



PHLN

Public Health Laboratory Network

Ornithosis or psittacosis (*Chlamydophila psittaci*)

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *chlamydophila psittaci*.

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1 PHLN Summary Laboratory Definition

1.1 Condition:

Psittacosis or Ornithosis

1.2.1 Definitive Laboratory Criteria

- Isolation of *Chlamydophila psittaci* from a respiratory specimen (e.g., sputum, BAL, pleural fluid or lung tissue), OR
- Detection of *C. psittaci* DNA in a respiratory specimen (e.g., sputum, BAL, pleural fluid or lung tissue) via polymerase chain reaction (PCR) assay, OR
- Fourfold or greater increase in antibody (Immunoglobulin G [IgG]) against *C. psittaci* demonstrated by micro-immunofluorescence (MIF) or complement fixation (CF) assays between paired acute - and convalescent - phase serum specimens obtained at least 2 weeks apart in those older than 5 years with a clinically compatible illness.

1.2.2 Suggestive Criteria

- A single high antibody titre against *C. psittaci* demonstrated by MIF, EIA or CF assays in a serum specimen obtained after onset of symptoms in those older than 5 years with a clinically compatible illness.

2 Introduction

2.1 The organism

Psittacosis (also known as parrot disease, parrot fever, and ornithosis) is a zoonotic disease caused by an obligate intracellular bacterium *Chlamydophila psittaci* (*Chlamydia psittaci* prior to 1999).¹ This species includes six avian serovars, designated A to F, and two mammalian strains (M56 and WC).² Sequenced *C. psittaci* genomes possess a single chromosome of approximately 1.1Mb and a conserved plasmid of ~8Kb containing 7-8 protein-coding sequences. The currently accepted *ompA* genotypes (A-F, E/B, M56 and WC) largely correspond to serovars and are distinguished by gene sequencing or genotype specific PCR. Genotypes A and B have been associated with psittacine birds and pigeons, respectively. Genotype C has been isolated primarily from ducks and geese, whereas genotype D was mainly found in turkeys. Genotype F was associated with infection in both turkeys and psittacine birds. The host range of genotype E is the most diverse (e.g., pigeons, ducks, turkeys). WC and M56 have been endemic in cattle and muskrats.²

C. psittaci bacteria are heat labile and killed at 56°C for 30 minutes. They are also killed by common disinfectants; however, they withstand desiccation for months.

2.2 The disease

Chlamydia (or *Chlamydophila*) causes oculogenital disease (*C. trachomatis*) or respiratory and systemic infections (*C. pneumoniae* and *C. psittaci*). Avian chlamydiosis refers to disease caused by *C. psittaci* in birds. This infection can occur in at least 460 bird species, spanning 30 different bird orders. The severity of disease in birds can range from mild to fatal disease. Infected birds may show signs of lethargy, anorexia, ruffled feathers, ocular/nasal discharge and diarrhoea. Antibiotic treatment is available for infected birds, but is prolonged (minimum 7 weeks of weekly injections, or oral treatment) and may be ineffective if the organism is in a dormant phase. Birds also do not develop protective immunity and, as a result, may become reinfected. *C. psittaci* can also infect mammals such as sheep, goats and cattle, causing chronic infection of the reproductive tract, placental insufficiency and abortion in these animals.³

Infection in humans usually manifests with 'flu-like' illness such as fever, chills, headaches, myalgias and dry cough. Breathlessness and chest tightness often accompany dry cough, and splenomegaly and rash may be present. The incubation period is usually 5-14 days, although periods of up to one month have been reported.³ Severity of infection can range from asymptomatic illness to systemic illness with severe pneumonia.^{4,5} Mortality rates of 15-30% were reported in the pre-antibiotic era although the disease is rarely fatal now. Chest radiograph findings include lobar or interstitial infiltrates.

Differential diagnoses of psittacosis include pneumonia due to other atypical infections such as *Coxiella burnetii*, the causative agent of Q fever, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Legionella species* or respiratory viruses such as human influenza virus. *C. psittaci* has been reported to cause infection in other organ systems including endocarditis, myocarditis, hepatitis, arthritis, keratoconjunctivitis, and encephalitis. Severe disease, including fetal deaths have been reported when infection has occurred in pregnancy.⁵⁻⁸

2.3 Transmission

C. psittaci can be transmitted to people exposed to birth fluids and placentas of infected animals. Human to human transmission has been suggested, but not proven.⁹ Infection due to *C. psittaci* in humans usually occurs through inhalation of aerosolised organisms from dried faeces or respiratory secretions, which can occur through mouth-to-beak contact or from handling infected birds. Pet birds such as parrots, parakeets, macaws, cockatiels, and poultry birds such as turkeys and ducks are most frequently involved in transmission to humans.^{1,3} Infected birds shed the bacteria through faeces and nasal discharges, which can remain infectious for several months. Infection can occur after brief exposures, and therefore patients may not always recall a definitive history of contact with birds.^{1,3,10,11} Veterinarians, bird breeders and animal shopkeepers are at particular risk. In addition, activities such as gardening and moving or trimming lawns without a grass catcher have been associated with human psittacosis.¹²

3 Laboratory Diagnosis / Tests

3.1 Bacterial culture

C. psittaci can be isolated by using cell culture method, although the procedure is not used for routine diagnostic purposes and only performed in reference laboratories. *C. psittaci* is a Biosafety Level 3 pathogen, and transmission of the organisms from patient specimens or infected cell cultures can occur through aerosolisation or splashes onto mucous membranes. Culture, when required, is time consuming and only performed in laboratories with a physical containment 3 facility.

3.1.1 Suitable specimen

Respiratory specimen including broncho-alveolar lavage and sputum are suitable specimens for *C. psittaci* cultures.

3.1.2 Media

A number of cell lines can be used to isolate chlamydiae from clinical specimens, including McCoy, HeLa 229, Hep-2, BGMK, Vero and L cells. Cultures are incubated for 48-72 hours in the presence of host cell protein synthesis inhibitor cycloheximide. A positive culture is confirmed through the presence of typical intracytoplasmic inclusions seen after immunostaining.⁸

3.1.3 Test sensitivity and specificity

There are no data available on sensitivity and specificity of culture as it is rarely done as a part of routine diagnosis and there is no test that is considered to be the 'gold standard' for diagnosis of psittacosis. The use of *C. psittaci* 6BC (ATCC VR-125, bird isolate) and Orni strain (human isolate) as controls is encouraged.

3.2 Serology

Serology remains the mainstay for diagnosis of *C. psittaci* infection, however, it can be prone to false-positive and false-negative results. The surface located antigens, such as chlamydial lipopolysaccharide, can cross-react with antibodies to other bacteria. Timely treatment with appropriate antibiotics may inhibit the development of detectable antibodies. The most commonly used serologic formats include recombinant enzyme linked immunosorbent assays (rELISA),

complement fixation test (CFT) and microimmunofluorescence test (MIF) to detect immunoglobulin M (IgM), IgG and IgA antibodies with either family, serotype or species specificity. Paired acute and convalescent serologic samples taken 10-14 days apart are preferred for diagnosis.

3.2.1 Recombinant ELISA

The recombinant ELISA is genus reactive and provides advantages of automation and objectivity for screening all requests for respiratory infection related chlamydia (*C. pneumoniae* and *C. psittaci*). Kits for rELISA utilise purified recombinant lipopolysaccharide. Anti-LPS antibody usually develops rapidly after the onset of infection; whereas MIF response is often delayed. Serologic testing for Chlamydia-specific IgG and IgA is often used to screen specimens prior to the more laborious MIF testing. Presence of IgG with a negative IgA indicates past infection, whereas the presence of both IgG and IgA or solitary IgA most likely indicates recent infection or a false positive IgA; repeat testing is indicated 10-14 days later to demonstrate IgG seroconversion or rising IgG titre.

3.2.2 Complement fixation test (CFT)

The complement fixation test can be used for serologic diagnosis of psittacosis. The test is based on antibody reactivity to the chlamydial LPS antigen common to all members of the *Chlamydiaceae*. Cross-reactivity can occur with other members of the *Chlamydiaceae* and titre above 1:16 can be interpreted as evidence of past or recent chlamydial infection.

3.2.3 Microimmunofluorescence test (MIF)

The microimmunofluorescence test is considered the method of choice for the confirmatory step for serologic diagnosis of Chlamydial infections. Species and serovar-specific antibody responses can be detected by using this method. Purified elementary bodies (EBs) of representative strains of *C. psittaci* are dotted in a specific pattern onto glass slides. Serial dilutions of patient sera are placed over the fixed antigen dots and incubated, and bound antibody is detected with fluorescein-conjugated anti-IgG or anti-IgM antibody. The MIF assay format is technically demanding, time-consuming and may be less suited for higher volume testing. Single titres greater than 32 or a four-fold rise in titre between acute and convalescent specimen are suggestive of acute infection. The performance of the test depends on several factors, including the antigen preparations used and the experience of the person reading the test.⁸

3.2.4 Quality Assurance

Laboratories performing Chlamydia serology participate in Quality Assurance Program organised by the Royal College of Pathologists of Australasia.

3.2.5 Test sensitivity and specificity

There are no data available on sensitivity and specificity of serology as there is no one test that is considered to be 'gold standard' for diagnosis of psittacosis.

3.3 Nucleic acid amplification tests (NAAT)

Given the limited specificity of serology testing due to the high prevalence of *C. pneumoniae* infection in humans, nucleic acid amplification based testing targeting divergent genetic targets, such as *ompA* gene, has been developed.

3.3.1 Suitable specimen

Sputum, throat swabs and bronchoalveolar lavage are the most commonly used specimens for NAAT. Blood, urine, CSF and occasionally environmental specimens can also be used.

3.3.2 Procedure

NAAT using genus specific and species specific sequences can be used to detect the *Chlamydia* genus and to speciate into *C. pneumoniae* and *C. psittaci*. NAAT has been based on MOMP gene (major outer membrane protein) and the 23S rRNA gene for genus and IncA gene and 16-23S rRNA interspacer region for *C. psittaci* species. The second step of PCR is species specific and discriminates between different *Chlamydia* species. Amplicons are detected using SYBR green and melt curve analysis and confirmed with gel electrophoresis.^{8,13}

3.3.3 Test sensitivity and specificity

There are no data available on sensitivity and specificity of serology as there is no one test that is considered to be the 'gold standard' for diagnosis of psittacosis.

3.4 Strain subtyping

Similarity of *C. psittaci* can be assessed by restriction fragment length polymorphism of *ompA* gene on isolates or PCR amplicons. Multiple loci variable number of tandem repeats (VNTR) analysis (MLVA) based on the detection of tandem repeat polymorphisms of eight loci has been described.¹⁴ MLVA analysis can be performed directly on DNA extracted from clinical specimens, however, the inter-strain discriminatory power of *C. psittaci* molecular typing is not fully understood.

4 Snomed-CT Terminology

SNOMED CT concept	Code
Psittacosis/ornithosis (disorder)	75116005
<i>Chlamydophila psittaci</i> (organism)	14590003
<i>Chlamydia psittaci</i> culture (procedure)	122401005

SNOMED CT concept	Code
Polymerase chain reaction analysis (procedure)	9718006
Chlamydia group complement fixation test (procedure)	315095005
<i>Chlamydia psittaci</i> IgG level (procedure)	134254001
<i>Chlamydia psittaci</i> IgA level (procedure)	395194001
<i>Chlamydia psittaci</i> IgM level (procedure)	134255000
<i>Chlamydia psittaci</i> antibody level (procedure)	315098007
Microbial antibody titer by immunofluorescence method (procedure)	104261003
Polymerase chain reaction analysis for genomic fingerprinting (procedure)	252370006

5 References

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