



Network for wildlife health surveillance in Europe Diagnosis Card

Avian chlamydiosis

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Etiology

Avian chlamydiosis is primarily caused by *Chlamydia* (*C.*) *psittaci*, an obligate intracellular bacterium belonging to the *Chlamydiaceae* family. It resides in the mucous epithelia of the respiratory and intestinal tracts of its hosts. The bacterium was first detected and described in the late 19th century following the zoonotic transmission of the disease from parrots to humans, resulting in severe atypical pneumonia with a fatal outcome. The disease was known as parrot fever or psittacosis in both humans and birds. Today, 'psittacosis' is a term used exclusively for symptomatic human infection with *C. psittaci*. In birds, the term 'psittacosis' has been replaced by 'avian chlamydiosis', which describes chlamydial infection in birds regardless of the species of *Chlamydia* or avian host or the presence of clinical features. Several other species in the *Chlamydiaceae* family have also been detected in wild and domestic birds, including *C. gallinacea*, *C. avium*, *C. buteonis*, *C. abortus*, and 'Candidatus *C. ibidis*', as well as species recently described as belonging to the *Chlamydiifrater* genus. Their pathogenic and zoonotic potential is not well understood, but appears to be low.

Affected species (wildlife, domestic animals, humans)

C. psittaci and other chlamydial species are widespread in wild birds, poultry, and domestic and captive ornamental birds, which serve as the primary reservoir for human infection. Severe clinical signs are most observed in captive psittacine birds, whereas wild and feral pigeons often show high infection and shedding rates without displaying any clinical signs. Relatively high prevalence rates have also been reported in seabirds, waterfowl, corvids and raptors.

Epidemiological characteristics and disease course

C. psittaci occurs worldwide with broad host diversity. Both diseased birds and asymptomatic carriers shed the pathogen in their droppings and ocular and respiratory secretions, where it remains viable for days or even months. Birds can acquire the infectious elementary bodies of *C. psittaci* by inhaling dust from dried feces or airborne particles, such as feathers, or by ingesting infectious material, including feces, prey or carcasses. Vertical transmission from parent birds to offspring has been reported (e.g. via eggshell penetration or in the nest), but appears to be infrequent. Factors influencing disease outbreaks and shedding levels include stress caused by nutrient shortages, overcrowding, breeding, migration, transportation or handling, and coinfections with other potential pathogens. Young birds are more severely affected. Avian chlamydiosis can manifest as an acute, subacute, protracted or chronic condition in birds. In adult wild birds, the most common form is subclinical and persistent.

Humans usually contract psittacosis by inhaling contaminated aerosols from dried bird feces and ocular or respiratory secretions often in an occupational context (poultry farmers) or a recreational context (pet bird owners, pigeon fanciers, wild bird feeders). The disease is rare, but underestimated. Symptoms can range from mild, non-specific febrile illness to a severe, life-threatening disease with atypical pneumonia and systemic organ failure.

Clinical signs

In birds, the spectrum and severity of clinical signs vary considerably depending on the virulence of *Chlamydia* strains, the host species and environmental conditions. Signs of disease in infected birds are non-specific and can include lethargy, drowsiness, paroxysmal tremors, signs of paralysis, nasal discharge, sinusitis, pneumonia, diarrhoea, anorexia, and conjunctivitis. However, infections in wild birds are most often subclinical, and the majority of reported clinical signs have been observed in captive or domestic birds.

Gross and histological lesions

Common necropsy findings include hepatosplenomegaly, fibrinous airsacculitis, pericarditis, serositis, rhinitis and enteritis. Histological lesions are characterized by a systemic histiocytic inflammatory response involving the liver, spleen and lungs. However, these lesions are not pathognomonic and require confirmation by immunohistochemistry, isolation of the agent, or molecular detection.

Differential diagnosis

As clinical signs and pathological lesions are non-specific, several differential diagnoses must be considered. For respiratory presentations, these include mycoplasmosis, pasteurellosis, aspergillosis and viral infections. For systemic and gastrointestinal diseases, these include salmonellosis, mycobacteriosis, listeriosis, campylobacteriosis and trichomoniasis.

Criteria for diagnosis

A case can be suspected based on clinical and pathological signs, antibody detection and epidemiological links. However, cases must be confirmed by isolating and identifying the agent or by directly detecting chlamydial DNA.

Recommended diagnostic method(s) and preferred samples (incl. recommended amount and appropriate storage)

Samples to be taken from live birds include ocular, pharyngeal and cloacal swabs, as well as fecal droppings. From necropsy, a mixture of lung, liver and spleen tissue can be used.

Isolation and cultivation of the pathogen have been established in embryonated eggs, but is more convenient when using cell lines such as Buffalo Green Monkey, Vero or McCoy cells. Proper handling of clinical samples is essential to preserve the infectivity of *Chlamydia*. A special medium consisting of sucrose/phosphate/glutamate/albumin (SPGA) is recommended for transport and storage. Before inoculating the cells, a mixture of antibiotics, typically streptomycin, vancomycin, amphotericin B and gentamicin is added to prevent bacterial and fungal contamination. Centrifugation is then used to enforce attachment of *Chlamydia* to the cells, after which the inoculated cells are incubated for 3–6 days. Chlamydial inclusions can be visualized using specific immunofluorescent staining on fixed cell monolayers. Cultivation of *Chlamydia* is not routine, but reserved for specialized laboratories due to challenging procedures and high biosafety requirements (level 3).

Molecular detection by polymerase chain reaction (PCR) is the most reliable and efficient method for confirming *C. psittaci* infections and conducting prevalence studies/surveillance. Samples are processed using commercial DNA preparation kits. Several conventional and real-time PCR protocols are available. For the detection and identification of *C. psittaci* and other avian *Chlamydia* species, a hierarchical approach is recommended, involving a 23S-rDNA-based pan-*Chlamydiaceae*-PCR followed by species-specific amplifications based on sequences of the *ompA* gene, which encodes for the major outer membrane protein, or other specific marker genes. Subtyping, which is particularly important for source tracing in cases of zoonotic transmission, given that genotypes are frequently host-associated and linked to specific avian species, can be performed using *ompA* sequencing, multi-locus sequence typing (MLST), or whole genome sequencing. The latter usually requires the agent to be isolated.

Serological detection does not play an important role in diagnosing avian chlamydiosis due to the general high prevalence of *Chlamydia* in birds, which is not necessarily disease-related, and the lack of species-specificity. Although in-house ELISA assays have been developed, no commercial kits are available.

EWDA proposed harmonized protocol (for harmonization at large scale)

PCR detection is best suited to standardization and harmonization between laboratories. A two-step approach is recommended: first, family-specific DNA sequences are detected using generic primers and probes; then, species identification is performed using specific amplification or sequencing (see WOAH Terrestrial Manual). This enables a wider range of chlamydial agents, including new species, to be detected, which is particularly relevant in unexplored wild bird populations.

Laboratories that can be contacted for diagnostic support

WOAH Reference Laboratories for avian chlamydiosis: Anses, Bacterial zoonosis unit, Animal health laboratory, Maisons-Alfort, France, Friedrich-Loeffler-Institut, Jena, Germany; Laboratory for Immunology and Animal Biotechnology Ghent University, Ghent, Belgium

Recommended literature

1. World Organization for Animal Health (WOAH). Chapter 3.3.1. Avian chlamydiosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 2018.
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