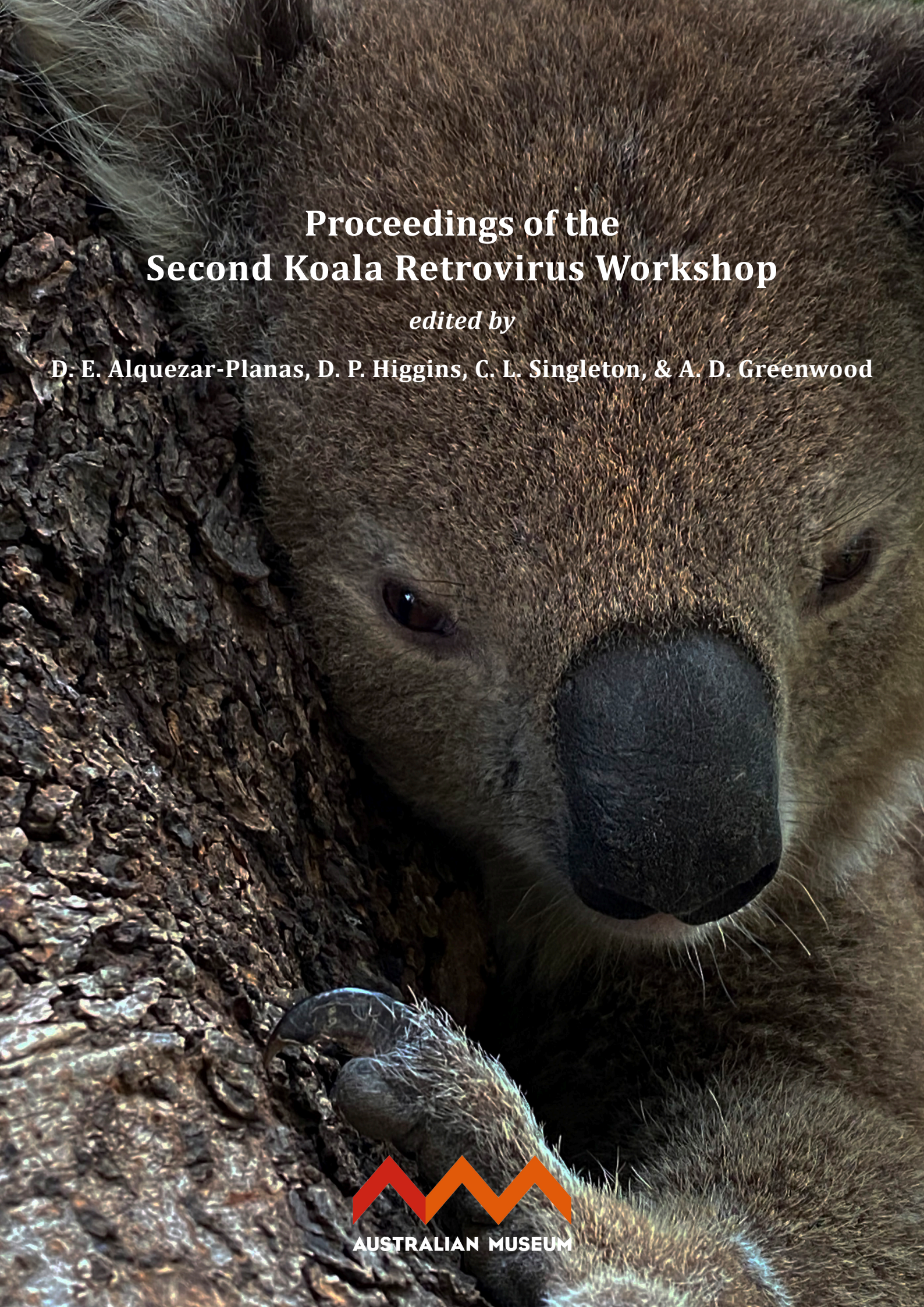
Proceedings of the Second Koala Retrovirus Workshop

edited by

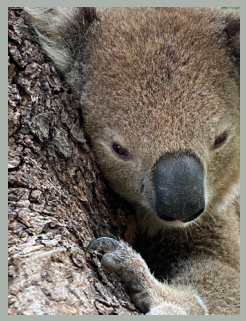
D. E. Alquezar-Planas, D. P. Higgins, C. L. Singleton, & A. D. Greenwood



AUSTRALIAN MUSEUM

Technical Reports of the Australian Museum Online **no. 38**

Table of Contents



Cover photo
by Damien P. Higgins

A series of peer-reviewed papers, edited by David E. Alquezar-Planas, Damien P. Higgins, Cora L. Singleton, & Alex D. Greenwood, and a discussion summary, from the *Second Koala Retrovirus Workshop* held online, 25–27 May 2021. Published 21 June 2023, in *Technical Reports of the Australian Museum Online* number 38, ISSN 1835-4211 (online).

The Australian Museum is a statutory authority of, and principally funded by, the NSW State Government.

Tech. Rep. Aust. Mus. Online
Number 38, pp. 49–52, 2023
https://doi.org/10.3853/j.1835-4211.38.2023.1841

Technical Reports of the Australian Museum Online
a peer-reviewed open-access journal
published by the Australian Museum, Sydney
communicating knowledge derived from our collections
ISSN 1835-4211 (online)

Koala Retrovirus Status and Putative Koala Retrovirus-Associated Diseases in Koalas (*Phascolarctos cinereus*) in North American Zoos

Cora L. Singleton and Chris Hamlin-Andrus

San Diego Zoo Wildlife Alliance, San Diego, CA 92101, United States of America

Abstract. The living koala population in North America is predominantly descended from koalas imported from a single Australian facility in 1976 and 1981, with several smaller imports from other facilities between 1985 and 2013. Koala retrovirus subtype B (KoRV-B) entered the North American population via imports in 2005, 2008, and 2013. The 2005 and 2008 KoRV-B positive lineages are deceased, but the 2013 KoRV-B positive lineage has seven surviving koalas, including one female of breeding age. Three koalas born to KoRV-B negative dams were documented as being KoRV-B positive at 15 months of age after nursing from a KoRV-B positive female. The prevalence of koala retrovirus subtype A (KoRV-A) detection in North America is 100% but the prevalence of KoRV-B detection is 17%. Lymphoid neoplasia is a common cause of mortality, dating back to founder koalas. Most cases of lymphoid neoplasia have occurred in presumptive KoRV-B negative koalas, and many cases have occurred between the ages of four and nine years. Familial clusters of lymphoid neoplasia are apparent. Additionally, myelodysplasia and fatal peripheral cytopenias are important putative KoRV-associated diseases that cause mortality in koalas in North America, with higher prevalence in koalas younger than two years of age. Since 2013, breeding of known KoRV-B positive koalas has been managed, to maintain separation from the remainder of the KoRV-B negative population.

Koalas in North America

The San Diego Zoo opened in 1916, after a small zoo exhibit was left behind at the end of the 1915–1916 Panama–California Exposition. Nine years later, in 1925, the zoo received international attention when Australia donated animals, including the first two koalas to arrive in the United States. In the 1950s, four koalas (two males and two females; 2.2) were imported but did not produce offspring. In the 1960s, two more importations occurred (three males and three females; 3.3). These koalas produced 11 offspring, three of which lived into the 1970s when this lineage ended. In 1976, six mature koalas (two males, four females, and one unknown sex pouch young; 2.4.1) were imported to San Diego Zoo. These koalas, plus an additional seven koalas (one male and six females; 1.6) imported in 1981 from the same facility in Australia, are the founders of the current North American koala population. Later, several other zoos imported smaller numbers of koalas from a variety of facilities in Australia. The last importation occurred in 2013.

The current North American koala population consists of 52 koalas living at 10 different zoos. San Diego Zoo Wildlife Alliance (SDZWA) cares for the largest colony of koalas outside of Australia (32 resident koalas) and manages the North American koala population through leadership of the Association of Zoos and Aquariums (AZA) Koala Species Survival Plan (SSP) program. All 10 zoos holding koalas participate in the Koala SSP program. In 1983, SDZWA established the Koala Education and Conservation Program (KECP) to support koala care, education, research, and

Keywords: koala retrovirus, KoRV, North America, leukaemia, lymphoma, myelodysplasia
ORCID iD: Cora L. Singleton https://orcid.org/0009-0007-1856-4073
Corresponding author: Cora L. Singleton csingleton@sdzwa.org
Submitted: 1 May 2022 **Accepted**: 5 May 2023 **Published**: 21 June 2023 (online only)
Publisher: The Australian Museum, Sydney, Australia (a statutory authority of, and principally funded by, the NSW State Government)
Citation: Singleton, Cora L., and Chris Hamlin-Andrus. 2023. Koala retrovirus status and putative koala retrovirus-associated diseases in koalas (*Phascolarctos cinereus*) in North American Zoos. In *Proceedings of the Second Koala Retrovirus Workshop*, ed. D. E. Alquezar-Planas, D. P. Higgins, C. L. Singleton, and A. D. Greenwood. *Technical Reports of the Australian Museum Online* 38: 49–52. https://doi.org/10.3853/j.1835-4211.38.2023.1841

Table 1. Koala retrovirus subtype B (KoRV-B) status of 52 koalas living in North America, March 2021.

	male		female		unknown		total	
	tested[a]	all[b]	tested[a]	all[b]	tested[a]	all[b]	tested[a]	all[b]
KoRV-B Positive	6	7	1	2	0	0	7	9
KoRV-B Negative	18	20	16	21	0	2	34	43
total	24	27	17	23	0	2	41	52
prevalence	25.0%	25.9%	5.9%	8.7%	0%	0%	17.1%	17.3%

[a] Laboratory tested using qPCR for KoRV-A and KoRV-B.

[b] Total number of koalas in each category, both laboratory-confirmed KoRV-B status and expected KoRV-B status based on KoRV-B status of the dam.

conservation. Five zoos in the United States and eight zoos in Europe are partners in the KECP.

History of koala retrovirus in North America

Based on the assumption of 100% transmission of koala retrovirus subtype B (KoRV-B) from dam to joey (Quigley *et al*., 2018), analysis of maternal pedigree of qPCR-confirmed KoRV-B negative and KoRV-B positive female koalas indicates that the founder koalas can be assumed to be KoRV-B negative. The first KoRV-B positive koalas (two full-sibling females) arrived in North America in 2005 and 2008. The female imported in 2005 and her four KoRV-B positive descendants over two generations died by 2016 from lymphoid neoplasia, age range 4.0 to 5.5 years (Xu *et al*., 2013). The female imported in 2008 and her single offspring died by 2014 at the ages of 7.5 years from mesothelioma and 2.5 years from pneumonia, respectively.

In 2013, two (1.1) of three (2.1) koalas imported from Australia were determined to be KoRV-B positive after arrival. The imported KoRV-B positive female died from lymphoid neoplasia at 11.5 years of age and six of her seven descendants are alive (4.2; age range 1.5–7.5 years). The imported KoRV-B positive male is still alive as of this writing.

Health surveillance and current koala retrovirus status

Health surveillance: Koalas receive regular comprehensive health examinations which include physical examination, computed tomography study, ultrasonographic exam, complete blood count, serum biochemistry panel, urinalysis, *Cryptococcus* spp. antigen test, cytological evaluation of a bone marrow aspirate (Dr Nicole Stacy, University of Florida), and koala retrovirus subtype A (KoRV-A) and KoRV-B qPCR (Molecular Diagnostics Laboratory, San Diego Zoo Wildlife Alliance) if status is undetermined. A complete post-mortem evaluation is conducted on every koala that dies, which includes gross examination, histopathological evaluation of a complete set of standardized tissue samples, and banking of selected biological samples. Gross and microscopic findings are recorded, and a cause of death is identified. For this discussion, lymphoid neoplasia includes lymphoma and lymphocytic leukaemia, and myelodysplasia refers to blood and bone marrow disorders ranging from subclinical nuclear dysplastic changes to fatal peripheral cytopenias.

KoRV testing: Testing for selected KoRV subtypes began in 2010 for research purposes, and this included testing of post-mortem samples dating back to 2008 in response to a familial cluster of mortalities due to malignant neoplasms (Xu *et al*., 2013). In 2014, diagnostic testing for detection of KoRV-A and KoRV-B was established at the SDZWA Molecular Diagnostics Laboratory. The KoRV assays (qPCR for KoRV-A and KoRV-B envelope genes) were developed based on protocols from Xu (Xu *et al*., 2013), William Switzer (Centers for Disease Control and Prevention, personal communication), and Maribeth Eiden (National Institute of Health, personal communication).

KoRV subtype prevalence: As of March 2021, 79% (41/52) of koalas living in North America had been tested for the presence of KoRV-A and KoRV-B using qPCR (Table 1). The 11 untested koalas were assigned expected KoRV-B status based on KoRV-B status of the dam. The KoRV-A subtype prevalence in North America was 100%. Overall, the KoRV-B subtype prevalence was about 17%, with a higher prevalence in males (25.0–25.9%) relative to females (5.9–8.7%).

Allonursing and KoRV-B transmission: Detailed breeding and parturition records are maintained for the North American koala population. One KoRV-B positive lactating female was living with three KoRV-B negative lactating females, all four with joeys of approximately the same age. Joeys were observed sharing dams often. Subsequently, all four joeys were confirmed to be KoRV-B positive by qPCR at 15 months of age. Since three of the joeys were born to KoRV-B negative dams and later tested KoRV-B positive, it is suspected that they acquired KoRV-B by nursing from the KoRV-B positive dam.

Sub-clinical myelodysplasia: Routine cytological evaluation of bone marrow aspirates began in 2017 to screen for sub-clinical myelodysplasia. Of 26 clinically healthy koalas evaluated to date, there was cytological evidence of cellular dysplasia in 77% (20/26) of koalas (Table 2). Dysplastic

Table 2. Koala retrovirus subtype B (KoRV-B) status and degree of myelodysplastic changes diagnosed by cytological evaluation of bone marrow aspirates from clinically healthy koalas living in North America.

	normal	minimal	mild	moderate	total
KoRV-B positive[a]	2	3	2	4	11
KoRV-B negative[a]	4	6	5	0	15
total	6	9	7	4	26

[a] Laboratory confirmed using qPCR.

Table 3. Crude and age-stratified mortality rates for lymphoid neoplasia (lymphoma and lymphocytic leukaemia) and myelodysplasia, two putative koala retrovirus-associated diseases, in koalas in North America 1959–2020.

	mortalities	necropsy reports	lymphoid neoplasia	myelodysplasia
1959–1975	21	12	8% (1/12)	0% (0/12)
1976–2020	247	195	21% (40/195)	7% (13/195)
pouch young (< 0.5 years)	63	24	0% (0/24)	0% (0/24)
immature (0.5–1.5 years)	29	28	0% (0/28)	21% (6/28)
mature (> 1.5 years)	155	143	28% (40/143)	5% (7/143)

changes ranged from minimal to moderate and were seen more frequently in the erythroid line than the myeloid line, and rarely in the megakaryocytic line. The presence and degree of dysplastic changes does not appear to be related to KoRV-B status. Assessment of cytological changes in individual koalas over time is in progress.

Mortality due to putative koala retrovirus-associated diseases

Two important putative koala retrovirus-associated diseases (PKAD) in the North American koala population are lymphoid neoplasia and myelodysplasia (Gillett, 2023). Between 1959 and 1975, 8% (1/12) of mortalities were attributed to lymphoid neoplasia (Table 3). Between 1976 and 2020, the cause of death of 21% (40/195) of koalas was attributed to lymphoid neoplasia and 7% (13/195) of koalas was attributed to myelodysplasia (Table 3). The mortality rate due to lymphoid neoplasia and myelodysplasia varies by age, KoRV status, and sometimes pedigree in the North American koala population.

Lymphoid neoplasia and KoRV status: Both KoRV-B positive and presumed KoRV-B negative koalas died from lymphoid neoplasia (Table 4). Of 171 immature (0.5–1.5 years of age) and mature (> 1.5 years of age) koalas that died between 1976 and 2020, 95% (162/171) were presumed KoRV-B negative and 5% (9/171) were confirmed KoRV-B positive. Of these 171 koalas, 23% (40/171) died from lymphoid neoplasia. Of the 40 koalas that died from lymphoid neoplasia, 82.5% (33/40) were presumed KoRV-B negative koalas, while 17.5% (7/40) were confirmed KoRV-B positive koalas. Mortality rate due to lymphoid neoplasia was 20% (33/162) in KoRV-B negative koalas and 78% (7/9) in KoRV-B positive koalas. Of the seven KoRV-B positive koalas that died from lymphoid neoplasia, five were from the same direct maternal lineage (Xu *et al*., 2013). It is not possible to determine if the mortality rate due to lymphoid neoplasia varies by KoRV status in the North American koala population based on this data, due to small sample size and confounding variables such as potential inbreeding effects and fixation of KoRV-A integration sites and dysregulation of oncogenes (McEwen *et al*., 2021).

Lymphoid neoplasia and age: The mortality rate due to lymphoid neoplasia varies by age in the North American koala population, with only mature koalas dying from lymphoid neoplasia (Table 3). Approximately 62% of deaths due to lymphoid neoplasia occurred in the age range 4–9 years. The highest mortality rate (82%) due to lymphoid neoplasia was age 4–5 years, which includes the familial cluster of five mortalities previously described (Xu *et al*., 2013). Mortality rate for lymphoid neoplasia ranged from 0% to 55% for other ages, with no cases of lymphoid neoplasia in koalas less than 2 years of age or greater than 18 years of age.

Lymphoid neoplasia and pedigree: Based on the assumption of 100% transmission of KoRV-B from dam to joey (Quigley *et al*., 2018), it appears that KoRV-B entered the North American koala population with importation of four positive koalas (full siblings 0.1 in 2005 and 0.1 in 2008; unrelated 1.1 in 2013). Therefore, there are two KoRV-B positive maternal lineages. Within one of these lineages, 15% (6/40) of the cases of lymphoid neoplasia occurred. In contrast, 45% (18/40) of lymphoid neoplasia can be traced back through two KoRV-B negative maternal pedigrees. Further analysis is required to evaluate the relationship of KoRV, lymphoid neoplasia, and pedigree in the North American koala population.

Myelodysplasia: The mortality rate due to myelodysplasia varied by age in the North American koala population (Table 3). Of the 13 koalas that died from myelodysplasia, 46% were immature koalas and 54% were mature koalas. From 1976–2020, myelodysplasia was a leading cause of death for immature koalas (6/28, 21%) but was an uncommon cause of death in mature koalas (7/143, 5%). In seven mature koalas that died from myelodysplasia, six were 5–9 years old and one was 12–13 years old. All 13 koalas that died from myelodysplasia were KoRV-B negative.

Husbandry and breeding management

Since 2013, breeding of confirmed KoRV-B positive koalas has been managed, to maintain separation from the remainder of the KoRV-B negative population. Between 2013 and 2018, most arranged breeding was between KoRV-B negative

Table 4. Koala retrovirus subtype B (KoRV-B) status and death due to lymphoid neoplasia (lymphoma and lymphocytic leukaemia) and myelodysplasia, two putative koala retrovirus-associated diseases, in koalas in North America 1976–2020.

	lymphoid neoplasia	myelodysplasia	other	total
KoRV-B positive[a]	7[c]	0	2	9
KoRV-B negative[b]	33	13	116	162
total	40	13	118	171

[a] Laboratory confirmed positive using qPCR.

[b] Both laboratory-confirmed negative using qPCR and presumed negative based on KoRV-B status of the dam.

[c] Five of seven cases from the same maternal lineage.

males and KoRV-B negative females. Prior to discovery of KoRV status, one KoRV-B positive female bred with two KoRV-B negative males. Starting in 2020, KoRV-B positive males were bred with the single remaining KoRV-B positive female.

Based on very low horizontal transmission of KoRV-B via casual contact (Quigley *et al*., 2018), the last remaining KoRV-B positive female koala was co-housed with several KoRV-B negative female koalas. After apparent KoRV-B transmission via allonursing, the KoRV-B positive breeding female was isolated during lactation.

Acknowledgements. The authors acknowledge the extensive contributions of the San Diego Zoo Wildlife Alliance staff including: Lisa Martin, Jennifer Moll, Jennifer Roesler, Lindsey King, Scott Rammel, Candis Malcom (Wildlife Care); Josephine Braun, Patricia Gaffney, Carmel Witte (Disease Investigations), and the Wildlife Health team. Special thanks to Nicole Stacy (University of Florida) and Cindy Stadler (Los Angeles Zoo) for valued long-term collaboration in koala care.

References

Gillett, Amber K. 2023. Defining putative koala retrovirus-associated disease in koalas. In *Proceedings of the Second Koala Retrovirus Workshop*, ed. D. E. Alquezar-Planas, D. P. Higgins, C. L. Singleton, and A. D. Greenwood. *Technical Reports of the Australian Museum Online* 38: 23–29.
https://doi.org/10.3853/j.1835-4211.38.2023.1836

McEwen, G. K., D. E. Alquezar-Planas, A. Dayaram, A. Gillett, R. Tarlinton, N. Mongan, K. J. Chappell, J. Henning, M. Tan, P. Timms, P. R. Young, A. L. Roca, and A. D. Greenwood. 2021. Retroviral integrations contribute to elevated host cancer rates during germline invasion. *Nature Communications* 12(1): 1316.
https://doi.org/10.1038/s41467-021-21612-7

Quigley, B. L., V. A. Ong, J. Hanger, and P. Timms. 2018. Molecular dynamics and mode of transmission of koala retrovirus as it invades and spreads through a wild Queensland koala population. *Journal of Virology* 92(5): e01871-17.
https://doi.org/10.1128/JVI.01871-17

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.
https://doi.org/10.1073/pnas.1304704110