Chlamydophila psittaci Diagnostics

David N. Phalen

Introduction

Chlamydophila psittaci is an intracellular bacterium that is widespread in nature. There are seven *C. psittaci* serovars and these serovars can also be distinguished genetically. By and large each serovar is associated with specific species of birds and the pathogenicity of these serovars to their natural host and humans varies considerably. Serovar A is the most common isolate from psittacine birds and it has a moderate degree of pathogenicity for humans. Serovar B is found world wide in the rock pigeon (*Columbia livia*) where infection prevalences can exceed 50%. This serovar is rarely associated with disease in people. Serovar C has been isolated from Anseriformes and less often Galliformes. Serovar D is common in turkeys and is a common cause of *C. psittaci* infection in humans. Serovar E has been isolated from a wide range of birds and historically was associated with pneumonitis in humans. Serovar F is rare and has been isolated from a "parakeet" and a turkey. A newly recognized serovar E/B has predominately been found in ducks, but was isolated from a lovebird (*Agapornis* sp.).[1,2]

C. psittaci is of concern for veterinarians and public health workers because of infection in poultry workers, the ability of some of its serovars to cause disease in cage birds, and the possible transmission of *C. psittaci* from pet birds to humans. More recently, it has become clear that *C. psittaci* is widespread in several species of wild birds where it may cause little disease, but becomes an issue for people consuming these birds or for scientists working with them. In addition to pigeons, *C. psittaci* has been found in fulmars chicks on the Faroe Islands, hawks and owls in Germany, cormorants in the Galapagos Islands, and in egrets on the Texas Coast.[3,4,5,6] It is likely that other studies of wild birds will also find *C. psittaci* infections. Some, but not all, *C. psittaci* serovars found in wild birds are likely to cause human infections and unless the organism is characterized serologically or genetically, its zoonotic potential is not known.

C. psittaci Infections in Cage Birds

C. psittaci infections can present in several different ways. Acute systemic disease is commonly seen in the larger species of parrots such as the Amazon and African grey parrots and cockatoos. These birds are depressed, stop eating, loose weight and spend much of their time sleeping. Respiratory signs may be present, but often are not. Typically they have yellow or lime green urates (biliverdinuria) and may have diarrhoea. Birds typically have a marked leukocytosis, heterophilia, and monocytosis and a marked increase in plasma aspartate aminotransferase concentrations. Serum electrophoretic changes are

common. Radiographs may show enlargement of the liver and spleen. Often the bird has recently been purchased or recently exposed to other newly purchased birds. None of these findings are specific for psittacosis as other systemic bacterial, parasitic, and fungal diseases and even some neoplasia may present similarly.

Systemic disease can also be seen in cockatiels. However, cockatiels more typically present with mild to moderate upper respiratory signs. Birds will have chronic nasal and ocular discharge and conjunctivitis. Distention of the infra-orbital sinus may occur, but is not specific for psittacosis. Psittacosis should also be included in the differential diagnosis of chronic upper respiratory disease in pigeons, doves and the larger species of parrots. Asymptomatic infection is common in cockatiels and pigeons.

The Organism and the Host Response

Several diagnostic assays have been developed to detect *C. psittaci* infections in birds. To understand how these work, it if first important to understand what happens to the bacteria once a bird is infected and how the bird's immune response reacts to infection. *C. psittaci* is most likely to enter the body by ingestion, inhalation, or by contact with the conjunctiva. It grows locally and then becomes systemic, probably traveling to other organs through the blood. The organism is then shed in oral, ocular, and respiratory secretions, and in the feces. *C. psittaci* can be found in ocular and oral secretions within 5 days of infection and in the cloaca by 10 days after infection. After a period of time (about 2 weeks) the immune system recognizes the bacteria and produces anti-*C. psittaci* IgM followed by IgG. IgM is generally present in birds with active infections. It has been the author's impression that in some birds with chronic infections IgM levels may decline to undetectable levels. IgM levels also drop rapidly following the onset of treatment. IgG levels persist in the blood as long as the bird is infected with *C. psittaci*. They decline over time once the infection has resolved. In some birds, anti-chlamydophila IgG may be found in the blood for many months after the infection has resolved.

Diagnostic Assays

Many diagnostic assays have been developed to detect birds infected with *C. psittaci*. These include antigen detection assays that detect the organism in oral or cloacal swabs, polymerase chain reaction assays that detect *C. psittaci* DNA in oral and cloacal swabs and blood and serologic assays that detect anti-*C. psittaci* antibodies (IgM, IgG or both).

Antigen detection assays include ELISA assays such as the Clearview assay (Inverness Medical, Cologne, Germany) that were originally developed to detect *Chlamydia trachomatis* infections in humans. Another is the immunofluorescence assay where cells collected from oral or cloacal swabs are stained with an antibody labeled with a fluorescent marker. A major draw back to these assays is that they are less sensitive than PCR and serology, also, anecdotal reports suggest that ELISA assays are prone to false positive results.

Multiple serologic assays have been developed to detect anti- *C. psittaci* antibodies. Two assays that have been used successfully in the USA include the complement fixation assay (CF) and the elementary body agglutination assay (EBA).[7,8,9] Both were developed by the late Dr. Jim Grimes. The CF assay was developed first and was found to detect only anti-*C. psittaci* IgG.[9] Because the CF only detects IgG, it will occasionally miss birds in a very early stage of infection and will remain positive in some birds after they have cleared the infection. It also is a very cumbersome, time consuming and labor intensive assay. The EBA was largely replaced the CF in the USA, but is not available in the rest of the world. The EBA is quick and easy to run, is highly sensitive, and remains the serologic test of choice. It detects anti-*C. psittaci* IgM.[9] Its only limitations are that it may become negative in birds with chronic psittacosis and may miss birds in the early stages of infection (infected less than 2 weeks). The EBA was designed primarily to identify parrots infected with *C. psittaci*. Its accuracy in non-parrot species remains unknown.

A number of indirect and blocking ELISAs have been developed to detect anti-*C. psittaci*. One of the first was a blocking ELISA, but this was found to cross-react with antibodies to other organisms and is no longer used (Alan Fudge, pers. com. 1998). The Immunocomb (Biogal-Galed Lab., Israel), a solid phase ELISA, has had better acceptance and is available in many countries in the world. This assay was compared to the EBA and the CF by the late Jim Grimes (Unpublished data). He found that if the positive control density was used as the cut off for a positive sample that the Immunocomb and the CF had the same level of sensitivity and specificity. Therefore a positive with this assay is strongly suggestive of a current or previous infection with anti-*C. psittaci*. It is expected that some birds will remain positive with this assay for some time, even after successful treatment.

A new blocking ELISA has been developed that RIDASCREEN *Chlamydia psittaci* ELISA (R-Biopharm, Darmstadt, Germany) and it has been applied to serum from rock pigeons.[3] A very high percent (95.6%) of the rock pigeons in this study were found to be seropositive. A carefully controlled study comparing this assay to other known serologic assays for anti-*C. psittaci* needs to be done before it is accepted as a valid test. Other serologic assays have been developed and marketed without adequate documentation of their accuracy and have subsequently been shown to highly inaccurate.[10] Therefore the veterinarian should carefully evaluate new assays and always consider the result in light of the clinical signs that the patient is showing.

The PCR assay detects *C. psittaci* DNA. This assay is used to detect *C. psittaci* in oral swabs and fecal swabs and blood of infected birds.[10,11] PCR has the advantage of being extremely sensitive and has the potential of detecting infected birds before they are seropositive. Its major disadvantage is that it may become negative shortly after the onset of treatment, making it necessary to collect samples prior to the onset of treatment. Not all PCR assays are equal and commercial laboratories should be using either a semi-nested, nested, or real time PCR to have the level of sensitivity necessary to detect infected birds. It should also be noted that the oral cavity is probably the best place to find the organism. Therefore, it is better to send in an oral swab, than to send in a cloacal swab covered with droppings. The material in droppings has the potential to interfere with the testing.

It is important to consider that the majority of tests currently available to detect birds infected with *C. psittaci* will still be accurate no matter with which genotype or serovar the bird is infected.

References

1. Andersen AA. Serotyping of US isolates of Chlamydophila psittaci from domestic and wild birds. *J Vet Diagn Invest* 2005;17:479-82
2. Geens T, Desplanques A, Van Loock et al. Sequencing of the Chlamydophila psittaci ompA gene reveals a new genotype, E/B, and the need for a rapid discriminatory genotyping method. *J Clin Micro* 2005;43:2456-61.
3. Prukner-Radovcic E, Horvatek D, Gottstein Z, et al. Epidemiological investigation of *Chlamydophila psittaci* in pigeons and free-living birds in Croatia. *Vet Res Com* 2005;29;17-21.
4. Herrmann B, Persson H, Jensen J-K, et al. *Chlamydophila psittaci* in fulmars, the Faroe Islands. *Emerging Infec Dis* 2006; http://www.cdc.gov/ncidod/EID/vol12no02/05-404.htm.
5. Schettler E, Langgemach T, Sommer, et al. Seroepizootiology of selected infectious disease agents in free-living birds of prey in Germany. *J Wildl Dis* 2001;37:145-152.
6. Travis EK, Vargas FH, Merkel J, et al. Hematology, plasma chemistry, and serology of the flightless cormorant (*Phalacrocorax harrisis*) in the Galapagos Islands, Ecuador. J Wildl Dis. 2006;42:133-141.
7. Grimes, J. E., D. N. Phalen, F. Arizmendi. Chlamydia latex agglutination antigen and protocol improvement and psittacine bird anti-chlamydial immunoglobulin reactivity. *Avian Dis*. 37:817-824, 1993.
8. Grimes JE, Tully, Jr, TN, Arizmendi A, Phalen DN. Elementary body agglutination for rapidly demonstrating chlamydial agglutinins in avian serum with special emphasis on testing cockatiels. *Avian Dis*. 38:822-831, 1994.
9. Grimes JE. Evaluation and interpretation of serologic responses in psittacine birds chlamydiosis and suggest complementary diagnostic procedures. *J Avian Med Surg* 10:75-83, 1996.
10. Phalen DN, M. Hofle M, Dahlhausen B, Radabaugh CS, Styles D. Diagnosis of *Chlamydia psittaci* infections in cockatiels and Columbiformes. *Proc Assoc Avian Vet*. New Orleans, LA. pp 13-17, 1999.
11. Dahlhausen B, Radabaugh CS. Detection of *Chlamydia psittaci* infection in pet birds using a molecular based diagnostic assay. Proc *Ann Meeting Assoc Avian Vet*. Reno, NV pp 191-198, 1997.