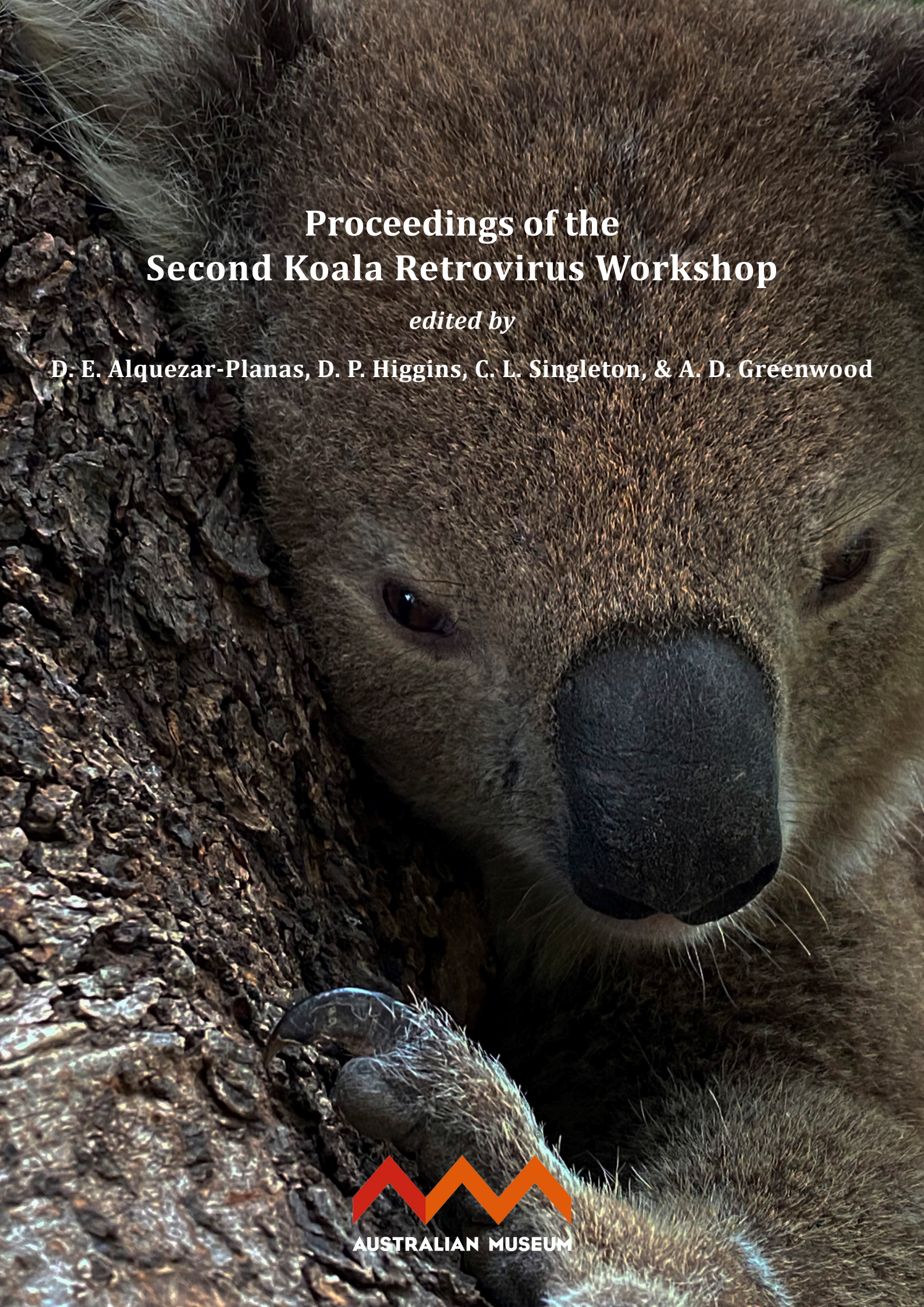
Proceedings of the Second Koala Retrovirus Workshop

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**

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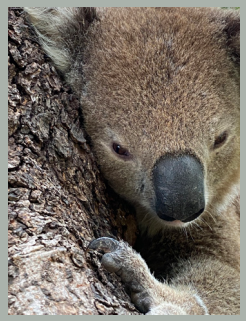

Cover photo
by Damien P. Higgins

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. 35–40, 2023
https://doi.org/10.3853/j.1835-4211.38.2023.1838

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)

Incidence, Trends, and Significance of Putative Koala Retrovirus-Associated Diseases in Monitored Wild Koala Populations in Southeast Queensland

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Abstract. Research indicates that northern koalas (*Phascolarctos cinereus*) are ubiquitously infected with koala retrovirus (KoRV). There is increasing evidence linking KoRV with neoplasia and a range of disorders associated with immunodeficiency, conditions observed at high rates in captive colonies, and sick koalas presenting to wildlife hospitals. However, less is known about the occurrence of these putative KoRV-associated diseases in wild populations. We analysed health data collected at the veterinary examinations of 691 koalas inhabiting three monitored wild koala populations in southeast Queensland between 2013 and 2020. At initial presentation, neoplasia and AIDS-like syndrome were detected at a prevalence of 1.16% (8/691; 95% CI 0.5–2.19%). Longitudinal data from koalas recruited into the monitoring programmes and receiving one or more subsequent examination revealed an incidence rate of 3.5 cases/100 koalas/year (95% CI 2.35–4.9). These findings indicate that a relatively small proportion of the populations studied were affected by these putative KoRV-associated diseases. However, the impact on individuals was severe, with high associated mortality in the diseased cohort. Furthermore, northern koala populations endure multiple threats, suffering severe declines in recent decades. We propose that the significance of putative KoRV-associated diseases on these populations should be considered within this context and that further research into the interactions between KoRV and other drivers of decline is warranted.

Introduction

Northern koala (*Phascolarctos cinereus*) populations in Queensland and New South Wales account for approximately two-thirds of the total range of this iconic native species and have suffered substantial declines in recent decades (McAlpine *et al*., 2015; Adams-Hosking *et al.,* 2016; Beyer *et al.,* 2018; Melzer *et al.,* 2000). Consequently, koalas in these regions were listed as "vulnerable" under the Australian Environment Protection and Biodiversity Act in 2012. Multiple threats have been implicated, including habitat loss and degradation, dog predation, vehicle strikes, bush fires, climate change and disease (Rhodes *et al.,* 2011; Beyer *et al.,* 2018; McAlpine *et al.,* 2015).

Koala retrovirus (KoRV) is highlighted as a major pathogen infecting koalas and receives ongoing attention for its suspected role in several diseases impacting populations (Quigley & Timms, 2020). KoRV-A is the endogenous form of this gammaretrovirus and is detected in 100% of northern koalas (Table 1). Other KoRV subtypes, designated KoRV-B through to KoRV-K, are believed to be exogenous, and have a much more variable prevalence geographically (Quigley & Timms, 2020; Joyce *et al.,* 2021). KoRV-B and KoRV-D are generally found to be the most predominant subtypes in southeast Queensland (SE QLD) koalas (Table 1).

Following infection, retroviruses insert into the host genome, with potentially mutagenic effects (Rabson & Graves, 1997). There is mounting evidence demonstrating

Keywords: koala retrovirus, KoRV, putative KoRV-associated disease, PKAD, lymphoid leukaemia, myelodysplasia, osteochondroma, Queensland
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Submitted: 13 January 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: McKay, Philippa A., and Brent D. Jones. 2023. Incidence, trends, and significance of putative koala retrovirus-associated diseases in monitored wild koala populations in southeast Queensland. 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: 35–40. https://doi.org/10.3853/j.1835-4211.38.2023.1838

Table 1. Prevalence of KoRV subtypes reported between 2012 and 2020 in wild koala populations in southeast Queensland (SE QLD). Dashes indicate that the relevant subtype was not tested for by that study. Koalas presenting to wildlife hospitals inhabited the Moreton Bay Region, Sunshine Coast, Gold Coast and Brisbane. *MBR* = Moreton Bay Region; *HV* = Old Hidden Vale Site located west of Ipswich.

Region in SE QLD	KoRV subtype % (number positive / number sampled)							References
	A	B	D	F	G	H	I	
Wildlife hospitals	100% (18/18)	78% (14/18)	94% (17/18)	44% (8/18)	11% (2/18)	6% (1/18)	6% (1/18)	Chappell *et al.,* 2017
MBR	100% (290/290)	29% (83/290)	—	—	—	—	—	Quigley *et al.,* 2018
MBR	100% (16/16)	25% (4/16)	88% (14/16)	25% (4/16)	0% (0/16)	0% (0/16)	0% (0/16)	Quigley *et al.,* 2019
Wildlife hospitals	100% (33/33)	100% (33/33)	100% (33/33)	0% (0/33)	33% (11/33)	0% (0/33)	97% (32/33)	Sarker *et al.,* 2019
MBR	100% (60/60)	40% (24/60)	97% (58/60)	77% (46/60)	2% (1/60)	0% (0/60)	0% (0/60)	Robbins *et al.,* 2020
HV	100% (20/20)	55% (11/20)	100% (20/20)	25% (5/20)	0% (0/0)	0% (0/0)	0% (0/0)	Robbins *et al*., 2020

a link between KoRV infection and neoplasia. Captive koala colonies suffer exceptionally high rates of neoplasia, particularly lymphoid neoplasms and leukaemia (Xu *et al.,* 2013; Gillett, 2014) and high rates are also reported in wild northern populations (Hanger & Loader, 2014; Gonzalez-Astudillo *et al.,* 2019; Fabijan *et al.,* 2020). Recently described KoRV proviral integration sites appear likely to influence the development of tumours (McEwen *et al.,* 2021). Plasma KoRV RNA levels are significantly higher in captive koalas with leukaemia or lymphoma (Tarlinton *et al.,* 2005) and a positive association has been demonstrated between neoplasia and both KoRV-B (Xu *et al.,* 2013; Quigley *et al.,* 2018) and KoRV proviral load (Sarker *et al.,* 2020). Associations have also been made between aspects of KoRV infection and certain chronic, severe diseases suggestive of dysfunction, dysregulation, or suppression of the immune system, including correlation between KoRV-B and overt chlamydial disease (Waugh *et al.,* 2017; Quigley *et al.,* 2018). An AIDS-like syndrome representing a suite of such conditions is recognized by clinicians and characterized in the literature (Gillett, 2014; Hanger & Loader, 2014; Quigley *et al*., 2018), although links to KoRV are currently largely putative.

Many of the studies investigating suspected clinical manifestations of KoRV infection have been conducted in captive koalas or sick individuals presenting to wildlife hospitals. However, there are far fewer data regarding the occurrence in longitudinally monitored, free-ranging koala populations. Our study seeks to quantify the potential impact of putative KoRV-associated diseases (PKAD) by examining veterinary records from 691 koalas inhabiting three monitored wild koala populations in SE QLD between 2013 and 2020. We estimate the initial prevalence detected when koalas enter the monitoring programmes. We then use longitudinal data to calculate incidence rates to establish the occurrence of KoRV-associated diseases over time. Finally, we examine variables of age, sex and familial trends and document outcomes for individuals in the diseased cohort. Our findings help to establish the significance of KoRV as one of many threats to the survival of koalas in SE QLD.

Materials and methods

Our study utilized three monitored wild koala populations in SE QLD. Moreton Bay sites 1 and 2 (MB1 and MB2) are located north of Brisbane approximately 20 km apart (27.2247°S 153.02°E and 27.3193°S 152.9571°E, respectively). Both are peri-urban/urban koala habitats composed of predominantly open eucalypt forest that has undergone varying levels of clearing and disturbance as part of infrastructure or extractive industry projects. The Old Hidden Vale site (HV) is geographically separated, located approximately 70 km away west of Ipswich (27.6594°S 152.4672°E). HV is rural koala habitat composed of grassland and open forest. All three are open populations, with new koalas recruited into monitoring programs over time as joeys or when moving into the area, and individuals are removed as they disperse or die.

Koalas were monitored using radiotelemetry and biotelemetry collars (K-Tracker, LX Group, Sydney) and tracked and sighted at least once every two weeks. Individuals underwent comprehensive veterinary examinations under anaesthetic approximately every six months, or more frequently for growing individuals or where health or welfare concerns were raised. Veterinary examinations were performed by veterinarians experienced with koalas and consisted of a full physical examination, including ultrasound of the urinary and reproductive tracts, and radiographs if indicated. Blood, urine, bone marrow aspirate, and peritoneal fluid were collected for cytological examination. All health data and clinical findings were recorded in a standardized database. Monitoring of activity data from biotelemetry collars and regular tracking of individuals enabled diseased koalas to be rapidly identified by field staff and recaptured for veterinary assessment. Similarly, deceased koalas were quickly located, and thorough necropsies performed.

We reviewed data for all koalas that had undergone at least one veterinary examination or necropsy. A case was considered positive for PKAD if consistent with one of the following categories: neoplasia, suspected but not confirmed neoplasia, and conditions suggestive of immune dysfunction or dysregulation (AIDS-like syndrome). Koalas were included in the AIDS-like syndrome cohort if they displayed two or more of the clinical signs outlined in Table 2. This is consistent with published inclusion criteria for this syndrome (Gillett, 2014; Hanger & Loader, 2014; Quigley *et al.,* 2018).

Initial prevalence was determined by the proportion of KoRV-associated disease cases identified at the initial veterinary examination upon entry into the monitoring program at each site. Incidence rate was then determined for the remaining susceptible koalas that underwent at least one subsequent veterinary examination, using the number of positive cases identified as the numerator and the total number of days all susceptible koalas were monitored for as the denominator. In the case of positive koalas, only the days monitored prior to being deemed positive were included in the calculation. The result was multiplied by 100 and 365 to give a rate of cases per 100 koalas per year. Sites were analysed as separate populations as well as the Moreton Bay region (MBR; MB1 and MB2) and SE QLD (MB1, MB2 and HV) combined.

Table 2. Clinical signs suggestive of immune dysfunction or dysregulation with examples. In our study, AIDS-like syndrome was diagnosed in koalas displaying two or more of these clinical signs.

Category of clinical signs suggestive of immune dysfunction / dysregulation	Examples of clinical sign
Dermatopathy	Generalized dermatitis, chronic otitis externa/media, paronychia
Oral lesions	Severe oral ulceration, severe periodontal disease
Chronic ill-thrift	Persistent or unexplained poor body condition
Fungal infections	Severe cryptococcosis
Severe, debilitating chlamydiosis	Severe chlamydiosis that fails to respond to treatment
Severe gastrointestinal disorders	Caeco-colic dysbiosis/typhlocolitis syndrome unrelated to antibiotic administration

The age and sex of koalas diagnosed with PKAD were analysed across all sites. Familial links were also explored in the MB1 population in a subset of koalas where the maternal lineage was known. The Winpepi software suite (Abramson, 2011) was used for all statistical analyses.

Results

Initial prevalence and incidence rate

The number of koalas subjected to an initial veterinary examination, those subjected to one or more subsequent examinations, and the duration of monitoring at each site are outlined in Table 3.

Six koalas at MB1 (n = 634) were diagnosed with PKAD at the first veterinary examination, giving an initial prevalence of 0.95% (95% CI 0.35–2.05%). Of these, one had lymphoma (16.7%), two had suspected myelodysplasia (33.3%) and three fell into the AIDS-like syndrome category (50.0%). Of the 541 koalas that went on to have subsequent examinations, PKAD was detected in 29 cases. These consisted of 11 cases of neoplasia (four lymphoma, one lymphoid leukaemia and lymphoma, three myelodysplasia, three osteochondroma), three cases of suspected neoplasia (two myelodysplasia, one lymphoma) and 15 cases of AIDS-like syndrome. The incidence rate was calculated as 3.4 cases/100 koalas/year (95% CI 2.29–4.89).

One koala at MB2 (n = 22) was diagnosed with neoplasia at initial veterinary examination, giving an initial prevalence of 4.55% (95% CI 0.12–22.84%). There were no cases of PKAD diagnosed in any of the koalas that went on to have subsequent examinations (n = 18).

One koala at HV (n = 35) was diagnosed with AIDS-like syndrome at the first examination, giving an initial prevalence of 2.86% (95% CI 0.07–14.92%). Of the 32 koalas that went on to have subsequent examinations, one was diagnosed with lymphoma and one with AIDS-like syndrome. The incidence rate was calculated as 5.3 cases/100 koalas/year (95% CI 0.73–18.98).

Excluding the geographically separate HV population, prevalence at initial examination (7/656) was 1.07% (95% CI 0.43–2.19%) and the incidence rate was 3.4 cases/100 koalas/year (95% CI 2.26–4.85) in the MBR (MB1 and MB2). Combining data from all three sites, prevalence at initial examination (8/691) was 1.16% (95% CI 0.5–2.19%) and the incidence rate was 3.5 cases/ 100 koalas/year (95% CI 2.35–4.9). The differences between the initial prevalence and incidence rates at each site were not statistically significant.

Age, sex, and familial variables

Across all three sites, there was no statistically significant difference between the number of male and female koalas presenting with PKAD (Upton's modified Chi-square test, $\chi = 0.004$; 1 degree of freedom; $p = 0.95$) (Campbell, 2007).

The mean age of diagnosis of neoplasia was 6.75 years across all sites. This was slightly lower for AIDS-like syndrome at 5.10 years. Koalas in this category presented over a wider range of ages (0.86–12.92 years) compared to koalas with neoplasia (3.83–11.00 years) (Fig. 1). The difference in means was not statistically significant.

Of the total 35 cases of PKAD identified in MB1, the dams of four individuals are known. The 21 female cases collectively had 29 joeys that were entered into the monitoring programs, and for which detailed health data are available. One potential familial link emerged from these data. An approximately five-year-old koala was diagnosed with abdominal lymphoma (Fig. 2) and her male offspring developed a pelvic osteochondroma at four years of age. As of the end of this study, her younger female offspring was alive, healthy, and continuing to be monitored. PKAD was not identified in any of the other 27 joeys, eight of which were still alive at the end of this study.

Outcomes

Of the 14 koalas diagnosed with neoplasia across all sites, 13 were humanely euthanized due to their disease presentation (92.9%). The mean survival time after diagnosis was 40

Table 3. The duration of monitoring and number of koalas sampled from each of the three sites (MB1, MB2, and HV).

Study site	Study duration	No. koalas given initial examination	No. koalas given one or more subsequent examinations	Mean no. of days monitored per koala
Moreton Bay site 1 (MB1)	03/2013–03/2021	634	541	574
Moreton Bay site 2 (MB2)	04/2019–05/2020	22	18	168
Hidden Vale site (HV)	05/2018–05/2020	35	32	433

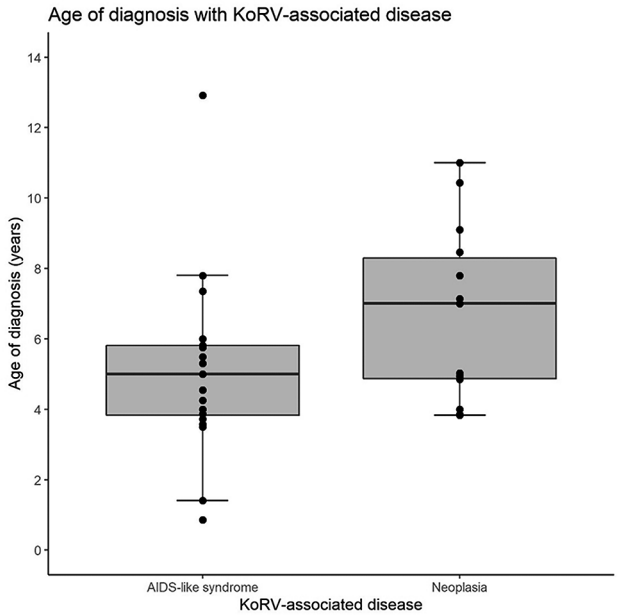

Figure 1. Graph showing the ages at which koalas from all three sites (MB1, MB2 and HV) were diagnosed with neoplasia and AIDS-like syndrome.

days, with 38.5% being euthanized within 14 days. The remaining koala was found dead. All five koalas with suspected neoplasia were also euthanized. Of the 25 koalas fulfilling the criteria for AIDS-like syndrome, nine were euthanized (45%), six were found dead, and outcomes for the remaining five are unknown as they were removed from the monitoring programs.

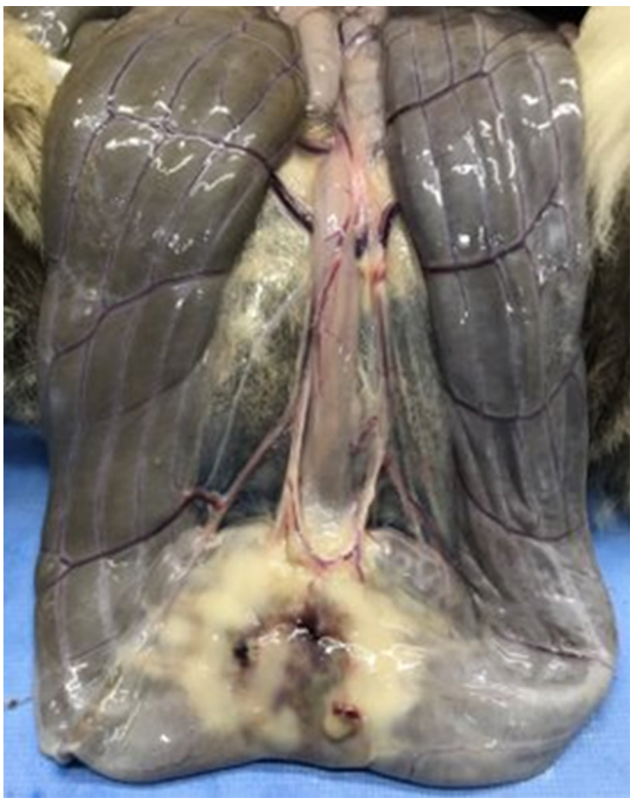

Figure 2. Photograph taken at necropsy of abdominal lymphoma diagnosed in an approximately five-year-old female koala from the Moreton Bay Site 1 (MB1). Photo credit to Endeavour Veterinary Ecology.

Discussion

Through an analysis of longitudinal health data, we sought to measure the occurrence of PKAD to determine their significance in wild koala populations in SE QLD. Previous studies have demonstrated high prevalence of endogenous and exogenous KoRV subtypes in this region, including koalas inhabiting our three study sites (Table 1). However, when surveying for potential clinical manifestations of KoRV infection, we found both prevalence and incidence rates to be relatively low. Combining data from all sites, we calculated prevalence of PKAD at initial presentation to be 1.16% (95% CI 0.5–2.19%), ranging from 0.95% (95% CI 0.35–2.05%) in MB1 to 4.55% (95% CI 0.12–22.84%) in MB2. The incidence rate for all sites combined was 3.5 cases/100 koalas/year, ranging from 3.4 to 5.4 cases/100 koalas/year between MB1 and HV, respectively. Differences in initial prevalence and incidence rate between sites were not statistically significant. This likely reflects the relatively small sample size from MB2 (n = 18) and HV (n = 32) when compared with MB1 (n = 541), and the variation in duration of monitoring at each site (7 years at MB1, 14 months at MB2, and 2 years at HV). This latter point is the most likely reason no further cases were identified at MB2 during the study.

As expected, our findings for the MB1 site were comparable to those reported by Quigley *et al.* (2018) in their study of 290 koalas from the same population. Between 2013 and 2017, 1.72% (5/290) of koalas presented with neoplasia and 2.41% (7/290) developed AIDS-like syndrome. Similarly, between 2013 and 2021, we reported neoplasia (including suspected cases) in 2.68% (17/634) and AIDS-like syndrome in 2.84% (18/634) of individuals. This is unsurprising given that in both studies, very similar inclusion criteria for diagnosing PKAD were employed (Quigley *et al.,* 2018) (Table 3).

In contrast, our calculations of PKAD occurrence in the MBR (MB1 and MB2) were lower than those determined by a previous analysis of a wild population in this area (Hanger & Loader, 2014). This study found prevalence at initial presentation to be 7.8% (23/296) between 2008 and 2013, compared to our detection of just 1.07% (95% CI 0.43–2.19%) between 2013 and 2021. In the previous study, longitudinal data were available for 126 koalas, in which the incidence risk was 12.5% per year. Again, this is notably higher than our incidence rate of 3.4 cases/100 koalas/year (95% CI 2.26–4.85), which converts to an incidence risk of 3.34% using the equation $CI = 1–e^{-I}$ where CI is incidence risk and I is incidence rate (Thrusfield *et al.,* 2018). These differences may reflect the use of more conservative criteria for diagnosing PKAD in our study. The aim of this was to avoid the inclusion of false positives in our analysis. This approach carries the risk of excluding cases that may be linked to KoRV and consequently, the true rate of disease in these populations is likely to be somewhere in between.

A single potential familial link was identified in those individuals in which dam and joey relationships were known. Given that this was limited to a very small subset of the population, further analysis was not pursued. Our study was conducted in a wild population and so information about the sires of individuals is unknown. However, this is unlikely to influence our analysis as endogenous KoRV-A is transmitted via germline DNA, and dam-to joey transmission is by far the most important mode of transmission for exogenous subtypes (Xu *et al.,* 2013; Quigley *et al.,* 2018; Joyce *et al.,* 2021).

Most of the data pertaining to PKAD in wild koala populations are collected from animals presenting to wildlife hospitals (Hanger & Loader, 2014; Fabijan *et al.,* 2020; Gonzalez-Astudillo *et al.,* 2020). In a retrospective survey of clinical records for the 10,082 koalas admitted to the Australia Zoo Wildlife Hospital, SE QLD, between 2004 and 2020, 10.9% were found to have presented with neoplasia or other PKAD (R. Booth, pers. comm.). While such datasets provide a valuable insight into the impact of disease on free-ranging populations, they represent a biased cohort constituting predominantly sick or injured individuals. This may explain the generally higher rates of neoplasia and other diseases falling under the umbrella of AIDS-like syndrome when compared to our study.

Individual animal welfare should also be considered when assessing the significance of PKAD on the health of koala populations. Neoplasia and AIDS-like syndrome commonly manifest as chronic, painful, and debilitating symptoms (Hanger & Loader, 2014; Fabijan *et al.,* 2020). In our populations, 72.26% of the diseased cohort were euthanized on welfare grounds upon veterinary intervention or found dead in the field. We found that individuals presented with symptoms over a very wide range of ages (Fig. 1). This was broadest for those with AIDS-like syndrome (0.86–12.92 years), likely reflective of the varied and often chronic nature of these conditions. These diseases lead to poor welfare outcomes for individuals, and may reduce reproductive success and life expectancy, particularly in koalas suffering from a young age.

Our findings suggest that neoplasia and other conditions suggestive of immune system dysfunction, dysregulation, or suppression impact a small percentage of the free-ranging populations that we studied in SE QLD. However, this should not diminish the potential impact of KoRV infection on wild northern koala populations. Rather, further research is needed to better understand the role of this virus within the framework of other threats and drivers of decline, such as high rates of chlamydial disease endured by these populations. Furthermore, studies that seek to contextualise the significance of KoRV infection will help to inform future management practices and ensure that the best health and welfare outcomes are achieved for our wild koalas.

Acknowledgements. We are grateful to all staff at Endeavour Veterinary Ecology for their efforts in monitoring the koalas and meticulously collecting and recording the data that were the subject of these analyses. Special thanks to Drs Jon Hanger and Amy Robbins for sharing their expertise on KoRV. The role of Moreton Bay Regional Council, the Queensland Government Department of Transport and Main Roads, CSR Ltd, the Turner Family Trust Foundation and Hidden Vale Wildlife Centre in enabling the collection and sharing of these valuable data is gratefully acknowledged.

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