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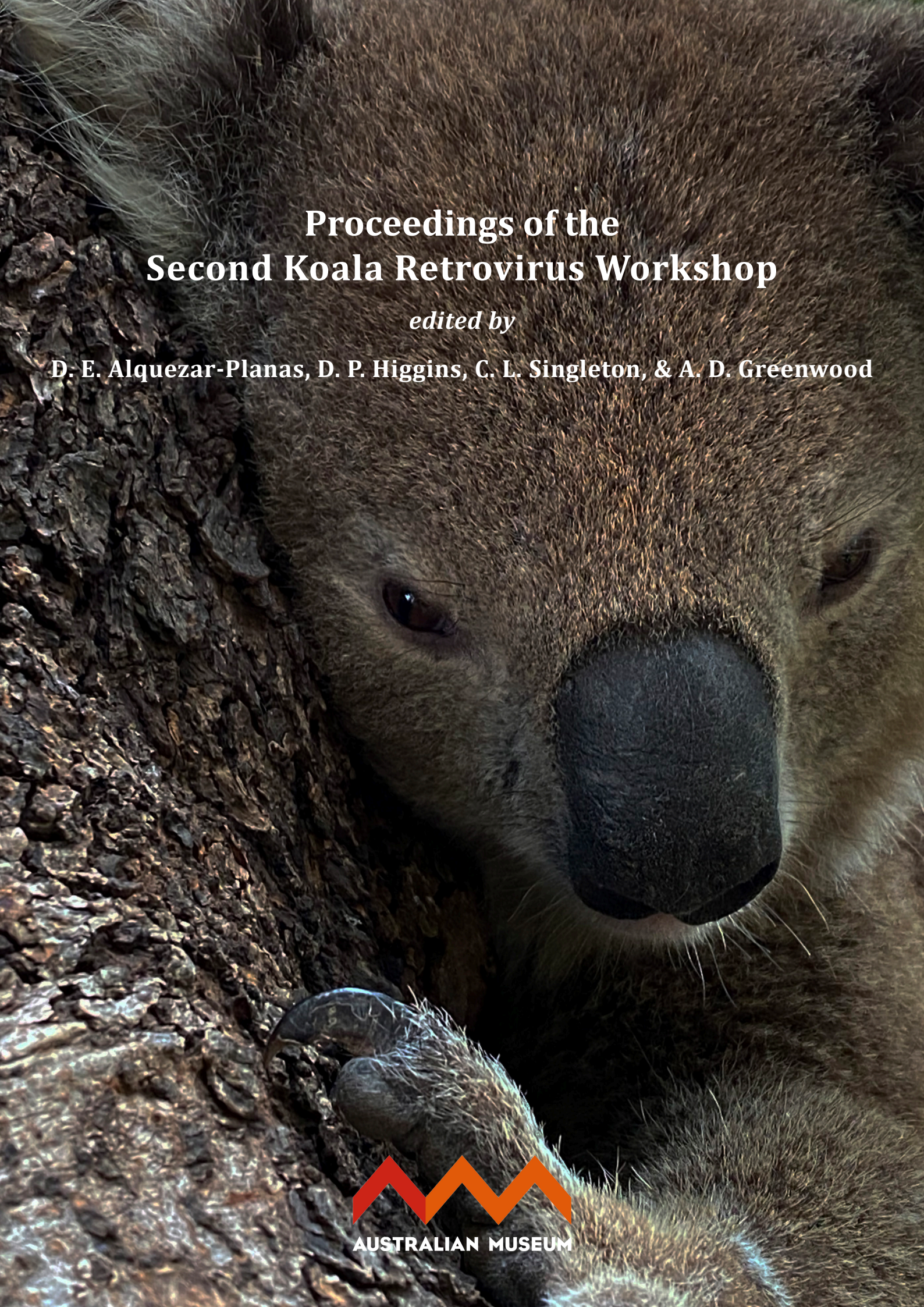

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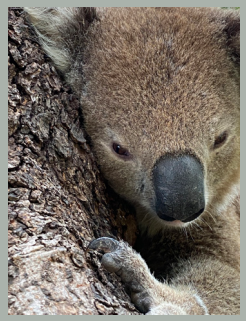

Cover photo
by Damien P. Higgins

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Koala Retrovirus Infection and Disease in South Australian Koala (*Phascolarctos cinereus*) Populations

Natasha Speight

School of Animal and Veterinary Sciences,
Faculty of Sciences, University of Adelaide, Roseworthy SA 5371, Australia

Abstract. Koala retrovirus (KoRV) infection, endogenous in all northern koalas (*Phascolarctos cinereus*), has been found to occur at lower, but increasing, prevalence in the Kangaroo Island and Mount Lofty Ranges koala populations in South Australia. Proviral and viral loads are also lower than in Queensland koalas, which may be due to exogenous spread of infection, or may be related to the variable presence of viral genes and fragmented expression that has been found in positive Mount Lofty Ranges koalas. However, high proviral loads and full expression across the KoRV genome in South Australian koalas has been found in individuals with neoplasia, particularly lymphoma, which can be as extensive and as severe as that observed in northern koalas. KoRV-A is the predominant subtype and no association with chlamydial status has been found except that high viral loads correlate with severity of chlamydiosis. Based on the complexity of KoRV infections in South Australian koalas, further research is needed to understand the differences in transmission and pathogenesis that occur.

Introduction

The understanding of koala retrovirus (KoRV) in koala (*Phascolarctos cinereus*) populations in South Australia (SA) has gradually increased over the past 15 years. Previous KoRV studies have focussed on Kangaroo Island, which holds one of the five geographically separated SA koala populations, the others being the Mount Lofty Ranges, Eyre Peninsula, the Riverland, and the lower southeast of the state. Kangaroo Island, at least prior to the 2019/2020 bushfires, and the Mount Lofty Ranges represent two of the largest SA populations and have generally been regarded as healthy, though genetically restricted. Recent KoRV research has been conducted with koalas from both of these populations, however the KoRV status of the other SA koala populations remains unknown.

Kangaroo Island

The Kangaroo Island koala population was founded from a small translocated group of koalas from French Island, Victoria, in the 1920s (Robinson, 1978), which subsequently expanded in numbers to the point of requiring population control measures (Duka & Masters, 2005). Their fecundity may be partly attributed to the recent finding that Kangaroo Island koalas are free of infection with *Chlamydia pecorum*, based on 170 koalas tested between 2014–2017 and analysis of over 13,000 veterinary records from a sterilization program (Fabijan *et al*., 2019). The total koala population, which was estimated at 50,000 in 2016, has now been reduced by approximately 80% in the recent bushfires to an estimated current population of 5,000–10,000 animals (DEW, 2020; Dunstan *et al*., 2021).

The earliest study of KoRV on Kangaroo Island in 2004 (n = 26) found no evidence of infection by end-point PCR (Tarlinton *et al*., 2006); however, a subsequent end point PCR based study conducted in 2007 found 15% prevalence within the animals sampled (n = 162) (Simmons *et al*., 2012). This low proportion of infected koalas, in conjunction with low proviral load, led researchers to conclude that transmission was exogenous, rather than endogenous, in this population (Simmons *et al*., 2012). However, a 2013 publication reported an updated prevalence of 30–35%

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ORCID iD: Natasha Speight https://orcid.org/0000-0001-5367-0241
Corresponding author: Natasha Speight natasha.speight@adelaide.edu.au
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within the animals sampled, based on unpublished data (Denner & Young, 2013), suggesting either a rapid increase in KoRV spread in Kangaroo Island koalas, a heterogenous distribution of KoRV in the island's population, or use of a more sensitive assay.

Our qPCR-based KoRV prevalence study in 2014–2017 targeted wild-caught Kangaroo Island koalas (n = 170) and detected KoRV in 42% of samples, with all positive animals positive for KoRV-A and negative for KoRV-B (Fabijan *et al*., 2019). The median proviral copy number was only 113 KoRV copies/10^3 β-actin copies; however, some koalas showed higher loads (maximum 12641 KoRV copies/10^3 β-actin copies), suggesting that in some individuals either exogenous KoRV infection was more extensive or that endogenous transmission was occurring. This mixed transmission pattern was further supported by the finding that of 19 mother-joey pairs, the infection status differed in five cases, with two pairs in which the mother was KoRV-positive and offspring negative, and three pairs where the offspring only was KoRV-positive (Fabijan *et al*., 2019). That several offspring were positive independent of their dam could represent dam infection below the detection limit, endogenous transmission from the sire, or exogenous transmission from other koalas.

Mount Lofty Ranges

The Mount Lofty Ranges koala population, near Adelaide, is principally derived from Kangaroo Island koalas translocated in the 1960s, with reported addition of individuals brought from New South Wales (Robinson, 1978) and Queensland (Lindsay, 1950). Little was known of the health status of this koala population, except that *Chlamydia pecorum* infection was common in the absence of clinical disease (Polkinghorne *et al*., 2013). Following this, high prevalence of the renal disease, oxalate nephrosis, was described at up to 55% in cohorts of necropsied individuals (Speight *et al*., 2013; Speight *et al*., 2018). In 2016, chlamydial infection was identified in 47% of wild-caught koalas (n = 75), associated in several cases with ocular and urogenital disease (Fabijan *et al*., 2019).

Our 2016 study of wild-caught Mount Lofty Ranges koalas (n = 75) identified a KoRV prevalence of 65% within the animals sampled, with a median proviral copy number of 35 copies/10^3 β-actin copies (maximum 574 KoRV copies/10^3 β-actin copies) (Fabijan *et al*., 2019). Only KoRV-A, not KoRV-B, was detected, and the likelihood of KoRV infection increased with age (Fabijan *et al*., 2019). KoRV was not found to be associated with chlamydial infection or disease, but periodontitis was more common in KoRV positive koalas (Butcher *et al*., 2020). Concurrent studies investigated putative KoRV-associated diseases, including the neoplastic conditions, leukaemia and lymphoma. The first documented case from 2014 was an older female koala initially presenting with hindlimb lameness, but found at clinical examination to have concurrent lymphosarcoma, reproductive chlamydiosis, and KoRV infection (Fabijan *et al*., 2017).

A large comparative study of KoRV in necropsied koalas from Queensland and SA found lymphoma in 4.3% (4/92) of the KoRV positive Mount Lofty Ranges koalas sampled (Fabijan *et al*., 2020). High proviral loads were found in both SA and Queensland koalas with neoplasia (Sarker *et al*., 2020); however overall, the SA koalas had lower proviral loads (median 2.71 × 10^3 KoRV DNA copies/10^3 β-actin copies) compared with Queensland koalas. Only 51% of SA koalas sampled had circulating virus detected, for which the load was also lower than koalas from Queensland (Sarker *et al*., 2020). However, high viral load in SA koalas was positively correlated with chlamydial disease severity, and in both populations, positively correlated with splenic lymphoid area, lymphocyte count, and metarubricyte count (Fabijan *et al*., 2020). KoRV-A was found to be the predominant subtype in the Mount Lofty Ranges cohort (Sarker *et al*., 2019).

As part of this large study, variable absence of KoRV DNA and RNA genes (Sarker *et al*., 2020) and defective expression (Tarlinton *et al*., 2017) was found in SA koalas in comparison with Queensland koalas, for which all genes were present (Sarker *et al*., 2020) with high expression (Tarlinton *et al*., 2017). A recent study comparing KoRV positive (all genes present on PCR) koalas, KoRV positive koalas with lymphoma, and KoRV negative (central KoRV genes absent on PCR) koalas in the Mount Lofty Ranges found only fragmented, or no expression, of central KoRV genes (*gag*, *pol* and *env*) in three positive and two negative SA koalas, respectively, despite expression of the terminal regions in all koalas (Stephenson *et al*., 2021). However, KoRV positive koalas with lymphoma showed high expression of all KoRV genes (Stephenson *et al*., 2021). These findings may explain the lower prevalence of KoRV in SA koala populations, the lower proviral and viral loads in positive animals, and the lower incidence of KoRV-associated neoplasia.

Conclusion

KoRV infection clearly shows a high level of complexity that differs among the koala populations across Australia. More research is needed to further understand the epidemiology and pathogenesis in both the Kangaroo Island and Mount Lofty Ranges populations of SA koalas. The infection profile of KoRV in southern koalas offers the ability for comparison with northern koala KoRV infections, and the hope that some koala populations in Australia can harbour koalas that are regarded as KoRV negative.

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