Journal of Avian Medicine and Surgery 13(1):45–49, 1999

Round Table Discussion

Roundtable on Chlamydiosis from the 1998 AAV Annual Conference

This roundtable is a synopsis of a panel discussion on the current status of chlamydial diagnostics held at the 1998 AAV Annual Conference and Expo, St. Paul, MN. Panelists included Dr. Carolyn Cray, Department of Comparative Pathology, University of Miami, Miami, FL; Dr. Gerry Dorrestein, Department of Veterinary Pathology, College of Veterinary Medicine, Utrecht, The Netherlands; Dr. Branson Ritchie, Psittacine Disease Research Group, University of Georgia, Athens, GA; Dr. Keven Flammer, Department of Companion Animal and Special Species Medicine, College of Veterinary Medicine, North Carolina State University, Raleigh, NC; Dr. Brian Speer, Oakley Veterinary/Bird Hospital, Oakley, CA; and Dr. Thomas Tully, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

Dr. Teresa Lightfoot (Avian and Animal Hospital, Largo, FL) served as moderator and initiated the session by reading a brief summary of available diagnostic tests for chlamydiosis. Then each participant was asked to make an opening statement. (Statements are not verbatim and have been edited.) Comments were added from the floor by Dr. Ross Babcock, Phoenix, AZ; Dr. Helga Gerlach, Munich, Germany; Dr. David Graham, College Station, TX; Dr. Ram Mohan, Reynoldsburg, OH; Dr. James Harris, Oakland, CA; and Dr. Alan Fudge, Citrus Heights, CA.

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Dr. Lightfoot:

A brief description of currently available tests is listed below and was the basis for the discussion by the panel and audience during the meeting. This list is certainly subject to change, as testing methods evolve. There are many unresolved issues concerning chlamydial testing; however, there is widespread agreement that diagnosis of illness associated with *Chlamydia psittaci* infection cannot be made simply by laboratory testing but must also include history, clinical findings, and results of routine blood tests, and radiographs. Legal issues surrounding the human zoonotic potential make this disease and its diagnosis a pressing concern in avian medicine.

Currently available diagnostic tests for *Chlamydia psittaci* include:

1. Culture of the organism

A positive finding is nearly 100% accurate. A negative finding, when feces or cloacal swabs are used, simply means the organism was not found and may be attributable to one of the following:

- The bird is not currently shedding the organism.
- Shedding is in insufficient numbers to be successfully detected by culture.
- The transport techniques cause the organism to die before viable culture.
- Negative tissue cultures at necropsy may also occur because of insufficient organisms, or because of improper handling. However, the odds of positive growth in an infected bird are good.

2. Antibody tests
   - Latex agglutination: may be unavailable in the future because of a lack of production of a suitable latex.
   - Direct complement fixation (DC): measures immunoglobulin (IgG), indicating previous exposure. Immunoglobulin G persists for a prolonged period of time in the bird. Species-specific problems occur with lack of antibody production, notably in cockatiels, budgerigars, and lovebirds.
   - Elementary body agglutination (EBA) test: detects IgM. It is a fairly sensitive test and may detect lower level of antibodies, therefore allowing detection in some cases in species with low antibody production. False negatives may still occur.
   - Immunofluorescent antibody (IFA): measures late-forming IgG antibody. It is a better test for birds with suspected "chronic" chlamydiosis than with an acute infection.
3. Antigen tests
   - Enzyme-linked immunosorbent assay (ELISA) monoclonal antibody: binds to a specific anti-

gen of *Chlamydia psittaci*. It can have false negatives if the amount of antigen available for interaction is low. (Antigen may be located within macrophages.) This assay can have false positives because of the interaction with *Staphlococcus* species or other bacteria.

- Immunofluorescent antibody (IFA): binds with antigen in feces or tissue. Same procedure as in ELISA. Potential that low level of antigen will give a false-negative result, and false-positive immunofluorescence may be associated with nonspecific bacteria. The advantage is that the organism does not need to be viable to be detected.

4. Polymerase chain reaction (DNA-PCR) assay
   - This assay detects DNA from the major outer membrane protein (MOMP) of the chlamydial organism.

Question: Would the panelists please comment on your views on current methods for diagnosis of chlamydiosis?

Dr. Cray:

I feel that a combination of tests is needed to best evaluate the state of disease in the patient. What is a DNA-positive bird? Is it infected or clinical?

I think it is currently difficult and likely will continue to be difficult to recommend a single test for chlamydiosis that will fit all possible clinical situations. We know that the different serologic, antigen capture, and DNA probe tests vary in sensitivity and specificity on the basis of the technology itself. The patient provides an additional level of variability. Variables include age, previous history, genetic resistance, infectious dose, concurrent disease, and previous or current treatment. These variables can alter the expected results. Care should be used in selecting an initial test(s) and in interpretating tests used to confirm positive results. It is important to realize that not all tests or combinations of tests will offer 100% definite proof of the chlamydia state of the patient.

As published in the *Proceedings of the 1998 AAV Annual Conference*, we found a variability between serologic versus antigen/culture/DNA results. Our serologic assay was limited to the IFA test. It should be noted that DNA probe assays offered greater sensitivity than antigen-capture tests and the use of blood samples for DNA probe assays resulted in the highest variability of correlation to other positive results. Cloacal, choanal, and/or fecal samples for DNA assays were more consistent throughout this study. Negative serostatus could be found in birds with acute signs, although positive antigen or DNA results were obtained. Follow-up samples reflected the later expected seroconversion. Once seroconversion occurred, positive serostasis continued for a prolonged period of time after treatment and in the absence of positive DNA or antigen results. This is reflective of the IGG sensitivity of the test. Please look for future publications from this laboratory concerning these issues.

Dr. Dorrestein:

In the Netherlands we use ELISA or PCR for antigen detection in cloacal swabs or the Immunocomb Test to demonstrate antibodies. For tissues we use the modified Macchiavello's stain (MMS) for cytology at necropsy. We find a 95% correlation with immunofluorescence and the MMS.

Dr. Ritchie:

Vaccination is the best tool currently available for controlling any infectious agent with a widespread distribution or that maintains itself in nature through reservoir hosts. Our research group has been working in conjunction with Dr. Tom Tully and a group of researchers at Lousiana State University to test a vaccine intended to reduce the uncontrolled spread of chlamydia among companion birds. Our data, as well as the data concerning the use of subunit chlamydial vaccines in other species, are encouraging.

Dr. Flammer:

No test or combination of tests will guarantee that a bird is free of chlamydia, but testing does have value. This is no different than many other diseases (eg, salmonellosis, polyomavirus, etc). A bird with positive test results is more likely to have chlamydia than a bird with negative test results, and vice versa. Combining an antibody and antigen test would potentially give the most accurate diagnosis.

Dr. Speer:

Chlamydial diagnostics is confusing. We must be able to differentiate between what we do for screening purposes versus diagnosis of individual clinical cases. We all look at the bird's history, physical examination, and results of hematologic tests and plasma biochemical analysis, and then we "guess" as to which specific confirmatory test to use. This approach strikes me as less than ideal in many ways. It is very difficult to screen flocks for chlamydia and obtain definitive "black and white" results.

Dr. Tully:

We need to understand the organism. If you understand the clinical presentation, you can better recognize the disease. We include chlamydiosis in our

differential based on history, physical examination, and complete blood count (CBC) results, and then try to confirm with other diagnostics. Subclinical cases are more difficult to diagnose. In the future, it is hoped that other tests will be helpful in diagnosing the disease and that vaccination can prevent the disease.

Question: Is it appropriate to treat chlamydiosis? Could treatment make the disease become subclinical?

Dr. Tully:

I recommend that we treat birds that have chlamydiosis. The problem is that many birds are treated "inadequately" by their owners. Veterinarians, therefore, need to communicate the importance and need for proper treatment.

Dr. Flammer:

If you treat chlamydiosis and improve the management, you get good results. Treatment does not guarantee that chlamydia will be eliminated from a flock, but many treated flocks do not have subsequent problems with chlamydiosis.

Dr. Ritchie:

One of our challenges in testing the efficacy of chlamydial vaccines was developing a model that could be used to demonstrate what level of protection a vaccine induced against either infection or disease. Some of our early studies were used to try and determine whether or not experimentally infected birds developed persistent infections following an appropriate course of antibiotics. Birds that are experimentally infected, treated with doxycycline, and then given what should have been immunosuppressive doses of steroids did not develop clinical signs and reactivation of an infection could not be demonstrated by using shedding assays or serologic tests.

As we face the clinical challenges of chlamydiosis, I think it is important to remember that our enemy is classified as a bacteria and it is not just one bacteria but some 60 genetically different organisms. Given that a strain of chlamydia has been shown to infect most species of animals, that this bacterium is widely distributed in nature, and that a bird that recovers from an infection is susceptible to reinfection, it is difficult to determine if a disease recurrence is a result of an endogenous organism or the result of exogenous organism with reexposure of a susceptible host.

Dr. Dorrestein:

In the Netherlands, we screen budgerigar flocks and when we identify positive birds, we treat the entire flock. In the beginning, we found 30% of the flocks were positive; now we find that only 5% are positive. We reduced the number of chlamydia-positive flocks that we could find. Flocks are checked semiannually.

Comment from floor (Dr. Babcock):

In Arizona, 10 large psittacine birds from each of nine large aviaries were tested and found to have a 30% positive rate by culture by the University of Arizona. Nonpsittacine birds in the same aviaries were 100% positve. The tested birds were not treated and not subsequently known to have become ill with chlamydia. Other aviaries with clinically normal chlamydia-positive birds were treated and some birds were lost during treatment. I treat any clinically ill bird for chlamydia, as well as any other bird with other specifically diagnosed disease if concurrent chlamydiosis is a possibility.

Dr. Flammer:

In North Carolina, we did a survey using five different tests on our birds. In a subsample of 900–1,300 samples, we found an incidence rate of 5%. At the state laboratory, chlamydiosis was identified in 15% of necropsy samples.

Dr. Babcock:

What's different about chlamydia and *Escherichia coli*? The difference is that chlamydia is an obligate intracellular bacteria, and that it is zoonotic. We are only discussing chlamydiosis because of its potential disease problems with humans.

Comment from floor (Dr. Gerlach):

When treating sick birds (ie, where the chlamydia are actively propagating and dividing), treatment is generally successful. If an infection is subclinical, most of the chlamydia are in macrophages. If their metabolism is not active, which is usually the case, treatment won't be successful because all tetracyclines need active protein metabolism of the bacteria to interfere with their propagation. In addition, an active immune system is necessary to remove the damaged forms of the chlamydia, which can be reconstructed if not removed. Meyers's choice of 45 days of treatment was based on the life span of a macrophage in humans. Chlamydia are already in the precursor cells of macrophages in bone marrow. If these divide, their load of chlamydia is divided as well to the daughter cells. So if macrophages are infected, we normally face a lifetime infection (I allow for exceptions).

Dr. Ritchie:

Chlamydia is a bacterium. The immune response is temporary. If reexposed in the flock the birds are susceptible to reinfection.

Dr. Gerlach:

Identification of serotypes may help to resolve the issue of chronic infection or reinfection.

Dr. Dorrestein:

We worked with a group of rosellas that were housed individually. We tested cloacal swab samples twice a week for 3 months. Each bird was a regular shedder, but not clinically ill. We started treatment with doxycycline. After we stopped treatment, we challenged with steroids and we could not detect shedding. In a group of different parrots housed in pairs, 4 to 5 months after antibiotic therapy, antibodies were not detectable. Chlamydia can be difficult to eliminate from the flock and from the environment.

Dr. Tully:

I don't advocate prophylactic treatment for *C. psittaci* in companion birds or for aviaries. I only treat birds that I suspect or have confirmed are infected with chlamydia.

Dr. Speer:

An individual bird is different from a flock. Treatment of flocks must be based on detection of a pathogen. If there is no disease and birds are made sick from our treatment, what are we doing? If we treat because of the test result or from paranoia, we are not serving our clients or our patients.

Dr. Ritchie:

Does chlamydia set up a latency state?

Dr. Cray:

In the literature for other species, chlamydia can establish a latency state.

Question: An HIV positive owner had two PCR-positive birds. The birds were treated and then were PCR negative. What should be done now?

Dr. Dorrestein:

Check for antibody in the birds and see if the titers go down after treatment.

Dr. Tully:

You must make sure the client knows the risks of chlamydiosis should they decide to keep the birds.

Question: What test should be used for a new bird examination?

Dr. Speer:

There is no single screening test for chlamydiosis that should be done for all avian or psittacine species. Chlamydiosis is a clinical diagnosis. If the test is negative and you tell them that it doesn't mean anything, then they ask why you did the test?

Comment from floor (Dr. Harris):

Although more people have died on golf courses on Sundays from lightning strikes since 1900 than from chlamydiosis, you must still be sure that you inform the client of the risks of this disease. Each of my clients gets a handout on chlamydiosis, we discuss it with the client, and give options for testing. Most people decline testing.

It would be appropriate to save a frozen serum sample from employees. If symptoms develop, they are sent to a physician and have paired serums that can be used to run titers rather than waiting for 3 weeks to have paired serum samples.

Comment from floor (Dr. Mohan):

The medical profession does not understand chlamydia. We treat it like any other bacterial disease. We do fluorescent antibody tests in our clinic. Although we used to find chlamydia frequently years ago, we currently run about 900 tests a year and only find a few cases. We test a choanal/cloacal swab sample and, if it is negative, we tell the owner the bird is not shedding.

Comment from floor (Dr. Graham):

Can chlamydia be latent? We don't know. It can be inapparent or subclinical, but what is latent? A single antibody titer is not diagnostic.

Dr. Ritchie:

For ease of understanding, I divide persistent infections into three subcategories: latent, chronic, and slow. Latent infections are then characterized by a "lifetime" infection with periods when an infectious agent is replicating and being shed followed by periods when the organism is inactive. Herpesvirus is the classic model. Most of the current data concerning chlamydia supports the induction of a chronic infection that is able to avoid immune system clearance for long periods in some hosts and not latency.

Question: What are you using for treatment of chlamydiosis?

Dr. Flammer:

A positive test triggers a regulatory event. The Compendium of Psittacosis, published in the *Journal*

of the American Veterinary Medical Association and available online (check the *Morbidity Mortality Weekly Report* from the Centers for Disease Control), covers this. Treatment is recommended for all the birds in the same airspace. Birds can be treated with chlortetracycline-medicated diets, intramuscular doxycycline, oral doxycycline, and doxycycline-medicated food or water. Sick birds, however, may not eat or drink enough to be adequately treated with medicated food or water. Because doxycycline disposition varies among species and is poorly researched, you should monitor the birds for hepatotoxicity if using this drug (look for yellowish urates and an increase in AST, LDH, and bile acid concentrations). Birds should also be monitored for secondary bacterial infections and treated if found.

It is also important to clean the facilities during and after treatment to eliminate chlamydia from the environment. Quaternary ammonium disinfectants are recommended, or use bleach at 1:32 dilution. Scrub the aviary and discard all organic materials, including nest material. Scrub the nest boxes and leave in the sun. Use appropriate diagnostics, but treat the bird.

Dr. Speer:

I'm not convinced of the effectiveness of enrofloxacin for treatment of chlamydiosis.

Dr. Dorrestein:

Some studies in Germany proved that there is activity against chlamydia using enrofloxacin, but it is not a reliable treatment regimen. Birds were treated for 2 weeks and they couldn't find chlamydia; however, there was no follow-up.

Dr. Tully:

Results of serial CBCs from birds infected with *C. psittaci* and being treated with fluoroquinolones maintain increased total white cell counts during the treatment period. However, the total white cell counts drop within a few days in birds treated with doxycycline.

Dr. Dorrestein:

Doxycycline is immunosuppressive and will make the bird feel better (ie, like a steroid).

Dr. Flammer:

I agree with the treatment failure with enrofloxacin. It has a variable response and the birds often relapse.

Question from floor (unknown): Why does the imported doxycycline preparation turn black?

Dr. Dorrestein:

Because it becomes oxidized. When a vial is opened, it should be put into a dark glass stoppered bottle and kept in the refrigerator at 4°C. Its antibacterial activity is still okay several weeks later.

Dr. Gerlach:

Tetracyclines (in particular doxycycline) can be changed by oxygen and/or light to 4-epi derivatives, which are toxic.

Dr. Flammer:

If pharmacist-compounded repository injections are used, you must be very careful. Batches of the drug may vary and cause problems. In our research with cockatiels, one manufacturer's product failed to maintain the desired 1-μg/ml blood levels after administration at 100 mg/kg body weight every 10 days.

Dr. Dorrestein:

We still use injectable doxycycline in large psittacine birds and stick with the original treatment. In flocks of small psittacine birds, we are treating successfully with medicated seeds. We use 250 mg/kg in dehulled seeds. Doxycycline is glued on the seeds. We treat budgerigars for 30 days, other small parrots for 45 days.

Dr. Flammer:

There is scant research information available to guide the duration of therapy in any bird except budgerigars. For lack of a better standard and because of regulatory standards, you should treat for 45 days.

Dr. Ritchie:

We couldn't find the organism after treating for 2 weeks, but we treated orally for 45 days.

Dr. Dorrestein:

If we suspect a case of chlamydiosis, we make impression smears of the conjunctiva and stain with modified Macchiavello's stain for cytologic examination. If positive, we then start treatment with doxycycline.

Comment from the floor (Dr. Fudge):

Last year the FDA (US Food and Drug Administration) did away with the investigational new drug permits requirement for the importation of Vibravenous so that it can be legally imported into the USA.

The session was closed.