Compendium of Measures To Control *Chlamydophila psittaci* Infection Among Humans (Psittacosis) and Pet Birds (Avian Chlamydiosis), 2010

National Association of State Public Health Veterinarians (NASPHV)

SUMMARY

Psittacosis, also known as parrot fever and ornithosis, is a bacterial infection of humans that can cause severe pneumonia and other serious health problems. It is caused by *Chlamydophila psittaci*, formerly known as *Chlamydia psittaci***.** From 2005 through 2009, 66 human cases of psittacosis were reported to the Centers for Disease Control and Prevention (CDC) through the Nationally Notifiable Diseases Surveillance System (NNDSS). In general, these cases occur after exposure to infected pet birds, usually cockatiels, parakeets, parrots, and macaws. In birds, *C. psittaci* infection is referred to as avian chlamydiosis. Infected birds shed the bacteria through feces and nasal discharges, and humans become infected from exposure to these materials. This Compendium provides information about psittacosis and avian chlamydiosis to public health officials, physicians, veterinarians, the pet bird industry, and others concerned with controlling these diseases and protecting public health. The recommendations in this Compendium provide standardized procedures to control avian chlamydiosis in birds, a vital step to protect human health. This document will be reviewed and revised as necessary.

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INTRODUCTION

Chlamydophila psittaci is a member of the family *Chlamydiaceae.* Currently there are at least eight serovars and nine genotypes described which in the future may prove to be of importance in the epidemiology of the disease in animals and humans.[1] In some cases, these obligate intracellular bacteria can be transmitted from birds to humans; the resulting infection is referred to as psittacosis (also known as parrot fever and ornithosis). *C. psittaci* typically causes influenza-like symptoms and can lead to severe pneumonia and nonrespiratory health problems. With appropriate treatment, the infection is rarely fatal. From 2005 to 2009, 66 human cases of psittacosis were reported (mean 13, range 8-21)[2] to the Centers for Disease Control and Prevention (CDC). This is likely an underrepresentation of the actual number of human cases as milder cases may not seek medical attention or be reported. Persons at risk include those exposed to pet birds, pigeons, and poultry and in specific occupations such as laboratory and wildlife workers. Human infection can result from even brief exposure to the contaminated excretions or secretions of infected birds.

In this Compendium, *C. psittaci* infection in birds is referred to as avian chlamydiosis. Chlamydial organisms have been isolated from over 460 bird species from 30 orders[3] but are most commonly identified in psittacine (parrot-type) birds, especially cockatiels and budgerigars (also called parakeets or budgies). Among caged, nonpsittacine birds, infection with *C. psittaci* occurs most frequently in pigeons and doves. Avian chlamydiosis can occur in canaries and finches but is infrequently diagnosed.[4] The recommendations in this Compendium provide standardized procedures for controlling avian chlamydiosis in the pet bird population, an essential step in efforts to control psittacosis among humans. This Compendium is intended to guide public health officials, physicians, veterinarians, the pet bird industry, and others concerned with the control of *C. psittaci* infection and the protection of public health.

INFECTION IN HUMANS (PSITTACOSIS)

Transmission

The disease resulting from *C. psittaci* infection in humans is called psittacosis. Most infections are typically acquired from exposure to psittacine birds, although transmission has also been documented from poultry and free-ranging birds including doves, pigeons, birds of prey and shore birds. Human infection with *C. psittaci* usually occurs when a person inhales organisms that have been aerosolized from dried feces or respiratory tract secretions of infected birds. Other means of exposure include mouth-to-beak contact and handling of infected birds' plumage and tissues. Even brief exposures to birds or bird waste can lead to symptomatic infection; therefore, certain patients with psittacosis might not recall or report having any contact with birds. Currently, pet birds are thought to pose a low risk to immunocompromized persons.[5] Person-to-person transmission has been suggested but not proven.[6] Standard infection-control practices and droplet transmission precautions[7] are sufficient for the medical management of humans with psittacosis, and specific isolation procedures (*e.g.*, private room, negative pressure air flow, and masks) are not indicated.

Clinical Signs and Symptoms

The onset of illness typically follows an incubation period of 5 to 14 days, but longer periods have been reported. The severity of the disease ranges from a mild, non-specific illness to a systemic illness with severe pneumonia. Before antimicrobial agents were available, 15% to 20% of humans with *C. psittaci* infection died; however, mortality has been extremely rare since the advent of antibiotics. Humans with symptomatic

infections typically have an abrupt onset of fever, chills, headache, malaise, and myalgia. A nonproductive cough is usually present and can be accompanied by breathing difficulty and/or chest tightness. A pulse-temperature dissociation (fever without increased pulse rate), enlarged spleen, and nonspecific rash are sometimes observed. Auscultatory findings may underestimate the extent of pulmonary involvement. Radiographic findings may include lobar or interstitial infiltrates. The differential diagnosis of *C. psittaci* pneumonia includes infection with *Coxiella burnetii, Histoplasma capsulatum, Mycoplasma pneumoniae, Legionella* spp, *C. pneumoniae*, and respiratory viruses such as influenza. *Chlamydophila psittaci* can affect organ systems other than the respiratory tract, resulting in endocarditis, myocarditis, hepatitis, arthritis, keratoconjunctivitis, encephalitis, and more recently, ocular adnexa lymphoma.[8] Severe illness with respiratory failure, thrombocytopenia, and hepatitis has also been reported.

Case Definition

The CDC and the Council of State and Territorial Epidemiologists (CSTE) have established national case definitions for epidemiologic surveillance of psittacosis. The updated case definitions were published in 2010.[9] These case classifications should not be used as the sole criteria for establishing a clinical diagnosis or determining medical management.

A patient is considered to have a confirmed case of psittacosis if clinical illness is compatible with psittacosis and the case is laboratory confirmed by one of two methods:

- Isolation of *Chlamydophila psittaci* from respiratory specimens (*e.g.*, sputum, pleural fluid, or tissue) or blood, or
- Fourfold or greater increase in antibody (Immunoglobulin G [IgG]) against *C. psittaci* by complement fixation (CF) or microimmunofluorescence (MIF) between paired acute- and convalescent-phase serum specimens obtained at least 2-4 weeks apart.

A patient is considered to have a probable case of psittacosis if the clinical illness is compatible with psittacosis and one of the two following laboratory results is present:

- Supportive serology (*e.g. C. psittaci* antibody titer [Immunoglobulin M, IgM] of greater than or equal to 32 in at least one serum specimen obtained after onset of symptoms), or
- Detection of *C. psittaci* DNA in a respiratory specimen (*e.g.* sputum, pleural fluid or tissue) via amplification of a specific target by polymerase chain reaction (PCR) assay.

Diagnosis

Most diagnoses are established by clinical presentation and positive antibodies against *C. psittaci* in paired sera using MIF methods. The MIF is more sensitive and specific than the previously used complement fixation (CF) tests; however, there is still some cross-reactivity with other chlamydiae (*C. pneumoniae*, *C. trachomatis*, and *C. felis)* so a titer result less than 1:128 should be interpreted with caution. Acute-phase serum specimens should be obtained as soon as possible after the onset of symptoms, and convalescent-phase serum specimens should be obtained at least two weeks after the first specimen. Because antimicrobial treatment can delay or diminish the antibody response, a third serum sample 4-6 weeks after the acute sample might help confirm the diagnosis. To increase the reliability of serologic results, acute and convalescent sera should be analyzed simultaneously at the same laboratory.

Chlamydophila psittaci can also be isolated from the patient's sputum, pleural fluid, or clotted blood during acute illness and before treatment with antimicrobial agents; however, culture is performed by few laboratories because of the technical difficulty and occupational safety concerns. Recently, real-time polymerase chain reaction (rt-PCR) assays have been developed for use in the detection of *C. psittaci* in respiratory specimens. These assays can distinguish *C. psittaci* from other chlamydial species and identify different *C. psittaci* genotypes.[10] While the assays appear to be highly sensitive and specific in avian samples, they have not yet been validated for use in human samples. Because proper sample collection techniques and handling are critical to obtain accurate test results, clinical laboratories performing these tests should be contacted directly for specifics on specimen submission.

Laboratories that Test Human Specimens for *Chlamydiaceae*

Information about laboratory testing is available from state public health departments. Few commercial laboratories provide MIF testing and manufacturing problems in 2009 resulted in limited availability of the MIF testing kits. Certain laboratories accept human specimens to confirm *C. psittaci* infection through culture, MIF or PCR **(**Table 1**)**. Other sources not included in this table may also be available.

Table 1: Laboratories that test human specimens for *Chlamydophila psittaci*

Laboratory	Tests Performed	Telephone Number Website
Focus Diagnostics Inc. (Quest subsidiary), Cypress, CA	Culture, MIF (IgM, IgA, IgG)	(800) 445-4032 www.focusdx.com
Laboratory Corporation of America, Burlington, NC	Culture, MIF (IgM, IgG)	(800) 222-7566 www.labcorp.com
Specialty Laboratories, Santa Monica, CA	MIF, (IgM, IgG, IgA)	(800) 421-4449 www.specialtylabs.com
ViroMed Laboratories Minnetonka, MN	Culture, MIF (IgG, IgM)	(800) 582-0077 www.viromed.com
Response and Surveillance Laboratory, Respiratory Diseases Branch, CDC Atlanta, GA**	MIF (requires paired sera), PCR, Culture, genotyping (multiple specimen types)	(404) 639-4921

*MIF = microimmunofluorescence, PCR = polymerase chain reaction

**CDC is a reference laboratory and samples must be submitted through State Health Departments

Treatment

Tetracycline antibiotics are the drug of choice for *C. psittaci* infection in humans. Mild to moderate cases can be treated with oral doxycycline (100 mg every 12 hours) or tetracycline hydrochloride (500 mg every six hours) for a minimum of 10 days. Severely ill patients should be treated with intravenous (IV) doxycycline hyclate (4.4mg/kg/day divided into two infusions, maximum 100 mg/dose). Antibiotic therapy should be continued for at least 10-14 days after fever abates. Most *C. psittaci* infections are responsive to antibiotics within 1-2 days; however, relapses can occur. Although in-vivo efficacy has not been determined, macrolide antibiotics are considered the best alternative agents in patients for whom tetracyclines are contraindicated (*e.g.* children <8 years of age, pregnant women, and persons allergic to tetracyclines).[11] Prophylactic antibiotics are not routinely administered after a suspected exposure to *C. psittaci*, but may be considered in some circumstances.

INFECTION IN BIRDS (AVIAN CHLAMYDIOSIS)

Transmission

C. psittaci is excreted in the feces and nasal discharges of infected birds. The organism is environmentally labile but can remain infectious for over a month if protected by organic debris (*e.g.*, litter and feces). Some infected birds can appear healthy and shed the organism intermittently. Shedding can be exacerbated by stress factors, including reproductive activities, rearing of young, relocation, shipping, crowding, and chilling.

Clinical signs

The usual incubation period of *C. psittaci* ranges from 3 days to several weeks. However, active disease can appear with no identifiable exposure or risk factor. Signs of avian chlamydiosis are non-specific and include lethargy, anorexia and ruffled feathers. Other signs include serous or mucopurulent ocular or nasal discharge, conjunctivitis, diarrhea and excretion of green to yellow-green urates. Severely affected birds may become anorectic and produce sparse, dark green droppings, followed by emaciation, dehydration, and death. Whether the bird has acute or chronic signs of illness or dies, depends on the species of bird, virulence of the strain, infectious dose, stress factors, age, and extent of treatment or prophylaxis.

Case Definitions

Clinical signs may be subtle or not always evident in infected birds.

A confirmed case of avian chlamydial infection is defined on the basis of **one** of the following:

- Isolation of *C. psittaci* from a clinical specimen.
- Identification of chlamydial antigen by use of immunofluorescence (fluorescent antibody) in the bird's tissues.
- A fourfold or greater change in serologic titer in two specimens from the bird obtained at least two weeks apart and assayed simultaneously at the same laboratory.
- Identification of *Chlamydiaceae* within macrophages in smears or tissues (*e.g.* liver, conjunctival, spleen, respiratory secretions) stained with Gimenez or Macchiavello stain.

A probable case of avian chlamydial infection is defined as compatible illness and **one** of the following:

- A single high serologic titer in a specimen obtained after onset of clinical signs.
- *Chlamydiaceae* antigen (identified by use of enzyme-linked immunosorbent assay [ELISA], PCR or fluorescent antibody) in feces, a cloacal swab specimen, or respiratory tract or ocular exudates.

A suspected case of avian chlamydial infection is defined as **one** of the following:

- A compatible illness that is not laboratory confirmed but is epidemiologically linked to a confirmed case in a human or bird.
- A bird with no clinical signs and a single high serologic titer or detection of chlamydial antigen.
- Compatible illness with positive results from a nonstandardized test or a new investigational test.
- Compatible illness that is responsive to appropriate therapy.

Diagnosis

Several diagnostic methods are available to identify avian chlamydiosis in birds (see Appendix 1).

Treatment

Treatment should be supervised by a licensed veterinarian (see Appendix 2).

PREVENTION AND CONTROL RECOMMENDATIONS

Aviary and pet shop owners are encouraged to implement recommendations such as those described in the Model Aviary Program.[12] Such programs encourage disease prevention and improve animal health and the human-animal bond. To prevent transmission of *C. psittaci* to humans and birds, specific control measures are recommended:

- **Educate persons at risk.** Inform all persons in contact with birds or bird-contaminated materials about potential health risks. By the time infection is recognized in a group of birds, a critical period for pathogen accumulation and possible dissemination to humans and other birds has already occurred. Bird caretakers with respiratory or influenza-like symptoms should seek prompt medical attention and inform their health care provider about bird contact.
- **Protect persons at risk.** When cleaning cages or handling potentially infected birds, caretakers should wear protective clothing, which includes gloves, eyewear, a disposable surgical cap, and an appropriately fitted respirator[13] with N95 or higher rating. Surgical masks might not be effective in preventing

transmission of *C. psittaci*. In addition, necropsies of potentially infected birds should be performed in a biological safety cabinet. The carcass should be moistened with detergent and water to prevent aerosolization of infectious particles during the procedure.

- **Maintain accurate records of all bird-related transactions for at least one year to aid in identifying sources of infected birds and potentially exposed persons.** Records should include the date of purchase, species of birds purchased, individual bird identification, source of birds, and any identified illnesses or deaths *among birds*. In addition, the seller should record the name, address, and telephone number of the customer and individual bird identification (*e.g.*, band or microchip number).
- **Avoid purchasing or selling birds that have signs consistent with avian chlamydiosis.** Signs are nonspecific and may include lethargy, ocular or nasal discharge, diarrhea, ruffled feathers or low body weight.
- **Avoid mixing birds from multiple sources**. To prevent epornitics (*i.e.*, disease outbreak in birds) and pathogen transmission to humans, additional control and prevention methods (*e.g.*, health screening, extended quarantine, and *C, psittaci* testing) may be required when birds from multiple sources are co-mingled.
- **Quarantine newly acquired or exposed birds and isolate ill birds.** Isolation should include housing in a separate air space from other birds and noncaretakers. Quarantine birds, including those that have been to shows, exhibitions, fairs, and other events, for at least 30 days after the event and test before returning or adding them to a group.
- **Test birds before they are to be boarded or sold on consignment.** House them in a room separate from other birds pending test results (see Appendix 1).
- **Screen birds with frequent public contact (*e.g.*, bird encounters, long-term care facilities, schools)**. Such testing may be used to reduce potential human exposure from birds. Specific protocols should be established in consultation with a veterinarian, recognizing that some birds may demonstrate persistent IgG antibodies in the absence of active infection (see Appendix 1). A negative *C. psittaci* diagnostic test result does not guarantee that the bird is not infected.
- **Practice preventive husbandry.** Position cages to prevent the transfer of fecal matter, feathers, food, and other materials from one cage to another. Do not stack cages and be sure to use solid-sided cages or barriers if cages are adjoining. The bottom of the cage should be made of a wire mesh. Substrate/litter that will not produce dust (*e.g.*, newspapers) should be placed underneath the mesh. Clean all cages, food bowls, and water bowls daily. Soiled bowls should be emptied, cleaned with soap and water, rinsed, placed in a disinfectant solution, and rinsed again before reuse. Between occupancies by different birds, cages should be thoroughly scrubbed with soap and water, disinfected, and rinsed in clean running water. Exhaust ventilation should be sufficient to prevent accumulation of aerosols and prevent cross contamination of rooms.
- **Control the spread of infection.** Care for healthy birds before handling isolated or sick birds. Isolate birds requiring treatment. Rooms and cages where infected birds were housed should be cleaned and disinfected thoroughly after removal of infected birds. Workers should wear appropriate protective clothing (see 'Protect persons at risk' above). When the cage is being cleaned, transfer the bird to a clean cage. Thoroughly scrub the soiled cage with a detergent to remove all fecal debris, rinse the cage, disinfect it (most disinfectants require 5-10 minutes of contact time) and rerinse the cage to remove the disinfectant. Discard all items that cannot be adequately disinfected (*e.g.*, wooden perches, ropes, nest material, substrate/litter). Minimize the circulation of feathers and dust by wet mopping the floor frequently with disinfectants and preventing air currents and drafts within the area. Reduce contamination from dust by spraying the floor with a disinfectant or water before sweeping it. A vacuum cleaner or pressure washer may aerosolize infectious particles and should be used with caution. Frequently remove waste material from the cage (after moistening the material), and burn or double-bag the waste for disposal. There is no documented transmission of *C. psittaci* via ventilation systems from pet bird aviaries or pet stores to humans, nor are there any studies specific for *C. psittaci* viability in these systems. Properly maintained ventilation systems are at low risk of harboring *C. psittaci*.[14] Theoretically, desiccation from forced air movement may reduce viability of the organism. Use of a high efficiency particulate air (HEPA) filter on air system return may be an option to reduce particulate matter in the air.

- **Use disinfection measures.** All surfaces should be thoroughly cleaned of organic debris before disinfection**.** *C. psittaci* is susceptible to many disinfectants and detergents as well as heat; however, it is resistant to acid and alkali. Examples of effective disinfectants include 1:1,000 dilution of quaternary ammonium compounds (*e.g.*, Roccal®, Zephiran®), 1% Lysol® or freshly prepared 1:32 dilution of household bleach (½ cup/gallon). Many disinfectants are respiratory irritants for both humans and birds and should be used in a well-ventilated area. Avoid mixing disinfectants with any other product.

Recommendations for Treating and Caring for Infected and Exposed Birds

Suspect birds not showing signs of illness should be isolated until their infection status is determined. Birds with confirmed or probable avian chlamydiosis should be isolated and treated under the supervision of a veterinarian (Appendix 2).

Responsibilities of bird owners, physicians, and veterinarians

Humans exposed to birds with avian chlamydiosis should seek medical attention if they develop influenza-like symptoms or other respiratory tract illnesses. The physician should consider psittacosis in ill patients exposed to known infected birds and collect human specimens for laboratory analysis if indicated. Psittacosis in humans is a Nationally Notifiable Disease[15] and most states require physicians to report cases of psittacosis to the appropriate state or local public health authorities. Early and specific treatment for psittacosis should be initiated. Timely diagnosis and reporting can help identify the source of exposure to *C. psittaci* thereby controlling infection spread. Local and state public and/or animal health authorities may conduct epidemiologic investigations and institute additional disease control measures. Birds that are suspected sources of human infection should be referred to veterinarians for evaluation and treatment.

Veterinarians should consider avian chlamydiosis in any lethargic bird that has nonspecific signs of illness, especially if the bird was recently purchased or stressed. If avian chlamydiosis is suspected, the veterinarian should submit appropriate laboratory specimens to confirm the diagnosis. Laboratories and attending veterinarians should follow local and state regulations or guidelines regarding case reporting. Veterinarians should work closely with governmental authorities on investigations and inform clients that infected birds should be isolated and treated. In addition, they should educate clients about the public health hazard posed by *C. psittaci* and the appropriate precautions that should be taken to avoid the risk of disease transmission.

Local and state epidemiologic investigations

Local health authorities should report cases to their state health or agriculture department, as appropriate.[16,17] Because of the potential zoonotic nature of this pathogen, public health and animal health authorities may need to conduct cooperative epidemiologic investigations to control the transmission of *C. psittaci* among humans and birds. An epidemiologic investigation should be initiated if a bird with confirmed or probable avian chlamydiosis was either:

- Procured from a pet store, breeder, or dealer within 60 days of the onset of signs of illness, or
- Linked to a person with confirmed or probable psittacosis, or
- Associated with several other suspect avian cases from the same source.

Other situations can be investigated at the discretion of the appropriate local or state public health department or animal health authorities.

Investigations involving recently purchased birds should include a visit to the site where the infected bird is located and identification of the location where the bird was originally procured (*e.g.*, pet shop, dealer, breeder, or quarantine station). Authorities should document the number and types of birds involved, the health status of potentially exposed persons and birds, locations of facilities where birds were housed, relevant ventilation-related factors, and any treatment protocol. Suspect birds should be tested as recommended (Appendix 1). Examination of sales records for follow up of other birds that had contact with the infected bird may be considered.

Quarantine of birds

Depending on the state's regulatory authority, animal or public health officials may issue a quarantine for all infected and exposed birds on premises where *C. psittaci* infection has been identified. The purpose of imposing a quarantine of birds within that facility is to prevent further pathogen transmission. Reasonable

options should be made available to the owners and operators of pet stores. Preferably, the owner of quarantined birds should treat the birds in a separate quarantine area to prevent exposure to the public and other birds. Alternatively, and with the approval of authorities, the owner can sell the birds after at least 7 days of treatment, provided that the new owner agrees in writing to continue the quarantine and treatment and is informed of the potential human health risk. After completion of the treatment or removal of the birds, quarantine can be lifted after the premises is thoroughly cleaned and disinfected. PCR-based environmental testing can be valuable in evaluating the effectiveness of cleaning and disinfection. The area can then be restocked with birds.

Bird importation regulations

Large-scale commercial importation of psittacine birds from foreign countries ended in 1993 with the implementation of the Wild Bird Conservation Act.[18] Limited importation of personal pets and avicultural specimens is permitted at this time. Illegally imported (smuggled) birds are a potential source of *C. psittaci* infection to domestic birds and people. The United States Department of Agriculture, Animal Plant Health and Inspection Service, Veterinary Services still regulates the legal importation of pet birds to ensure that exotic poultry diseases are not introduced into the United States.[19] These regulations are set forth in the Code of Federal Regulations, Title 9, Chapter 1. Current minimum treatment protocols under these regulations are not always sufficient to resolve infection in all birds.

Appendix 1

TESTING METHODS FOR *C. PSITTACI* IN BIRDS

Bacteria are classified as *Chlamydophila psittaci* based on shared biochemical characteristics and genome composition. The individual chlamydial organisms that meet these classification criteria are not identical and represent life forms that have evolved, and continue to evolve, through infection of both ancient and naïve hosts. Diversity in the organism, the level of exposure, and the host response may cause spurious test results in some individual animals.

Diagnosis of avian chlamydiosis can be difficult, especially in the absence of clinical signs. A single testing method might not be adequate. Therefore, use of a combination of culture, antibody-detection and antigen-detection methods is recommended, particularly when only one bird is tested. Although there is no epidemiologic evidence of increased disease risk to young, elderly, or immunocompromised humans, more rigorous testing should be considered for birds in contact with these individuals. Consultation with an experienced avian veterinarian may help when selecting tests and interpreting results. Because proper sample collection techniques and handling are critical to obtain accurate test results, clinical laboratories should be contacted for specifics on specimen submission.

Pathologic diagnosis

In birds with avian chlamydiosis, cloudy air sacs and enlargement of the liver and spleen may be observed, but no specific gross lesion is pathognomonic. Chromatic or immunologic staining of tissue or impression smears can be used to identify organisms in necropsy and biopsy specimens.

Bacteriologic culture

Use of culture is recommended to avoid limitations associated with other tests. Tissue specimens from the liver and spleen are the preferred necropsy specimens for culture. In live birds with suggestive clinical signs of chlamydiosis, a combined conjunctival, choanal and cloacal swab specimen or liver biopsy specimen is recommended for testing. Swabs of conjunctival and choanal tissues may be most sensitive for detecting nucleic acid in subclinically infected birds. Depending on the stage of infection and affected tissue, infected birds might not shed detectable levels of *C. psittaci* in feces.[20] If feces are chosen as a site for attempted detection of *C. psittaci*, serial fecal specimens should be collected for 3 to 5 consecutive days and pooled for submission as a single sample.

Chlamydophila species are obligate intracellular bacteria that must be isolated in tissue culture or embryonating chicken eggs. Specialized laboratory facilities and training are necessary for reliable identification of chlamydial isolates and adequate protection of microbiologists. The diagnostic laboratory should be contacted for specific procedures required for collection and submission of specimens. The proper handling of specimens is critical for maintaining the viability of organisms for culture, and a special transport medium is required. Following collection, specimens should be refrigerated and sent to the laboratory packed in ice but not frozen.

Tests for antibodies

A positive serologic test result is evidence that the bird was infected by *Chlamydiaceae* at some point, but it might not indicate that the bird has an active infection. False-negative results can occur in birds that have acute infection when specimens are collected before seroconversion. Treatment with an antimicrobial agent can diminish the antibody response. However, IgG titers may persist following successful treatment.

When specimens are obtained from a single bird, serologic testing is most useful when signs of disease and the history of the flock or aviary are considered and serologic results are compared with white blood cell counts and serum liver enzymes. A fourfold or greater increase in the titer of paired samples or a combination of a titer and antigen identification is needed to confirm a diagnosis of avian chlamydiosis.

- **Elementary-body agglutination (EBA)** - The elementary body is the infectious form of *C. psittaci*. Elementary-body agglutination is commercially available and detects IgM antibodies, an indication of early infection. Titers greater than 10 in budgerigars, cockatiels, and lovebirds and titers greater than 20 in larger birds are frequently detected in cases of recent infection. However, increased titers can persist after treatment is completed.

- **Indirect Fluorescent Antibody Test (IFA) -** Polyclonal secondary antibody is used to detect host antibodies (primarily IgG). Sensitivity and specificity varies with the immunoreactivity of the polyclonal antibody to various avian species. Low titers may occur because of non-specific reactivity.

- **Complement fixation (CF**) **-** Direct CF is more sensitive than agglutination methods. False-negative results are possible in specimens from parakeets, young African gray parrots, and lovebirds. High titers can persist after treatment and complicate interpretation of subsequent tests. Modified direct CF is more sensitive than direct CF.

Tests for antigen

Tests for antigen detect the organism. These tests give rapid results and do not require live, viable organisms; however, false-positive results from cross-reacting antigens can occur. False-negative results can occur if there is insufficient antigen or if shedding is intermittent. As with all nonculture tests, results must be evaluated in conjunction with clinical findings.

- **Enzyme-Linked Immunosorbent Assay** (**ELISA) -** ELISA tests were originally developed for identification of *Chlamydia trachomatis* in humans. The exact sensitivity and specificity of these tests for identifying other *Chlamydiaceae* are not known. They are now occasionally used to identify suspected *C. psittaci* in birds. If a bird has a positive ELISA result but is healthy, the veterinarian should attempt to verify that the bird is shedding antigen via isolation of the organism. When a clinically ill bird has a negative ELISA result, a diagnosis of avian chlamydiosis cannot be excluded without further testing (*e.g.*, culture, serologic testing, or polymerase chain reaction [PCR] assay).

- **Fluorescent Antibody Test (FA) -** Monoclonal or polyclonal antibodies, fluorescein staining techniques and fluorescent microscopy are used to identify the organism in impression smears or other specimens. These tests have similar advantages and disadvantages to ELISA. This test is utilized by some state diagnostic laboratories.

Tests for DNA

Numerous laboratories offer diagnostic testing using polymerase chain reaction assay (PCR). PCR amplification can be sensitive and specific for detection of target DNA sequences in collected specimens (*e.g.*, combined conjunctival, choanal and cloacal swab specimens, and blood). Results differ between laboratories because there are no standardized PCR primers and laboratory techniques and sample handling may vary. Because of the sensitivity of the assay, samples for PCR must be collected using techniques to avoid contamination from the environment or other birds. PCR does not differentiate between viable and nonviable microorganisms. Test results must be interpreted in light of clinical presentation and other laboratory tests.

Additional tests

Additional diagnostic techniques are in use or under development. Readers are encouraged to research peer-reviewed reports on such tests before use.

Laboratories that test avian specimens for *C. psittaci*

Table 2 lists government and university laboratories that perform chlamydial diagnostic tests. There are numerous private laboratories that provide similar services. Inclusion in Table 2 does not imply endorsement by the National Association of State Public Health Veterinarians or constituent institutions.

Table 2: Laboratories that test avian specimens for Chlamydiaceae

Laboratory	Tests Performed*	Telephone Number Website
Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI	Culture, PCR	(517) 353-1683 www.dcpah.msu.edu
Comparative Pathology Laboratory, University of Miami, Miami, FL	ELISA (antigen), IFA, PCR	(305) 585-6303 www.pathology.med.miami.edu
Infectious Diseases Laboratory (IDL), University of Georgia College of Veterinary Medicine, Athens, GA	Culture, PCR, IFA	(706) 542-8092 www.vet.uga.edu/sams/idl
Texas Veterinary Medical Diagnostic Laboratory (TVMDL), College Station, TX	Culture, PCR, DCF	(979) 845-3414 http://tvmdlweb.tamu.edu/
Diagnostic Virology Lab, National Veterinary Services Laboratories (NVSL), VS, APHIS, USDA, Ames IA**	Culture, CF	(515) 663-7551 http://www.aphis.usda.gov/animal_health/lab_info_services/about_dvl.shtml

*CF = Complement fixation, ELISA= Enzyme-linked immunosorbent assay, IFA = Indirect fluorescent antibody, PCR =Polymerase chain reaction assay, DCF – Direct compliment fixation,
**NVSL is a USDA reference laboratory and samples must be submitted through State Veterinary Diagnostic Laboratories

Appendix 2

TREATMENT OPTIONS FOR BIRDS WITH AVIAN CHLAMYDIOSIS

Routine prophylactic antibiotic treatment is highly discouraged as it may cause adverse affects and could generate resistant strains of *C. psittaci* and other bacteria. Although antibiotic resistant *C. psittaci* has not yet been reported in birds, resistant *Chlamydia suis* has been documented in swine.[21] Therefore, potential development of resistant strains of *C. psittaci* is a concern for avian patients.

Treatment of avian chlamydiosis can be challenging. Although treatment protocols are usually successful, knowledge is evolving and no single protocol ensures safe treatment or complete elimination of infection in every bird. Therefore, treatment for avian chlamydiosis should be supervised by a licensed veterinarian after consultation with an experienced avian veterinarian.

General Recommendations for Treating and Caring for Infected and Exposed Birds

- Isolate birds that are to be treated in clean and uncrowded cages.
- Protect birds from undue stress (*e.g.*, chilling, unnecessary relocation), poor husbandry, and malnutrition. These problems reduce the effectiveness of treatment and promote the development of secondary infections with other bacteria or yeast.
- Sick birds may consume inadequate amounts of medicated food or water, so they should initially be treated with medication delivered directly by mouth or injection.
- The recommended treatment period for avian chlamydiosis has historically been 45 days, except in budgerigars where 30 days of treatment can be effective. Continue medication for the full treatment period to avoid incomplete resolution of the infection.
    - Birds may have reduced chlamydial shedding within days of treatment initiation.
- Observe the birds daily, and weigh them every 3 to 7 days. If the birds are not maintaining weight, have them reevaluated by a veterinarian.
- Remove all oyster shell, mineral blocks, and cuttlebone during treatment. High dietary concentrations of calcium and other minerals inhibit the absorption of tetracyclines. In hand-fed neonates where dietary calcium is required, the calcium and tetracycline should be given at least 4 to 6 hours apart.
- Good husbandry practices should be followed to prevent opportunistic infections.
    - Clean up all spilled food promptly.
    - Wash food and water containers daily.
    - Provide appropriate vitamins daily.
- Treated birds can be reinfected; therefore, contaminated aviaries should have a final thorough cleaning and disinfection several days before treatment ends.
- Post-treatment testing should be conducted no sooner than two weeks after treatment is completed.
- Avian facility managers should provide employees with simple, concise written treatment procedures to ensure treatment success.

Treatment Using Doxycycline

Doxycycline is presently the drug of choice to treat birds with avian chlamydiosis. It is better absorbed and more slowly eliminated than other tetracyclines thus, allowing lower drug doses (improving palatability with food or water-based administration) or less frequent administration (improving ease of treatment). Treated birds should be monitored for signs of doxycycline toxicosis to include:

- General signs of illness (signs of depression, inactivity and decreased appetite), or
- Green- or yellow-stained urine, or
- Altered results of hepatic tests (high serum activities of aspartate aminotransferase and lactate dehydrogenase and high serum concentration of bile acids).

If toxicosis occurs, administration of doxycycline should be stopped and supportive care provided until the bird recovers. Treatment with a different regimen or lower doxycycline dose can be tried after the bird no longer shows signs of toxicosis. Below are several options for treatment. Options should not be combined in the same day.

- **Doxycycline medicated feed for budgerigars and cockatiels** - It is critical to use the recommended doxycycline formulation and dietary ingredients to achieve safe and effective results. The following medicated diet[22] can be used to treat avian chlamydiosis:

    1. Mix 1 part cracked steel-cut oats with 3 parts hulled millet seed (measured by volume).
    2. To each kilogram (kg) of oat-millet mixture, add 5 to 6 milliliters (mL) of sunflower oil. Mix thoroughly to coat all seeds.
    3. Add 300 milligrams (mg) of doxycycline hyclate (from capsules) per kilogram of oat-millet-oil mixture, and mix thoroughly to ensure that oats and millet seeds are evenly coated.

    Prepare fresh medicated oat-millet-oil mixture daily because doxycycline stability in this diet is unknown. Feed as the sole diet. The oats and hulled millet seed are available at health food stores. Small-sized millet should be selected. Sunflower oil is available in grocery stores. Doxycycline hyclate capsules are available in 50- and 100-mg sizes.

- **Doxycycline medicated water** - Results of pharmacologic studies indicate that doses of 200 to 400 mg of doxycycline hyclate/liter of water for cockatiels, 400 to 600 mg/liter for Goffin's cockatoos, and 800 mg/liter for African gray parrots will maintain therapeutic concentrations.[23,24] Research data are lacking for other species, but empiric use of 400 mg/liter of water has been successful for many psittacine birds. Medicated water should be prepared daily and provided in clean bowls, rather than water bottles. Do not use medicated water for budgerigars as it will **not** maintain therapeutic concentrations.[16]

- **Orally administered doxycycline** - Doxycycline is the drug of choice for oral administration; either the monohydrate or calcium-syrup formulations can be used. Dosage recommendations are as follows: 25 to 35 mg/kg every 24 hours for cockatiels, 25-50 mg/kg for Senegal parrots, blue-fronted and orange-winged Amazon parrots; and 25 mg/kg every 24 hours for African gray parrots, Goffin's cockatoos, blue and gold macaws and green-winged macaws. Precise dosages cannot be extrapolated for other species; however, 25 to 30 mg/kg every 24 hours is the recommended starting dosage for cockatoos and macaws, and 25 to 50 mg/kg every 24 hours is recommended for other psittacine species. If the bird regurgitates or refuses the drug, another treatment method should be used.

- **Injectable doxycycline** - The only suitable doxycycline formulation for intramuscular (IM) injection is Vibramycin SF IV[a] (Vibrovenos®),[a] a specific European formulation that can be imported in small quantities into the U.S. (Table 3). It is effective if administered at doses of 75 to 100 mg/kg, IM, every 5 to 7 days for the first 4 weeks and subsequently every 5 days for the duration of treatment. This formulation can cause irritation at the injection site, but it is usually tolerated. Other injectable doxycycline hyclate formulations may cause severe tissue reactions if given IM and should not be used.

Alternative treatment regimens

Injectable Oxytetracycline - Limited information exists to guide the use of an injectable, long-acting oxytetracycline product LA-200.[b] Current dosage recommendations are as follows: subcutaneous (SC) injection of 75 mg/kg every 3 days in Goffin's cockatoos, blue-fronted and orange-winged Amazon parrots, and blue and gold macaws.[25] This dosage might be suitable for other species but has not been tested. This product causes irritation at the site of injection and is best used to initiate treatment in ill birds

a. Pfizer Laboratories, London, England
b. Pfizer Laboratories, Exton, PA

or those that are reluctant to eat. After stabilization with oxytetracycline treatment and the birds are eating and drinking normally, the birds should receive another form of treatment to reduce the irritation that is caused by repeated oxytetracycline injection.

- **Chlortetracycline (CTC) Medicated Feed** - Chlortetracycline medicated feed has historically been used for flock treatment, however doxycycline regimens are preferred. If used, CTC medicated feed should be the **only** food provided to the birds during the entire treatment. Birds' acceptance of medicated feed is variable, thus food consumption should be well monitored. Acceptance can be enhanced by first adapting the birds to a similar, nonmedicated diet. Treatment begins when the birds accept the medicated feed as the sole food in their diet. The following options are available:
  - Medicated mash diets (i.e., >1% CTC with <0.7% calcium) prepared with corn, rice, and hen's scratch.[26]
  - Pellets and extruded products containing 1% CTC can be used. They are available and appropriate for use with pet birds. Select a pellet size appropriate for the size of bird being treated.[27, 28]
  - A special diet might be necessary for lories and lorikeets, which feed on nectar and fruit in the wild.[29]

Treatment Methods Not Recommended

Use of water medicated with chlortetracycline (Aureomycin®), oxytetracycline (Terramycin®) or other tetracycline products (except doxycycline) is not recommended. These products may reduce water consumption, are not likely to be effective and may interfere with subsequent disease testing.

Sources of Medications

The following sources (Table 3) are not listed as an endorsement of the companies or products. Other sources might be available.

Table 3: Sources of medication for treatment of avian chlamydiosis

Contact	Product	Telephone Number Website
	DOXYCYCLINE	
Local pharmacies	1.Docycycline hyclate capsules 50 & 100 mg 2. Doxycycline calcium oral suspension 3. Doxycycline monohydrate oral suspension 4. Vibramycin 50 & 100 mg capsules	
Dr. Gerry M. Dorrestein Wilhelminalaan 19A 5512BJ Vessem The Netherlands	Vibramycin SF I.V.*	Tel: 000 316 11057602 Fax: 000 313 02533131 dorresteingm@planet.nl
	MEDICATED FEED	
Avi-Sci Inc. St. Johns, MI	CTC Medicated Diet (Chlortetracycline, 1%)	Tel: 800.942.3438 mike@avi-sci.com
Roudybush Paso Robles, CA	Medicated Diet 1% CTC (Chlortetracycline 1%)	Tel: 800.326.1726 www.roudybush.com
Zeigler Brothers Inc. Gardners, PA	Chlortetracycline, 1% (special order, 50# minimum)	Tel: 800.841.6800 www.zeiglerfeed.com

*Importation must be in accordance with FDA Regulatory Procedures Manual, Chapter 9-2, Coverage of Personal Importations, pg 12-15. http://www.fda.gov/downloads/ICECI/ComplianceManuals/RegulatoryProceduresManual/UCM074300.pdf. For questions or clarification, contact the Food and Drug Administration, Center for Veterinary Medicine, Division of Compliance at (240) 276-9200.

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Additional Resources

General Public (Fact Sheets)

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