The Koala and its Retroviruses:
Implications for Sustainability and Survival

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KoRV and *Chlamydia*: Are they Co-culprits?

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Abstract. There are two main infectious disease threats for the koala; *Chlamydia* and KoRV. A major question is whether or not KoRV predisposes koalas to more severe chlamydial disease. In the only study to date that has examined co-infections, KoRV load (as determined by qPCR) and chlamydial load (as determined by qPCR) and chlamydial disease were examined in wild koalas. While there was a statistically significant correlation between *Chlamydia* infection load and *Chlamydia* clinical disease score, there was no significant correlation between KoRV load and either *Chlamydia* infection load or *Chlamydia* clinical disease score, however the groups were not ideally constructed and hence additional comparisons are needed. If KoRV does predispose koalas to more severe chlamydial disease, one would expect it to do this via an effect on the koala immune system. A series of *Chlamydia* vaccine trials in captive as well as wild koalas are showing that koalas in fact appear to make perfectly normal antibody and cytokine responses to vaccine antigens, even if they have high circulating KoRV loads, arguing against an immune suppressive effect by KoRV.

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In Australia, wild koala (*Phascolarctos cinereus*) populations in many areas, particularly Queensland and NSW, are declining for many reasons. One of the main causes of these declines is infection and disease due to *Chlamydia* (Polkinghorne et al., 2013). While *Chlamydia* cause similar disease syndromes in their non-koala hosts, the koala seems to have a higher than expected level of disease. This raises the question as to whether or not KoRV is somehow contributing to chlamydial disease. This brief overview will focus first on what we know about *Chlamydia* in koalas and then look at the very limited data regarding KoRV and *Chlamydia*.

Overview of *Chlamydia*

Chlamydia is an obligate intracellular bacterium with a unique two-phase developmental cycle. Immunity to chlamydial infections requires both a strong, neutralising antibody response as well as an interferon-gamma directed T cell response. Of the nine species present in the genus *Chlamydia*, two, *C. pecorum* and *C. pneumoniae*, cause infections in koalas (Jackson *et al*., 1999; Deveraux *et al*., 2003). The frequency of chlamydial infections (measured by a range of techniques, but utilizing PCR mostly of late) varies between populations, ranging from nil (on just a few island populations) to 90% in several populations (Polkinghorne *et al*., 2013). Disease levels also vary, but usually represent 25% or so of the infection level at any time point sampled. Animals are infected at ocular and urogenital sites mainly. Of the two chlamydial species, *C. pecorum* is by far the most common and is thought to be the species responsible for the symptoms observed (Glassick *et al*., 1996).

Even though it is *C. pecorum* that is responsible for most infection and disease in koalas, there is considerable genetic diversity between sub-strains (Jackson *et al*., 1997). A range of gene markers have been used to assess *C. pecorum* strain diversity and while there are some minor differences, they all show that the various koala *C. pecorum* genotypes cluster together, but show considerable strain diversity.

Chlamydia infection and clinical disease

A key question that is still unanswered is how chlamydial infection or strain variation, relates to overt clinical disease. We know from other species of *Chlamydia* that different strains and sub-strains account for differences seen in pathogenicity (although this is still a new area of research for all *Chlamydia*). Therefore, given that we have considerable strain diversity within the strains of *C. pecorum* infecting koalas, it is also quite conceivable that this strain diversity also explains the virulence difference observed.

Chlamydia and KoRV

Finally, is there any evidence linking KoRV infection to adverse pathogenicity for chlamydial co-infections ? There is very limited data on which to make any comments. The Queensland University of Technology (QUT) group is developing an anti-*Chlamydia* vaccine and this has been used to vaccinate a significant number of koalas now. In several of these trials, in a captive koala colony, the vaccinated animals were tested for exogenous KoRV levels and found to have high KoRV copies per ul (greater that 10^6 and even up to 10^8). Despite these high levels of exogenous KoRV, all animals produced a very strong B and T cell response to the chlamydial vaccine.

The only other study was conducted as a collaboration between researchers at University of Queensland and QUT. Log KoRV load (measured as exogenous KoRV via PCR) was analysed against *Chlamydia* infection load (as measured by a quantitative PCR assay) and *Chlamydia* disease score. While there was a statistically significant correlation between *Chlamydia* infection load and *Chlamydia* clinical disease score, there was no significant correlation between KoRV load and either *Chlamydia* infection load or *Chlamydia* clinical disease score. This study however did have several limitations and deserves to be repeated.

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