

Chlamydia in Australian wild birds **Fict.sheet**

Introductory statement

Chlamydia psittaci causes disease in bird species the world over. It is a significant pathogen in wild birds as well as in commercial poultry flocks and is zoonotic, having the potential to cause significant and even fatal disease in humans. In this fact sheet, avian chlamydiosis refers to the disease in birds and psittacosis refers to disease caused by *C. psittaci* in humans.

Aetiology

Chlamydia psittaci of the *Chlamydiaceae* family, is a non-motile, gram-negative, obligate intracellular pathogen that causes contagious, systemic, and occasionally fatal disease of birds.

The family *Chlamydiaceae* comprises nine known species in the genus *Chlamydia*: *C. trachomatis*, a causative agent of sexually transmitted and ocular diseases in humans; *C. pneumoniae*, which causes atypical pneumonia in humans and is associated with diseases in reptiles, amphibians, and marsupials; *C. suis*, found only in pigs; *C. muridarum*, found in mice; *C. felis*, the causative agent of keratoconjunctivitis in cats; *C. caviae*, whose natural host is the guinea pig; *C. pecorum*, the etiologic agent of a range of clinical disease manifestations in cattle, small ruminants and marsupials; *C. abortus*, the causative agent of ovine enzootic abortion and *C. psittaci*, comprising the avian subtype and aetiologic agent of avian chlamydiosis in birds and psittacosis, a zoonotic illness in humans (Andersen and Franson 2008).

Natural hosts

All bird species are susceptible to *C. psittaci* infection, however, the nature of disease in infected birds will depend on the host and strain of bacteria. *Chlamydia psittaci* contains 9 genotypes (A to F, E/B, M56 and WC) with the strains from A to F being isolated from birds, and the M56 and WC strains identified in mammals (Lent et al. 2012). The avian genotypes cluster according to host species with A and B associated with psittacine birds and pigeons respectively. Ducks and geese are infected with genotype C while D has been isolated from turkeys. Genotype E has a wider host range, which includes pigeons, ratites, ducks, turkeys and occasionally humans. Psittacine birds and turkeys have also been infected with genotype F, while the final

avian genotype E/B is mainly isolated from ducks (Andersen et al. 1998; Geens et al. 2005; Pannekoek et al. 2010). *Chlamydia trachomatis, C. abortus* and *C. pecorum*, as well as a variety of unclassified *Chlamydia* organisms, are also reported as infecting avian species in a single study in Europe (Sachse Konrad et al. 2012). These unclassified organisms, identified in pigeons (Sachse Konrad et al. 2012), poultry (Gaede et al. 2008), ibis (Vorimore et al. 2013) and Antarctic seabirds (Isaksson et al. 2015) have recently been found to be closely related. Two new species have been proposed in the family *Chlamydiaceae*; *C. avium* sp. nov. comprising previously uncharacterised strains from pigeons and psittacine birds and *C. gallinacea* sp. nov. comprising previously uncharacterised strains from poultry (Sachse K. et al. 2014).

World distribution

The geographic distribution of Chlamydiae in wild and domestic birds is worldwide. Infection has been detected in at least 30 orders and 460 species of birds (Kaleta and Taday 2003). Infection is most commonly reported in *Psittaciformes* (parrots). This probably reflects the frequency of sampling because of the popularity of parrots as cage and aviary birds. Based on the diverse range of wild bird species in which *C. psittaci* infections have been documented, it can be assumed that all wild birds are susceptible to avian chlamydiosis (Kaleta and Taday 2003).

Surveillance for *C. psittaci* is often carried out opportunistically alongside other disease investigations. The presence of concurrent disease and sampling of potentially unhealthy populations makes these data difficult to interpret. The true prevalence in healthy, wild bird populations is therefore unknown. However, it is generally considered that the prevalence of infection of wild birds with *C. psittaci*, under natural conditions and in their natural environment, is low (Blomqvist et al. 2012).

Occurrences in Australia

Historical studies reveal that *C. psittaci* was present in Australia as early as the 1930s (Burnet 1935) and, based on direct and indirect compliment fixation testing, was widespread in South Australia in both wild birds and aviculture as early as 1951 (Beech and Miles 1953). Based on experiences of avian veterinarians, *C. psittaci* is widespread in Australian captive bird population (Alex Rosenwax pers. comm. 2017).

Two studies examining prevalence of avian chlamydiosis in Australia looked at both captive and wild birds. A study of 409 birds in Victoria sampled birds (both wild and captive) that had been presented to a wildlife hospital, with 3.6% of the captive birds positive for *C. psittaci* on PCR. The source of birds (submitted to a rehabilitation hospital) may have biased prevalence rates, as only 0.67% of the wild birds tested were PCR positive for *C. psittaci* (Amery-Gale et al. 2017).

A second survey that tested pigeons and *anseriformes* (waterfowl and magpie geese) found that 10 to 57% of pigeons in pigeon lofts, but none of the *anseriformes* were shedding the organism. The same study recorded a prevalence of between 5 to 42% in a selection of domestic psittacine aviaries. Only a limited number of wild birds (n=10) were sampled and none returned positive for *C. psittaci* (McElnea and Cross 1999).

These studies suggest that the prevalence of avian chlamydiosis in wild birds in Australia is relatively low.

Overseas, studies examining feral pigeons in 11 European countries revealed seropositivity to *C. psittaci* in populations ranging from 19.4% to 95.6% (Magnino et al. 2009). Despite appearing to be a major potential source of disease, there currently appear to be no data available for feral pigeons in Australia.

Epidemiology

Information about the routes of transmission in wild birds is limited, but knowledge is extrapolated from domestic species. Infection via the eye or transmission by arthropod vectors is possible. Transmission via the egg has been reported in numerous domestic species (Kordová et al. 1972; Wittenbrink et al. 1993) but this is not considered an efficient mode of transmission within a flock. In high-density production flocks, circulating air may hold significant amount the bacteria (Dickx and Vanrompay 2011).

Despite the lack of information, ingestion and inhalation are thought to play the major role in transmission in wild birds. Persistent infections and extending shedding may occur from both gastrointestinal tract and nasal mucosa. Inoculation via the respiratory tract results in a high rate of infection, rapid spread and a relatively high mortality rate in turkeys (Page 1959), pigeons and captive psittacines (Meyer 1965). Inhalation of *C. psittaci* may be acquired from nasal exudates, aerosolised faeces (dry or wet) and aerosolised air droplets. High density and environmental accumulation of faeces favours inhalation transmission. Pathogenesis after ingestion has also been studied in turkeys (Page 1959) and ducklings (Thierry et al. 2016). In turkeys, the disease process was similar to that seen following aerosol exposure with the birds displaying mild signs of diarrhoea. The ducklings that were not inoculated (but kept in the same pen as those that were experimentally infected) also showed signs of disease at day 10 post-infection and the bacterial load based on PCR was similar to those birds that were experimentally infected, suggesting that rapid bird-to-bird transmission had occurred. It is now accepted that in some species (e.g. waterfowl), faecal-oral transmission is most likely the main route of infection, and the reason *C. psittaci* is endemic on many duck farms (Vorimore et al. 2015; Hulin et al. 2016).

In young birds, regurgitant feeding may transfer infection from parents to young. High concentrations of Chlamydiae have been reported in the crop and crop fluids in turkeys (Page et al. 1975), pigeons (Meyer 1965) and herons and egrets (Moore et al. 1959). Species who crop feed young or feed chicks in the nest have a greater opportunity to directly transfer infectious organisms. Precocial species are more likely to self-infect through oral transmission, especially in species that feed together in flocks. Water birds feeding in warm, shallow wetlands that are heavily contaminated by faecal material are also at high risk for infection. Predators and scavengers may become infected through consumption of infected carcasses (Brand 1989).

The ability to of the organism to evade host defences and be persistently shed by the host, combined with its resistance to desiccation outside the host, means that apparently healthy birds can infect both the environment and in-contact birds. Studies in feral pigeons and Eurasian collared doves (*Streptopelia decaocto*) reveal a variable infection rate, with higher prevalence reported in densely populated areas in these species (Magnino et al. 2009; Donati et al. 2015). Because the organism is shed in faeces, conjunctival or respiratory secretions (often intermittently with few clinical signs) it is difficult to assess the risk of transmission of *C. psittaci* to other animals, including humans. Shedding of *C. psittaci* may increase during times of stress. Increased population density, concurrent infections or breeding may also increase the risk of infection and shedding (Andersen and Vanrompay 2009).

Persistent infections have been reported in birds (Ward 1999) and, when stressed, these birds may resume shedding. Mortality events in the pet bird trade are common when introducing new birds to a pet store, or when sourcing wild-caught birds for aviculture (Meteyer et al. 1992).

Clinical signs

Avian chlamydiosis in domestic psittacines and production poultry causes three types of disease: acute, subacute and chronic. In all cases, signs are non-specific but include anorexia, diarrhoea, lethargy, weight loss, biliverdinuria and ruffled feathers. In more severe cases, dark green faeces are accompanied by anorexia, dehydration, emaciation and death, if left untreated (Andersen and Vanrompay 2009; Yin et al. 2013; Cong et al. 2014). Mucopurulent or serous oculonasal discharge may be the only clinical sign.

Most infected feral pigeons are asymptomatic. Clinical signs of depression, serous conjunctivitis, blepharitis, rhinitis and diarrhea have been reported (Andersen and Vanrompay 2009). Conjunctivitis is often seen in one or both eyes in domestic and feral pigeons and pigeon fliers refer to this as a "one-eyed cold".

However, there may be no clinical signs and many chronically infected birds and show no signs until stressed. Persistent infections normally result in a bird that is either clinically normal or shows only mild signs. Psittacine birds often shed Chlamydiae and develop clinical signs after transportation and introduction to new environments.

Diagnosis

Live bird

Diagnosis of avian chlamydiosis can be difficult, especially in the absence of signs and must be confirmed by laboratory tests. Haematology, blood biochemistry, radiology and endoscopy all provide supportive evidence of clinical chlamydiosis. Radiographically and endoscopically, there are often signs of pneumonia and air sacculitis. Splenomegaly may be significant.

A single testing method may not give a definitive answer. A combination of tests, specifically antibodydetection and PCR, is recommended, particularly when only one bird is tested.

The most commonly used antibody test in Australia is the Enzyme-Linked Immunosorbent Assay (ELISA). However, it has not been approved for use in birds. The Immunocomb antibody detection test is commonly used in veterinary practice as a point of care diagnostic tool. There is currently very little peer-reviewed literature assessing the Immunocomb as a diagnostic tool and its sensitivity and specificity for most species is unknown.

Polymerase chain reaction (PCR) is a highly / moderately sensitive and specific test for the detection of *C. psittaci.* It is recommended that samples collected from live birds include pooled conjunctival, choanal and cloacal swabs. However, in the live bird, sample contamination may lead to false positives, especially where a large number of birds are being sampled simultaneously. If they are to be tested, samples should be collected from birds prior to their beginning treatment as many will become PCR-negative shortly after the commencement of anti-chlamydial treatment.

Dead bird

Historically, the preferred method for diagnosis was by isolation and identification of the living organism. However, this is now rarely undertaken. The prolonged turn-around time for results, the flawless sampling and transport technique required and the possible risks to laboratory personnel mean that fast, reliable and accurate molecular techniques have superseded this technology. Special stains may be used on impression smears or histopathology of liver or spleen to highlight elementary bodies (Schmidt et al. 2015). Immunofluorescent antibody staining also utilises impression smears of fresh tissue but requires an anti-chlamydial antibody in order to be effective. Formalin fixed tissues can undergo immunohistochemical staining to increase sensitivity, however, cross reaction with some bacteria and fungi are expected and an experienced interpretation is important for accurate analysis (Schmidt et al. 2015).

The preferred approach because of some of these challenges is now PCR, which has replaced traditional diagnostic methods as a fast, reliable and sensitive diagnostic tool. PCR assays targeting a variety of chlamydial genes are available and a range of sensitivities and specificities has been reported. Pan-chlamydial PCR is available that targets the 16s rRNA-23S rRNA region, however conservation between species makes subsequent speciation difficult (Everett et al. 1999).

Clinical pathology

Birds with chlamydiosis often show anaemia (haematocrit <30%), marked leucocytosis (white blood cells >30,000) and absolute heterophilia and monocytosis.

Pathology

In systemic disease with multiple organ involvement, gross lesions are fairly consistent across all avian species (Suwa et al. 1990). The severity and distribution of the lesions, however, depends on factors including host species and susceptibility, virulence of the strain, concurrent infection and route of exposure. In overwhelming infections with virulent strains, lungs show diffuse congestion and the pleural cavity may contain fibrinous exudate. The pericardium may be thickened, congested and coated with fibrinous exudate. The heart may be enlarged, and its surface may be covered with thick fibrin plaques or encrusted with yellowish, flaky exudate. In most species, the liver is enlarged and discoloured and may be coated with thick fibrin. The spleen is enlarged, dark and soft, and may be covered with grey-white spots (Andersen and Vanrompay 2009).

Histopathological lesions also vary between strains and susceptibility of host. Psittacines consistently show multifocal hepatic and splenic necrosis with splenic lymphocytes being markedly depleted and replaced by swollen, reactive macrophages. Fibropurulent air sacculitis may be mild to severe and may be seen in conjunction with conjunctivitis and pericarditis (Suwa et al. 1990).

Differential diagnoses

There are a number of differential diagnoses, including respiratory infections (of both the upper and lower respiratory tract), caused by bacteria, fungi or viruses; vitamin A deficiency; and juvenile wasting disease.

Laboratory diagnostic specimens

In live birds, the best sites for the collection of samples are the conjunctiva, choana and cloaca in combination, as well as the peritoneal or air sac exudate (Andersen 1996). If bacterial isolation is the goal, faeces, choanal and cloacal samples should be collected for 3 to 5 consecutive days and the samples should be pooled and sent to the laboratory (CDC 2010). A full sample set should be collected from dead birds for

histology as well as fresh liver, lung and spleen for culture. Impression smears should be made from the cut surface of the liver and spleen?

Treatment

There are a variety of treatment options available in birds, some more suitable for flock treatment, whilst others are suitable only for cage birds. Options include oral doxycycline liquid given at 35 mg/kg daily or azithromycin 40 mg/kg every second day for 21 days (Guzman et al. 2010). Traditionally, injectable doxycycline administered at 50 mg/kg once a week for seven weeks was used. Bleeding from the injection site and muscle necrosis associated with the viscous injectable doxycycline are common. Studies reported that doxycycline administered in the water at 800 mg/l for 42 days and 500 mg/l for 45 days resulted in adequate blood levels in psittacines and doves respectively (Flammer et al. 2001; Padilla et al. 2005) and that doxycycline mixed with seed at 500mg/kg provided therapeutic drug levels in cockatiels (*Nymphicus hollandicus*) (Powers et al. 2000). Because of the variability in water intake between and within species, especially at different times of the year, oral dosing is recommended over in-water or in-feed medication, however, in a flock situation, these modalities may be the only practical alternative.

Prevention and control

Prevention of infection in wild birds is not possible given the wide spread and endemic nature of the disease. Controlling disease in wild bird populations is similarly difficult, however, it is possible to decrease stress and help to limit the spread of the disease amongst populations. Encouraging natural foraging behaviours and preventing congregations of large flocks of wild birds at feeding stations or single point water sources, ensuring cleanliness of the birds' environment and removing diseased birds from the flock will all help to control the spread of disease amongst wild birds.

Mass treatment of wild birds is not recommended or feasible, however there may be merit in the treatment of at risk populations, birds being brought into captivity or feral pigeons free-ranging in city parks if they come in frequent contact with humans at high risk of infection. In this situation, medicated feed or water may be considered to reduce the public health risk of *C. psittaci*. Chlamydiae are susceptible to most disinfectants including quaternary ammonium compounds, alcohol, benzalkonium chloride, bleach and hydrogen peroxide (WB 2000). Frequent, routine, disinfection appears to be the most suitable means of controlling the disease spread (Hulin et al. 2016).

In outbreaks involving free-flying birds, control methods will depend on the species involved and federal and state regulations. The outbreak area may be closed to the public and in general, it is advisable to reduce the amount of infective material in the area by collecting and incinerating carcasses. However, on-site activities must be weighed against the likelihood of scaring infected birds away from the area and thus spreading the pathogen to new locations. Field personnel should wear protective gear and handle carcasses appropriately to prevent contamination of the environment and mechanical transmission of the organism on equipment and vehicles.

Surveillance and management

Wildlife disease surveillance in Australia is coordinated by the Wildlife Health Australia. The National Wildlife Health Information System (eWHIS) captures information from a variety of sources including Australian government agencies, zoo and wildlife parks, wildlife carers, universities and members of the public. Coordinators in each of Australia's States and Territories report monthly on significant wildlife cases identified in their jurisdictions. NOTE: access to information contained within the National Wildlife Health Information System dataset is by application. Please contact <u>admin@wildlifehealthaustralia.com.au</u>.

There are currently no formal surveillance programs for *C. psittaci* in birds in place in Australia. Results of previous studies are reported above. Wild birds infected with chlamydia are of interest to the Wildlife Health Australia (WHA) and we encourage those with laboratory confirmed cases of this condition in native Australian or feral animals to submit this information to the national system for consideration for inclusion in the national database. Please contact us at <u>admin@wildlifehealthaustralia.com.au</u>.

Statistics

There are a large number of cases of avian chlamydiosis recorded from all areas of Australia in the National Wildlife Health Surveillance Database (eWHIS – see <u>www.wildlifehealthaustralia.com.au</u>). Host species reported in eWHIS include a wide range of native psittacines, red-browed finch (*Aegintha temporalis*), Gouldian finch (*Erythrura gouldiae*), Australian magpie (*Gymnorhina tibicen*), magpie-lark (*Grallina cyanoleuca*), feral and native pigeons and doves, superb lyrebird (*Menura novaehollandiae*), tawny frogmouth (*Podargus strigoides*), white-faced heron (*Egretta novaehollandiae*), bush stone-curlew (*Burhinus grallarius*) and various ducks, geese and swans.

NOTE: access to information contained within the National Wildlife Health Information System dataset is by application. Please contact <u>admin@wildlifehealthaustralia.com.au.</u>

Research

Research is required into the prevalence and strains of *C. psittaci* in wild birds in Australia, and the risk and its management, especially where human disease outbreaks are possible.

Human health implications

The avian strains of *C. psittaci* can infect humans. Disease can vary from mild to potentially fatal systemic disease with severe pneumonia (CDC 2010; Branley et al. 2014). Most human infection occurs through inhalation of infectious aerosols. Secondary spread of avian strains between humans has been documented but is not a significant mode of transmission (Smith et al. 2005). Personal protective equipment should be worn when handling infected or suspected infected birds or contaminated materials. At-risk groups include bird owners, pet shop employees, veterinarians, poultry-processing workers, zoo workers and taxidermists. Infectious aerosols are readily created while handling birds or working in confined areas where dried bird droppings are present (Hulin et al. 2016). Postmortem examination of infected birds and handling of infected cultures or eggs pose a particular human health risk (Wobeser and Brand 1982; Williams et al. 1998; Telfer et al. 2005; Laroucau et al. 2015). Human infections can result from transient exposures such as entering rooms where infected birds had been held or cleaning infected cages; this has been reported in the Australian pet bird trade when clients at a pet store became infected after visiting the bird room (Monaghan et al. 2007; Branley et al. 2008; Kalmar et al. 2014; Vorimore et al. 2015).

In Australia risk factors for psittacosis include exposure to aerosolised particles from gardening activities (leaf blowing, lawn mowing) or to pet or wild birds (through feeding activities or pet stores) (Burnet 1935; Williams et al. 1998; Telfer et al. 2005; Monaghan et al. 2007; Patrick et al. 2008; Branley et al. 2014; Branley et al. 2016). The majority of the literature concerning Chlamydia in wild Australian birds has been published

because of its direct association with zoonotic disease outbreaks in humans. These outbreaks highlight that direct bird contact is not necessary for the development of psittacosis (Williams et al. 1998; Telfer et al. 2005; Monaghan et al. 2007; Branley et al. 2014). Until recently, little was known about the identity of the different strains of *C. psittaci* found in Australia, but In 2006, an outbreak of five human cases and a wild crimson rosella were sampled from Wentworth Falls, NSW, the strain was found to cluster into the 6BC clade, the most pathogenic of *C. psittaci* stains (Branley et al. 2016).

Early detection and diagnosis is important. In appropriately treated humans, the disease is rarely fatal (Smith et al. 2005). Symptoms typically include an abrupt onset of fever, chills, headache, malaise, and myalgia. A non-productive cough accompanied by breathing difficulty and tightness of chest is common.

Conclusions

Little is known about the prevalence of this disease in wild Australian birds. Despite an apparently low prevalence in free-living birds, the ability of *C. psittaci* to cause serious and sometimes fatal disease in humans, as well as the potential for wild birds to infect captive birds, or endanger threatened species, makes it a pathogen of interest for future research (Telfer et al. 2005).

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To provide feedback on this fact sheet

We are interested in hearing from anyone with information on this condition in Australia, including laboratory reports, historical datasets or survey results that could be added to the National Wildlife Health Information System. Negative data are also valuable. If you can help, please contact us at admin@wildlifehealthaustralia.com.au.

Wildlife Health Australia would be very grateful for any feedback on this fact sheet. Please provide detailed comments or suggestions to <u>admin@wildlifehealthaustralia.com.au</u>. We would also like to hear from you if you have a particular area of expertise and would like to produce a fact sheet (or sheets) for the network (or update current sheets). A small amount of funding is available to facilitate this.

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