# Avian Chlamydiosis

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**Abstract:** Avian chlamydiosis, sometimes also referred to as psittacosis or ornithosis, is an important infectious disease of companion birds, especially psittacines, domestic poultry and wild birds. The infection usually becomes systemic and is occasionally fatal. Its main causative agent is the obligate intracellular Gram-negative bacterium Chlamydia (C.) psittaci. The infection is widespread and, due to carcass condemnation at slaughter, decrease in egg production, mortality and the expense of antibiotic treatment, represents a major factor of economic loss in birds commercially raised for meat and egg production, as well as posing a permanent risk for zoonotic transmission to man.

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**Introduction:**

Chlamydiaceae are Gram-negative obligate intracellular bacteria and the species Chlamydia psittaci (C. psittaci) causes respiratory disease in birds. Chlamydia psittaci infections have been demonstrated in at least 465 different bird species, spanning 30different bird orders (Kaleta and Taday, 2003). The symptoms may vary from in apparent to severe, depending on the chlamydial strain, stress condition, age and health status of the avian host. The symptoms in birds include rhinitis, conjunctivitis, nasal discharge, dyspnoea, diarrhoea, polyuria, anorexia, lethargy and dullness (Vanrompay et al., 1995). Chlamydia psittaci is a well-known zoonotic agent causing psittacosis or parrot-fever in humans. During the last three decades, psittacosis outbreaks were reported in the United States (Grimes and Wyrick, 1991; Newman et al., 1992), China (Ni et al., 1996), India (Chahota et al., 2000), Australia (Tiong et al., 2007) and European poultry industries (Ryllet al., 1994; Vanrompay et al., 1997; Van Loock et al., 2005a; Sting et al., 2006; Laroucau et al., 2009). Zoonotic transferoccurs through inhalation of contaminated aerosols originating from feathers, faecal material and respiratory tract exudates. Handling the plumage, carcasses and tissues of infected birds and in rare cases, mouth-to-beak contact orbiting also carry a zoonotic risk (Beeckman and Vanrompay 2009). Psittacosis in humans may vary from in apparent to fatal in untreated patients (Kova´cova´ et al., 2007). Symptoms include high fever, chills, headache, myalgia, non-productive coughing and difficult breathing (Beeckman and Vanrompay, 2009). C. psittaci infections mostly occur on turkey or duck farms. However, C. psittaci infections are emerging in European and Asian chickens. Recently, Dickx et al. (2010) examined Belgian broiler breeder, broiler and layer farms by a C. psittaci recombinant major outer-membrane protein (MOMP)-based antibody ELISA (Verminnen et al., 2006) and found98%, 95% and 95% seropositive layers, broilers and broiler breeders, respectively. Moreover, they demonstrated C.psittaci genotype D in the air of chicken hatching chambers and in slaughtered Belgian and French broilers. Zoonotic transmission to hatchery and abattoir employees did occur (Dickx et al., 2010; Dickx and Vanrompay, 2011) Vertical or transovarial transmission of C. psittaci during formation of the egg in the ovary/oviduct of the breeder has been described for chicken eggs (Wittenbrink et al., 1993). It leads to infection of1-day-old birds. Nevertheless, vertical transmission of C. psittaci is thought to be rare (Harkinezhad et al., 2009).

**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 atypicalpneumonia 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 closelyrelated. 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 et al. 2014).

**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 isnot 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 nasalmucosa. 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).

**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). Most infected feral pigeons are asymptomatic. Clinical signs of depression, serous conjunctivitis, blepharitis, rhinitis and diarrhea have been reported (Andersen and Vanrompay 2009).

**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 hostspecies 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 thickfibrin. 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).

**Diagnosis**

***1-Isolation***

Isolation of *C. psittaci* is currently regarded as the standard method for the determination of active infections of birds. It is important that fresh samples are taken for isolation. A special buffer containing sucrose, phosphate and glutamase (SPG) is used for transporting, storing and freezing samples [Spencer and Johnson, 1983]. It is recommended that, where practicable, pharyngeal swabs, cloacal swabs and faeces samples are taken from live birds and preferably sampling should be repeated over a number of days to increase the likelihood of detecting intermittent excreters [Andersen, 1996]. *C. psittaci* can be isolated in embryonated fowls. eggs, but more commonly celllines are used. Buffalo green monkey (BGM) cells are considered the mostsensitive for isolating *C. psittaci*, but Vero and L929 cells are often used and the organism will grow in other cell lines [Vanrompay et al., 1992; Andersen, 1998].

***2-Polymerase chain reaction (PCR)***

A number of reports on the use of PCR techniques to detect *C. psittaci* have appeared in the literature [McElnea and Cross, 1999] and several different strategies have been used. These9tests are reported as able to detect *C. psittaci* DNA in samples of tissues, faeces and choanal and cloacal swabs and are sensitive, rapid and have performed better than traditional tissue staining methods and culture when employed for specimens that that have not been taken properly or mishandled [Moroney et al., 1998]. At present, data validating the PCR tests in comparison with *C. psittaci* isolation, such as those reported by Hewinson et al. (1997) and Messmer et al. (1997). McElnea and Cross [1999] suggest that these tests can be especially suited to the detection of *C. psittaci* in avian samples; however, more work in this area is desirable for corroborating this evidence and standardizing techniques among laboratories. In the absence of conclusive validating data, PCR still seems to be the test of choice based on its simplicity, sensitivity and comparability with validated tests used for other chlamydial species.

***3-Serological tests***

The complement fixation [CF] test remains the most widely used test for detecting antibodies to *C. psittaci* despite its complexity and the need to overcome the anticomplementary effect of most avian sera (usually by the addition of normal chicken serum). Diagnosis of an active infection in individual birds can be made by demonstrating a four-fold rise in CF titre (in paired sera). Other tests such as the elementary body agglutination test [Grimes et al., 1994] and the latex agglutination test [Arizmendi and Grimes, 1993] have been developed, but lack proper validation. Conventional ELISA tests have been developed for detecting antibodies to *C. psittaci* in birds [Evans et al., 1983; Ruppanner et al., 1984], but Andersen [1998] reports a lack of specificity, probably because of cross reaction with gram-negative bacteria. A blocking ELISA kit is available commercially and has been reported to be highly sensitive [Gerlach, 1999].

**Vaccination**

No commercial vaccine is available for avian chlamydiosis. Recently, anexperimental plasmid DNA vaccine containing the gene coding for the major outer membrane protein of chlamydiae was shown to give protection in turkeys [Vanrompay et al., 1999a; 1999b].

**Treatment of infected birds**

Antibiotic treatment of birds is the usual response to known infections. Tetracyclines are usually considered the drugs of choice although quinolones (enrofloxacin) or macrolides (azithromycin) have also been used. Chlortetracycline (CTC) is given on food at levels of 500-5,000 ppm depending on the bird species to be treated and type of food [Gerlach, 1999]. One of the main problems is that birds are often reluctant to eat food treated with tetracyclines and achieving sufficiently high blood levels may take some time. Intramuscular injection of oxytetracycline has been used for larger birds, butpossible side effects include severe muscle necrosis at the site of injection [Gerlach, 1999]. With all tetracycline treatments, problems may ensue due to the elimination of the normal gut flora. Doxycycline has also been used for injecting and in food (1000 mg/kg) with some reported success [Gerlach, 1999]. Doxycycline medicated drinking water (200-800 mg/litre, depending on the species and environmental conditions) has also proved effective [Flammer, 2000].

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