Proceedings of the Second Koala Retrovirus Workshop

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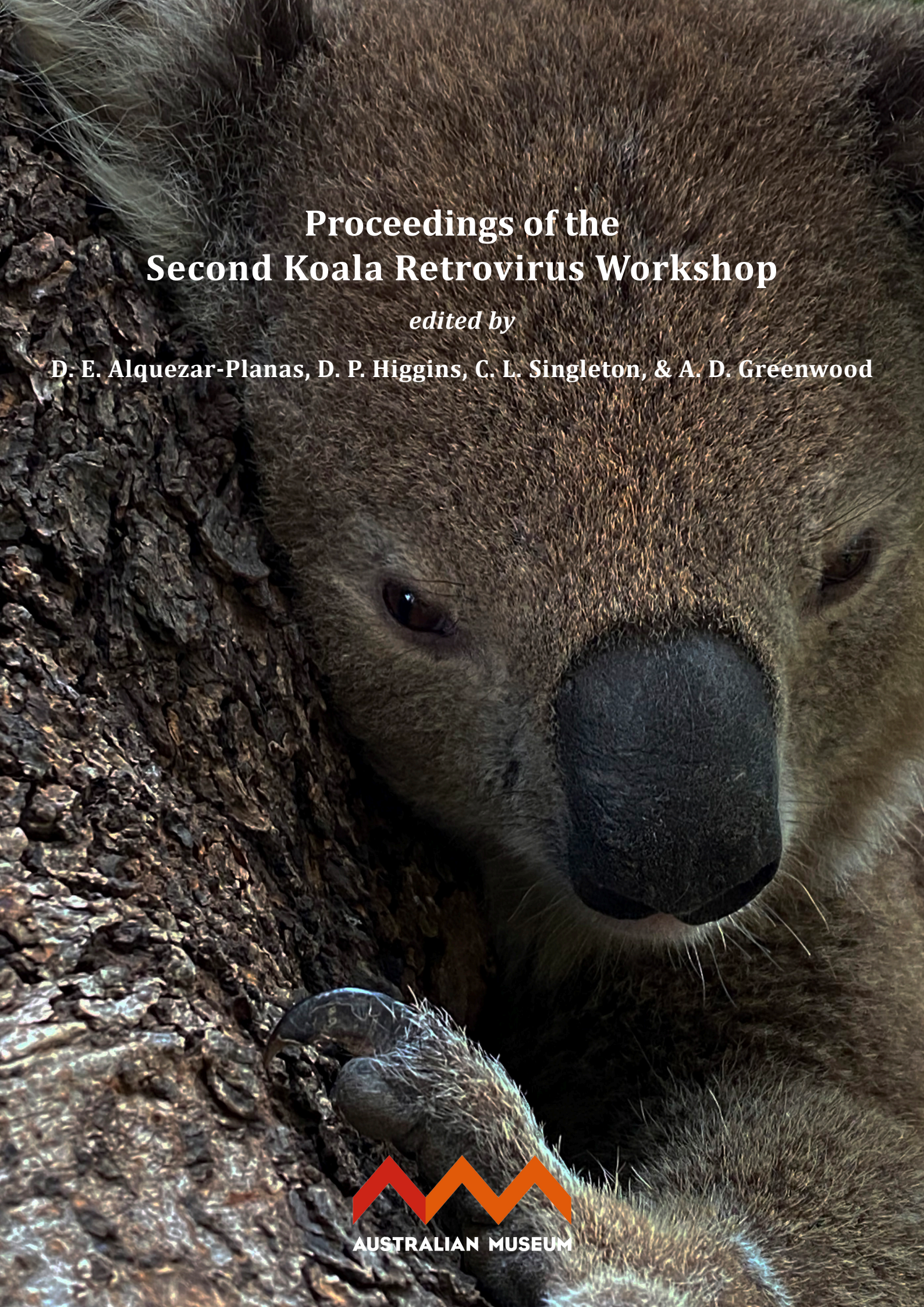

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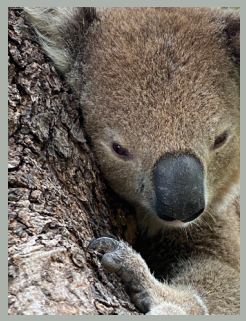
Table of Contents



Cover photo
by Damien P. Higgins

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Putative Koala Retrovirus-Associated Diseases in the Japanese Captive Koala (*Phascolarctos cinereus*) Population

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Abstract. Japan began housing koalas (*Phascolarctos cinereus*) in 1984, increasing from six individuals in 1984 to a peak of 96 koalas in 1997. However, the number of koalas has almost halved since and as of 2020, 54 koalas remain in zoos in Japan. Although records of 330 koala deaths have been accumulated over 37 years, there have been no comprehensive reports on the relationship between the causes of death and koala retrovirus (KoRV) in the Japanese captive population. Based on the koala studbook updated by the Japanese Association of Zoos and Aquariums, we have investigated causes of death in the Japanese captive koala population. The most common cause of death was joeys falling. When combined with stunted joey growth, one-third of the koalas died within a year of birth. Deaths due to malignant neoplasms and opportunistic infections cannot be directly associated with KoRV infection because no test for KoRV had been performed before or during disease onset. It is suspected that KoRV may be associated with deaths due to the large number of cases of neoplasms, which accounted for 16.4% of all deaths.

Introduction

Captive koala (*Phascolarctos cinereus*) breeding began in Japan when three zoos introduced six koalas from Australian zoos in 1984. The number of koala individuals and institutions increased subsequently through further imports and reproduction. A total of 81 koalas have been imported so far, all but one from Australia. Eight koalas have been exported overseas to the United States, the United Kingdom, Australia and other countries. A total of 311 koalas have been born in Japan, and 330 koalas have died since 1984. After reaching a peak of 96 individuals in 1997 and 10 institutions in 1998, the number of koalas has halved in 15 years. As of the end of December 2020, 54 koalas were living at seven institutions in Japan.

The purpose of this study was to investigate the more than 300 cases of koala deaths that occurred in Japanese captive populations from the point of view of KoRV, which is thought likely to cause immunosuppression and malignant neoplasms (Tarlinton *et al*., 2005; Quigley *et al*., 2018; Zheng *et al*., 2020), and to search for a relationship between KoRV infection and mortality.

The Japanese koala studbook, started in 1984 and updated by the Japanese Association of Zoos and Aquariums in 2020, contains information on a total of 392 koalas, including 331 koalas that were born in Japan and 330 koalas that died in Japan. The results categorizing these koalas by cause of death are shown in Table 1. In cases where two or more causes of death were recorded together, malignant neoplasms were prioritized as the cause of death.

Deaths of 102 joeys less than 1 year old accounted for 30.9% of all deaths. Of these deaths, 66 cases were due to "joey falling," and 36 cases were due to "stunted growth of joey" including five cases of joey loss. About one third of the 311 koalas born in Japan died before the age of one year old. Although this is a very high mortality rate, the European

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Table 1. Causes and rates of death of koalas in the Japanese captive population 1984–2020.

causes of death	all koalas	northern koalas	southern koalas
	(n = 330)	(n = 299)	(n = 31)
Malignant neoplasms	54 (16.4%)	54 (18.1%)	0
lymphoma	29 (8.8%)	29 (9.7%)	0
leukaemia	14 (4.2%)	14 (4.7%)	0
other neoplasms	11 (3.3%)	11 (3.7%)	0
Opportunistic infections	13 (3.9%)	13 (4.3%)	0
cryptococcosis	12 (3.6%)	12 (4.0%)	0
pyocyanic disease	1 (0.3%)	1 (0.3%)	0
Immunodeficiency	2 (0.6%)	2 (0.7%)	0
Septicaemia	11 (3.3%)	11 (3.7%)	0
Joey falling	66 (20.0%)	60 (20.1%)	6 (19.4%)
Stunted growth of joey	36 (10.9%)	34 (11.4%)	2 (6.5%)
Old age	33 (10.0%)	24 (8.0%)	9 (29.0%)
Others	115 (34.9%)	101 (33.8%)	14 (45.2%)

captive population also shows a similar trend (Mulot, 2014).

In this studbook, 33 mortalities are recorded as "old age" for koalas showing no particular cause of death other than weakness of various body functions associated with old age. Mortality categorized as "others" includes 18 deaths of unknown cause (all older than 1 year), and diseases clearly unrelated to retroviruses such as intestinal torsion, traumatic shock, respiratory obstruction and spinal curvature, as well as various inflammations where it is not clear from the studbook whether the diseases were infectious or not.

A total of 80 mortalities (24.2%) were attributed to putative KoRV-associated diseases, which are divided broadly into two groups: 1) bone marrow dysplasia and neoplasia and 2) putative immune dysfunction disorders (Gillett, 2023). Mortalities resultant from "malignant neoplasms" accounted for 54 cases. Among those, 29 cases were lymphoma and 14 cases were leukaemia, both of which are thought to be associated with aspects of KoRV infection. Other neoplasms recorded in 11 cases include liver, uterine and ovarian tumours, colorectal cancer, squamous cell carcinoma, and peritoneal mesothelioma. Retroviruses are known to be directly associated with different types of cancers, sarcomas, and lymphomas in mammals including humans (Miyazawa, 2009). Therefore, the association of KoRV with neoplasms other than lymphoma and leukaemia cannot be excluded.

Some retroviruses cause immunosuppression in some animal species (Miyazawa, 2009). Hence, in addition to the two cases of suspected immunodeficiency, 12 cases of cryptococcosis and one case of pyocyanic disease, both of which are opportunistic infections, are suspected to be related to KoRV infection. Furthermore, 11 cases of mortality due to septicemia were recorded. Although the studbook has no record on the details of the causative organisms, it is possible that opportunistic bacteria, or bacteria that entered the bloodstream from the host's intrinsic flora, especially the intestinal flora, contributed to their development as a result of immunosuppression.

Of the 392 koalas listed in the studbook, 354 individuals are of northern lineage, 38 individuals are southern, and there are no hybrids. Koalas of northern and southern lineages have been separated and have not been kept in the same zoo except for exceptional cases. Therefore, although KoRV transmission could have occurred within the same koala lineage, it is unlikely that the infection has occurred between northern and southern koalas. When the causes of death of koalas from northern and southern lineages are compared, different trends are observed. Although the number of deaths among southern koalas is not large, accounting for only 31 cases or less than 10% of the total, none of the deaths were due to malignant neoplasms such as lymphoma or leukaemia. Northern koalas have a significantly higher rate of death from malignant neoplasms than the southern koalas (Fisher's exact test, $p = 0.0029$).

In a PCR-based study of 648 wild southern koalas, none were found to be infected with KoRV-B (Legione *et al*., 2017). Similarly, of 51 koalas in Japanese zoos in 2008, 27 of 40 northern koalas were found to be infected with KoRV-B, while all 11 southern koalas were found to be uninfected (Shojima *et al*., 2013). In light of reports finding a significant association of KoRV-B infection in wild koalas with other neoplasms (Quigley *et al*., 2018) and finding significantly higher proportions of leukaemia, lymphoma and other cancers in koalas infected with KoRV-B, -E and -F than those infected with only KoRV-A (Zheng *et al*., 2020), the fact that there are 54 deaths from malignant neoplasms only in the northern koalas of the Japanese captive population and none in the southern koalas further strengthens the suspicion of an association between these diseases and KoRV, especially KoRV-B. However, as KoRV-B negative koalas suffer high rates of neoplasia, it cannot be excluded that the koalas of northern Australian origin did not suffer from neoplasms caused by KoRV-A.

In conclusion, 80 of the 330 (24.2%) koala deaths in the Japanese captive population were due to putative KoRV-associated diseases (malignant neoplasms and possible opportunistic infections). However, since the viral expression of KoRV before and at the time of disease onset or even the presence of infection of KoRV has not been evaluated in all but a few cases, it is not possible to definitively link these causes of death to the virus.

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