

## **Avian Clinical Pathology**

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Recognizing a bird is sick is one of the most difficult aspects of avian medicine. Sick birds hide their signs. When birds do become overtly ill, it is not unusual for the bird to have been sick for weeks or even months before signs were obvious. Acute illness does occur in birds. The veterinarian's job is to try to determine if the disease signs are the result of a manifestation of a chronic disease or acute illness. This may change prognosis, diagnostics and treatment.

Recognition of a sick bird is not always so easy. Besides the typical history, one must ask the owner a series of questions that may elucidate if the bird is sick. Non-specific signs of a sick bird include not perching, not eating, not drinking, weakness, "fluffed", and lethargy. More specific signs of disease include crop stasis, watery and loose droppings, yellow to green urates, and regurgitation. Birds with traumatic events will present just as any other animal. Fractures, bleeding, open wounds, and punctures into body cavities all can occur.

One of the challenges of working with avian patients is the reliance we have on diagnostic testing as opposed to using our physical examination to lead us to the etiology of a problem. The common presentation to the avian veterinarian of the "sick bird syndrome" is the "fluffed" bird that is sitting on the bottom of the cage. Not only don't we know how long the bird has actually been sick, but we may not even know which organ system to start investigating. Therefore, in avian medicine, more than with dogs and cats, we rely on our minimum database to point us in the right direction in terms of treatment and further diagnostics.

Interpretation of clinical pathology values in avian patients begins with proper sample collection. This is true in all species but of immediate importance in birds because there may only be one chance to get your sample. Many avian patients are critically ill by the time the owner seeks veterinary assistance. Without appropriate blood collection technique, incorrect interpretation will ensue with a possible incorrect diagnosis; you do not have time for a second chance. Proper collection technique includes inciting the least amount of stress to the patient, using appropriately sized collection material, and placing samples into suitable containers. It is also essential, for proper interpretation, to send samples to a laboratory that is familiar with avian patients or to do your clinical pathology in-house with appropriate methodology.

Interpretation of the CBC and biochemistry panel in birds is an evolving science. There is much that is known but even more that is not known. Many of the same interpretations of results that are used in dog and cat medicine are used with avian patients. Differences of physiology between birds and mammals are reflected in the interpretation of the biochemistry results. Obviously, the term "avian medicine" covers thousands of species. The veterinarian, concentrating just on pet birds, still may likely see a few hundred different species. At our level of understanding, it is not possible yet to identify unique CBC and biochemistry characteristics of each of these species.

The white blood cell count (WBC) is interpreted in birds much the same way it is in mammals. An elevation of the total white count most commonly represents inflammation, infection, or neoplasia.

A stress leukogram causing a mild increase in the total WBC count is reported by some to be present in birds. A lower than normal WBC count is not always a cause for concern. A dramatically lower WBC count could represent a viral infection or damage to the immune system. Heterophils are the white blood cells most likely to increase when an infection is present. Typically, there are more heterophils relative to lymphocytes in the WBC count but some species or individuals normally will have a reverse heterophil:lymphocyte ratio.

Bacterial infections in pet psittacine birds are probably overdiagnosed. Spontaneous, primary bacterial infections in adult pet birds are likely not common. Bacterial infections in chicks that are still being hand fed primarily occur due to poor husbandry practices. Bacterial infections as a result of a traumatic injury (i.e., dog bite) are a more common source of infection in adult birds. In these cases of documented bacterial infections, the white blood cell count usually does not go above 20,000 to 25,000  $\times 10^3/\text{mm}^3$ . It is typically a heterophilic response. If the bacterial infection is chronic both an absolute and relative monocytosis is observed. In severe, acute infections, band cells can be seen. Band cells are also seen in septic conditions although band cells are rare in birds.

Along with the WBC count, an electrophoresis panel (EPH) is a useful indicator of bacterial infections, inflammation, and humeral immune response. Both the beta (acute infection) and the gamma (chronic infection) fractions can be elevated due to bacterial infections. It is expected in the first stages of a bacterial infection, the beta section of the EPH will be elevated. In fact, it is possible that only the beta section will be elevated in the face of a normal WBC count when a bacterial infection is present. In chronic bacterial infections, the gamma section may be elevated with or without an increase in the beta section. This may also correspond to a monocytosis in the WBC.

Chronic infections are not common in pet birds. Therefore, there are only a few differentials for a chronically elevated WBC count in avian patients. The most likely causes are fungal infections (usually aspergillosis), chlamydiosis, mycobacteriosis, or neoplasia. Species that are more prone to fungal infections include African greys, macaws, amazons, pionus, and jardines. Usually, the WBC count will show dramatic increase in the face of an aspergillus infection. The white blood cell count may go over 100,000  $\times 10^3/\text{mm}^3$  cells. This is usually a heterophilic response and a relative and absolute monocytosis can be present. Birds infected with mycobacterium can show the same type of leukogram as birds suffering from aspergillosis. The same could be stated about psittacosis but in many birds, the leukogram may not be as dramatically increased. In chronic cases of psittacosis, there may be a normal leukogram or a slight increase above normal with an increased monocyte count.

Due to the difficulty of performing an automated analysis and the small sample size most veterinarians obtain from birds, many practitioners will only use the PCV to describe the red blood cell count. It is uncommon to see on laboratory reports the mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV0) or the hemoglobin concentration (Hgb). Reported normal reference ranges for avian PCV's are quite variable. The PCV value may depend on age, species, sex, and time of year the sample is taken. Therefore, the practitioner is urged to develop their own reference range of normal or to use a commercial laboratory that has developed their own normal ranges. It appears that younger birds have a lower PCV. Anemia is common in sick birds. It is important, just as it is in mammals, to classify the anemia as to regenerative or nonregenerative. It is slightly more challenging in birds as their blood cells are already nucleated. Characteristics such as immaturity, size, color, and shape of the red blood cells can be used. But a more objective method is by using red cell distribution width percentage

(RDW%). Polycythemia is uncommon in pet birds but does occur. Repeatable, elevated PCV's in birds that are not dehydrated is suspicious for a diagnosis of polycythemia.

What tests should be included in the biochemistry panel? This depends on the sample size, the analyzer, and what information is required. One may use different panels if a health check is being performed, if the bird has a history of liver disease, or if only a few drops of blood could be obtained. There are many analytes that can be measured on avian samples but not all have been validated for use in birds. In some cases, the relationship between abnormal values and disease status is not totally elucidated.

The measurement of total protein in avian patients represents the addition of pre-albumin, albumin and globulin concentrations. In birds, chemistry analyzers may not be able to adequately quantify albumin and globulin. The most accurate method to quantify the albumin and globulin concentrations is with an EPH. Commercial albumin reagents will return a lower albumin concentration as compared to that of an EPH. Total protein abnormalities represent in birds many of the same disease conditions as is seen in mammals. For example, an elevated total protein could represent dehydration. Or a low total protein could signify protein loss.

Evaluating liver disease and liver function in birds is extremely important as liver disease is common. Analytes such as alanine aminotransferase (ALT), serum alkaline phosphatase (SAP), and bilirubin are not useful in birds. ALT and SAP are not sensitive or specific in birds for liver disorders. Birds do not produce bilirubin but rather biliverdin. At present, there are no reliable tests to quantitate biliverdin. One of the best measurements of liver disease in birds is AST. This is sensitive but not necessarily specific for liver disease. Damaged muscular tissue releases both AST and creatine phosphokinase (CPK) into the blood. To help determine if AST is elevated from hepatic or muscular disease, the CPK should be examined. A high CPK along with a high AST probably is evidence of muscular disease while a high AST alone probably represents liver disease. Bile acids appear to be a good measurement of liver function in birds. A single sample appears to be sufficient; pre and post prandial samples are not necessary in psittacine patients. One analyte that in the past may have been used to measure liver disease, lactate dehydrogenase (LDH), appears to not be useful. Elevations in LDH may only represent sample degradation rather than organic disease.

It is more difficult to evaluate renal disease in birds than in mammals because there are less analytes and tests that are useful in avian patients. Uric acid concentration represents renal function. Increases in the plasma uric acid concentration probably signifies renal dysfunction. Since uric acid is actively secreted in the renal tubules, it is unlikely that uric acid concentration rises significantly with dehydration. There can be renal disease (i.e., glomerular disease) without increases in the uric acid. Creatinine is not produced in birds and cannot be used to measure renal function. Blood urea nitrogen is not produced in significant quantities in birds and is not used to measure renal function. There are reports that BUN may be useful to measure hydration status in birds. The avian urinalysis is difficult to interpret. Urine is directly deposited into the cloaca (there is no bladder) and can mix with intestinal and genital products. Plus, retropulsion of urine into the rectum is known to occur further changing the constituents of urine. Therefore, it is unlikely urine assays accurately represent renal function or dysfunction. It is reported that one of the most reliable aspects of avian urine is the presence of renal casts as they only come from the kidney and are not seen in the normal urinalysis. In chronic renal disease, there may be an increase in phosphorus with a low to normal total calcium. Some have also reported a high total calcium with chronic renal disease. The urinalysis in birds is of almost no use in determining the health of the kidneys. Urine is mixed with contents from the gastrointestinal and genital tracts. Also, urine can be retropulsed

into the gastrointestinal tract and water absorbed causing urine concentration. The most useful aspect of a urinalysis is to look for casts as a sign of urinary tract disease.

Calcium concentration is an important analyte to measure in avian plasma as numerous disease states will alter the total calcium concentration. There are a number of aspects of calcium measurement that need to be elucidated before more accurate interpretations can be made regarding this analyte. It is important to submit as good a quality sample as possible for calcium determination. Calcium is affected by hemolysis and calcium does not dilute accurately if the sample is small requiring dilution for measurement. In mammals, it is now recognized that ionized calcium concentrations are a better indication of calcium homeostasis than total calcium concentration. Since there is not an accurate correction factor for calcium and albumin for most avian species, being able to measure ionized calcium would allow better interpretation of changes in the plasma calcium concentration. Logistically, at present, it is not feasible for many practices to measure ionized calcium. Once more analyzers are designed to measure ionized calcium and validation studies are done, ionized calcium may become a useful analyte. Presently, the most common causes for changes in calcium concentration reflect nutritional deficiencies, egg production, and renal disease.

Electrolytes such as sodium, chloride, potassium, and phosphorus are measured in avian patients just as they are in mammals. These analytes are measured less often in birds so unfortunately our database is limited as to the different diseases associated with abnormalities in these analytes. Imbalances can be caused by organ dysfunction, gastrointestinal disorders, and especially iatrogenic causes.

Cholesterol has not been well studied in pet birds. Sedentary birds on all seed diets may have elevated values. It would be valuable to have reference ranges for cholesterol in pet birds as an objective measure of nutritional improvement in pet birds. Egg laying birds may also have elevated values.