

CHLAMYDIA AND KOALAS: PROGRESS TOWARDS THE DEVELOPMENT OF A VACCINE AND UNDERSTANDING THIS DEBILITATING DISEASE

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Abstract

Virtually every wild population of koalas in Australia is infected with *Chlamydia*, and in many cases, the level of disease is a severely threatening process. Of the nine currently recognised species of *Chlamydia*, two infect koalas, *C.pecorum* and *C.pneumoniae*. Recent epizootiological studies in SE Queensland show that approximately 50% of animals are infected, with *C.pecorum* being the most common and most virulent species. The question of how long koalas have had chlamydial infections and where they got them from, is still unanswered, but we are moving closer to this by exploring the genetic relationships between *C. pecorum* strains in koalas and those that are found in Australian livestock. Of the processes threatening koala populations (habitat destruction, road accidents, domestic dogs, disease) modelling suggests that only disease efforts can have significant effects in declining populations. We have therefore begun a program to develop a chlamydial vaccine for koalas. Using different vaccine combinations and delivery methods, we have shown that healthy koalas can mount a strong immune response to vaccination. We have also shown that the vaccine is safe to administer to both healthy and diseased koalas. While there are still many hurdles to overcome, the development of an effective chlamydial vaccine looks promising.

***Chlamydia* and the decline of Australia's free-range koala populations**

The koala (*Phascolarctos cinereus*), a native Australian arboreal marsupial, is the only surviving family member of *Phascolarctidae*. Many koala populations throughout Australia are under significant threat of collapse with serious declines leading to localised extinction. The expeditious nature of these declines can be largely attributed to confounding pressures of man such as: 1) land clearing and habitat fragmentation; 2) motor vehicle trauma; and 3) dog attacks. Despite the presence of these factors, modelling has revealed that chlamydial disease is the most significant of these processes and the only factor that, if reduced, could stabilise a peri-urban wild koala population (Rhodes et al., 2011).

Chlamydia, an obligate intracellular bacterial pathogen, is the main aetiological agent of disease in the koala. Koalas are infected with two species of *Chlamydia*, *C. pecorum* and *C. pneumoniae*, which have been detected in most screened wild koala populations to date. *C. pecorum* is consistently the most widespread and more pathogenic of the two species (Jackson et al., 1999; Devereaux et al., 2003). The clinical impact of chlamydial disease on the koala is profound. Clinical manifestations of koala disease include ocular infections, resulting in either acute or chronic active keratoconjunctivitis that can lead to blindness if left untreated (Cockram and Jackson, 1981; Wan et al., 2011). Urinary tract infections are also prevalent and can cause cystitis, which manifests as a discoloration and ulceration of the koala's rump ("dirty tail" or "wet bottom") due to the animal's incontinence.

Perhaps the most important manifestations of chlamydial disease are linked to the effects of upper reproductive tract infection in female koalas. In these animals, chlamydial infection and the resulting inflammation of the upper reproductive tract organs can cause structural changes which, in severe cases, cause sterility (Girjes et al., 1988; Wan et al., 2011). Importantly, many koalas carry *Chlamydia* without displaying symptoms while others can show chronic and permanent signs of disease long after the chlamydial infection has resolved (Wan et al., 2011). In these cases, laboratory diagnosis using molecular methods will usually be required.

An update on chlamydial epidemiology

Epidemiological studies have revealed that chlamydial infections in this host species are widespread with prevalence rates of chlamydial infection in studied populations on Australia's mainland ranging from 6 – 100% (McColl et al., 1984), although the exact rate is heavily influenced by the detection method used and the individual population studied. While sexual transmission is clearly a major mechanism of transmission for koala chlamydiae, data of Jackson et al. (1999) also suggest that young animals can be infected by their mothers, possibly by engaging in pap feeding of their young.

The origin of *Chlamydia* in koalas?

It is currently unclear whether chlamydial disease in the koala is an example of a “natural infection”. Molecular epidemiological investigations of koala *C. pecorum* have previously revealed that genetically related strains of this bacterium could be found in Australian cattle and sheep as well as in geographically-distinct koala populations (Jackson et al., 1997), supporting ongoing cross-host transmission between domestic livestock and koalas. More recently, doubts have surfaced over these observations with new sequence data emerging that koala *C. pecorum* strains, while genetically diverse, appear to be distinct from cattle and sheep strains, suggesting a rapid diversification and expansion of the koala lineage, possibly supporting isolated cross-host transmission and selection in a “new host” (Marsh et al., 2011), however, this analysis did not include samples from Australian livestock. Koala *C. pneumoniae* strains conversely show a different evolutionary trajectory, supporting evolution of this microorganism with the koala host and the suggestion that these and related animal strains are ancestral to *C. pneumoniae* strains in humans (Mitchell et al., 2010a; Mitchell et al., 2010b).

Progress towards developing a chlamydial vaccine

While some treatment options exist for koalas with identifiable chlamydial infections and disease, a chlamydial vaccine is still likely the best option for controlling this problem. We have already made excellent progress towards developing a safe prototype vaccine for koala *C. pecorum*. Initial trials of our chlamydial vaccine for the koala evaluated the immunological response of healthy koalas to a multi-subunit chlamydial vaccine derived from non-koala chlamydial species (Carey et al., 2010). The observed high levels of antigen-specific peripheral blood mononuclear cell (PBMC) proliferation (> 1 year) and sustained plasma antibody levels in immunised animals. Further, koala IgG antibodies were able to inhibit *in vitro* infection of McCoy cells with *C. muridarum*, the strain from which the vaccine antigens were derived. More recently healthy koalas, alongside koalas with significant ocular or urogenital tract disease, were immunised with a chlamydial major outer membrane protein (MOMP)-based multi-subunit vaccine derived from koala *C. pecorum* (Kollipara et al., 2012). This study

showed that koala vaccination is safe and induces an immune response that may provide ongoing protection from natural *C. pecorum* infections in the future. An important aspect of this latter work was the observation of cross-reactivity of MOMP antibody responses from vaccinated healthy and diseased koala cohorts to different MOMP proteins. To date our results provide strong evidence that development of a chlamydial vaccine to protect this iconic marsupial is feasible. While these results are promising, much work still needs to be performed. Future work planned will further evaluate cross-protection offered by our prototype MOMP-based recombinant protein vaccine in koala *C. pecorum* strains detected outside of the South-East Queensland region and may also soon include trials of the vaccine in wild koalas and those presenting to wildlife care groups. The work and collaboration of the latter groups will be essential to the success of this koala *Chlamydia* vaccine.

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