Virginia Cooperative Extension Service

VIRGINIA VETERINARY NOTES

VIRGINIA-MARYLAND REGIONAL COLLEGE OF VETERINARY MEDICINE

May-June 1991 No. 51

WHAT'S INSIDE!

Kent C. Roberts, DVM
Extension Veterinarian

Virginia Tech and Virginia State • Virginia's Land-grant Universities

THE EXPANDED HEMATOCRIT

There is a wealth of information available to the trained eye and mind in the centrifuged microhematocrit tube. So starting from the top down...

Icterus Index: Comparison of plasma color to potassium dichromate standards allows an objective comparison (a number assignation).

This is an inexpensive method of ascertaining progression or regression in diseases producing icterus. A numerical comparison of plasma color is also useful when talking to a colleague over the telephone. Less than 7.5 is normal for dogs and cats. Less than 20 is normal for horses and cattle. (Jain NC: Schalm's Veterinary Hematology, Fourth Edition, Lea and Febiger, Philadelphia, 1986.)

Total Protein: By breaking the microhematocrit tube in the plasma layer and placing several drops of plasma on the glass plate of a refractometer (total solids meter) total protein may be determined.

Fibrinogen: The difference between total protein in the plasma column before and after heat precipitation (56.C, 3-5 minutes+) and centrifugation is a clinically satisfactory method to quantitate fibrinogen increases. No, you cannot subtract total protein of serum from that of plasma as other proteins come down in the clot and fibrinogen is always overestimated. (Sorry folks!)

Heartworms: Heartworms may be found most easily by examining the area of the interface of plasma and buffy coat under 100X (low power) magnification. These parasites may be visualized elsewhere in the buffy coat and the red cell pack.

Trypanosomes: Trypanosomes concentrate at the interface of the buffy coat and the plasma layer. Direct examination for these parasites may be accomplished (see Heartworms above) or the microhematocrit tube may be broken just above the buffy coat and the buffy coat/plasma interface spilled onto a glass slide, smeared and stained for microscopic examination.

Platelet Quantitation: At the interface of the buffy coat and the plasma is a cream-colored layer (you may need a magnifying glass), much different from the grey buffy coat. These are platelets. Presence of this layer almost invariably suggests adequate platelet numbers. This layer is especially visible in feline blood.

White Blood Cell Quantitation: The first single percent (1% of the microhematocrit tube) of the buffy coat represents 10,000 white blood cells/µl. The second single percent represents 20,000 white blood cells/µl. For example a buffy coat that is 3% of the microhematocrit tube represents a 50,000 white blood cell count/µl, 10,000 cells from the first percent and 20,000 cells for each of the remaining two percents. There is good correlation between buffy coat counts and automated white blood cell counts. A buffy coat less than 0.5% generally represents leucopenia while a buffy coat of greater than 1.5% represents leucocytosis.

Buffy Coat Smears: Breaking the microhematocrit tube just above the buffy coat and smearing and staining the buffy coat is routine whenever Ehrlichia inclusions in white blood cells are sought. A buffy coat smear is also used to examine large numbers of white blood cells when there is clinical concern about an occasional unclassifiable cell form noted on the blood smear or when a myeloproliferative process is suspected. The uses of buffy coat smears are virtually endless.

Reticulocytes and Nucleated Red Blood Cells (NRBCs): Any pinkish tinge to the lower portion of the buffy coat suggests the presence of red blood cells of low specific gravity. Included among red cells with low specific gravity are leptocytes, reticulocytes and nrbcs. Noting a pinkish tinge in the buffy coat suggests examination of the peripheral blood smear for the presence of the cells listed above and their quantitation.

Red Blood Cell Age: Individual red blood cell position in the red blood cell pack is determined by intracellular specific gravity. Therefore the youngest red blood cells are found in or just below the buffy coat and the oldest red blood cells are at the bottom of the red blood cell pack. In general, the oldest red blood cells are more vulnerable to red blood cell parasitism than younger cells. Examination of concentrated older red blood cells, cells obtained and smeared by breaking the microhematocrit tube near the bottom, allows examination for Hemobartonella, Eperythrozoon and, Cytauxzoon.

Examination of Erythrocyte Glycolytic Pathway Intermediates: The age difference between erythrocytes is a consideration when attempting to ascertain erythrocyte glycolytic pathway deficiencies. Pyruvate kinase deficiency, a nonspherocytic hereditary hemolytic anemia, has been described in Basenjis, Beagles and West Highland White Terriers. In order to correctly quantitate erythrocyte pyruvate kinase in a suspect patient, blood from the middle of the red blood cell pack to the bottom must be used. Pyruvate kinase will be deficient in those cells which have lost their nuclei weeks to months previously. Younger cells, recently having lost their nuclei, often will have "normal" to increased concentrations of pyruvate kinase, masking the deficiency. Many pyruvate kinase deficient patients have enormously high reticulocyte counts (some exceed 50%). Unless these cells are removed from examination, diagnosis will be extremely difficult. **...and, the microhematocrit may be determined. --Bernard Feldman, DVM, PhD, Clinical Pathology, VA-MD Regional College of Veterinary Medicine, Blacksburg, VA.**

COLOSTRUM ALTERNATIVE IN PUPPIES

Investigators at the University of Missouri (Bouchard, et al, 1990 Annual Conference for the Society for Theriogenology, Toronto, Ontario) evaluated various methods to provide neonatal puppies with immunoglobulins in the event of colostrum deprivation. Puppies administered pooled canine serum orally and subcutaneously were compared to two control groups of puppies. The two control groups were puppies that were allowed to suckle their dam and puppies that were fed only milk replacer. These two control groups of puppies were compared to groups of puppies that were given canine pooled serum orally at birth and at 12 hours after birth and parenterally by subcutaneous administration. The immunoglobulin concentrations of IgG, IgM, and IgA were measured at birth and 24 hours after birth. The control puppies that were allowed to suckle their dam had significantly higher levels of IgG and IgA than any other group. It was shown that intestinal absorption of immunoglobulins is minimal after 12 hours postpartum.

The most effective means of providing immunoglobulins to colostrum-deprived neonatal puppies was found to be the injection of 16 mls of canine serum subcutaneously. The puppies tolerated this procedure with minimal pain if the serum was administered slowly. The levels of immunoglobulins obtained with subcutaneous administration of serum were considerably lower than those obtained from puppies that suckled their dam, but were higher than those levels obtained from oral administration of canine serum given at birth or at 12 hours post-whelping. This procedure may be helpful in managing neonatal puppies that are deprived of colostrum. **--Beverly J. Purswell, DVM, PhD, Diplomate A.C.T., Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA.**

SUDDEN ACQUIRED RETINAL DEGENERATION

Sudden Acquired Retinal Degeneration (SARD) is a syndrome characterized by the sudden onset of blindness in adult dogs who appear to be otherwise in good health. Vision loss occurs over a period of less than three to four weeks, but the owner may perceive vision loss to occur over as little as 24 hours. The pupils are moderately to widely dilated with a sluggish or absent pupillary response to light. SARD-affected dogs will have a normal ophthalmoscopic examination and an extinguished electroretinogram (ERG). No cause, prevention, or treatment for SARD is currently known.

The typical SARD-affected dog is middle-aged, in good health, often moderately obese, and is presented because of recent and sudden onset of blindness. SARD is not a breed-related disease. Often these dogs have a history of polyuria, polydipsia and polyphagia from approximately the same time that blindness became apparent. These systemic signs along with characteristic laboratory abnormalities (elevated serum alkaline phosphatase, serum aspartate aminotransferase, serum alanine aminotransferase, serum cholesterol and/or serum bilirubin) are suggestive of hyperadrenalcorticism with subclinical secondary steroid-induced hepatopathy. (1,2,%) Some dogs studied had elevated basal serum cortisol levels and/or abnormal responses to ACTH stimulation and dexamethasone suppression tests which were sufficiently abnormal to prove hyperadrenalcorticism. It has been suggested that the hyperadrenalcorticism in these dogs is physiological, that is, the body is responding to some stress yet to be identified. (1,2,)

SARD is an acute, irreversible retinal degeneration characterized histologically by an initial, rapid loss of the photoreceptors which affects rods and cones equally and all areas of the retina simultaneously. This is in contrast to most hereditary retinal degenerations where rods and cones may be affected differentially. A recent paper suggests that a neurotoxic action would best explain the pathology of SARD. (6) The initial rapid phase of degeneration occurs over less than three to four weeks and is followed by a slow degeneration of the remaining retinal layers.

It is very important to obtain an ERG on dogs who present with sudden vision loss in order to distinguish SARD from optic neuritis. Optic neuritis can be treated with high levels of systemic corticosteriods and should be regarded as an emergency, since vision may be saved. Dogs with retrobulbar optic neuritis may present with a normal ophthalmoscopic examination, but the ERG should be normal.

If there has been a delay of several months between the onset of blindness and the presentation of the animal for examination, some degree of retinal degeneration may be apparent in SARD. At this point, it may be difficult to distinguish SARD from the different forms of Progressive Retinal Atrophy (PRA). In both instances, the ERG may be extinguished and thus non-diagnostic. Careful questioning of the owner and characterization of the onset of vision loss may provide important clues in differentiating SARD and PRA. Other differential diagnoses for sudden onset of blindness in the dog include: retinal detachment (due to inflammation, hypertension, vitreal dysplasia, etc.), intraocular hemorrhage, neoplasia, trauma, and uveitis. **--Jill L. Wagner, Denise M. Lindley, DVM, MS, Diplomate ACVO, Purdue University Notes, No. 170, January 1991, Purdue University.**

THOUGHT FOR THE MONTH

As long as there are exams, there will always be prayer in schools.

DEVELOPMENT OF IMMUNITY IN THE NEONATAL DOG AND CAT

Of the stages in life where the possession of effective protective systems is required, the period following birth is most critical. The newborn animal must leave the protection of a sterile uterus and enter an environment populated with a vast array of microorganisms, may of which are potential pathogens. Thus, it is essential that all the body's protective mechanisms be brought effectively and rapidly into play.

It is generally considered that, on average, the level of passively acquired antibodies to most canine viral pathogens will drop to non-interfering levels by 10 to 12 weeks. However, for other pathogens such as canine parvovirus, there is evidence that maternal immunoglobulins may interfere with vaccination for as long as 16 weeks and vaccination prior to that time may still be ineffective.

The effectiveness of a vaccine in the presence of maternal antibodies is directly proportional to the level of this antibody. Very low maternal antibody levels may interfere with poorly antigenic vaccines (low antigen mass or excessively attenuated) to a much greater extent than a highly antigenic vaccine. This may be demonstrated by the cases of canine parvovirus (CPV) and feline panleukopenia (FPL).

In the case of CPV vaccine, any detectable maternal antibody will effectively interfere with successful immunization. Indeed, it may do so even after it has dropped to undetectable levels. For example, the response of puppies to CPV vaccine may be blocked until 2-4 weeks after maternal antibody is undetectable by a hemagglutination inhibition test. Similar considerations apply to FPL vaccines, i.e. the response to vaccination is a more sensitive indicator of the presence of maternal antibody than are in vitro serologic tests. Thus, in kittens exposed to FPL, antibodies with a virus neutralization titer of 1:32 are needed to prevent infection, but antibodies with a titer of 1:8 or greater suppresses the response to vaccination. The window of susceptibility between the loss of protective immunity and the ability to be successfully vaccinated is, therefore, about 2-5 weeks for FPL.

Thus, a simpler procedure is to vaccinate all puppies over six weeks of age when presented, and ask the owner to return for revaccination at 12 to 14 weeks. There are many similar alternative procedures, all aimed at conferring early protection while leaving as few puppies as possible unprotected for as short a time as possible. **--Ian Tizard, Texas A&M, College Station, TX, Proceedings, Soc for Theriogenology, 1988 as reported in DVM News, Vol. 3, No.1, South Dakota State University, Brookings, SD.**

SCROTAL CESTODIASIS IN A DOG

Scrotal cestodiasis was diagnosed form a surgical biopsy specimen from an 8-year-old Miniature Poodle. Peritoneal cestodiasis with secondary scrotal cestodiasis was suspected and could be explained by migration of the parasite along the vaginal tunics. Subsequent necropsy confirmed severe peritoneal cestodiasis due to Mesocestoides sp. It appears that scrotal cestodiasis may be an early indicator of peritoneal cestodiasis in male dogs and diagnostic pathologists and clinicians should be aware of this condition. **--Cornell Veterinarian 78:273-279, 1988.**

TOPICAL CORTICOSTEROID THERAPY

Topical corticosteroids should be considered more frequently in the treatment of inflammatory dermatoses in veterinary medicine. Topical steroid therapy, as with systemic steroid therapy, may cause adverse effects and should not be considered an innocuous form of therapy.

The activity of steroids is thought to result from binding with a steroid receptor. Studies have demonstrated two distinct functions of the receptor (glucocorticoid and DNA binding) and each resides in discrete domains of the protein. Steroid binding to the glucocorticoid receptor is believed to trigger a conformational transformation which "unmasks" the DNS-binding domain of the receptor. An ensuing cascade of events leads to translocation of the glucocorticoid-receptor complex from the cytoplasm to the nucleus and activation of gene transcription. The production of mRNA results in the synthesis of specific proteins that are believed to mediate the steroid-induced effects. Glucocorticoids have been shown in-vitro systems to inhibit the release of arachidonic acid metabolites, mainly prostaglandins, leukotrienes and platelet activating factors, by the production of a phospholipase A_2 inhibitor protein, lipocortin. Phospholipase A_2 is a key enzyme for the release of arachidonic acid, the precursor of various inflammatory mediators, from membrane phospholipids.

Much information exists in the literature concerning topical steroid therapy in man. Although systemic corticosteriods are more effective in the treatment of inflammatory dermatoses, topical therapy is preferred due to the decreased development of adverse systemic side effects. Although topic glucocoritcoids are considered safer than most forms of systemic steroid therapy, it has been well-documented that cutaneous absorption of topically applied steroids can result in suppression of the hypothalamic-pituitary-adrenal axis (HPA axis) in some patients. HPA axis suppression has been demonstrated after intranasal, ocular and cutaneous steroid administration.

Compared to man, very few reports exist in the veterinary literature regarding topical steroid therapy. HPA axis suppression has been reported following the use of ocular, otic and topical steroid preparations in the dog. HPA axis suppression has been produced after a 7 day application of topical otic products (Panalog®, Tresaderm®). HPA suppression was still present 14 days after discontinuation of therapy. Daily application of triamcinolone acetonide (Panalog®), fluocinonide (Lidex®) and betamethasone (Topagen®) to the skin of normal dogs for 5 days produced HPA suppression which was evident for 3 to 4 weeks after discontinuation of therapy. HPA axis suppression has also been reported following the daily use of ophthalmic preparations containing 0.1% dexamethasone and 1% prednisolone acetate.

Local adverse effects have also been reported following the use of topical steroid preparations in humans and animals. In humans, they include atrophy, steroid acne, telangiectasias, striae, contact allergies, hypertrichosis and hypopigmentation. In animals, they include atrophy, alopecia, pigmentary disturbances, contact dermatitis and dermatitis incognito.

Topical steroid therapy, primarily creams and ointments, has been used less frequently in animals as compared with man due to the drawbacks associated with haircoat, owner compliance and patient cooperation. Of the steroid products currently available for use in humans, a shampoo preparation has the most application for use in animals. Bathing is a common and beneficial method of therapy. The use of a steroid shampoo preparation could potentially minimize the disadvantages associated with other steroid formulations (i.e., creams, ointments).

A steroid shampoo would be an ideal topical therapy for allergic and seborrheic conditions, especially when generalized in nature. Topical or systemic adverse effects associated with the use of a steroid shampoo, a 0.01% fluocinolone acetonide preparation (F/S shampoo®, Hill's Dermaceutical's Inc.) was evaluated in dogs in a study at North Carolina State University, School of Veterinary Medicine. The findings from this study showed no evidence of HPA axis suppression, systemic or local side effects following the daily application of this shampoo on normal dogs for 5 days. **--Animal Health Beat, abstracted from Trettien, A.L., The Veterinary Allergist, The Acad, of Vet. Allergy, Summer 1989 as reported in Iowa State University Extension Newsletter, September, 1989.**

VIRGINIA-MARYLAND REGIONAL COLLEGE OF VETERINARY MEDICINE CONTINUING EDUCATION OPPORTUNITIES FALL 1991

Date	Program	Location	Contact Hours
August 29	Lyme Disease Symposium	Charlottesville	6
September 26	Small Animal Medicine Update	Charlottesville	4
*October 4-5	Practical Eye Surgery	Blacksburg	10
*October 11-12	Orthopedic Surgery - Canine Hindlimb	Blacksburg	10
*November 1-2	Gastrointestinal Endoscopy	Blacksburg	10

*Limited enrollment course

Note: Program brochures are mailed six-eight weeks prior to the course date. For CE course information, please contact:

Kent Roberts, DVM
VA-MD Regional College of Veterinary Medicine
Blacksburg, VA
(703) 231-7181

NEWSLETTER FUNDING

This newsletter is printed and distributed with Virginia Cooperative Extension Service funds which will be unavailable on or before July 1, 1991, because of state budget reductions. The editor is searching for funding alternatives rather than charge a subscription fee for continued publication. Possible sources of newsletter funding are presently being explored and the results of this search will be known by early June.

Your interest in Virginia Veterinary Notes is appreciated.

Virginia-Maryland Regional College of Veterinary Medicine Extension Staff:

Dr. J.M. Bowen - Extension Specialist - Equine
Dr. C.T. Larsen - Extension Specialist - Avians
Dr. K.C. Roberts - Extension Specialist - Companion Animals
Dr. W. Dee Whittier - Extension Specialist - Cattle

K.C. Roberts, Editor
Maura M. Wood, Production Manager of VIRGINIA VETERINARY NOTES

VIRGINIA POLYTECHNIC INSTITUTE
AND STATE UNIVERSITY
VIRGINIA COOPERATIVE EXTENSION
BLACKSBURG, VIRGINIA 24061-0512

NON-PROFIT ORG
U. S. POSTAGE
PAID
BLACKSBURG, VA 24
PERMIT #28