PROCEEDINGS OF THE

NORTH AMERICAN VETERINARY CONFERENCE

VOLUME 20

JANUARY 7-11, 2006

ORLANDO, FLORIDA

SMALL ANIMAL EDITION

UNDERSTANDING CLINICAL PATHOLOGY IN RABBITS

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Over the past several years, the popularity of the domestic rabbit (*Oryctolagus cuniculus*) as a pet has risen exponentially. As a result, the numbers of these animals presented to the veterinary practitioner has grown as well. Unfortunately, this rise in popularity has not been accompanied by an increase in the clinical pathology database as it applies to the companion animal. Most of the reference values are still based upon rabbits maintained within laboratory settings. To this date, many texts still comment on the lack of current reference values for the rabbit.

In order for any laboratory data to be valid, the samples must be collected in a fashion that is reproducible and avoids artifactural changes. Blood collection from the rabbit depends upon the clinician's ability to adequately restrain the patient with minimal stress placed upon it. In most instances, physical restraint is adequate; however, there are occasions that may mandate judicious use of chemical restraint, typically inhalant anesthesia.

BLOOD COLLECTION FROM THE RABBIT

The blood volume of the rabbit is estimated to be 55 to 65 ml/kg. In most cases, one may safely collect 6 to 10% of the blood volume, or 3.3 to 6.5 ml/kg. Such volumes will permit a variety of clinical laboratory procedures. The author prefers to collect blood for complete blood counts (CBC) in EDTA tubes and samples for biochemical evaluation in lithium heparin tubes. Rabbit blood clots relatively quickly at room temperature; therefore, the clinician must add the whole blood sample to the EDTA tube quickly to avoid artifactural changes associated with clot formation. The sample size utilized may be smaller than the EDTA tube was designed to accommodate, thereby causing blood dilution by the liquid EDTA. This dilution may be prevented by using tubes that match the sample size, or at a minimum shaking out the liquid EDTA from the larger tube prior to introduction of the specimen. When serum, rather than plasma, is required, a firm clot is typically formed in 30 to 45 minutes.

A variety of sites have been advocated for the collection of blood from the rabbit. In the pet rabbit, the preferred sites are the cephalic vein, the lateral saphenous vein and the jugular vein. Cardiac puncture, while described for exsanguination of laboratory rabbits, is probably inappropriate for the pet rabbit. Similarly, numerous techniques for use of the marginal ear veins and central ear artery have been advocated. These techniques typically involve cannulation of the vessel with an appropriately sized needle, often after vasodilatation has been stimulated by a topical irritant, such as xylene or d-limonene. Unfortunately, these techniques may be associated with thrombosis of the vessel and subsequent avascular necrosis and sloughing of the skin or even entire pinna. This sequela is most common in breeds with small ears, such as the dwarfs.

Use of the **cephalic vein** is similar to that for most mammals. In many rabbits, however, the antebrachium is short making occlusion of the vein difficult. The rabbit may be restrained in sternal recumbency and the vessel occluded by encircling the limb at the elbow. The vein is easily visualized by moistening the fur with alcohol. Only small samples may be collected from this site, and it is often preserved for use for placement of an indwelling venous catheter.

One of the mostly easily accessed sites is the **lateral saphenous vein**. With the rabbit held in lateral recumbency, the leg is extended and the vessel occluded by encircling the leg just proximal to the stifle joint. The saphenous vein is then easily palpated coursing in a diagonal direction across the mid-tibia. Visualization may be improved by gently plucking, not shaving, fur from the area. Blood should be collected relatively slowly with a 1 cc syringe to avoid collapsing the vein with excessive suction. Following withdrawal of the needle, pressure should be maintained to prevent hematoma formation.

Larger blood samples are best collected from one of the paired **jugular veins**. The stress associated with jugular phlebotomy typically mandates use of some form of chemical restraint. The positioning in the awake rabbit may result in an acute respiratory arrest, especially in stressed or dyspneic rabbits. When use of chemical restraint is not possible, collection from the jugular is performed in a manner similar to that of the cat with the rabbit restrained at the edge of the table, head extended upward and front legs held down. In does with a large dewlap, the thumb should be used to force it ventrally to visualize the jugular vein.

In most instances, however, the rabbit will be chemically sedated. With the rabbit in dorsal recumbency at the edge of the table, the front legs are pulled caudally and the head tipped slightly towards the floor. This will expose the ventral cervical region and the readily apparent jugular veins. Shaving or plucking fur will facilitate visualization. Typically, the vessel is relatively superficial, but it may lie underneath a substantial amount of fat in obese rabbits.

ARTIFACTUAL CHANGES

While tremendous advances have been made in pet rabbit medicine, much of the currently available reference data is based upon controlled laboratory settings. As such, interpretation of data must be made with this in mind. One must always take those steps necessary to control artifactural changes that are within the control of the clinician.

While not truly artifacts, one must evaluate much of the laboratory data bearing in mind numerous intrinsic factors that will affect various parameters. In the rabbit, age, sex, breed, and circadian rhythms all affected the hemogram. As expected, young rabbits had significantly lower RBC and WBC parameters than adults.

Interestingly, time of day has dramatic effects on the leukocyte population. Total and lymphocyte counts were lowest in late afternoon and evening; neutrophils highest at the same time, and eosinophils were highest in the afternoon, lowest in the morning.

In the rabbit, the stress of immobilization and transportation may affect glucose levels. The catecholamine release associated with stress may result in dramatic elevations in the leukocyte count, as well as a neutrophilia and lymphopenia.

Several muscle enzymes, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and creatinine kinase (CK), elevate significantly following physical restraint of the rabbit. Other enzymes not associated with skeletal muscle, such as alanine aminotransferase (ALT) and alkaline phosphatase (AP), tend not to rise under similar circumstances. It has been suggested that the influence of restraint may be decreased in rabbits that are handled frequently by their owners.

While not specifically described for the rabbit, it is anticipated that hemolysis of the blood specimen will create substantial artifactural changes. One would expect a decrease in the RBC and amylase values and an increase in AST, ALT, LDH, CK, TP, and K. It is therefore critically important to minimize hemolysis by the proper selection of needle/syringe, minimize force when transferring blood into tubes, and immediate separation of blood cells from serum or plasma.

COMPLETE BLOOD COUNT

While many of the concepts typically utilized in companion animal hematology are the same for rabbits, there are some idiosyncrasies of rabbit hematology that warrant discussion. First, rabbits are subject to a number of potential artifacts in their CBC, as previously described. The rabbit red blood cell typically demonstrates significant variability in size (anisocytosis), and therefore an elevation in the red cell distribution width (RDW) is not necessarily indicative of reticulocytosis.

The rabbit neutrophil may exhibit a variety of appearances. While the cytoplasm is typically clear, variable coloration of intracytoplasmic granules is expected. For that reason a number of different names, including heterophils, pseudoeosinophils, and acidophils, for this cell type have appeared in the literature.

Substantial evidence exists to support the fact that the rabbit lymphocyte responds differently to the effects of catecholamine, cortisol, and infection. Catecholamines typically result in movement of lymphocytes from the spleen and bone marrow into the peripheral blood. Cortisol has the opposite effect, thereby decreasing circulating lymphocyte counts. In the face of infection, a lymphopenia typically occurs and is associated with a neutrophilia. Total white cell counts rarely change in the face of disease; however, there may be substantial changes in the differential counts. In most cases, a decreased blood cellularity (anemia and lymphopenia) is a nonspecific change associated with disease in the rabbit.

SERUM CHEMISTRY.

As previously indicated, the serum chemistry panel is subject to a variety of artifacturally induced changes. For that reason, it is important that the clinician collect samples in such a fashion as to assure minimal artifact and therefore provide reproducible results.

Alkaline phosphatase (AP). AP is a cell membrane-associated enzyme with three isoenzymes in the rabbit. Significantly higher levels are associated with osteoblasts, renal tubular epithelium, intestinal epithelium, liver, and placenta. As a result of this wide distribution, elevations of AP are typically non-specific, but are often associated with bony lesions, young growing animals, enteric disease, and biliary obstruction.

Alanine aminotransferase (ALT). In most companion animals, ALT elevations are typically associated with hepatocellular damage. In the rabbit, ALT is much less specific, as the liver contains less ALT activity, and the enzyme is also present in cardiac muscle. In addition, the serum half-life of ALT is substantially less in the rabbit (5 hr) when compared with the dog (45–60 hr). Elevations are often seen in cases of hepatocellular inflammation, hepatic lipidosis, *Eimeria* infection, and some hepatic neoplasms. It has been observed that slight elevations of ALT in asymptomatic rabbits may be associated with exposure to aromatics, such as those found in wood shaving beddings.

Aspartate aminotransferase (AST). AST is found in a number of tissues in the rabbit, including liver, skeletal muscle, kidney, and pancreas, the first two being the highest. Elevations of AST may be found in cases of liver inflammation, skeletal muscle damage, or associated with physical exertion.

γ-glutaryltransferase (GGT). GGT is found in both the liver and the kidney. While the greatest concentration is found in the renal tubular epithelium, circulating levels do not rise in association with tubular disease, as the enzyme is apparently eliminated in the urine. Therefore, elevations identified in the blood are from hepatic origin, specifically bile duct epithelium. Hepatobiliary disease rather than hepato-cellular damage therefore is associated with elevations of GGT. It must be noted, however, that this change is not terribly sensitive.

Bilirubin. The rabbit has low biliverdin reductase activity, and therefore the predominant bile pigment excreted by the rabbit is biliverdin. Some bilirubin is, however, produced (approximately 30% of the total), and elevations indicate cholestasis.

Creatinine kinase (CK). As in other mammals, CK is an enzyme associated with muscle, cardiac, skeletal, and smooth, and brain. In general, elevations are noted in conjunction with myocyte damage or inflammation.

Lactate dehydrogenase (LDH). LDH activity is widely distributed through a large variety of tissues in the rabbit, and is therefore of limited diagnostic use in this species.

Total protein (TP). TP levels may vary depending upon the rabbit's age, reproductive state, and breed. For example, Polish breeds had higher TP than New Zealand Whites or Dutch breeds. Elevations of TP generally indicate dehydration, chronic disease, or

hyperthermia. Decreased values suggest loss, either via the urinary or digestive system, nutritional disease (eg, malnutrition or starvation), or decreased liver production.

Cholesterol. While changes in circulating cholesterol may suggest a variety of metabolic aberrations in many species, such is not necessarily the case in the rabbit. A number of normal physiologic variables may influence circulating cholesterol. First, males tend to have a lower level than females. Second, there is a definite circadian fluctuation with higher levels in late afternoon and evening. Finally, there is a significant postprandial effect upon measured cholesterol. Unfortunately, cecotrophy makes collection of a "fasting" sample problematic. Hypercholesterolemia may be associated with arteriosclerosis, liver disease, hypothyroidism, and hypercortisolemia.

Glucose. Interpretation of this serum chemistry parameter requires an understanding of the normal physiology of the herbivore, particularly as it differs from that of the carnivore. As a result, hypoglycemia is rarely seen in the rabbit. When seen, it is typically associated with a poor prognosis in conditions such as hepatic lipidosis, sepsis, starvation, and severe gastrointestinal disease. Hyperglycemia, on the other hand, may be associated with a variety of conditions. Elevations may occur secondary to the effects of restraint and handling. The diagnosis of diabetes mellitus in the rabbit has been controversial. For the purposes of this discussion, suffice to say diagnosis cannot be based upon a single sample. Other causes of hyperglycemia include hepatic disease, GI stasis, shock, and hyperthermia.

Blood Urea Nitrogen (BUN). In traditional pet species, BUN elevations are associated with renal dysfunction, either via renal disease or decreased perfusion. In the rabbit, urea concentrations may vary depending upon a variety of physiologic factors. Circadian fluctuations are present with highest levels found in late afternoon and early evening. Additionally, the quantity and quality of protein in the diet may influence the BUN. In addition, there is an influence on BUN exerted by cecal microflora, either catabolism or protein excesses. Therefore, slight changes in BUN are difficult to interpret. In general, however, elevations may be seen with dehydration, renal compromise, *E. cuniculi* infections, and urolithiasis. Interpretations must be made with caution and in conjunction with other clinical parameters.

Creatinine. Creatinine, the product of muscle metabolism, is freely filtered through the glomerulus without subsequent tubular resorption. As a result, elevations may be the result of dehydration or renal disease.

Calcium. The rabbit's calcium metabolism is unique in domestic animal species in that most of the dietary calcium is absorbed from the intestine independent of vitamin D. Therefore, serum levels are directly related to dietary levels. The kidney plays a more significant role in the elimination of calcium than in other species. Hypercalcemia may be associated with high levels of dietary calcium, renal disease and subsequent compromise in the ability to excrete calcium, and is often seen associated with thymoma. Hypocalcemia is uncommon, but may be seen in lactating does.

Phosphorus. Interpretation of abnormalities of the circulating phosphorous levels is difficult, at best, and should be made in conjunction with other clinical and laboratory parameters. In general, there is little information regarding the interpretation of hyper/hypophosphatemia in the rabbit.

As one can readily appreciate, hematology and serum chemistry parameters are valuable adjuncts to the diagnostic process in the rabbit. One must remember, however, that much of the reference data is acquired from a laboratory setting, with rabbits of limited genetic range, limited physical activity, and on a controlled diet. In addition, a paradigm shift is required for the typical small animal clinician, as one is dealing with the herbivorous rabbit, not the carnivore generally presented in the small animal practice.

Suggested Reading

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