Clinical pathology of rabbits and ferrets

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The principles of proper clinical pathology transcend a particular species and what is appropriate for dogs and cats, is usually appropriate for rabbits and ferrets. Following these principles gives one the best chance of obtaining interpretable results. It is true that incorrect results are worse than no results at all. Wrong results can lead one down the incorrect diagnostic path and the diagnosis may be missed and ultimately, the patient is not treated properly. In all species, venipuncture techniques are the key element in determining how meaningful the results will be. As it would be "bad form" to clip a dog's toenail and use that sample to determine the health of your patient, so it is bad form to do the same for the small mammal patient. Why is it worth the effort to perform venipuncture in these species? It is because toenail clips cause so much tissue damage that abnormalities cannot be discerned from artifacts. Toenail clips allow tissue "juices" to enter the sample. This fluid can dilute the sample or add electrolytes into the sample and cause difficulties in interpretation. The PCV is difficult to interpret due to possible dilution of the sample. Microclots enter the sample and they render the CBC and biochemistry panel results suspect. Red blood cells can lyse with this method. This will cause an artifactually low PCV and will also cause abnormalities in the biochemistry panel. Lysis of the red blood cell membrane can increase the phosphorus and potassium values. This can pose a problem when interpreting blood results when considering diseases such as renal failure and hypoadrenocorticism.

Always use blood tubes appropriate for the size of the specimen. Successful venipuncture and sample collection are wasted if blood is placed in a tube meant for a larger sample. In larger tubes, the anticoagulant amount can dilute a small sample. Place small blood samples in microtainers. Small blood samples are those under 2 ml of blood. There are three general types of microtainers: those with no anticoagulant (serum microtainers), K-EDTA anticoagulant, and heparin anticoagulant. It is uncommon to use serum microtainers with small mammals. Almost all biochemistry tests have the same results whether run on serum or plasma. The advantage of the use of plasma is that per volume of sample, more plasma than serum can be obtained from the same volume of blood. And, since in exotic animal medicine, small samples are the rule, it is preferable to use plasma rather than serum for biochemistry panels. Therefore, it is preferable to place

biochemistry samples in heparin microtainers rather than serum microtainers. Although, plasma can also be obtained from K-EDTA tubes, if possible, only use K-EDTA tubes for CBC results. The potassium in K-EDTA tubes immediately exchanges with the calcium of the blood sample. This results in a biochemistry panel report of a very high potassium concentration (over 10 mEq/dl) and a very low calcium concentration (close to 0 mmol/L).

It may seem convenient to use microhematocrit tubes to collect blood from nail clips or the controlled laceration of a vessel. The blood is allowed to drip into the tubes. The tubes are spun down, broken open and the plasma collected. This method usually results in a great amount of cell lysis. The plasma biochemistry results may not reflect what is actually found in the patient. With use of the proper blood collection techniques, microhematocrit tubes do not need to be used for biochemistry analysis.

Once the blood sample is placed in the microtainer, the next thing you do may alter the results you get and significantly change the direction of your treatment. Ideally the plasma should be separated from the cells. Allow the blood tube to sit for a few minutes and then spin the sample. Once it is spun, decant off the plasma and place the plasma in a tube without anticoagulant. If the plasma is placed back in a tube with heparin, this may cause sample dilution. If the plasma is not separated from the blood cells and if it is sent to the lab unspun, at least three analytes of the chemistry panel will be affected, blood glucose, potassium and phosphorus. Blood glucose is decreased when the plasma sits on the blood cells. The active cells use the glucose in the plasma. The laboratory will read a blood glucose concentration lower than what was in the sample originally. In ferrets, when an insulinoma is suspected, this value is critical. If the sample cannot be separated, then measure blood glucose immediately at patient-side. Both potassium and phosphorus can elevate in samples when the red blood cells are in prolonged contact with the plasma. Damage to the red blood cell membrane causes potassium and phosphorus to leak through the membrane or leak around a broken membrane (hemolysis). Certain disease states increase the fragility of the red blood cell membrane and leakage may be unavoidable. Elevated potassium or phosphorus values may not reflect poor technique or handling, but rather a disease condition. That is why correct handling is critical for proper interpretation. If the results reveal an elevated potassium or phosphorus and proper technique was followed, one may then pursue a diagnosis characterized by increased fragility of red blood cell membranes. There are some drawbacks when separating the plasma from the cells in the hospital. It does take extra time and effort to spin and pipette the sample. With very small samples it is technically difficult to adequately retrieve all of the plasma unless micropipettes are used. If a

very small blood sample is retrieved, it may best to leave the pipetting of the sample to the laboratory. Finally, if the same tube is used to evaluate the CBC and the biochemistry profile, then the tube cannot be spun as this will render the cells useless for the CBC.

Even before your sample is in the blood tube, the integrity of the RBC membrane can be compromised. It is important to realize that potassium and phosphorus concentrations will increase in plasma even without gross hemolysis; they leak through damaged membranes. Damage can happen from the venipuncture itself. In the smaller exotic patients, one must use small bore needles that are more prone to causing hemolysis than the larger gauge needles. If possible, remove the needle from the syringe when placing the sample into the tubes to decrease the chance of cell lysis. Many of the routine chemistry analytes will be effected by hemolysis but not as greatly as potassium and phosphorus. Both bilirubin and bile acids are falsely elevated with hemolysis. Bile acids elevate not only with hemolysis but also with lipemia. Lipemia, in exotic animals, is most commonly noted in birds but has been occasionally seen in ferrets. Lipemia can cause RBC membranes to rupture, even with the best technique. Lipemia will also effect every aspect of the CBC and biochemistry analysis. When the sample is lipemic, none of these values are reliable indicators of patient health. It is possible to overcome the effects of lipemia by using a machine known as a dry chemistry analyzer. Most commercial labs and veterinary hospitals use wet analyzers.

Clotting of the blood sample can be a significant problem during venipuncture of small mammal patients. It is not that these species clot faster, but the venipuncture technique most veterinarians use on these animals is not as “perfected” as their technique is in dogs and cats. Therefore, since it may take longer to get a sample, there is more of a chance of the blood clotting. To prevent clotting, the needle and syringe can be heparinized. With this method, there is the potential to change the staining characteristics of the cells or to dilute the sample with too much heparin. This is where experience is important. If you feel too many of your samples are clotted then it is worth the risk of over-heparinization of the sample. Clotting of blood samples changes both the biochemistry and CBC results. The CBC may show falsely elevated or depressed cell values. Also, platelets are decreased. Clotting causes hemolysis therefore altering the biochemistry values.

Samples for the complete blood count are submitted both in slide form and in microtainers. In all species, it is ideal to make two blood smears immediately after venipuncture. These slides are the best representation of the complete blood count of the patient at the time of the sample. This is even true in dogs and cats. In some exotic patients, there is not enough blood to make smears and also fill a K-EDTA microtainer. In these patients, the smears will be the laboratory's only measurement of the CBC. In patients where larger quantities of blood can be taken, some blood can be placed in K-EDTA tubes for CBC analysis. In mammals, this blood can be analyzed through an automated system. In many cases, the hematologist prefers both fresh blood smears and whole blood to analyze. That allows them to make their own smears if the submitted smears are not perfect. It appears that both heparin and K-EDTA will preserve blood cells for later analysis. K-EDTA will give less staining artifacts but may not preserve the integrity of the cells as long as heparin will. Heparin causes many staining artifacts but may preserve the cells for a longer amount of time.

No matter where you practice, it is important to have a working relationship with the laboratory and the clinical pathologists at that laboratory. Each laboratory may have their own preferences for sample submission and each laboratory should provide you with normal ranges for the particular species. During this section, generally accepted references ranges will be discussed but the specific ranges may vary between laboratories. The WBC count in ferrets ranges from 2500 to 8,000 x cells /mm^3. Typically, it is neutrophilic with relative lymphocyte counts usually below 50%. Both absolute and relative increases in the lymphocyte count might indicate the presence of lymphosarcoma. Severe, acute infections can elicit a left shift. The normal RBC count and hematocrit can be higher than what is expected in other mammals. In ferrets, isoflurane anesthesia can cause a significant reduction in indices of the erythron. Forty-five minutes after anesthesia the values return to normal ranges. Care should be exercised when subjecting anemic, geriatric, or debilitated ferrets to isoflurane-induced anesthesia.

Biochemistry values in ferrets are much like what is found in other mammals with few exceptions. Kidney function tests are more difficult to interpret than they are in the dog or cat. Blood urea nitrogen concentrations range as high as 21.4-25 mmol/L(30-35 mg/dl) in normal ferrets. Creatinine is difficult to interpret in ferrets. Normally, it is below 44 mmol/L (0.5 mg/dl), frequently even at 8.8 mmol/L (0.1 mg/dl). Elevations in ferret creatinine concentration with acute renal disease are subtle or even non-existent. It appears that when ferret creatinine concentration is

elevated, renal disease is severe and long standing. Elevations in phosphorus are commonly observed with chronic renal disease. This can be used to determine if chronic renal disease is present when the creatinine concentration is equivocal. Pancreatic beta cell tumors are common in older ferrets and, as such, accurate measurement of the blood glucose concentration is essential. Measurement of the liver enzymes appears similar to that of dog and cats. Alanine aminotransferase (ALT), serum alkaline phosphatase, and total bilirubin concentrations will increase with liver disease and/or liver function derangements. Alanine aminotransferase is found in cytoplasm and mitochondria. In ferrets, ALT is most concentrated in the liver.

Insulinoma is a very common disease of older ferrets. This disease is characterized by hypoglycemia due to a presumed increased production of insulin. The CBC is usually normal in these ferrets. The universal finding is a low blood glucose 3.6-3.9 mmol/L (65-70 mg/dl). Other causes of hypoglycemia such as septicemia and liver disease should be ruled out by history, signs, and diagnostic tests. If the blood glucose measurement is between 3.6 and 4.4 mmol/L (65 and 80 mg/dl) and the diagnosis of an insulinoma appears equivocal, then perform a fasting blood glucose concentration. Fast the ferret for 2 - 4 hours and then perform venipuncture. If the blood glucose is lower than 80 mg/dl, then the diagnosis of an insulinoma is made. It appears that the insulin concentration is not always elevated in ferrets with insulinoma. It is not known why this occurs. Likely, insulin is produced episodically and when it is assayed, the concentration may be normal at that time. There are no other clinical pathology results needed to diagnose this disease. Definitive diagnosis is made on histopathology of diseased pancreas.

Adrenal gland disease is another common problem in ferrets. Clinical signs are usually used to diagnose this disease. Typical adrenal gland tests will not aid in the diagnosis of the disease. The CBC and biochemistry panel will usually show no abnormalities attributable to this disease. The urinalysis is usually normal. The typical assays used to diagnose adrenal gland disease in dogs are also normal. The best clinical pathology test for adrenal gland disease in ferrets is the adrenal gland androgen profile. It measures four hormones and at least one of which is elevated in almost all cases of adrenal gland disease in ferrets. These four hormones are estrodial, androstenedione, 17-OH-progesterone, and DHEAS. The cortisol:creatinine ratio is not specific for adrenal gland disease and may not be useful to diagnose this disease. Histopathology is definitive method of diagnosis. There are two conditions related to adrenal gland disease in ferrets that will cause changes, some dramatic, in the clinical pathology. Rarely, male ferrets with adrenal gland disease develop prostatic disease. The prostate may become infected and the urinalysis may show

excessive mucus, crystals, bacteria, and red and white blood cells. An inflammatory leukogram is common and band cells may even be seen. In very rare cases, adrenal gland disease can cause a suppression of the bone marrow leading to a non-regenerative anemia. If this continues, both a thrombocytopenia and a leukopenia will be observed.

Epizootic catarrhal enteritis (ECE) is a common gastrointestinal disease and can cause distinct clinical pathology abnormalities. This disease appears to be highly infective, spreads rapidly amongst a group of ferrets, and can be spread by fomites. Older ferrets (over a year of age) appear to develop a more severe form of this disease as younger ferrets may show no clinical signs of disease. A coronavirus has been postulated as the etiology of this disease. Hematology may reveal a slightly elevated WBC. In prolonged cases, a relative and absolute monocytosis can be observed. Biochemistry results may be abnormal depending on severity of disease. The more severe the disease, the more deranged the biochemistry results are. In severe forms of this disease, the ALT is greatly elevated. Values above 1000 U/l are not uncommon. As the disease progresses, the SAP may also be elevated. SAP has been shown to go as high into the 200 U/l range.

Lymphosarcoma (LSA) is not an uncommon disease in pet ferrets. The CBC in some cases of LSA will show evidence of disease. The relative and absolute lymphocyte counts may be elevated. The important aspect is that the circulating lymphocytes show bizarre appearances, have mitotic figures, and are very young cells. The biochemistry panel is typically normal unless LSA has invaded an internal organ and resulted in dysfunction. Diagnosis of LSA in ferrets is usually dependent on histopathology. Cytology is performed on a biopsy of a lymph node or mass and examined. It is sometimes difficult even for the most experienced pathologist to discern lymph node hyperplasia from neoplasia.

Rabbits normally have a WBC that range from 4,000 to 9,500 cells/mm^3. Neutrophils are commonly referred to as heterophils in rabbits. It is not unusual for up to 70% of the WBC's to be lymphocytes although typically there are equal numbers of lymphocytes and heterophils. A number of studies have shown and clinical experience concurs that when rabbits have acute bacterial infections the total WBC count infrequently increases significantly but the differential count changes. A heterophilia and lymphopenia are seen with acute bacterial infections. Also, nucleated RBC's are observed in the beginning stages of infection. Because bacterial infections in rabbits are a common occurrence, it is important to realize that an associated leukophilia is not always present.

Band cells are also rarely seen even in acute, severe bacterial infections. The hematocrit in rabbits is much like that in dogs and cats.

Plasma biochemistry values in rabbits are much like what is found in other mammals. Renal disease will increase both BUN and Cr concentrations. In chronic renal disease, an increase in the phosphorus concentration can be observed. Interpreting high calcium concentrations can be difficult as clinically normal rabbits can have calcium concentrations as high as 16 mg/dl. Alanine aminotransferase (ALT) concentration may not be as good an indicator of hepatocellular disease in rabbits as it is in other mammals. Alanine aminotransferase concentration is reduced in rabbits and there is less organ specificity as significant quantities exist in both the liver and cardiac tissue. It appears that only after chronic and/or severe liver disease are there elevations in rabbit ALT activity. There are studies that show that as the degree of hepatic necrosis increases, so does the plasma ALT concentration. Aspartate aminotransferase (AST) activity is found in many tissues in the rabbit including the liver, heart, skeletal muscle, kidney and pancreas. For these reasons, AST is not a specific indicator of liver disease in rabbits. Plasma increases in Lactate dehydrogenase (LDH) are seen with liver disease but cannot be used to assess liver health for a number of reasons. Lactate dehydrogenase is found in a wide variety of tissues so is very nonspecific. Also, since there is a high amount of LDH activity in RBC's, even a small amount of hemolysis can lead to an increase in plasma LDH concentrations. Plasma glutamate dehydrogenase (GDH) activity is likely a useful indicator of liver health in rabbits but GDH is not measured by all veterinary laboratories. Normal reference intervals for GDH are likely between 5-7 iu/L. Serum alkaline phosphotase is a useful indicator of liver health but is not overly sensitive. Concentrations rise only after chronic or severe liver disease. Total bilirubin activity in rabbits is normally very low and severe liver disease must be present before elevations are seen. Rabbits produce little or no amylase resulting in very low plasma amylase activities. Hepatic lipidosis is common in pet rabbits. Yet, biochemistry changes do not always reflect the degree of hepatic lipidosis. Ultrasonography is a more sensitive and specific test for hepatic lipidosis than the biochemistry panel. Common rabbit conditions do not always cause changes in the biochemistry profile. Gastrointestinal ileus is a common presentation yet few changes are seen on the biochemistry profile in these rabbits. Rabbits produce little or no amylase resulting in very low plasma amylase activities.

Two infections in rabbits that may or may not be endemic in the rabbit population are *Pasteurella multocida* and *Encephiltozoon cunuculi* infections. *P. multocida* can cause a severe bacterial

infection in rabbits. In other animals, this organism can cause minor disease and is easily treated. It is thought to cause significant morbidity and mortality in pet rabbits. Many infectious disease conditions are commonly attributed to *P. multocida* but it is really unknown if this bacteria is responsible for as much disease as it is blamed for. It can be difficult to diagnose definitively as bacterial cultures are not always performed in rabbits, cultures may show no growth, or the infection may be in a place inaccessible to culture. There are *P. multocida* antibody assays that can be performed on rabbit blood. These tests are probably not very useful in the pet rabbit. Even a rising titer (the best way to interpret antibody tests) does not prove that *P. multocida* is responsible for the signs of disease. Probably the best use of this test is as a herd health indicator when specific-pathogen-free rabbits are kept in a laboratory situation. In pet rabbits, it is difficult to justify using this test.

The same could be said of the *E. cuniculi* test. *E. cuniculi* is a protozoal parasite of rabbits. It is shed in the urine. It may or may not cause clinical disease in rabbits. Some have attributed neurologic disease to *E. cuniculi*. Others believe it is a cause of renal disease in rabbits. No definitive studies have been performed in pet rabbits to prove or disprove these theories. It is difficult to find this organism in the live rabbit. Even in post mortem examination, this protozoan is usually not found. An assay exists to measure the antibody titer to *E. cuniculi*. The significance of a positive or negative titer in a pet rabbit is unknown. Again, in a rabbitry setting, rising titers might mean contamination has occurred. In pet rabbits, there may be little reason to perform this test as the results are difficult to interpret in terms of clinical significance.