

Chlamydia in koalas

Fact sheet

September 2023

Key points

- Chlamydiosis is an extremely common and important bacterial disease of both wild and captive koalas and occurs throughout their geographic range.
- Clinical signs include keratoconjunctivitis (inflamed eyes) and brownish staining and wet fur around the rump (wet bottom). Reproductive disease is often asymptomatic.
- Treatment is possible but requires courses of antibiotics and captive care.
- Chlamydiosis has the potential to cause significant population declines, when combined with other pressures, particularly in naïve koala populations.

Aetiology

The *Chlamydiaceae* are a family of Gram-negative, obligate intracellular bacteria. They have two forms: an infectious elementary body and a replicating reticulate body. *Chlamydia pecorum* and *C. pneumoniae* are the causative agents of chlamydiosis in koalas ^[1, 2]. *Chlamydia pecorum* is the main cause of chlamydial infection and disease in koalas, while *C. pneumoniae* appears to be less pathogenic and is detected infrequently ^[1, 3-5].

One Health implications

Wildlife and the environment: chlamydial disease has a detrimental impact on individual koala health and welfare. The reduction in fertility caused by this disease has the potential to impact population viability. Both *C. pneumoniae* and *C. pecorum* have been found in other Australian species, however there are no confirmed reports of transmission between koalas and other wildlife ^[6].

Domestic animals: both *C. pneumoniae* and *C. pecorum* have been found in domestic animals. Some koala *C. pecorum* genotypes are genetically similar to genotypes found in livestock. However, there have been no confirmed reports of transmission between koalas and domestic animals ^[6].

Humans: there is no evidence of transmission of *C. pecorum* or *C. pneumoniae* from koalas to humans^[7].

Natural hosts

As well as koalas, *C. pecorum* has been found to infect birds, marsupials and mammals, specifically bandicoots, gliders, quolls, possums and various species of livestock ^[8-12]. Some koala genotypes have been found in other Australian native species ^[9, 10].

Chlamydia pneumoniae has also been isolated from humans, horses, reptiles, amphibians and other marsupials ^[13]. However, animal strains of *C. pneumoniae* appear genetically distinct from human strains ^[14].

World distribution

The disease is reported in captive koalas held outside Australia^[15].

Occurrences in Australia

Chlamydia has been detected in wild koalas across their geographic range, in Vic, NSW, Qld and SA ^[13]. Specific *C. pecorum* genotypes are geographically distinct and are found in only northern or southern koala populations ^[16].

Epidemiology

Most cases of chlamydiosis are caused by *C. pecorum* and it is the more pathogenic of the two chlamydiosis-causing species in koalas. Disease due to *C. pneumoniae* infection is rare, although it should still be considered in cases that are negative for *C. pecorum*^[17]. Both species of *Chlamydia* can be isolated from ocular and urogenital sites in koalas.

Infection with *C. pecorum* often occurs without evidence of clinical disease. This may be because the infection does not cause clinical disease or the infection may be causing inapparent disease, requiring specialised equipment for detection ^[4, 18, 19]. It is likely that a combination of factors such as host immune response, pathogen, environment and co-infection influence the development of chlamydial disease ^[13].

The prevalence of chlamydial infection in different free-ranging koala populations ranges from 0-89% ^[17, 20], whereas the prevalence of overt chlamydial disease ranges from 4% to 44% ^[2, 21]. Studies suggest that two thirds of koalas infected but with no clinical signs, will go on to develop signs of disease over the next four years ^[22]. Typically, northern koala populations are considered to suffer more severe chlamydial disease, compared to southern populations ^[2, 17, 20, 23, 24].

Transmission is generally by direct contact (during mating) or via aerosol. This can include direct transfer of infected discharges from the eyes and urogenital tract, venereal transmission via infected semen and faecal-oral transmission during pap feeding by dependent young. Spread to juveniles may also occur during birth ^[7, 15, 25]. After experimental inoculation the incubation period was 7-19 days for the ocular form of the disease and 25-27 days for the urinary tract form ^[15].

Chlamydia are relatively resistant organisms and can survive outside the host in appropriate environmental conditions ^[26].

Clinical signs

Infection can be clinical or subclinical (meaning disease is not apparent at clinical examination). Clinical disease causes keratoconjunctivitis, urinary tract infection, and/or reproductive tract infection ^[7, 27-29]. Koalas acutely affected with ocular disease may have blepharospasm (squinting or closed eyes), and a serous discharge that progresses to become mucopurulent. The conjunctival tissues become red and begin swelling within two weeks after the onset of clinical signs with the discharge becoming purulent. Over time the conjunctival tissues may proliferate and project beyond the lid margin completely obscuring the eye. Blindness or a secondary keratitis may develop.

Koalas with urinary tract disease develop brownish staining and wet fur around the cloaca and rump due to constant wetting with urine. This is commonly known as 'wet bottom'. There may also be evidence of pain (crying) and straining when urinating.

Reproductive tract infections are often asymptomatic, the only indication being reduced fertility, particularly in females. Palpable changes to the testis or ovaries may be present ^[7, 18, 30-32].

Diagnosis

Diagnosis is based on clinical signs in conjunction with laboratory tests. Palpation and ultrasonography can be used to detect ovarian bursal cysts, bladder wall thickening, pyometra, hydronephrosis and renal fibrosis. Diagnosis may be difficult in koalas due to the lack of bacterial shedding in some infected koalas, and the absence of clinical signs of disease ^[4].

Laboratory diagnostic specimens and procedures

PCR is the preferred method for confirming chlamydial DNA, as it allows for detection of non-pathogenic and inapparent infections ^[13]. For live koalas, the conjunctiva and urogenital sinus or penile urethra are swabbed. Viable organisms are more reliably obtained on a second swab. The first swab removes exudate and surface debris while the second swab is more likely to remove viable epithelial cells ^[7, 32]. Using correct sample collection techniques, PCR is a sensitive and reliable method that can be used to test swabs. The cost and laboratory requirements of PCR testing can restrict its use. The use of PCR on faecal samples is being assessed as a non-invasive method for the detection of chlamydia in free-ranging populations ^[33-35].

Loop mediated isothermal amplification (LAMP) assays can be used for point-of-care testing in koala hospital and rehabilitation facilities in Australia ^[36-39]. This type of testing, is rapid but may be of lower sensitivity ^[6].

An immunohistochemistry test has been used on formalin-fixed material, although this method may have poor sensitivity ^[40, 41]. There is no commercially available serological test available ^[7].

A complete necropsy should be performed on all dead koalas. A range of tissues should be collected, including any obvious lesions, and submitted in formalin for histopathology.

Pathology

Ocular chlamydiosis presents histologically as a conjunctival accumulation of plasma cells and neutrophils along with villous hypertrophy and hyperplasia of the conjunctival epithelium.

Urinary tract disease involves the bladder, kidneys and urethra. Cystitis is characterised by mucosal hyperplasia and degeneration of the epithelium with mixed inflammatory cells and fibrosis of the

lamina propria and submucosa, depending on the chronicity of the disease. In severely affected bladders, hyperplasia and hypertrophy may result in a polypoid appearance. Renal changes include tubular dilatation and degeneration, protein cast formation, hydronephrosis, chronic interstitial nephritis, pyelonephritis and fibrosis.

Male koalas with prostatitis may have degeneration of the glandular epithelium with large accumulations of necrotic debris and inflammatory cells in the ducts and gland parenchyma. Fibrosis and mononuclear infiltration of the lamina propria and submucosa of the prostatic urethra may also occur. Issues with sperm morphology, motility and viability may also be seen ^[42].

Female koalas may have evidence of vaginitis, cervicitis, metritis, salpingitis and ovarian diverticulitis. These are characterised by a mixed inflammatory infiltrate and, in more chronic cases, squamous metaplasia of the uteri and cystic dilation of the uterine glands along with submucosal fibrosis causing the walls of the genitalia to be thickened and distorted. Endometrial glands may be destroyed. There may be fibrous adhesions between the ovaries and bursal walls and cyst formation in the ovarian bursae ^[13, 32].

Differential diagnosis

Differential diagnoses include other agents that can cause conjunctival, urinary or reproductive tract disease in koalas. *Streptococcus, Staphylococcus, Corynebacterium* spp., *E. coli, Bacillus* spp., *Pasteurella* spp., *Aspergillus* spp. and *Klebsiella oxytoca* have all been isolated from koala reproductive tracts ^[32]. Phascolarctid herpesvirus has been recently associated with reproductive disease, specifically paraovarian cysts in koalas ^[43].

Treatment

Treatment generally involves the administration of antibiotics such as enrofloxacin, florfenicol, azithromycin, marbofloxacin, chloramphenicol or doxycycline. Each antibiotic has certain issues regarding either availability, side effects, treatment success or gut dysbiosis. For the treatment of chlamydiosis in koalas, chloramphenicol and doxycycline are commonly used ^[6], although no significant difference has been found between the choice of antibacterial treatment and koala survival ^[44]. The optimal duration of treatment has not yet been determined, so current recommendation is that koalas with severe disease are treated for 28 days or more ^[13, 44]. Topical antimicrobials, usually combined with topical corticosteroids, flushing and systemic treatment, are used to treat ocular disease. For severe conjunctival proliferation, surgical intervention should only be considered if vision is obscured at the end of treatment. Analgesia and anti-inflammatories should be provided, using meloxicam, buprenorphine, carprofen or prednisolone ^[13].

Because antibiotics may affect the koala's gastrointestinal microflora, nutritional support and prevention is important. Koala faecal matter is commonly used as a gut microflora supplement to reduce the risk of dysbiosis, although more research is required to evaluate its efficacy ^[45-47].

Antibiotic treatment improves the koala's clinical status and reduces shedding, but may not actually eliminate the organism, due to the chronic status of most of the infections encountered, the intra-

cellular nature of the bacteria and the low blood concentrations achieved following administration of enrofloxacin and chloramphenicol ^[48, 49].

For severely debilitated animals, or those suffering co-morbidities, successful treatment is generally not possible, and the decision to euthanise is based on animal welfare concerns ^[50].

Prevention and control

Captive populations: to maintain chlamydia-free populations all new koalas should be isolated and held in quarantine for a minimum of 45 days. They should be anaesthetised, examined and tested by PCR twice, at least 21 days apart. See the Australian Government "Conditions for the overseas transfer of koalas" document (2009) <u>www.environment.gov.au/system/files/resources/e54cd87f-22b5-46e9-8a21-5e16f7c645cb/files/koala-export-conditions.rtf</u>.

Biosecurity practices are important for preventing chlamydial transmission within captive settings and when reintroducing captive individuals into the free-ranging population. Important practices include isolating animals from different locations while in care, preventing cross-contamination through appropriate decontamination, disinfection, personal protective equipment and equipment use practices and releasing rehabilitated individuals at their location of origin ^[51, 52].

Free ranging populations: prevention and control of *Chlamydia* in wild populations is challenging and generally involves capture and treatment of diseased koalas ^[13]. *Chlamydia* infection status must be ascertained prior to embarking on any translocation programs.

Vaccination is a potentially valuable tool in the control of infected koala populations. The current recombinant vaccine formulation (research use only) is effective in reducing disease in koalas. Novel formulations utilising chlamydial peptides, and a combined Chlamydia and KoRV vaccine can also reduce chlamydial burden in koalas ^[53].

Research

Further work is recommended in:

- understanding the importance of chlamydial infection and disease to koala populations throughout their range.
- investigating the impacts of treating infected koalas and releasing them back into the wild.
- the role that host, pathogen and environmental factors play in prevalence of infection and disease among populations, and in individual disease outcomes.
- improved understanding of the potential risk of *Chlamydial* transmission between koalas and other species.
- continued investigation into vaccine development.
- further understanding and development into the use of faecal samples as a non-invasive method for the detection of chlamydia in free-ranging populations.
- building a national framework of standards for the assessment, treatment and biosecurity practices for chlamydial disease in koalas ^[6].

Surveillance and management

There is no targeted surveillance program for koala chlamydia. However, population surveillance of *Chlamydia* in koalas occurs through wildlife organisations and research groups. The National Koala Monitoring Program provides an Australia-wide approach to improving understanding of koala population status and trends (www.nkmp.org.au).

Wildlife Health Australia administers Australia's general wildlife health surveillance system, in partnership with government and non-government agencies. Wildlife health data is collected into a national database, the electronic Wildlife Health Information System (eWHIS). Information is reported by a variety of sources including government agencies, zoo based wildlife hospitals, sentinel veterinary clinics, universities, wildlife rehabilitators, and a range of other organisations and individuals. Targeted surveillance data is also collected by WHA. See the WHA website for more information: https://wildlifehealthaustralia.com.au/ProgramsProjects/eWHIS-WILS-WildlifeHealthInformationSystem.aspx

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Wildlife Health Australia recognises the Traditional Custodians of Country throughout Australia. We respectfully acknowledge Aboriginal and Torres Strait Islander peoples' continuing connection to land, sea, wildlife and community. We pay our respects to them and their cultures, and to their Elders past and present.

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