Pre-analytical factors affect your chemistry results!

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- Plasma handling for best chemistry results
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Comparison of biochemical values in serum and plasma, fresh and frozen plasma, and hemolyzed samples from orange-winged Amazon parrots (Amazona amazonica).


Summary: Blood samples were obtained from Amazon parrots and handled in very controlled and reproducible steps to produce serum and plasma and also two grades of hemolyzed samples. Additional nonhemolyzed plasma was frozen at -80°C for 24 hours. Although results from serum and plasma samples fell within published reference intervals for this species, there were significant differences between the two sample types including CO2, phosphorus, calcium, total

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What is the Avian Digest?

The *Avian Digest* is prepared and published by the Division of Comparative Pathology of the University of Miami Miller School of Medicine.

The division’s faculty has access to many journals that publish papers of interest and importance to the avian veterinary community. The goal of the digest is to briefly review these papers and add a “Note from the Lab”. The latter is why we found the particular paper interesting to present to you.

This is our first issue to focus on this interest. Please contact the lab with questions and comments about how we can improve future issues. Also, if you come across an interesting article, please send it to us!
Possible human-avian transmission of *Mycobacterium tuberculosis* in a green winged macaw (*Ara chloroptera*).


**Background:** *M. tuberculosis* infections in birds are considered rare in comparison to more common agents of *M. avium* and *M. genavense*. Infections in humans are more common. Antemortem diagnosis in birds is clearly difficult but practitioners should be aware of signalment and potential for zoonoses.

**Summary:** A 5 year old green winged macaw presented with swelling in his right leg and progressive lethargy. A cytology prepared from an aspirate of the swelling was suggestive of spindle cell sarcoma. The WBC count was markedly increased although serum biochemistry analyses were normal. Histologic exam of a biopsy of the area revealed acid fast positive rods. The bird was euthanized and the necropsy revealed infection in the lungs and liver. Cultures and PCR were positive for *M. tuberculosis*. In investigation of the epidemiology of this infection, the owner confirmed his own previous infection with pulmonary tuberculosis a few years previously during which time he was in very close contact with the bird including mouth-to-beak feeding. Other birds in his collection were euthanized and no evidence was found of further infection. The two owners had previously been treated for pulmonary tuberculosis and had completed treatment between 6 and 15 months before the euthanasia of the bird. The owners stated that they would pre-chew food for the bird. The isolates of *M. tuberculosis* from the bird were compared to those of the owners using several different molecular techniques including RFLP analysis. The isolates were found to be identical.

**Clinical Impact:** This paper joins previous publications and proceedings that *M. tuberculosis* can be contracted by birds from their owners. The authors recommend followup of any bird with cutaneous swellings and leukocytosis and note that their initial investigation suggested a tumor rather than infection in this case.

**Note from the lab:** Although acid fast staining is still considered the standard for the diagnosis of mycobacterial infections, practitioners should strongly consider submission of samples for culture and PCR for increased sensitivity and specificity to diagnose this potential zoonotic agent.

*Possible human-avian transmission of Mycobacterium tuberculosis in a blue-fronted Amazon parrot (Amazona aestiva aestiva).*


**Background:** Close contact can result in transmission of infection from humans to birds. DNA analysis provides the tool to prove these zoonotic infections.

**Summary:** An adult blue fronted Amazon parrot presented with skin proliferations and a sublingual nodule. The bird was emaciated and subsequently euthanized. The skin proliferations were analyzed by histology and found to contain acid fast bacteria. Numerous granulomas were also found in the nodule, the eye conjunctiva, lungs, liver, spleen, and kidney. *M. tuberculosis* was identified by culture and PCR. The two owners had previously been treated for pulmonary tuberculosis and had completed treatment between 6 and 15 months before the euthanasia of the bird. The owners stated that they would pre-chew food for the bird. The isolates of *M. tuberculosis* from the bird were compared to those of the owners using several different molecular techniques including RFLP analysis. The isolates were found to be identical.

**Clinical Impact:** This paper is the first report utilizing advanced molecular technology to definitely link transmission of *M. tuberculosis* from humans to birds.
protein, and CPK. Notably, frozen plasma contained many changes including a marked 48% increase in LDH values. This is a novel finding which is contrary to many reports in mammals.

The induction of hemolysis increased potassium levels up to 85%. Marked changes were also observed in LDH, alkaline phosphatase, and phosphorus. Other analytes including AST, total protein, and CPK were also altered but within 10% of starting levels. Interestingly, this study documented that hemolysis can be present but not easily visualized; that is, even apparently non-hemolyzed samples may have some artifactual changes.

The authors also confirmed anecdotal reports that a higher volume of plasma vs. serum is obtained from Amazon parrots. Per 0.5ml blood draw, 0.28ml of plasma was obtained vs. 0.24ml of serum.

Clinical Impact: While most practitioners routinely use plasma for biochemical analyses, this paper does demonstrate serum will produce different results although these changes will likely not be clinically significant in normal birds. Plasma is demonstrated to be the sample of choice for superior sample volumes. Hemolysis has been known to affect many chemistry results but this paper is the first clear documentation in psittacines. Notably, even trace hemolysis that may not be visualized may have an impact on results. Freezing can affect results – most notably with increases in LDH.

Note from the lab: This paper demonstrates the importance of proper sample handling. Clinics should adhere to their own in-house SOP which will dictate tube type, length of time before centrifugation, time and speed of centrifugation, and packaging of samples for transport.

Nearly half of the samples received in this laboratory are plasma transported in green top tubes that contain gel. Even if centrifuged with a gel to pack the RBCs, the transport of plasma on the separated RBCs will still result in changes in many analytes including glucose and potassium. The gel can also become dislodged during transport or the RBCs may be exposed to temperatures that cause lysis and result in sample hemolysis.

Small snap cap microfuge tubes for transport of separated plasma are preferable and available from the lab at no additional charge. Contact Client Services.

High levels of corticosterone in feather-plucking parrots (Psittacus erithacus).


Background: Several different etiologies for feather damaging behavior (FDB) including behavior, parasitism, bacterial or fungal infection, nutritional deficiencies, allergy, or an unrelated medical condition have been proposed. If stress is involved in this process, there is an expectation that corticosterone levels would be different in FDB birds.

Summary: Two groups of parrots were studied which represented 7 normal African grey parrots and 10 with FDB. The control birds were housed at a separate location from the FDB birds in a large aviary. Significantly higher levels of fecal corticosterone were observed in FDB parrots (p=0.032).

Clinical Impact: Although limited sample size and differences in the housing and husbandry of study birds were present, this study does support the premise of a potential link between stress and FDB.

Note from the lab: We have investigated the link between stress and FDB in a study 51 African grey parrots soon to be published in the Journal of Avian Medicine and Surgery (Clubb, Cray, Goodman, and Arheart) examining corticosterone levels in the blood. No significant differences were observed. Fecal corticosterone provides a non invasive tool to study stress and may reflect differences not apparent in blood testing. Further comparative studies are warranted.
**Chlamydophila psittaci** genotype E/B transmission from African grey parrots to humans.


Background: There are six serovars of *Chlamydophila psittaci* and all can possibly infect humans. They can be distinguished using monoclonal antibodies to their unique major outer membrane protein (MOMP) or genetic analyses including PCR. The genotypes follow certain host species prevalence. Genotype A is commonly found in psittacines, B in pigeons, C and D in poultry, and E in a variety of non-psittaciforms. In 2005, a new genotype called E/B was described and isolated from ducks, pigeons, and turkeys.

Summary: Birds at a parrot rescue group were tested for the presence of *C. psittaci*. This included 20 African grey parrots, 14 blue and gold macaws, and 2 green winged macaws. The macaws were housed in a separate room. The facility manager, who was regularly in close contact with the African grey parrots, complained of shortness of breath. The macaws (which were kept as breeding birds) were regularly treated with doxycycline. 25% of the African grey parrots exhibited feather loss, depression, weight loss, and mild dyspnea. All birds as well as the manager were sampled and tested using a nested PCR reaction for a gene of the MOMP which could be further probed to distinguish the specific genotype. The analyses revealed that 25% of the African grey parrots as well as the manager were positive for *C. psittaci* genotype E/B. The manager elected not to be treated and clinical symptoms remained. The birds were treated and distributed among other breeding facilities. Interestingly, the two individuals that visited the rescue group to obtain samples also tested positive and demonstrated antibody titers against *C. psittaci*. They remained asymptomatic, were not treated, and became negative (by PCR) with time; antibody titers remained nearly 3 months after exposure.

Clinical Impact: This is the first report of the transmission of *C. psittaci* genotype E/B from psittacines to humans. It is notable that the strain appeared not to cause severe symptoms in either the birds or humans in contrast to that which has been observed with genotype A.

*Note from the lab:* These three articles (see the next two pages) open discuss several issues of importance.

Read on about more zoonotic incidences of Chlamydyphilosis...
An outbreak of psittacosis due to *Chlamydophila psittaci* genotype A in a veterinary teaching hospital.


**Background:** Occupational exposure to bird species makes for the potential transmission of a number of zoonotic agents although infection with *C. psittaci* is considered one of the primary agents of concern. In addition to the general population that may have incidental or direct exposure to birds, veterinarians and their staff need to use safe handling practices with birds and be aware of the clinical signs of human infection.

**Summary:** Three patients who were staff members of a veterinary school were admitted to a hospital within one month of each other. One patient was hospitalized with multi organ failure. *C. psittaci* was cultured from each patient and these samples also tested positive by PCR. In total, there were 10 staff members or students that were identified as diagnosed with psittacosis. Of the remaining 26 exposures, others were either asymptomatic or complained of a brief fever/headache. The reported symptoms included muscle aches, severe headache, fever, dyspnea, and chills. Infection was confirmed by either a rising IgG titer and/or positive PCR test. Notably, one asymptomatic person remained PCR positive for 2 months. Amazon parrots that which were used in a laboratory class at a veterinary teaching facility had been recently exposed to cockatiels which were housed in the same facility for a short period. The asymptomatic parrots routinely tested negative by antigen testing until the investigation of the source of human infection. Infection after the human exposure was confirmed by PCR to be genotype A.

**Clinical Impact:** The broad spectrum of reaction after exposure – from extreme (multi-organ failure) to the lack of symptoms supports the premise that infection with *C. psittaci* might be more common in humans having contact with birds than previously assumed. The authors recommend the necessity of a clear management of birds in the academic setting where multiple human exposures could easily occur. Prophylactic treatment is suggested as is keeping records for all bird transactions for one year for easier followup in the event of an outbreak.

**Note from the lab:** This paper demonstrates that both infected humans and birds may be asymptomatic. Either population may represent a poorly defined carrier state during which time they may be infectious. Screening tests can be helpful but negative results can be obtained during the incubation period before the organism is excreted in significant numbers for detection by conventional assays.

Infection by *Chlamydophila avium* in an elderly couple working in a pet shop.


**Background:** Occupational exposure to bird species makes for the potential transmission of a number of zoonotic agents although infection with *C. psittaci* is considered one of the primary agents of concern. A number of review articles from human medicine report infection in mostly 30 to 60-year-old adults although the elderly population should be considered when exploring the etiology of unknown pneumonias due to changes in immune function and the presence of secondary disease.

**Summary:** An elderly couple (76 and 77 years of age) ran a pet shop. continued on page 6.
Chlamydiae and atherosclerosis: can psittacine cases support the link?


**Background:** *Chlamydophila pneumoniae* has been associated with atherosclerosis in humans although studies in this area have been of much contention. While similar histologic changes have been described in psittacines and other avian species, there has been no association of such changes with clinical signs and the etiology of these lesions remains undetermined.

**Summary:** Tissues from 103 pet birds necropsied over a 14 year period were graded for the presence of atherosclerosis and DNA was screened for chlamydial DNA by PCR. Those tissues found suspect by PCR were further screened by immunohistochemistry. African grey and Amazon parrots showed the highest incidence of atherosclerotic histologic changes. Just over 20% of the cases demonstrated a PCR product consistent with chlamydial DNA but on further testing, only 4% of the cases were positive for *C. psittaci*. Immunohistochemistry corroborated half of the PCR reactive cases although the authors reported a high level of nonspecific staining. Differences in the results from the two techniques may be related to degradation of the DNA during formalin fixation.

**Clinical Impact:** This paper is the first to describe atherosclerosis and testing for chlamydial DNA in a large number of psittacines. Chlamydial DNA could not be consistently detected in the lesions. The authors conclude that there is no link between infection and atherosclerosis in psittacine birds.

*Note from the lab:* While some animal models support the association between infection with *C. pneumoniae* and the development of atherosclerosis, current reviews of human literature place the association at under 15%. There has not been a standard of testing in human studies which has led to quite varying results regarding this association. For more information on the link in avians, likely look for upcoming information from Pilny et al. who presented their preliminary findings at AAV in 2007.

**Infection by Chlamyphila avium in an elderly couple working in a pet shop.**

*Continued from page 5*

The man was admitted to a hospital with lethargy and fever in the absence of a cough. Radiographs revealed infiltrates in both lobes of the lung. Routine blood work was unremarkable with the exception of elevated liver enzymes. Testing by CF and MIF revealed high IgG titers to *C. psittaci*. Antibiotics were given and the patient remained hospitalized for 17 days. His wife was admitted one day later also with a fever. Similar radiographic and lab work changes were present. She responded to antibiotic treatment was hospitalized for 16 days. The couple did not consent to test any birds at their pet shop.

**Clinical Impact:** It is possible that the patients became infected by birds within their pet shop. There are also two reports in the literature that indicate possible nosocomial transmission of *C. psittaci*. Bird exposure to the elderly population should represent a concern to both physicians and veterinarians.

*Note from the lab:* This paper was followed by publication of a letter from a group of veterinarians that importantly noted that chlamydo-

philosis in avian species can be asymptomatic (*JCM* 43:5410-5411, 2005). They reported an outbreak at a pet shop that had recently received lovebirds which were now exhibiting severe clinical signs. All the birds died within two weeks and fecal PCR revealed *C. psittaci*. An additional 14 birds present in the shop were assessed. All were asymptomatic but two parrots were found to be fecal PCR positive for two weeks after the loss of the lovebirds. None developed any clinical signs and all have remained PCR negative at the time of the writing.
Background: Avian chlamydotheliosis is a concern of veterinary and human medicine. Infection of avian species can result in morbidity and mortality and result in serious economic losses in the poultry industry. To date, there has not been a successful development of a vaccine for bird species.

Summary: The MOMP or major outer membrane protein has been described to be the immunogenic protein of *C. psittaci* and that protein which would confer protection. The MOMP gene was amplified by PCR, inserted into a plasmid, and transfected into an adenovirus. Seven day old chickens were inoculated with the virus and anti-MOMP antibodies were measured at several time points. Antibody titers were significantly higher than control chickens with titers peaking at day 21 and declining thereafter. These birds also demonstrated significantly higher levels of lymphocyte proliferation to the immunogen.

A second study was conducted challenging the chicks with a *C. psittaci* isolate. While non vaccinated birds show changes in clinical condition, 9 of 10 vaccinated birds had no changes or histological lesions through the termination of the experiment 6 months later.

Clinical Impact: This study shows promising data that a MOMP based recombinant vaccine would provide protection against *C. psittaci* infection in chickens.

Note from the lab: Similar vaccines have been successfully used in animal models of *Chlamydia* infection in goats, guinea pigs, and sheep. A single report in turkeys reported only partial conferment of protection. Studies regarding the application and efficacy of such vaccines are pending in other species including psittacines. Look for more information upcoming from Tom Tully’s research group.

**Listeriosis in a cockatiel (Nymphicus hollandicus).**


Background: Listerosis, caused by *Listeria monocytogenes*, is a disease of concern in ruminants with various clinical symptoms. Infection has been reported in bird species but is thought to be rare in psittacines.

Summary: A 4-year-old cockatiel from a large outdoor aviary presented was submitted for necropsy after several days of lethargy and weight loss. The liver was enlarged and microscopically was found to have large numbers of gram positive rod-shaped bacteria. Changes were also observed in the spleen, bone marrow, and kidneys. Cultures and other diagnostic testing confirmed the presence of *L. monocytogenes*. No lesions were found in the heart or brain that represent two primary target tissues of infection in chickens. No additional cases of infection were found in the aviary. It was hypothesized that the bird acquired the infection orally via the soil or contaminated feed or water. The serotype identified in this paper is common to infections with humans and animals.

Clinical Impact: This paper is the first report describing detailed histological changes and the identification of a serovar of *Listeria* in a cockatiel.

Note from the lab: Infection with this incredibly hardy bacteria is still believed rare with less than 500 human deaths per year.

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**Construction and immunogenicity of recombinant adenovirus expressing the major outer membrane protein (MOMP) of Chlamydia psittaci in chicks.**


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