



Chlamydial infection and disease in the koala

Chlamydiae are obligate intracellular bacterial pathogens able to infect and cause serious disease in humans, birds and a remarkably wide range of warm and cold-blooded animals¹. The family *Chlamydiaceae* have traditionally been defined by their unique biphasic developmental cycle, involving the inter-conversion between an extracellular survival form, the elementary body and an intracellular replicative form, the reticulate body. However, as with many other bacteria, molecular approaches including 16SrRNA sequence are becoming the standard of choice. As a consequence, the chlamydiae are in a taxonomic state of flux. Prior to 1999, the family Chlamydiaceae consisted of one genus, *Chlamydia*, and four species, *Chlamydia trachomatis*, *C. psittaci*, *C. pecorum* and *C. pneumoniae*. In 1999, Everett *et al*² proposed a reclassification of *Chlamydia* into two genera (*Chlamydia* and *Chlamydophila*) and nine species (*Chlamydia trachomatis*, *C. suis*, and *C. muridarum* and *Chlamydophila psittaci*, *C. pneumoniae*, *C. felis*, *C. pecorum*, *C. abortus*, and *C.*

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caviae). While some of these species are thought to be host specific (*C. suis* – pigs, *C. muridarum* – mice, *C. felis* – cats, *C. caviae* – guinea pigs) many are known to infect and cause disease in a wide range of hosts.

Chlamydial disease in the koala continues to be one of the most common infectious diseases affecting this unique marsupial. Infections lead to four major disease syndromes in the koala; keratoconjunctivitis, respiratory tract disease, genital tract disease eventually resulting in infertility, and urinary tract infection causing incontinence which is sometimes known as 'dirty tail' or 'wet bottom'³. The original isolates made

from koalas were designated as *C. psittaci*, however as the genus was expanded in the 1990s, the koala strains were re-designated *C. pecorum* and *C. pneumoniae*. *C. pecorum* appears to be the species responsible for conjunctivitis, cystitis, nephritis and genital inflammation. *C. pecorum* infections are widespread in most free-range koala populations and, depending on the diagnostic test used, infection levels range from 10-90%^{4,6}. In a few geographically isolated populations, such as French Island, Magnetic Island and Kangaroo Island, no chlamydiae have been detected, however *C. pecorum* are present in all other populations that have been tested. *C. pneumoniae* was once thought to be a specific pathogen of humans, but has now been found in association with pneumonia, rhinitis and conjunctivitis in the koala⁷. Infections caused by the koala biovar of *C. pneumoniae* are usually low grade, especially in comparison to *C. pecorum* infections within the same population⁶.

The increasing use of family-wide PCR and sequencing approaches has now shown that additional types of chlamydiales are present in the koala. Devereaux *et al*⁸ showed that in addition to *C. pecorum* and *C. pneumoniae*, a wide range of novel Chlamydiales were present in diseased koalas. In the free-range population they studied, there was 72% total chlamydial prevalence, which compromised 52% prevalence of *C. pecorum*, 12% prevalence of *C. pneumoniae* and greater than 50% prevalence of the novel chlamydiae. The novel koala chlamydiae themselves cluster together with other *Chlamydia*-like bacteria but are within a second lineage separate from the more traditional

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Chlamydiaceae species. Because of the overall high prevalence levels in this particular population, not surprisingly, many koalas were infected with more than one chlamydial genotype. The novel chlamydial genotypes were found at both ocular and urogenital anatomical sites, including evidence for ascending infections within the female genital tract and hence these novel chlamydiae could potentially cause disease in their own right.

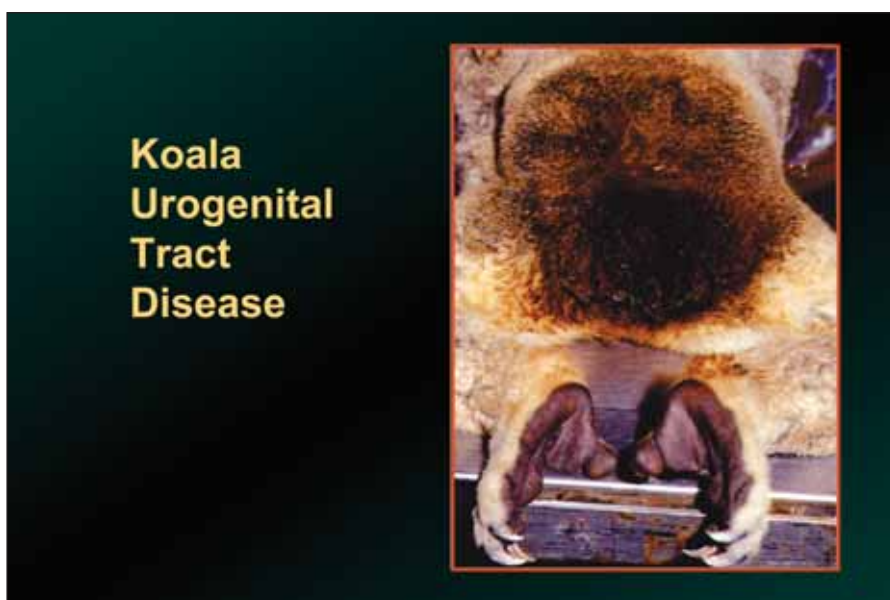
One question that still remains unanswered is – where did the chlamydial strains infecting koalas come from. It is now clear that the genotypes of chlamydiae infecting the koala are primarily not host restricted. Work by Jackson *et al*⁹ showed that several genotypes of *C. pecorum* isolated from koalas were genetically identical to those found in sheep/cattle from Australia, some of which are also present in Europe. This suggests that at least some of the strains of *Chlamydia* present in koalas today possibly came via infections in domestic species such as sheep and cattle when they came to Australia with European settlers. There is good evidence that *Chlamydia* infect the gastrointestinal tract of sheep and cattle and are shed in the faeces. Given that

sheep/cattle in Australia often inhabit similar land to koalas, this mode of cross-host transmission is certainly feasible.

Other genotypes of 'koala' chlamydiae, however, do not appear to have similar histories. This suggests that the koalas probably shared their chlamydiae with a range of other hosts some time in the past and indeed some 'koala' genotypes may well be restricted to the koala. The relatively recent finding of *C. pneumoniae* in koalas is also of significant interest. *C. pneumoniae* was designated as new species in 1989 and the strains isolated from humans (respiratory and cardiovascular) are all remarkably similar, genetically. The strain of *C. pneumoniae* from koalas is very similar to the human strains. However, as analysed by Wardrop *et al*¹⁰ using four gene markers, the koala strains usually show some minor polymorphisms compared to the human strains, suggesting that they have a different origin. However, a recent study by Cochrane *et al*¹¹ using atherosclerotic plaque material from patients with carotid artery disease and genotyping the *C. pneumoniae* isolates by sequencing fragments of two genes, identified genotypes in these patients that were apparently the same as isolates previously

obtained from koalas. Because all *C. pneumoniae* isolates are very similar genetically, caution should be used in interpreting this data. Nevertheless, it does appear that *C. pneumoniae* is clearly not restricted to humans and has now been identified in a range of warm and cold blooded hosts including amphibians, reptiles, other Australian wildlife (eg bandicoots and gliders) and also koalas.

The final aspect relates to the impact of chlamydial disease in the koala and its importance in the potential downfall of particular koala populations. *Chlamydia* seems to have many common features in all the hosts it infects. It is often extremely good at transmitting itself between hosts, despite its apparent fragile nature outside its own host cell environment. This quality also holds for the koala, because given the relative infrequency of contact between koalas in the wild, high infection levels are not uncommon. Chlamydial infections in most hosts are not uncommon and are usually chronic, often staying with the host for many years, if not for life. This is true for genital *C. trachomatis* infections in humans as well as *C. pneumoniae* respiratory and possibly cardiovascular infections in humans. A similar characteristic is probably also true for koalas and their chlamydial infections. While chlamydial infections might be common, chlamydial disease is much less common, with perhaps only 10-20% of infections manifesting as disease at any point in time. This is true for genital *C. trachomatis* and respiratory *C. pneumoniae* infections in humans and again a similar trend is seen in koalas. An open question, though, is whether particular strains of *Chlamydia* are virulent and, therefore, these strains always cause disease or whether all strains of *Chlamydia* are capable of causing chronic infections and, subsequently, disease. It is known from human and other



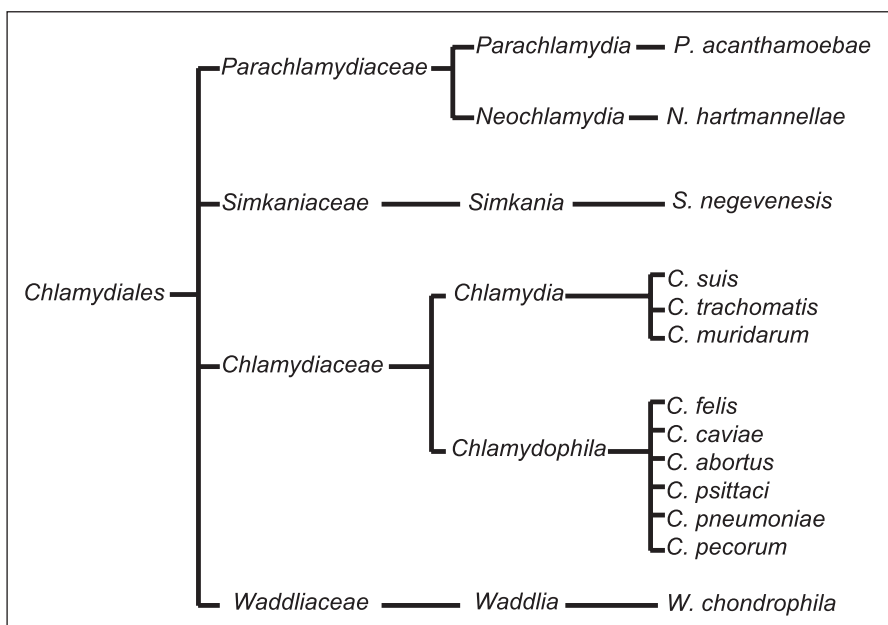
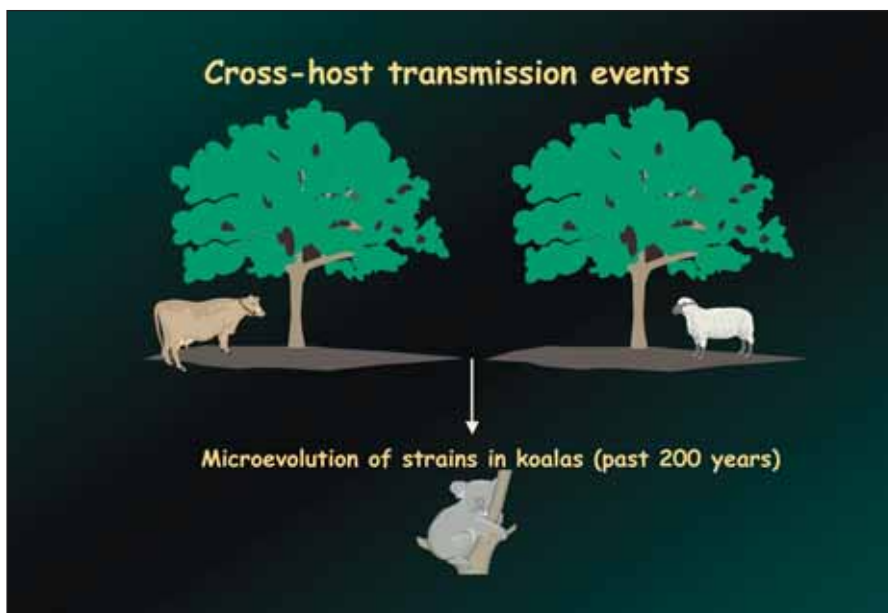


Figure 1. Taxonomic tree of the order *Chlamydiales* based on 16S and 23S rRNA sequences².

animal studies that individuals with apparently harmless or chronic chlamydial infections, can lapse into disease if their immune status is perturbed, either by other infections or by stresses of various types. It is likely that the same *Chlamydia*-wide phenomenon occurs in the koala and that, even though most populations have medium to high levels of chlamydial infection, that is not a major threat to the individual animal when they are in good health. However, if their health is

perturbed for any reason (eg stress due to lack of adequate food trees, stress due to overcrowding or human impact, immunosuppression due to retroviral infection) then the balance is tipped in favour of the parasite and individual populations can be under the combined threat. *Chlamydia* is currently undergoing a whole genome sequencing explosion and this new wealth of information should shed more light on this intriguing parasite and its interaction with all its hosts, including the koala.

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