Question 1: Why are the results of Psittacosis testing difficult to interpret and understand?

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Answer 1: There are currently five tests available for Psittacosis (Chlamydioidosis) testing in the live bird, three of which are performed on blood. The BELISA, the Complement Fixation (CF), and the Latex Agglutination (LA) measure the antibody response to the Chlamydia. A positive response can be an indication of active infection or previous exposure. False positives have been found with the BELISA test.

The fourth test, the ELISA test, is an antigen test performed on the stool, measuring the antigen being shed from the body. Since infected birds will intermittently shed antigen, it is possible to have a negative result in a positive bird, because the test was performed at a time when the bird was not shedding the antigen.

The fifth test is a test that examines fecal material under the fluorescent microscope or is stained with one of the fluorescent techniques to detect the presence of the organism. These tests are dependent upon intermittent shedding periods.

Though an antigen test is negative, it is possible that the bird actively has the disease. If an antibody test is positive, it is possible that the bird has had previous exposure resulting in an elevated blood titer, but the bird does not have active disease. It is necessary in birds with positive antibody titers to retest them four to six weeks after the first test to determine if there is a rising titer. A rising titer indicates active disease. A stable or falling titer indicates exposure. As a result of these variables, interpretation becomes difficult in many cases and it is sometimes necessary to rely on a combination of tests to prove that a bird is actively sick with Psittacosis.

The complement fixation test is not accurate in all species and its validity cannot be accepted in cockatiels and African Greys and possibly in other species.

As one can see, because of the variables and limitations of each of the tests, interpretation is not simple. Factors such as active clinical signs and exposure potentials must be considered as well as evaluating test results.

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Answer 2: A number of laboratory procedures are used in the diagnosis of Psittacosis.

1) Procedures that isolate the agent: Culturing droppings, nasal discharge or tissue samples that isolate the disease producing agent is a confirming test. Making preparations of tissues or feces and applying special stains that are specific for the disease producing agent are confirming tests if the stained organism is found in the preparations. These tests can be performed on samples from live birds obtained by biopsy or dead birds during a post mortem examination.

Cultures will be negative if no live organisms are available when the sample arrives at the lab, so rapid shipment in special liquids under refrigeration is important. Special stains can be negative if the sample used does not contain organisms dead or alive. This can occur if the sample does not contain organisms when it is obtained, such as droppings which may only contain organisms on occasion or a tissue that may not be involved when the sample is collected while other tissues at the same time are infected.

2) Procedures that combine with the agent and identify that combination: The ELISA test is one such procedure. The organism, dead or alive, or a portion of the organism present produces a positive reaction. These tests are dependent on infective material being present in the sample. Since birds often only shed the organism from time to time, a negative test is not a guarantee that the bird is not infected. Multiple samples over a period of days are better than one sample, but still may be falsely negative. On the other hand, false positives can occur from other agents in the sample.

3) Procedures that measure the body's response to the organism: When a body is exposed to an infectious agent, the immune system responds by producing antibodies against the agent. Antibody levels are a method of diagnosing psittacosis. A negative titer (no or low level of antibodies) is considered a negative test. Some species such as African Greys, budgies and cockatiels do not respond by producing large levels of antibodies even when infected so this test is only useful in some species. Antibody levels do not necessarily reflect current health status of a bird. For example, macaws that were infected, treated and cured can have very high antibody titers for long periods of time. A confirming positive antibody titer involves two samples obtained three weeks apart that demonstrate a considerable rise in the level of antibodies between the first and second sample. A fourfold rise is confirming of psittacosis (1:16 titer to 1:128 titer, for example).

4) Clinical signs: Although not diagnostic in themselves, clinical signs of disease and supporting blood counts and radiographic signs are helpful in the diagnosis. Malaise (a state of ill feeling), loose droppings with lime green urine, jaundice (yellowing of the skin), high white blood cell counts and enlargement of the liver and spleen and cloudiness of the air sacs are suggestive of psittacosis.

In my experience, the examination by a pathologist of any bird that dies, gives the best chance of diagnosing this disease. I would encourage all clients to have this done on any bird in their possession that dies.

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Answer 3: Psittacosis, a disease caused by the bacterial organism, Chlamydia psittaci, continues to elude accurate diagnosis for several reasons. The problem is that birds...
may appear clinically normal and yet still be incubating the disease.

First, the psittacosis organism is an obligate intracellular parasite. This means that the bacteria survives within the cells of the host, which is considered a "privileged" position because the organism evades the surveillance of the immune system. This bacteria has the ability to lie dormant within the cell and not result in disease for months or years.

If the organism is undetected by the immune system, then no immune response can be mounted and the bacteria remains uncleared from the host. Normal immune response includes production of antibodies specific to the invading organism; but this requires that the immune system be "exposed" to the invader. Unfortunately, some of the psittacosis tests rely upon the presence of the specific chlamydial antibodies which may not be produced because the organism is "hidden" within the host's cells unexposed to the immune system. Thus the bird may actually have psittacosis, but the test will be negative since inadequate antibodies are being produced for purposes of detection.

Second, other tests rely upon the detection of bacterial antigens or whole organisms. Antigens are the components of the bacteria which stimulate an immune response. Again, if the psittacosis organisms are "hidden" within the cells, then no antigen would be "shed" into systemic circulation and would go undetected. This would again result in a negative test even though the bird is carrying the psittacosis bacteria.

Finally, some of the tests available are specifically developed for *Chlamydia trachomatis*, a human pathogen. Although these tests may have some cross-reactivity with the psittacosis organism *Chlamydia psittaci*, they are not specifically designed to detect it.

There are more sensitive tests under development but they are not yet commercially available. The tests we currently have access to, rely upon either antibody production or the presence of antigen (bacterial components) in the samples. If the psittacosis bacteria is latent, i.e., lying dormant within the host cells, then antibodies will not be produced in quantities sufficient to be detected and antigens will not be shed into systemic circulation in a quantity adequate for detection. Usually, when the bird is ill, one or both types of tests will be positive, but unfortunately not always. Therefore, diagnosis of psittacosis is a combination of testing, history, clinical signs, and laboratory results, experience and luck.

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Question 2: Should the bacteria *E. coli* cultured from the cloaca of psittacine birds be treated in every situation?

R. Halwell Washington

Answer 2-1: The gram negative bacterial organism *E. coli*, is considered pathogenic when isolated from the gastrointestinal tract of seed-eating birds. However, it is possible to isolate this organism from normal, healthy birds. Some avian veterinarians feel that if there is any evidence of *E. coli* or any of the other pathogenic gram negative bacteria, the bird should be treated with an appropriate antibiotic in an attempt to eliminate the organism from the bird's system. The most universally accepted practice is to place a bird that has cultured positive to *E. coli* on antibiotics only if the bird is showing clinical signs of illness or if, quantitatively, the bacterial count on culture is very high.

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Answer 2-2: *E. coli* isolated from the cloaca of psittacine birds may or may not need to be treated. The decision to treat must be based on a number of findings. Is the number of bacteria large or small? Is the strain of bacteria highly resistant to antibiotics or sensitive to all of them? Is the bird clinically sick? Does the bird have a concurrently high white blood cell count with the presence of toxic and reactive cells?

If sick, bacteria present in large numbers which are highly resistant, the white blood cell count is high, and toxic cells are present, I would treat the bird with an appropriate antibiotic chosen from sensitivity tests. If normal with none of the above criteria, I would not treat the bird, but would repeat the culture and blood count in 10 to 14 days, and re-evaluate the situation.

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Answer 2-3: Treatment of *E. coli* is one of the most controversial subjects in avian medicine. There are numerous opinions, each of which is supported by valid arguments. In my clinical experience, treatment of *E. coli* is dependent upon the species, age, and clinical presentation of the bird.

In some species, such as cockatoos, *E. coli* is frequently recovered from the cloaca of clinically normal birds. Therefore, if the species is one in which *E. coli* is regularly recovered, and the bird is clinically normal, I consider the bacteria a commensal organism (i.e. normal flora). However, this is not to suggest that the bacteria does not have the potential for being pathogenic, i.e. the ability to cause disease. But, *E. coli* recovered from locations other than the gastrointestinal tract, such as the respiratory tract, should be considered pathogenic and treatment is necessary. In South American species, *E. coli* is not often cultured from the cloaca. Frequently, recovery of the bacteria can be associated with clinical illness either directly due to the presence of the bacteria or, secondarily, as an opportunist in a bird debilitated from other causes. Therefore, I do treat under these circumstances.

In pre-weaning psittacine babies, I usually treat *E. coli* regardless of the species. Treatment in this instance seems to expediate growth and weaning.

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Answer 2-4: Gram negative organisms, including the bacteria *E. coli*, are generally considered to be potential pathogens in the intestinal and respiratory tracts of avian patients. Presence of this organism does not necessarily indicate presence of infection and, in many instances, may be transiently part of the normal flora of that individual bird. *E. coli* is frequently isolated from various portals in avian patients, and needs to be interpreted as part of the entire clinical picture rather than necessarily as a disease causing and producing agent. Prime examples of this would be the routine culturing of *E. coli* from the crop and/or cloacal area of many species of macaws, cockatoos, lories, and toucans. In many of these individuals, the bird is clinically normal and appears to be maintaining a healthy, host-inhabited relationship.

In other situations and individuals, presence of gram negative bacteria, including *E. coli*, can be associated with disease. Considerable controversy exists as to the significance of gram negative bacteria in the body of avian adult and pediatric patients. This author disagrees with the viewpoint that large numbers of gram negative bacteria obtained from any site in the bird are to be considered abnormal, and suggests that interpretation is quite complex and may vary tremendously with the individual. In general though, presence of gram negative bacteria isolated or identified from healthy birds should be considered on an individual basis. Many birds will thrive and prosper with abundant growth of members of the bacteria family Enterobacteriaceae (of which *E. coli* is a member), if the individual is clinically healthy.

Age and species of the bird in question often is an important factor in determining pathogenicity of *E. coli*. For example, isolation of large numbers of *E. coli* from the crop and cloaca of an eight week old clinically healthy Umbrella Cockatoo chick would not be considered a treatable problem by this author, where as isolation of the same bacteria from the crop and cloaca of a clinically healthy Rose-breasted Cockatoo would be considered significant and a treatable problem. This serves as an example demonstrating species variation with microflora growth and could be additionally used to differentiate age, where this same bacteria in a week old Umbrella Cockatoo would be watched very closely or possibly even treated, as would be undertaken with a similar aged Rosie.

Part of the difficulty in deciding when to consider treatment of a bird when *E. coli* has been cultured, is the large number of different strains of *E. coli* which are present and the inability of the current available and affordable technology for the veterinarian to differentiate between the pathogenic and non-pathogenic strains. Therefore, depending on the species of bird, the age of the bird, the entire clinical picture in a specific situation, the other available laboratory data, and the experience and opinion of that particular veterinarian, the decision to treat a bird with antibiotics for the bacteria *E. coli*, will continue to be a complex one.

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