The Koala and its Retroviruses:
Implications for Sustainability and Survival

edited by

Geoffrey W. Pye, Rebecca N. Johnson, and Alex D. Greenwood

nature culture **discover**

Australian Museum science is freely accessible online at
http://australianmuseum.net.au/journalfinder
6 College Street, Sydney NSW 2010, Australia

Australian museum

Technical Reports of the Australian Museum, Online (2014) No. 24, pp. 39–45.
ISSN 1835-4211 (online)
http://dx.doi.org/10.3853/j.1835-4211.24.2014.1612

An Examination of Disease in Captive Australian Koalas *(Phascolarctos cinereus)* and Potential Links to Koala Retrovirus (KoRV)

Amber K. Gillett

Australia Zoo Wildlife Hospital, Beerwah Queensland 4519, Australia

Abstract. Koalas *(Phascolarctos cinereus)* are known to suffer from a range of neoplastic and immunodeficiency-related disorders but the importance of these conditions to captive koala populations has not previously been thoroughly examined. This study aimed to improve our understanding of disease in captive koalas by conducting a detailed questionnaire survey across most facilities that house koalas in Australia. Responses were received from 16 facilities across five Australian states that resulted in disease information for a total of 264 koalas. The collated data indicated that neoplasia is the major type of diagnosed disease affecting captive koalas, with lymphoma clearly the most common (c. 40%). A variety of other disorders were reported including bone marrow disease (especially leukaemia), cryptococcosis and dermatitis, the latter of which was the only condition reported from all five states. These data suggest a higher incidence of disease in facilities in Queensland and New South Wales, which are predominantly comprised of northern koalas. Mortality records spanning up to 28 years were received from six of the surveyed facilities which indicated that of 303 deceased captive koalas, 32% of deaths were attributable to the diseases mentioned above. It is likely that the prevalence of disease reported here is an underestimate due to the lack of, or inconsistent application of, appropriate diagnostic investigations amongst facilities from all states. Given that previous research suggests that northern koalas are ubiquitously infected with koala retrovirus (KoRV) and that they have higher viraemic loads than their southern counterparts, there may be a link between KoRV and the higher disease expression among northern koalas postulated here. Further research is required to determine if there is a causal link between KoRV and the predominant diseases among captive koalas reported in this study.

Gillett, Amber K. 2014. An examination of disease in captive Australian koalas *(Phascolarctos cinereus)* and potential links to koala retrovirus (KoRV). In *The Koala and its Retroviruses: Implications for Sustainability and Survival*, ed. Geoffrey W. Pye, Rebecca N. Johnson and Alex D. Greenwood. *Technical Reports of the Australian Museum, Online* 24: 39–45.

Wild koala *(Phascolarctos cinereus)* populations are found across a broad geographic range in eastern and south-eastern Australia and occur in the states Queensland (QLD), New South Wales (NSW), Australian Capital Territory (ACT), Victoria (VIC) and South Australia (SA) (Martin & Handasyde, 1999). Koalas are generally referred to as either "northern" or "southern", a description which is largely determined by state borders. Northern koalas are distributed from north QLD to south of the NSW/VIC border and southern koalas are distributed through most of VIC and SA (Carrick, 2013). Variations in appearance between northern and southern koalas are evident and most notably include longer fur length and larger size in southern compared to northern koalas. In many parts of the species range, populations of wild koalas are declining at alarming rates and local extinctions have already occurred across considerable areas. Declines are largely attributed to habitat loss, trauma (road and domestic/wild dog attacks) and disease within populations (principally chlamydiosis). A further potential threat to koalas that is receiving increased scientific attention

is koala retrovirus (KoRV). Koala retrovirus (KoRV) has become endogenized in koalas and appears to be 100% prevalent in wild (and captive) QLD and NSW koalas and its presence is being increasingly identified amongst wild koalas in the southern states of Australia VIC and SA (Ávila-Arcos *et al*., 2013). Retroviruses are known to affect many vertebrate species and can lead to immunosuppression and in some cases neoplasia such as leukaemia and lymphoma (Rosenberg, 2011). These conditions are prevalent in wild and captive koalas and there is speculation that KoRV may play a role in inducing neoplastic and immunosuppressive disorders in koalas.

While KoRV appears to be widespread in koalas, the circulating viral load may be of greatest importance in understanding the virus' impacts in koalas. An association appears to be evident between the presence of high circulating levels of KoRV and immunodeficiency and neoplastic disorders in both wild and captive koala populations throughout Australia (Hanger *et al*., 2000; Tarlinton *et al*., 2005). Further research is currently being conducted to better understand the role of KoRV and virus load upon infected individuals, and the potential management implications for captive animals.

The most recent tally of captive koalas in Australian facilities from the Zoo and Aquarium Association (ZAA) identified 514 northern koalas and 112 southern koalas currently being held across six of Australia's seven states. All northern animals, except four, are held in QLD, NSW and WA facilities and almost 80% of the southern animals are listed in VIC and SA. Captive populations originated from wild gene pools of both southern and northern koalas but now captive breeding for zoological collections is commonplace throughout Australia. Captive koalas are also routinely moved between Australian zoos and even exported from Australia to international zoological institutions. Occasionally, wild animals are also incorporated (through government-approved species management programs) into established captive colonies within Australia to use as display animals or in captive breeding programs. Wild koalas approved for such placements have often sustained injuries deeming them not fit to return to the wild, are orphaned hand-raised individuals that have not demonstrated wild instincts or are infertile due to *Chlamydia*-related reproductive disease. In the latter case, certain state governments prohibit the re-release of infertile koalas to the wild so after chlamydial treatment and ovariohysterectomy, to remove the diseased reproductive tract, these koalas can be placed in a captive management situation for display and education purposes.

Across the facilities that house captive koalas there are increasing reports of neoplastic and potential immuno-deficiency disorders. Previous investigations of some captive populations have identified as many as 55% of deaths were attributable to lymphoid neoplasia (Hanger, 1999). Lesions appear to be more frequently reported in the northern koalas and include leukaemia, myelodysplasia, tumours (Fig. 1) and immunodeficiency-like syndromes that tend to include generalized dermatitis and/or stomatitis/oral ulceration (Fig. 2). Anecdotal reports of recognized patterns in the above syndromes from one generation of captive koala to the next have also been observed, with some facilities having seen more than three generations succumb to the same form of neoplastic diseases (leukaemia and lymphoma) at similar ages (M. Panayiotou, pers. comm.). Other conditions such as cryptococcosis are also encountered in captive populations as an opportunistic infection likely related to immunosuppression (Hanger *et al*., 2003) and overwhelming environmental load of the organism (Krockenberger *et al*., 2002).

To date, very limited information has been available on the prevalence of specific neoplastic and immunodeficiency-like syndromes in captive koalas throughout Australia. The aim of this manuscript was to collate detailed information on the prevalence of specific neoplastic and potential immunosuppressive disorders within these facilities, examine currently employed diagnostic techniques and discuss the potential role of KoRV in the expression of identified diseases.

Materials and methods

A questionnaire survey was developed to elicit detailed information from relevant captive koala facilities on the type and prevalence of neoplastic and potential immunosuppression-related syndromes in captive koalas. The survey was electronically distributed to veterinary and wildlife network email server lists including the Australian Wildlife Health Network (AWHN), Wildlife Disease Association (WDA) Australasian section and ZAA (Australasian section). As such, the survey should have been received by representatives of all 39 Australian facilities that house captive koalas and are members of the ZAA. The survey was also directly emailed to individual veterinarians, curators and other wildlife facilities known to the author. The audience captured by this approach was in the order of 750 people/facilities (500 AWHN, 158 WDA, 82 ZAA and 10 personal contacts).

Questionnaire recipients were asked a series of questions regarding captive koalas at their facility. Firstly, they were asked the total number of captive koalas currently at their facility and if they had acquired wild koalas for display or breeding purposes. Participants were then asked to state the number of koalas affected by leukaemia, myelodysplasia, erythroid dysplasia, chronic dermatitis or other signs of immunosuppression (including chronic ill thrift, recurrent or persistent stomatitis, oral ulceration and severe debilitating chlamydiosis), cryptococcosis, osteochondroma, lymphoma or other neoplasia. For ease of reporting in this manuscript, leukaemia, myelodysplasia and erythroid dysplasia have been categorized as "bone marrow conditions", dermatitis, stomatitis and signs of immunosuppression are categorized as "AIDS-like conditions", cryptococcosis remained as "cryptococcosis" and osteochondroma, lymphoma, and other neoplasia are categorized as "tumours".

Finally, participants were asked if they had recognized any of the mentioned syndromes in multiple generations of the same genetic lineage, and what procedure/s they routinely perform when examining an koala ill from an unspecified cause (survey options included blood smear, bone marrow, abdominocentesis or none at all).

Once all data regarding the diseases of interest was collated from returned surveys an additional information request was sent to each of the 16 facilities. The requested information included a tally of total koala numbers housed during the history of their facility, the time frame covering the period of provided records, and mortality records during the reported time frame. Mortality records were requested for all of the disease conditions listed in the initial survey, defined as diseases of interest (DOI), as well as for mortalities due to other causes, so that the proportional contribution of disease-related deaths could be calculated.

All returned surveys were received electronically via email. Participants were not asked within the survey to define what facility they were from, however this

Figure 1. Basal cell carcinoma on the face of adult 10 year old male northern koala *(Phascolarctos cinereus)*.

information was obtained once surveys were returned in order to separate findings by state. Participants were not asked to separate koalas into numbers of northern versus southern koalas in their facility.

Results

The total number of facilities/individuals that responded to the initial survey was 16. Information from one facility was provided via the Australian Registry of Wildlife Health (ARWH) and not the facility itself, whilst 15 facilities provided direct responses. Six participants were from NSW, five from QLD two from VIC, two from SA and one from Western Australia (WA). The total number of koalas presently held in the participating facilities totalled 414 with 282 in QLD, 52 in NSW, 46 in SA, 24 in WA and 10 in VIC.

Disease information from the initial survey was provided for a total of 264 koalas, comprising 189 from QLD (71.5%), 64 from NSW (24.3%), six from WA (2.3%), three from VIC (1.1%), two from SA (0.8%). Tumours were overwhelmingly the most prevalent condition noted by participants (56% of 264 koalas). Within this category lymphoma was the most common diagnosis (Fig. 3), followed by "other neoplasia", and almost all tumours were in QLD and NSW animals (Fig. 4). Seventeen "other tumours" were reported in the "other neoplasia" category and are listed in table 1. Some conditions listed under "other tumours" were not specifically identified by tumour type, including ovarian cancer, papilloma and unspecified neoplasia, but these were included in the final dataset. In QLD and NSW, which in combination accounted for more than 95% of disease cases examined here, lymphoma was clearly the most prevalent tumour representing approximately 40% of all diseases in these states (Fig. 5).

AIDS-like conditions appeared to account for the next most prevalent disease processes (20% of 264 koalas), with most facilities noting dermatitis as a common finding. The percentage for dermatitis represented in Fig. 3 is an underrepresentation because one facility commented that they saw "lots" of animals with this condition, but they did not assign a numerical value.

Bone marrow conditions represented the third most prevalent category (14% of 264 koalas). However, cases of leukaemia represented the vast majority of reported diseases in this category, being more than five times more common than other reported forms of bone marrow disease (Fig. 3). Virtually all cases of bone marrow disease were diagnosed from QLD and NSW facilities (Fig. 5). Cryptococcosis was noted in 29 (11%) cases.

Two facilities noted a potential hereditary pattern of disease (specifically lymphoma and lymphoma with leukaemia). Both facilities were from Queensland and housed majority northern subspecies of koalas. The remaining facilities reported that no pattern was noted and a response was not received from one facility as the information about its koalas was obtained through the AWHN archive and not the facility itself.

Fifteen facilities provided information regarding which procedures they routinely performed when examining a

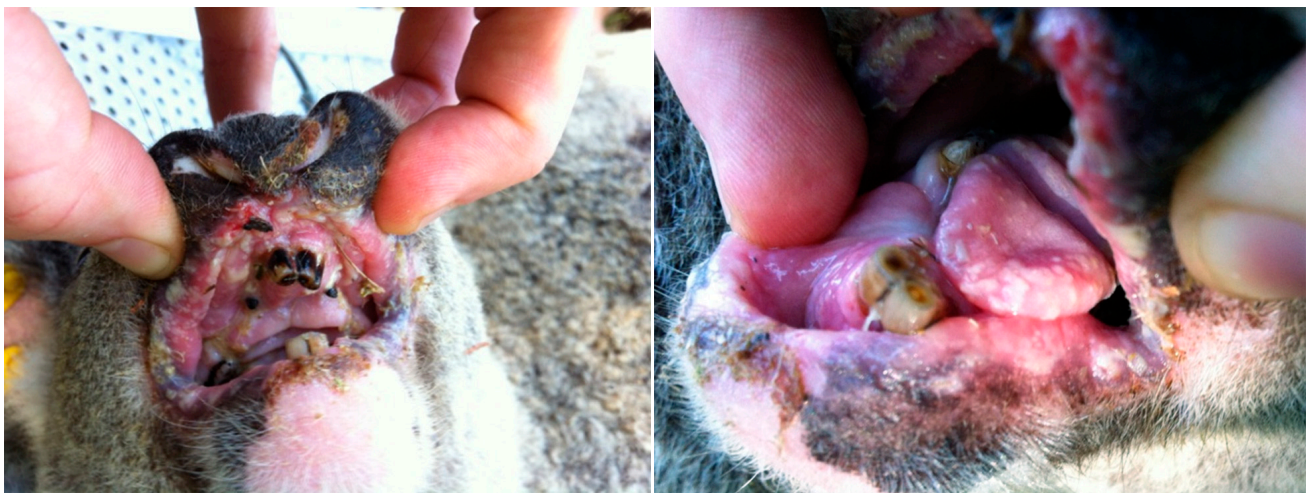

Figure 2. Stomatitis and oral ulceration in an adult (exact age unknown) female northern koala *(Phascolarctos cinereus)*.

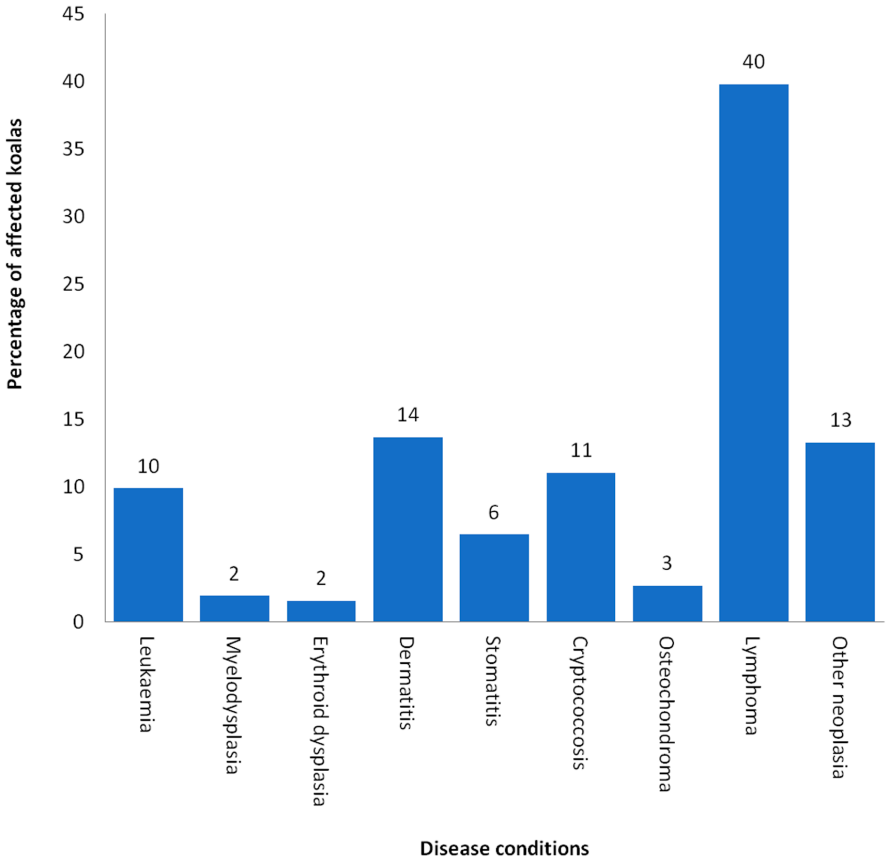

Figure 3. Percentage of captive koalas *(Phascolarctos cinereus)* (n=264) affected by conditions reported from 16 surveyed facilities in Australia.

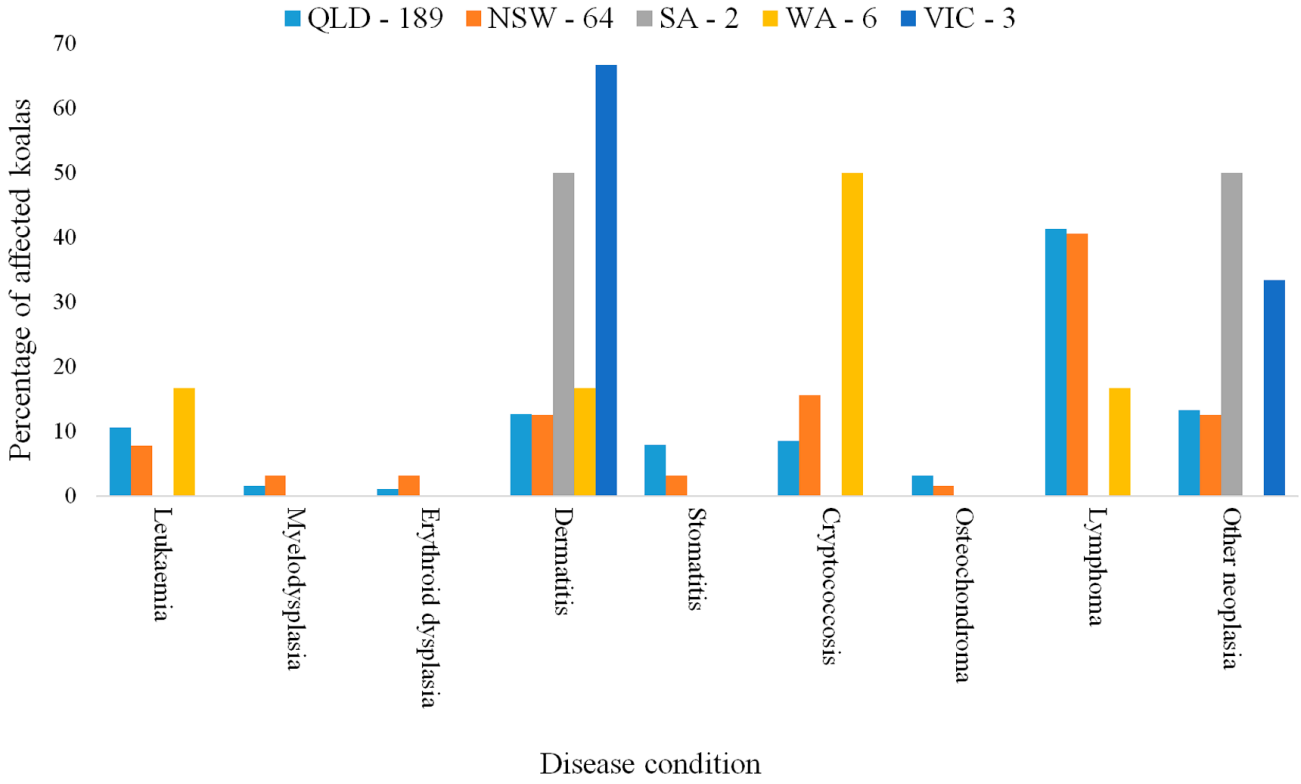

Figure 4. Percentage of disease in 264 captive koalas *(Phascolarctos cinereus)* identified by Australian state.

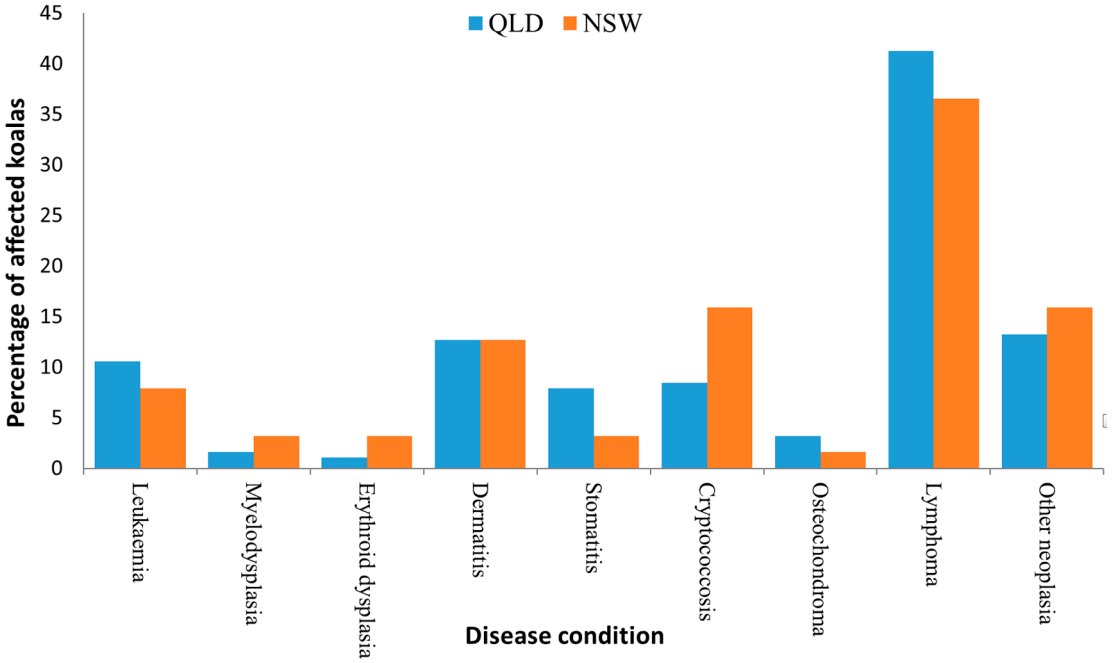

Figure 5. Comparison of disease prevalence in captive koalas between QLD (n=189) and NSW (n=64) as reported from 11/16 facilities (QLD 5, NSW 6).

koala ill from unspecified cases. One facility reported that they did not routinely conduct any diagnostic procedures. Of the remaining 14 facilities, all but one routinely conducted blood smears; two facilities stated they routinely utilized one additional diagnostic technique (bone marrow aspirate or abdominocentesis), whilst only two facilities routinely conducted all three procedures.

Responses to the additional information request on koala mortalities were obtained from six facilities across three states (QLD 2, NSW 3 and WA 1). The period of time reported on by each facility varied from five years (NSW) to 28 years (WA) with four of the six facilities able to provide data for at least a 10 year period. The total number of koalas housed collectively amongst the facilities for the time frames outlined was 533 with mortality records available for 303 animals. When analysed as a combined dataset, 32% of these mortalities were attributed to the DOI whilst the remaining 68% consisted of deaths due to other causes (including pouch mortality, gastrointestinal conditions, septicaemia and age related death). Of the DOI the most common causes of death were lymphoma (53%), other neoplasia (15%) and leukaemia (11%).

When examined on a state basis 192 mortality records were available from QLD, 84 from NSW and 27 from WA. When expressed as a percentage, mortality rates due to the DOI were similar between QLD and NSW (35% and 32% respectively) but considerably lower from WA (7%) (Fig. 6).

Table 1. List of different types of neoplasia identified under "other neoplasia".

description	count
cholangiocellular carcinoma	1
fibrosarcoma	2
haemangiosarcoma	3
intestinal adenocarcinoma	1
leiomyoma	1
mesothelioma	7
myxosarcoma	1
nephroblastoma	1
osteosarcoma	4
ovarian cancer (type not described)	1
papilloma	1
renal cystadenocarcinoma	1
periosseous giant cell tumor	1
phaeochromocytoma	1
sarcoma	1
squamous cell carcinoma	3
other—unspecified	4
tally	34

Discussion

Although neoplasia has been well documented in wild and captive koalas, this study provides the first comprehensive examination of the types and prevalence of neoplastic and potential immunodeficiency based disease in captive koalas throughout Australia. The results of the study indicate that tumours, particularly lymphoma, are the main form of disease identified in the sampled captive populations, which is similar to what previous studies have found in wild and captive koalas (Canfield, 1990; Canfield *et al*., 1990; Connolly *et al*., 1998; Hanger, 1999). The study has also shown a high number of other neoplasias occurring in captive koalas (see Table 1).

Dermatitis was the only reported condition in common between all five examined states, although it represented only a relatively small proportion of all disease (14%). This condition alone is generally non-fatal but may be indicative of underlying immunosuppression. Interestingly, lymphoma, osteochondroma, cryptococcosis, stomatitis and all forms of bone marrow disease were only reported from QLD, NSW and WA.

The vast majority of data collated in this study was derived from koalas in QLD and NSW facilities. Interestingly, these data show the relative prevalence of each disease is very similar across these two states, as highlighted in Figure 5.

Collectively, historical mortality rates due to the diseases of interest amongst the two QLD and three NSW facilities were similar, despite QLD providing more than double the number of koala mortality records than NSW, 192 and 84

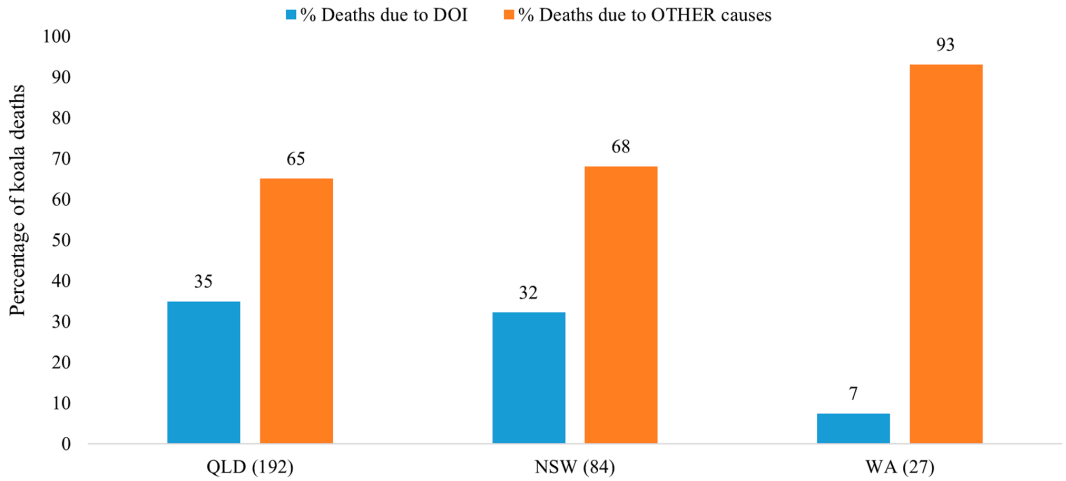

Figure 6. Comparison of causes of mortality amongst captive koalas in QLD (2 facilities), NSW (3 facilities) and WA (1 facility) for up to a 28 year reporting period.

respectively. Mortality rates due to the diseases of interest could be much higher than that represented here for QLD as unfortunately two of the four largest koala facilities were unable to provide the requested mortality record information. Of the QLD facilities that did respond, all housed a majority of northern koala species with reporting periods of up to 15 years. The maximum reporting period for NSW facilities was also 15 years whilst WA provided 28 years of mortality data and both states housed predominantly northern koalas. When all six facilities were analyzed collectively the mortality rate of captive koalas attributed to the diseases of interest in this manuscript was in the order of 32% with lymphoma identified as the most common disease resulting in death (53%). This is relatively consistent with findings in North America and Europe (G. Pye, pers. comm.).

No mortality records were available for the examined facilities from SA and VIC. However, although there are currently more than 50 captive koalas held in these states and there are likely to have been considerably more than this in the history of the facilities, only five cases of disease were reported from these states in this study. This small number of disease cases makes it difficult to draw any meaningful conclusions about the main cause of disease or the level of mortality due to disease, but it does suggest a low level of disease amongst captive koalas in SA and VIC, which are almost exclusively southern koalas. It has previously been suggested that southern koalas suffer less from all forms of disease (including chlamydiosis) than their northern (QLD, NSW) counterparts.(Simmons *et al*., 2011).

It has been suggested that KoRV may directly induce neoplastic or immunosuppressive disease in koalas, especially among those individuals with high viral loads (Tarlinton *et al.*, 2005). Recent research into the prevalence of KoRV across the distribution of wild koalas revealed that all examined koalas from QLD (n = 277) and NSW (n = 100) tested positive for KoRV as identified by Hanger *et al*. (2000) and that proviral copy number was much higher in these animals than southern counterparts (Simmons *et al*., 2011). Although thorough sampling for KoRV has not been conducted on captive koalas in Australia, it is reasonable to presume that captive koalas bred from wild QLD or NSW koala lines will have similar levels of viraemia and proviral copy number. Therefore, it is possible that the presumed higher incidence of disease among northern koalas in this study may infer a relationship between high levels of KoRV and the prevalence of disease.

Queensland was the only state where a potential hereditary pattern of disease was reported. Two facilities, both housing northern koalas, reported successive generations dying from the same disease, namely lymphoma or leukaemia. One facility noted these conditions occurring in at least three generations of koalas, with all animals succumbing to disease at similar ages, suggesting there may be a heritable susceptibility to disease expression.

This study has identified what disease conditions are present in captive populations and what diagnostic tools are routinely used at the examined facilities. It is suggested that the prevalence of all the listed conditions of interest may be underestimated for a number of reasons. Firstly, the presence of bone marrow diseases can only be definitively diagnosed if bone marrow is assessed. This can be done antemortem or post-mortem but requires a core marrow sample to be examined cytologically or histopathologically. Only three facilities (18%) reported using a bone marrow sampling technique when assessing ill koalas. This does not exclude the use of histopathology for diagnosis post-death but does indicate that antemortem techniques for bone marrow assessment are infrequently used by most facilities. Also, bone marrow may be less commonly sampled at necropsy unless there is suspicion of bone marrow disease. For example, in an animal that is aged or that may not have significant and obvious abnormalities in their blood film consistent with marrow disease, sampling of bone marrow may not be routinely done.

Diagnostic investigation may also be constrained where veterinary and laboratory assistance is extremely limited (for example, due to financial constraints and location/access difficulties). This may be especially true of smaller facilities. Therefore, it is highly plausible that many conditions may be missed or under diagnosed due to the lack of thorough diagnostic investigation and post mortem examination.

Further information regarding the prevalence of disease in captive koala populations can only be gained by the development of, and adherence to standardized diagnostic investigation and post mortem techniques in the future. This, in conjunction with routine screening of animals for KoRV and determination of viraemic loads may improve our knowledge on whether animals with high viraemic loads, more commonly develop neoplastic and immunosuppressive syndromes. Given the reports from two facilities of potential hereditary patterns of neoplastic disease this area needs further investigation. As KoRV is

an endogenized virus, accumulated amplification of virus through generations is a potential concern. If KoRV is found to definitively induce neoplasia then it is plausible that increasing reports of neoplasia in captive animals from the same genetic lineage will occur. This may be a much greater issue in the management of captive of koalas than in wild populations. If standardized diagnostic techniques, record keeping and necropsy protocols can be implemented throughout all institutions that house koalas then more accurate information can be gathered on the real prevalence of neoplastic and potential immunosuppressive disorders, and their relationship with KoRV. With improved knowledge in this area perhaps the gap between KoRV as a causative factor in these diseases can be closed and strategies essential to the future management of captive koala populations can be implemented.

Acknowledgments. I would like to especially thank all the participants that responded to this survey. I would also like to thank Dr Jon Hanger for his assistance with developing the questionnaire and his knowledge on the subject of KoRV, the reception staff at the Australia Zoo Wildlife Hospital and Australia Zoo for their unwavering dedication to retrieving the volumes of information required for this article. I would also like to thank Tiggy Grillo for her assistance and advice on disseminating the survey and Dr Sean FitzGibbon for his invaluable advice on how best to present this information.

References

Ávila-Arcos, M. C., S. Y. Ho, Y. Ishidam, N. Nikolaidis, K. Tsangaras, K. Hönig, R. Medina, M. Rasmussen, S. L. Fordyce, S. Calvignac-Spencer, E. Willerslev, M. T. Gilbert, K. M. Helgen, A. L. Roca, and A. D. Greenwood. 2013. One hundred twenty years of koala retrovirus evolution determined from museum skins. *Molecular Biology and Evolution* 30(2): 299–304.
http://dx.doi.org/10.1093/molbev/mss223

Canfield, P. 1990. Diseases affecting captive and free-living koalas and their implications for management. In *Koala Summit: Managing Koalas in New South Wales, 7–8 November 1988*, pp. 36–38. University of Sydney: Sydney, Australia.

Canfield, P. J., W. J. Hartley, and G. L. Reddacliff. 1990. Spontaneous proliferations in Australian marsupials—a survey and review .1. Macropods, koalas, wombats, possums and gliders. *Journal of Comparative Pathology* 103: 135–146.
http://dx.doi.org/10.1016/S0021-9975(08)80170-3

Carrick, F. 2013. National perspective on the current status of koalas: setting the scene for central Queensland koala conservation. In *Conserving Central Queensland's Koalas*, pp. 4–10. Central Queensland Univeristy: Rockhampton, Australia.

Connolly, J. H., P. J. Canfield, S. Hemsley, and A. J. Spencer. 1998. Lymphoid neoplasia in the koala. *Australian Veterinary Journal* 76: 819–825.
http://dx.doi.org/10.1111/j.1751-0813.1998.tb12337.x

Hanger, J. 1999. *An Investigation of the Role of Retroviruses in Leukaemia and Related Diseases in Koalas*. Ph.D. thesis, The University of Queensland, Australia.

Hanger, J., J. McKee, R. Tarlinton, and A. Yates. 2003. Cancer and haematological disease in koalas: a clinical and virological update. In *Proceedings of the Australian Association of Veterinary Conservation Biologists Annual Conference, Cairns*, pp. 19–30.

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: 4264–4272.
http://dx.doi.org/10.1128/JVI.74.9.4264-4272.2000

Krockenberger, M. B., P. J. Canfield, J. Barnes, L. Vogelnest, J. Connolly, C. Ley, and R. Malik. 2002. *Cryptococcus neoformans* var. *gattii* in the koala *(Phascolarctos cinereus)*: serological evidence for subclinical cryptococcosis. *Medical Mycology* 40: 273–282.
http://dx.doi.org/10.1080/mmy.40.3.273.282

Martin, R., and K. Handasyde. 1999. *The Koala: Natural History, Conservation, Management*. Second edition. UNSW Press Australian Natural History series, University of New South Wales Press Ltd: Sydney.

National Koala Conservation and Management Strategy. 2009–2014. Natural Resource Management Ministerial Council, Canberra, Australia, p. 37.

Rosenberg, N. 2011. Overview of retrovirology. In *Retroviruses and Insights into Cancer*, ed. Jaquelin Dudley, pp. 1–30. Springer eBooks, 363 pp.

Simmons, G., P. Young, J. McKee, J. Meers, and T. Mizuno. 2011. The epidemiology of koala retrovirus. *Journal of Veterinary Epidemiology* 15: 1–9.
http://dx.doi.org/10.2743/jve.15.1

Tarlinton, R. E., J. Meers, J. Hanger, and P. R. Young. 2005. Real-time reverse transcriptase pcr for the endogenous koala retrovirus reveals an association between plasma viral load and neoplastic disease in koalas. *Journal of General Virology* 86: 783–787.
http://dx.doi.org/10.1099/vir.0.80547-0