Summer 1998

FROM THE ASSISTANT DIRECTOR

Since our last newsletter, the Equipment Committee of the ADDL has met and decisions have been made to purchase new equipment for our laboratory. Of these equipment purchases, perhaps one that everyone is most excited about is the Gas Chromatograph/Mass Spectrometer for the Toxicology Laboratory. Called a "GC-Mass Spec" for short, this equipment will allow us to more precisely evaluate samples for toxins with greater sensitivity than we have ever been able to do in the past. Prior to the purchase of this instrument, we have relied on our colleagues on other parts of the West Lafayette campus and at other diagnostic labs to assist us with these analyses by using their equipment. This equipment purchase and others were possible by using monies from fee incomes. We look forward to being able to serve you better once this new equipment is up and running.

Since some disease situations or conditions are seasonal, we are reprinting two articles in this newsletter which appeared in our newsletter last summer. Both of these articles deal with aquaculture species. The number of phone calls regarding questions about aquaculture species continues to increase, indicating the seasonality of some of these diseases.

As always, we enjoy hearing from you. If you have any comments, suggestions or ideas regarding the Animal Disease Diagnostic Laboratory, or this newsletter, please contact us.

Feline Hyperthyroidism

Feline hyperthyroidism is found mainly in elderly cats, 9-22 years old, of both sexes. Clinical signs usually develop gradually and include loss of weight despite hyperphagia, frequent bowel movements with abundant soft or liquid stool, polydypsia, polyuria, nervousness, and poor haircoat. Findings of physical examination may include tachycardia murmurs (200-300 dpm), gallop murmurs, atrial premature contractions, and occasionally, a mass in the area of thyroid gland may be palpated. Clinical diagnosis may be confirmed by serum T3 and/or T4 levels which are elevated.

A variety of proliferative thyroid lesions have been associated with pathology of feline hyperthyroidism. Most lesions are benign, including thyroid nodular hyperplasia and adenomas. Nodular hyperplasia can be manifested as adenomatous hyperplasia or multinodular goiter. These lesions are usually bilateral and only slightly enlarge the gland. Adenomas are usually unilateral and markedly enlarge and distort the glandular structure. Thyroid carcinoma (follicular or papillary) can occur and also cause hyperthyroidism.

Nevertheless, discovery of a thyroid mass does not necessarily indicate that the cat is hyperthyroid because many of these lesions are non-functional. The other important point is that cardiomyopathy is often a serious complication of feline hyperthyroidism. The heart is enlarged with left ventricular hypertrophy that may progress to dilatation of ventricles.

- by Tsang Long Lin, DVM, PhD

Fatal Human Herpes Simplex Virus Encephalitis in a Domestic Rabbit

After minor head trauma, a 2.5 year old male rabbit (*Oryctolagus cuniculus*) exhibited seizures followed by coma and natural death. Two days elapsed between the onset of seizures and death. One day prior to death, an ophthalmic examination revealed papillary edema and hemorrhage. At necropsy, a small amount of hemorrhage was observed around the optic chiasm. The brain was examined histologically and revealed extensive inflammation and neuronal necrosis with intranuclear inclusion bodies. Because domestic rabbits are used as an experimental model for (human) herpes simplex virus (HSV) encephalitis and because a naturally occurring case of HSV type I - encephalitis in a domestic rabbit was reported in Europe (Weissenbock *et al*. Vet Pathol 1997;34:44-47), HSV-encephalitis was suspected in this rabbit. DNA extracted from paraffin-embedded sections of brain from this rabbit was subjected to the polymerase chain reaction (PCR) using HSV type I/II - specific primers, and a 3+/3+ signal was detected. Among the various human herpes virus infections (e.g., varicella-zoster, Epstein-Barr), this PCR technique is specific for HSV. This case demonstrates the susceptibility of domestic rabbits to HSV and emphasizes that humans with active lesions of herpes labialis or herpetic stomatitis (i.e. "cold-sores" or "fever blisters") should not contact domestic rabbits.

- Evan B. Janovitz, DVM, PhD
- Cindy Fishman, DVM
- Sarah Zimmerman
- Steve Thompson

Porcine Hemorrhagic Syndrome

Porcine Hemorrhagic Syndrome is a coagulopathy of swine which is observed sporadically in Indiana and throughout the Midwest. In the US, it most commonly occurs approximately two weeks after a change in feed. In the majority of cases, vitamin K (as menadione) has been absent from the ration. However, even when vitamin K was present in the feed, outbreaks have been reported which resolved when additional vitamin K was added.

This syndrome is characterized by internal and/or external hemorrhage (from bite wounds, castrations, injections, etc.), prolonged bleeding times (prolonged PT and APT), anemia, lameness (bleeding into joints), and anorexia with rapid response to vitamin K therapy. Porcine Hemorrhagic Syndrome most often affects recently weaned pigs, but can affect swine of any age. Morbidity is often high and mortality can range from 4% to 88%.

The cause of Hemorrhagic Pig Syndrome is still unknown almost 30 years after it was first reported. This is probably due in large part to the sporadic incidence of the syndrome, because of the widespread supplementation of swine rations with vitamin K (as menadione). However, a feed-related toxin, perhaps of fungal origin, is thought to be a possible culprit, although dietary deficiencies, use of combinations of antibiotics,

and inadequate intestinal synthesis are also possibilities.

Diagnosis of this condition is based on the history and clinical signs, prolonged coagulation times, and rapid response to: vitamin K therapy and removal of the incriminated feed.

Within the last year, one case of vitamin K-responsive Hemorrhagic Syndrome in a herd of pigs was reported in Indiana. From the liver of one of these pigs, the Assistant Chemist of the ADDL Toxicology Laboratory was able to identify a possible causative agent. Fortunately for the swine herd, the case was very rapidly resolved by vitamin K supplementation and taking the pigs off of the new feed. Unfortunately for the purposes of identifying the causative agent, the feed was destroyed and vitamin K was administered before clinical evaluations could be made, or the feed and other samples could be further analyzed to confirm the presence of the suspect agent. Therefore, if any cases of suspected Porcine Hemorrhagic Syndrome are seen, we would greatly appreciate it if the ADDL Toxicology Laboratory could be notified as soon as possible for a thorough work up to attempt to identify the causative agent of this syndrome.

If there are any questions, or to report a suspected outbreak of Porcine Hemorrhagic Syndrome, please call the Animal Disease Diagnostic Laboratory - Toxicology Section at (765) 494-7440.

- by Jennifer Harms, B.S.
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Prevention and Control of BVD Virus Infection

Introduction

Bovine viral diarrhea (BVD) is caused by an RNA virus. Two distinct types of BVDV, Type 1 and Type 2, can be differentiated in the laboratory, based on their RNA makeup, the structure of their protein capsules and the antibodies that are made in response to their infection. Within each BVDV type, several different strains have also been identified. Furthermore, a specific virus may be either cytopathic or non-cytopathic, indicating its ability to cause visible damage to experimentally infected cells in the laboratory. BVD virus has a worldwide distribution, with serum antibody prevalence in cattle ranging from 50-90 percent. However, the incidence of acute clinical disease in the general cattle population is less than 5 percent. Disease problems associated with BVD virus include bovine viral diarrhea, immunosuppression, repeat breeding, abortion and mummification, congenital defects, and persistent infection.

Types of BVD Infection

Persistent Infection:

Persistent infection (PI) with BVDV can develop when a fetus is exposed to a non-cytopathic strain of the virus before day 125 of gestation. PI animals are immunotolerant to the homologous strain of the virus. However, they can still make antibodies to different (heterologous) strains of BVDV. Animals under 3 months of age should have whole blood (buffy coat cells) submitted for testing because maternal colostral antibodies can neutralize the virus in their serum and lead to a false negative test. Fetal infection in the first trimester of gestation can result in abortion or the development of a mummified fetus. Congenital defects can result when the fetus is infected with BVDV in late first, second and early third trimester of gestation. The stage of fetal development determines the type of defect that occurs. The most common defect is cerebellar hypoplasia. Infection late in the pregnancy usually results in the birth of clinically normal calves.

Acute Infection:

Bovine viral diarrhea refers to a mild disease caused by a non-cytopathic BVD virus infection in immunocompetent cattle. In general, animals develop acute BVD 10-12 days post-infection with viremia starting approximately 4 days after exposure. At the time animals develop clinical disease they are usually starting to make neutralizing antibodies. These neutralizing antibodies lead to a false negative serum test. Since BVDV is also leukocyte associated, whole blood (buffy coat) is the sample of choice for isolation of BVDV from clinically ill animals.

Mucosal Disease:

Mucosal disease is the classic disease syndrome that people often associate with BVDV. The prevailing hypothesis is that mucosal disease occurs when immunotolerent, PI cattle are subsequently infected with a homologous strain of cytopathoic BVDV. Superinfection with cytopathic BVDV may result in acute mucosal disease or may persist as chronic mucosal disease. The source of the superinfecting cytopathic virus is from another animal or from a

mutation of the persistently infecting noncytopathic virus. Animals with mucosal disease usually die in a few days of severe diarrhea and dehydration. Chronic form is usually manifested as intermittent diarrhea, oronasal and interdigital ulcerations.

Laboratory Diagnosis

Acute Infection:

Virus isolation must be done in the first 3-10 days post-infection. Some animals may be virus isolation positive for only 2-3 days post-infection. A whole blood sample is the best sample for BVDV isolation from acutely infected animals. In addition, swabs from mucosal and nasal surfaces can be collected and submitted for virus isolation. Paired acute and convalescent samples collected 30 days apart are required to identify four fold increase in serum antibody titers following convalescence. Many BVDV-associated abortions are virus isolation negative. The detection of antibodies in the fetus will confirm intrauterine infection. If the dam on the other hand, is antibody negative, BVDV can be ruled out as a cause of abortion. The diagnosis of BVDV-induced congenital defects in calves should include both virus isolation and serology to detect BVDV-specific antibody prior to uptake of colostrum.

Persistent Infection:

The identification of persistently infected animals is routinely made by virus isolation. In most cases serum is adequate for virus isolation. Due to colostral antibody, in young calves less than 3 months of age the best sample is whole blood in which the mononuclear cells are separated for virus isolation. Persistent infections should only be determined by identification of BVDV by virus isolation in sequential samples collected 30 days apart. By testing the animal 30 days apart it is possible at the same time to test for a four-fold increase in antibody titer should the first virus isolation have been due to acute infection.

Herd Screening:

In herds with BVD problems, blood should be collected from all breeding animals more than six months old, and samples should be submitted for virus isolation. Virus isolation using a microtiter immunoperoxidase detection is the most commonly used method for testing such large numbers of samples. In addition, any calves born for the next 9 months must be tested to ensure that no additional persistently infected animals are born that were in utero at the time of testing. All animals from which BVD virus is isolated should be culled from the herd. Animals that are kept for their genetic value should be re-tested in 30 days and should be culled if the second test result is still positive. Young stock should be kept isolated from the breeding herd until they are old enough to be tested or sold. Once vaccination programs are introduced, virus isolation becomes the only reliable method of identifying vaccinates that are persistently infected with BVD virus.

- by Zuhair Bani Ismail, DVM
- edited by Luvan Anothayanontha, DVM

Organophosphate and Carbamate Insecticide Poisoning

Organophosphate and carbamate insecticides are commonly used for small animals as flea and tick powders, sprays, foggers, shampoos and dips, flea collars, and formerly, as systemic insecticides. They are also frequently used as household, garden, and farm insecticides. Chlorpyrifos, parathion, diazinon, famphur, phorate, terbufos, and malathion are examples of organophosphates while carbofuran, aldicarb, and carbaryl, are carbamates. They are all marketed under a wide variety of trade names.

Both organophosphate and carbamates are highly toxic to all animals, including pets, livestock, and humans although some are far more toxic than others. All OP/Carbamate insecticides are fat soluble and therefore are easily absorbed through the skin and then transported throughout the body. These chemicals kill insects and cause poisoning in animals by inhibiting the enzyme, acetylcholinesterase (AChE) which normally functions to degrade acetylcholine in nerve synapses. Inhibition of AChE in the nerves results in a buildup of acetylcholine (ACh) and overstimulation of ACh receptors. Since all organophosphate and carbamate insecticides have the same mechanism of action and can be long-lasting, the effects of multiple exposures (for example: flea dip, flea powder, flea collar, and home and lawn flea treatment) are additive.

There are two types of ACh receptors, muscarinic and nicotinic. Overstimulation of muscarinic receptors gives rise to the characteristic SLUDD signs of OP/Carbamate poisoning: salivation, lacrimation, urination, defecation, and dyspnea (due to increased bronchial secretions and bronchoconstriction), plus bradycardia and miosis. Overstimulation of nicotinic ACh receptors produces muscular fasiculations and tremors initially followed by flaccid paralysis. Death in acute poisonings is

frequently due to respiratory failure resulting from inhibition of central (medullary) respiratory drive, excessive bronchial secretions, and bronchospasms coupled with depolarizing blockade at neuromuscular junctions (diaphragm and intercostals).

The diagnosis of OP/Carbamate poisoning is made based on:

(1) History of exposure to one or more OP/Carbamates.

(2) Signs:

(a) Muscarinic signs: SLUDD + bradycardia and miosis.

(b) Nicotinic signs: muscle stiffness, muscle fasiculations, tremors, weakness, flaccid paralysis.

(c) CNS signs: restlessness, hyperactivity, seizures.

(d) Animals often found dead.

NOTE: Not all animals read the book and can present with any combination of the signs listed above.

(3) Blood ACh activity: usually less than 25% of normal with OP/Carbamate exposure. Whole blood must be submitted with an anticoagulant such as EDTA or heparin.

(4) Clinical Pathology: Some organophosphates have been associated with an increase in CPK and AST.

(5) Necropsy: There are no definitive gross or histological lesions in acute poisonings. Brain cholinesterase is significantly decreased. Stomach contents and liver are frequently used to diagnose OP/Carbamate poisoning and identify which chemical was responsible for the poisoning.

If OP/Caramate poisoning is suspected, the samples which should be taken and submitted for diagnosis are:

1. Whole, unclotted blood, refrigerated as quickly as possible. Do not freeze.
2. Frozen brain (one half of the brain frozen as quickly as possible.).
3. Vomitus or stomach contents, frozen. This is often the best sample to positively identify which chemical is responsible for the poisoning.
4. Liver, 25g, frozen.
5. Eyeball for retinal cholinesterase activity. Remove and freeze as soon as possible. (Used primarily in large animal cases when no other samples are available.)
6. Skin is not routinely tested, but can sometimes be used if exposure was dermal and no other samples are available.

NOTE: A full necropsy should always be performed and a full set of tissues submitted for histology to rule out other causes of death, particularly if legal action is a possibility.

Organophosphate and carbamate poisoning is relatively common in pets and livestock. Animals can present with any combination of the muscarinic and/or nicotinic signs listed above or are frequently found dead. Diagnosis in live animals is based on a history of appropriate clinical signs, depressed blood acetylcholinesterase activity, and the identification of an OP/Carbamate in vomitus or stomach contents, if available. Diagnosis at necropsy is based on history, depressed brain or retinal acetylcholinesterase activity, and the identification of a specific OP/Carbamate in stomach contents and/or liver.

- by Jennifer Harms, B.S.
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Feline Infectious Peritonitis

Feline infectious peritonitis (FIP) is an infectious, life threatening disease of primarily young cats, with all ages of cats being susceptible. The death losses usually begin shortly after weaning and peak at 8-18 months of age. Progression of FIP may be facilitated by concurrent infection with feline leukemia virus or feline immunodeficiency virus.

The causative agent is a coronavirus which can be of two types; feline infectious peritonitis virus (FIPV) or feline enteric coronavirus (FECV). Depending on which virus is involved, the clinical signs may range from asymptomatic, to gastrointestinal disease or two widespread multi-organ disease. FIPV and FECV have been referred to as biotypes because they are morphologically and antigenically identical except for the disease potential.

Entry of FIPV can be initiated by respiratory, fecal-oral (predominantly), or intra- uterine route. The virus replicates in epithelial cells of oropharynx or intestine. Antibodies are elicited and virus-antibody complex is formed. Virus-antibody complexes can be engulfed by monocytes or macrophages and also circulate until deposited in the small blood venules on the serosal or pleural surface, meninges, ependyma,

and uveal tissue. This results in disseminated immune-mediated vasculitis and perivasculitis leading to vascular endothelial damage, leakage of fibrin and immunoglobulin, and pyogranulomatous lesions. If severe damage occurs, clotting abnormalities, thrombocytopenia, increased fibrin degradation products (FDP's), decreased clotting times, and development of DIC may be inevitable.

Feline infectious peritonitis can be catergorized into effusive (wet) and non-effusive (dry) forms. The effusive form may present with nonspecific signs (fever, anorexia, weight loss, etc.), but the most noticeable sign is the progressive abdominal distention or ascities. Pleural effusion can occur and leads to respiratory distress, decreased exercise tolerance, dyspnea, and muffled heart and lung sounds. Peritoneal or pleural fluid is secondary to vasculitis and leakage of fluid from the vasculature. The fluid is usually a "straw colored" pyogranulomatous inflammatory exudate with diffuse granular fibrinous exudation covering serosal surfaces or floating in the effusion. The non-effusive form lacks specific clinical signs, but may be associated with specific organ dysfunction resulting from pyogranulomatous inflammation. Clinical signs range from fever, weight loss, anorexia, respiratory distress to renal, hepatic, pancreatic, CNS, or ocular disease. Kidneys may be enlarged as "lumpy-bumpy" kidneys due to pyogranulomatous lesions in the cortex and the cat may have polyuria, polydypsia, lethargy, vomiting, proteinuria, and azotemia. The liver may have focal necrotizing pyogranulomatous inflammation involving hepatic capsule and adjacent hepatic parenchyma. Affected animals may have increased ALT, GGT, alkaline phosphatase, or hyperbilirubinemia. The brain and spinal cord may have meningoencephalitis and myelitis, with vasculitis. Central vestibular signs, seizures, ataxia, depression, nystagmus, or personality changes may be evident. The eyes may have bilateral anterior uveitis, keratitis, iritis, aqueous flare, corneal edema, fundic pyogranulomas, and engorgement of retinal vessels with perivascular cuffing. Lungs may have granulomatous pleuritis and pneumonia. Microscopic lesions consist of multiple foci of necrosis and pyogranulomatous inflammation, usually extending from and incorporating the wall of a blood vessel. The lesions appear to be a primary vasculitis and the evidence suggests that it is mediated through circulating immune complexes.

Clinical pathology laboratory findings include normocytic, normochromic anemia, leukocytosis with neutrophilia and lymphopenia. Changes in clinical chemistry panel depend on which organ(s) are involved. Peritoneal or pleural fluid can be clear, slightly opaque to pale yellow, or golden with fibrin strands and flakes. Cellularity usually ranges between 1600 to 25,000 cells per microliter. Cells are mostly a mixture of nondegenerate neutrophils, macrophages and lymphocytes.

Clinical diagnosis is made by history, physical examination, laboratory findings, and coronavirus antibody titers. Tissue biopsy with pyogranulomatous inflammation is still the preferred diagnostic procedure that will definitely confirm FIP. Serum tests include virus isolation, IFA, ELISA, and agar gel immunodiffusion. Care needs to be taken when evaluating antibody titer results. Coronavirus titers have been found in serum of apparently healthy cats, in cats with FIP, and in cats with a disease other than FIP. The antibody titers do not identify the strain of coronavirus responsible for seroconversion, so the presence of a titer only indicates that the cat has been infected with a coronavirus such as FIP, FECV, canine coronavirus, TGE, or bovine or human coronavirus. Most healthy cats with a positive titer probably have been infected, but it is impossible to predict accurately the long-term prognosis. In general, a high titer (>1:3200) and clinical signs point to the diagnosis of FIP, but this is not absolute. And if a cat is healthy, symptom free and has a negative titer, it does not mean that the cat does not have FIP. A few cats with histopathologically confirmed FIP have been seronegative. Possible reasons include disappearance of the virus in advanced stages; formation of immune complexes that leave little or no free coronavirus antibodies to react with the test or the test is not sensitive enough.

Most treatments center on supportive care, such as antibiotics to decrease secondary infection, IV fluids, nutritional support, and systemic corticosteroids to decrease disseminated antibody-mediated vasculitis. Antiviral and immunosuppressive agents have not been effective. Prognosis for cats with definitively diagnosed FIP is extremely poor.

It is recommended that all cats with signs of FIP be isolated, cats that are infected with FeLV and FIV be removed, overcrowding does not occur, improvement of hygiene and nutrition, proper removal of feces and admitting only cats that have negative coronavirus antibody titers.

Removal of health cats with positive coronavirus antibody titers from multiple-cat residences is justified only if there is strong evidence that the cat is a source of FIP infection for the other cats. The litter box should be shared by no more than two cats, cleaned and disinfected regularly, and should be kept away from the feeding area. Vaccinations are available but are not universally recommended and should only be used in cats that are at risk, which include multi-cat households or catteries with confirmed FIP problems.

- by Mark Schlatter, Class of 1998
- edited by Tsang Long Lin, DVM, PhD

Aquaculture Submissions to ADDL - Purdue University

Aquaculture case submissions range from one fish submitted by a fish hobbyist to numerous fish from large private or zoological collections, food-fish producers, or pet-fish suppliers. With the increasing number of hobbyists as well as the emerging aquaculture industry in Indiana, the number of aquaculture case submissions is rapidly increasing.

It is imperative to note that the condition of the fish sample submitted dictates the outcome of our diagnostic investigation in many instances. Care must be taken in collecting an adequate sample and transporting this sample to our lab.

The best sample for submission is an acutely affected, live fish exhibiting clinical signs or having gross lesions of disease. Clinical signs of disease are usually limited to anorexia, lethargy, abnormal swimming or position in the water column, "flashing" (rubbing of the body against a substrate in the aquatic environment), and the loss of fright response. Gross lesions include exophthalmia (unilateral or bilateral), ascites, skin erosions or ulcers, missing scales, frayed fins, or hemorrhage of the skin, eyes or fins. If small fish (less than 4 inches, head to tail length) are involved in the outbreak, 6-8 fish should be submitted. If these fish represent a single disease process, as determined by the pathologist, then tissues are commonly pooled, and a single case accession fee is assessed.

The best and most assured method for transporting fish to the lab is hand delivery with the fish in a clean bucket, plastic or styrofoam cooler with water from the environment from which the fish originated. If transportation time is greater than 1-2 hours, it is recommended that a small battery operated aerator be used for supplemental oxygen. For shipment of fish, place fish in a large thick transparent plastic bag filled approximately 1/3 full with water. An "air cap" or oxygen should be present immediately above the water surface, occupying at least 1/3 to 1/2 of the plastic bag. The bag should be sufficiently tied and placed inside another bag to prevent leakage. This bag should be placed within a thick, wax-coated cardboard box for shipping. This box, along with the submission form, can be shipped via UPS or another appropriate overnight carrier company.

Since many aquaculture cases are associated with water quality problems, it is a good practice to submit a water sample with the fish submission. We are able to perform all of the standard water quality tests on water samples except for the dissolved oxygen (DO) concentration. This water quality parameter is best evaluated "on site" since it changes rapidly in a sample container. THIS WATER SAMPLE MUST BE SUBMITTED SEPARATELY, i.e., without fish. This water sample should be submitted in a clean one quart jar with a screw top lid (i.e. canning jar) with a layer of aluminum foil placed between the water sample and the lid. Water samples shipped in this manner are also satisfactory for pesticide or herbicide analysis as well as testing water quality parameters.

If recently deceased fish are the only sample available to the veterinarian for diagnostic evaluation, the best results are obtained by having the veterinarian take tissue samples for submission. Culturette swabs, with transport media, of the liver and spleen are the best bacteriologic samples for submission. The internal organs including gills, liver, spleen, gastrointestinal tract, integument, skeletal muscle, etc. can be submitted for histopathology in 10% formalin solution. Alternatively, if the fish is less than 3 inches in length, it may be submitted "whole" in formalin after opening the abdominal cavity to expose the internal organs to fixative.

If herbicides or pesticides are suspected, it is imperative to take a water sample from the pond or lake IMMEDIATELY. Detection of certain herbicides and pesticides can be made from frozen fish fillets, so filleting of the fish and freezing before shipment is the best way to prepare samples for shipment. The following table emphasizes the important points of this article.

A short history should be included with each submission. Husbandry, water quality problems, stocking densities, size of the tank/pond/lake,

approximate mortality and morbidity, algal bloom history, origin of the fish, date and time of onset of current problem are all very pertinent issues to remember when completing the history portion of the submission form. Please feel free to contact us if you have questions regarding aquaculture submissions.

- by Tim Muench, DVM, MS
- edited by M. Randy White, DVM, PhD

Suspected Problem	
If you have live fish:	If you have dead fish:
Water Quality	Water Quality
Parasitism	Parasitism
Bacterial Agents	Bacterial Agents
Viral Agents	Viral Agents
Environmental Contaminants	Environmental Contaminants

Best Sample To Submit	
If you have live fish:	If you have dead fish:
Water sample in clean glass quart jar	Water sample in clean glass quart jar
Acutely affected, live, ill, non-treated fish	Culturettes in transport media
* if < 3" long - submit 12-15 fish	Tissue sections of pertinent organs in 10% formalin solution
* if > 3" long - submit 6-8 fish	Frozen fillets (muscle and skin only)

Catastrophic Oxygen Depletion and How to Avoid It

During the warm spring and summer months, we receive an increased number of phone calls regarding sudden fish kills in ponds. The typical history includes observing a very large number of fish dead in an otherwise normal pond following a rainstorm or summer thunderstorm. Usually the owner is very concerned that the fish may have died due to "run-off" of farm chemicals into the pond. Most of the time, these fish kills are a result of a phenomenon known as "pond stratification." Pond stratification is somewhat of a misnomer, since the stratification can also occur in lakes, creeks and some rivers. The stratification leads to a catastrophic depletion of oxygen which almost always results in a very high mortality of aquatic animal life within 24-48 hours following the "de-stratification."

The scientific reasoning behind this phenomenon of pond stratification relates to the temperature of the pond. In the early spring, while the temperature of the pond is still relatively low, the dissolved oxygen is uniformly distributed throughout the pond. As the atmospheric temperature increases, the pond begins to stratify, that is, become layered, with the surface water becoming warmer and lighter while the cooler and denser water forms a layer underneath. Circulation of the colder bottom water is prevented because of the difference in densities between the two layers of water. Dissolved oxygen levels decrease in the bottom layer since photosynthesis and contact with the air is reduced. The already low oxygen levels are further reduced through the decomposition of waste products, which settle to the pond bottom. After a rain, or any other event which disrupts the two layers, a "de-stratification" or "turn-over" of the pond occurs. This has the effect of releasing all of the dissolved oxygen from the upper layer of the pond into the atmosphere, hence, a catastrophic oxygen depletion.

Once stratification of a pond occurs, there is nothing that can be done to alleviate the situation. However, pond stratification can be very easily prevented by the use of supplemental aeration. Aerators come in all sizes and shapes as well as different power sources, i.e., tractor p-t-o, electrical, mechanical, etc. It is important to aerate the pond properly, i.e. match the size of the aerator to the pond, since over-aeration is wasted and may even lead to oxygen supersaturation, known as "gas-bubble" disease and under-aeration will not prevent stratification. In those cases where we suspect catastrophic oxygen depletion, all other possible pathogens including bacterial, viral, parasitic agents are eleminated from the differential diagnosis list. However, the history of several days to weeks of

warm weather followed by a sudden rainstorm are highly suggestive of this condition. If you suspect that you are dealing with a pond turn-over situation following pond stratification, it is imperative to have the pond owner take a water sample and have the dissolved oxygen (DO) concentration evaluated immediately. This water sample should be collected in a clean glass jar or bottle with a screw-top lid and should be completely filled by completely submersing the sample and container and placing the lid on the container while it is still under water.

- by: Tim Muench, DVM, MS
- edited by: Randy White, DVM, PhD

Equine Endometrial Biopsy

Management of the infertile broodmare requires the performance of a breeding soundness examination (BSE). The BSE consists of transrectal palpation and ultrasonography, evaluation of vulvar conformation, vaginoscopy and digital vaginal examination, endometrial culture and cytology and endometrial biopsy. The three uterine techniques are interpreted in light of each other and should be performed at the same time to provide an accurate diagnosis.

Indications for breeding soundness examination include: repeat breeders, barren mares, prepurchase examinations, habitual aborters, vaginal discharge, urine poolers, cervical lacerations, rectovaginal tears and fistulas (prior to surgical repair), suspected luteal insufficiency, irregular cycles, physiological anestrous, chronic uterine infection, pyometra, hydometra, mucometra, multiple endometrial cysts, palpable uterine abnormalities (neoplasia), and mares over 12 years of age who have not had a foal within the last year.

Breeding soundness examinations can be performed during any stage of the estrous cycle, however, it is easiest during estrus when the cervix is relaxed and immune function is highest. In this way, should any contamination of the uterus occur due to a break in technique, the mare is more able to clear the infection. After the mare's perineum has been prepped (tail wrapped and tied to the side), the operator uses sterile technique to obtain the biopsy specimen (performed following culture and cytology). An alligator type biopsy rod is used to obtain the specimen which should be at least 5 x 12 mm in diameter. If inadequate tissue is obtained, a second biopsy is taken. A single biopsy is representative of the entire endometrium if no palpable abnormalities are noted. A repeat biopsy is often taken following uterine therapy to evaluate the response to treatment. The specimens are placed in 10% formalin or Bouin's fixative before processing. Samples placed in Bouin's fixative should be transferred to 70% ethanol or 10% formalin after 3-4 hours of fixation to prevent hardening of the tissue, which results in poor staining. The biopsy specimen is processed routinely and stained with hematoxylin and eosin.

Kenney established a grading system for endometrial biopsies in 1978. The system was revised in 1986 by both Kenney and Doig. This system takes into consideration inflammation and fibrosis of the endometrium and then provides an estimation of the mare's ability to conceive and maintain a pregnancy until term. The uterus is graded in the following manner:

Grade I: normal endometrium or mild, focal inflammation or fibrosis = > 80% chance of conceiving and maintaining until term.

Grade IIA: mild - moderate inflammation and/or multifocal fibrosis with 1-3 layers of fibroblasts surrounding glands or < 2 fibrotic nests per 5 mm linear field = 50-80% chance of conceiving and maintaining until term.

Grade IIB: moderate inflammation and/or multifocal - diffuse fibrosis with 4 or more layers of fibroblasts surrounding glands or 2-4 fibrotic nests per 5 mm linear field = 10-50% of conceiving and maintaining until term.

Grade III: severe inflammation and/or diffuse fibrosis with 5 or more fibrotic nests per 5 mm linear field = < 10% chance of conceiving and maintaining until term.

The biopsy is evaluated for the nature and severity of the inflammation. Neutrophils are seen during the acute inflammatory stages of infection; being replaced by lymphocytes, plasma cells and macrophages as the process becomes more chronic. Eosinophils are seen associated with fungal infections, pneumovagina and urine pooling. Stromal cells produce collagen in response to chronic inflammation or as a result of normal aging processes. Fibrosis is seen initially along the vasculature and endometrial glands and then spreads to the stratum compactum and spongiosum as the disease process progresses. As the level of fibrosis increases, the glands form nests. Fibrosis of the basement membrane is indicative of severe disease. Dilated lymphatics (lacunae) are often noted with moderate to severe fibrosis when drainage from these vessels becomes diminished.

The luminal epithelium is assessed for the presence of a continuous layer of cells and for the height of the epithelium. In estrus the cells are tall cuboidal to low columnar; these cells progress to high columnar during diestrus. The endometrial glands are straight during estrus and highly convoluted during diestrus. During winter anestrus, the epithelium is low cuboidal with minimal convolution of the glands. Endometrial atrophy occurs during winter anestrus as a normal finding. When this occurs during the physiologic breeding season, it is indicative of severe pathology and is most commonly seen in aged mares with diminished ovarian activity.

The endometrial biopsy is an integral part of the BSE. Not only does the biopsy allow assessment of pathologic changes of the endometrium, it provides the clinician with an accurate prognosis as to the mare's future reproductive potential. It is important to remember however, that the biopsy is only one part of the BSE and must be interpreted in light of the other diagnostic findings during the infertility examination.

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