosuppression, unusual opportunistic infections, e.g., coccidioidomycosis, other neoplasia).
d San Diego Zoo origin koalas.

e Los Angeles Zoo origin koalas.

f Includes koalas on loan to other non-named zoological institutions.

Table 2. KoRV-B testing of living koala	s (Phascolarctos cinereus) at US zoos (Febr	ruary 2013).

institution	number of koalas tested	number of koalas not tested	KoRV-B	total %
San Diego Zoo	14	5 (incl. 3 joeys)	0	0
San Diego Zoo Quarantine	3	0	1	33
Los Angeles Zoo	6	1 (joey)	0	0
San Francisco Zoo	4	0	0	0
Riverbanks Zoo and Garden	2	2 (incl. a joey)	0	0
Cleveland Metroparks Zoo ^a	1	5	0	0
Columbus Zoo and Aquarium	^a 2	0	0	0
Miami Metro Zoo ^a	0	2		
Lowry Park Zoo ^a	1	0	0	0
Albuquerque Zoo ^b	2	0	2	100
Dallas Zoo ^a	2	0	0	0
Palm Beach Zoo ^b	2	0	1	50
total	39	15	4	7.4

a San Diego Zoo koalas

b Los Angeles Zoo koalas

Discussion

As is the situation in some zoo-based koala populations in Australia, KoRV is likely a significant cause of mortality in koalas in the US with it associated with up to 41% of all mortalities. These results highlight the clinical importance of this virus and that the sustainability of the population could be greatly increased if measures to reduce the expression of KoRV-related disease could be discovered.

The significance of the presence of KoRV-B in some zoos in the US is unknown at this time. Further work is required to determine the pathogenicity of KoRV-B and whether it is of any more concern than KoRV-A. Extensive testing in Australia coupled with mortality reviews may answer these questions. The findings could determine the relative importance of a KoRV-B-negative koala population and could negatively impact the sustainability of the US koala population if there is a need to keep KoRV-B-positive and KoRV-B-negative koalas separated. ACKNOWLEDGMENTS. The authors acknowledge the support of the Association of Zoos and Aquariums Koala Species Survival Plan member institutions and their staff in providing samples and mortality data for this study.

References

- Hanger, J. J., L. D. Bromham, J. J. McKee, T. M. O'Brien, and W. F. Robinson. 2000. The nucleotide sequence of koala (*Phascolarctos cinereus*) retrovirus: a novel type C endogenous virus related to gibbon ape leukemia virus. *Journal of Virology* 74(9): 4264–4272.
 - http://dx.doi.org/10.1128/JVI.74.9.4264-4272.2000
- Xu, W., C. K. Stadler, D. Kim, M. Alemaheyu, W. Switzer, G. W. Pye, and M. V. Eiden. 2011. Identification of a novel gammaretrovirus in koalas (*Phascolarctos cinereus*) in US zoos. 23rd Workshop on Retroviral Pathogenesis, Montpellier, France.
- Xu, W., C. K. Stadler, K. Gorman, N. Jensen, D. Kim, H. Zheng, S. Tang, W. M. Switzer, G. W. Pye, and M. V. Eiden. 2013. An exogenous retrovirus isolated from koalas with malignant neoplasias in a US zoo. *Proceedings of the National Academy of Sciences, USA* 110(28):11547–11552. http://dx.doi.org/10.1073/pnas.1304704110