Chlamydia psittaci Infections in Birds: A Diagnostic Dilemma?

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Psittacosis, or infection with *Chlamydia psittaci,* is a world wide problem for wild and captive birds. The spectrum of disease produced by infection with *Chlamydia psittaci* is extremely variable and ranges from unapparent infections, to chronic upper respiratory infections, to acute rapidly fatal systemic disease. Although historical, clinical pathologic, and physical findings are often suggestive of *Chlamydia psittaci* infection many other diseases will produce similar signs and ancillary diagnostic assays are essential to confirm the diagnosis. Ancillary diagnostic assays are also extremely important in screening for unapparently infected birds, as these birds are a potential source of *Chlamydia psittaci* for other birds and humans.

Assays that have been used to diagnose *Chlamydia psittaci* infections in live birds include direct culture of the organism,[1] visualization of the organism with special stains,[2] antigen detection in oral and cloacal swabs, protein gel electrophoresis of serum (EPH),[4] detection of chlamydial DNA by polymerase chain reaction (PCR),[5,6] and serology.[1,26,7] While direct culture of *Chlamydia psittaci* is the most specific diagnostic method, recovery of the organism is often difficult and takes several days. Visualization of the organism has been said to be a sensitive technique, but there is little published data to support this claim (Dorrenstein G, pers. com.). Changes in the EPH are said to suggestive of *Chlamydia psittaci* infection, but other diseases may induce similar changes. PCR is a new assay and has yet to be compared to more traditional diagnostic methods.[5,6]

In the United States, serology has been a time honored method for diagnosis of *Chlamydia psittaci* infections. Three serologic assays, the latex agglutination assay (LA), the complement fixation assay (CF), and the elementary agglutination assay (EBA), have been developed by Dr. Jim Grimes. The EBA has been shown to detect antichlamydial IgM, the CF to detect antichlamydial IgG and the LA to detect both antichlamydial IgG and IgM. Due to problems preparing antigen for the LA, the LA is no longer offered commercially.[1,2,7]

Both the EBA and CF have their distinct advantages and disadvantages. Following experimental infection, the EBA has been shown to become positive before the CF. However, in most naturally occurring infections both assays will be positive in birds exhibiting clinical signs of disease. Rarely a bird will exhibit clinical signs before either assay is positive. Surveys of naturally infected birds suggest that, with long standing infections, the EBA will become negative but the CF will remain positive. Following treatment the EBA is the first to become negative, while the CF will positive, sometimes for many months. Therefore the presence of a positive CF antibody titer, in an apparently healthy bird, cannot be used to differentiate between a previously infected bird and an unapparently infected bird. The CF has another disadvantage, in that serum from some birds is occasionally anticomplementary and as a result cannot be used in this assay.[1,2,7]

Recently, an immunofluorescent assay for the detection of anti-chlamydial antibody (IFA) has been developed.[6] This assay is said to detect chlamydia specific antibody, but was shown to be negative in some birds that were positive on PCR.

In this report, we compare PCR, EPA, and three serologic assays (CF, EBA, and IFA) as ancillary diagnostic assays in a flock of cockatiels (*Nymphicus hollandicus*) naturally infected with *Chlamydia psittaci*.

MATERIALS AND METHODS

The study flock of 38 cockatiels was chosen when a bird from the flock was presented with a severe chronic upper respiratory infection. The bird was found to be positive for antibody based on the EBA. A visit to the flock revealed that several birds were showing similar signs and many were thin. The owner reported that the problems had first been noticed a few weeks after new birds purchased at a bird mart had been introduced

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to the flock, approximately 8 weeks earlier.

A second flock was used as a control. This flock of nine birds were caged raised and maintained in a wire bottomed cage in isolation for 5 years. At no time during the 5 years did the birds exhibit signs of illness.

Birds were bled once from the right jugular vein and aliquots of the blood were submitted for testing (EBA and CF) to the Texas Veterinary Medical Diagnostic Laboratory (College Station, TX, USA) and for IFA and EPA to the University of Miami (Comparison Reference Services, Miami, Florida, USA). Each bird's oral cavity and cloaca was swabbed with a single swab. The swabs were submitted for PCR to Research Associates (Milford, Ohio, USA).

RESULTS AND DISCUSSION

None of the nine isolated birds were positive on EBA, CF or PCR. IFA and EPH results are pending. Five samples submitted for CF (11%) from the aviary were anticomplementary. A comparison between the CF and EBA showed agreement 80% of the time. The majority of birds for which there was disagreement were EBA negative and CF positive. These birds may have been birds with chronic infections and IgM titers that had fallen below detectable levels. A single bird had a positive EBA but was negative on the CF. This bird may have been in the early stages of infection.

A comparison between the EBA and PCR showed a 83% agreement. Two birds were EBA positive and PCR negative. The largest discrepancy was in EBA negative birds (6) that were PCR positive. When all birds that were either seropositive on the EBA, the CF or both, were compared with PCR positive birds there was 89% agreement. Four PCR negative and seropositive birds were swabbed a second time a week later and two were positive on the second sampling.

The EPH was scored as either positive or negative. Negative samples were those that showed no evidence of systemic disease. EPH changes suggestive of hepatitis, nephiritis, or a infection with an inflammatory disease (*Chlamydia psittaci*, aspergillus, or tuberculosis) were considered positive. A comparison between the EPH and EBA showed a 81% agreement.

IFA was compared to EBA. They were found to be in agreement 41% of the time. Most agreement was associated with samples negative on both IFA and EBA. Only 2 of 17 samples that were EBA positive were also IFA were positive. A similar result was found when the IFA was compared to the CF where there was only 27% agreement and the all of the disagreement could be accounted for by IFA negative CF positive samples.

Conclusions

There can be no doubt that many birds in the cockatiel aviary were infected with *Chlamydia psittaci*. High EBA and CF antibody titers were found in most birds and following treatment with doxycycline the birds signs resolved. The results of the CF, EBA, and PCR were very similar. Discrepancies in the results of these assays are best explained by early infections prior to antibody production (PCR positive, EBA negative), and chronic infections where IgM titers have fallen below detectable levels (EBA negative, but CF and PCR positive). Two PCR negative birds were also EBA negative and had low CF titers. These birds may not have been actively infected, but may have had a residual antibody from a previous infection.

EPH detected the majority of the EBA positive birds. Four of five birds that were EBA negative and EPH positive were also PCR positive, suggesting that the EPH was even more sensitive than the original data suggested. Unfortunately, the EPH was very nonspecific. Nearly all the positive reports indicated that more than one disease should be considered in the differential.

The IFA correlated poorly with the EBA and CF. If this assay truly does detect antichlamydial antibody, it is extremely insensitive, at least in cockatiels.

Based on the above data, we conclude that the EBA is a sensitive and specific diagnostic assay. Early infections may be missed, but most will be detected. The PCR is also sensitive and specific. Suggesting that the use of a combination of these assays may be an excellent means of diagnosis in the clinically ill bird and

detecting unapparently infected birds.

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