Spring 1999

FROM THE ASSISTANT DIRECTOR

The transition in ADDL leadership has been smooth and, hopefully, inapparent to you. Many thanks to Dr. Randy White for his 2 1/2 years as Interim Director of the ADDL. He has been very effective as Interim Director and in focusing on diagnostic pathology and research.

In May, Dr. Brad Njaa will be joining the ADDL as a staff pathologist. His primary role will be support of the ADDL necropsy and biopsy services. Dr. Njaa comes to us from the University of Saskatchewan where he is completing his MS degree in Veterinary Pathology and preparing for the certification examination in veterinary pathology. He will be accompanied by his wife and three children. We look forward to having Brad's expertise in the ADDL.

Y2K is real! We are working to update our computer system and some of our laboratory equipment that will shut down on January 1, 2000. If all goes according to schedule, we should have our new programs operational by September. We will be seeking the advice of a select group of our users for improvements in reporting and billing. As we rewrite the computer programs, you will see new, easier to read formats for reports and bills.

It was a pleasure to visit with many of you at our booth during the IVMA meeting in March. I received excellent suggestions to improve the ADDL diagnostic service, most of which we are implementing. I encourage you to contact me (billvan@addl.purdue.edu or 765-494-7472) with any suggestions or concerns about ADDL services.

Do We Or Do We Not Have Infectious Pancreatic Necrosis Virus in Indiana's Prize Skamania Steelhead and Rainbow Trout?

This is the question that has been asked by many. Unfortunately, there is no precise answer, but, the following information may shed some light on this issue.

In late October, 1998, in order to comply with the Great Lakes Fish Health Committee's control policy and model program, Mixsawbah State Fish Hatchery in Walkerton, Indiana, of the Indiana's Department of Natural Resources, Fish and Wildlife Division, submitted tissue samples from winter run steelhead trout for annual fish health inspection evaluation to the Animal Disease Diagnostic Laboratory (ADDL), Purdue University, West Lafayette, Indiana. Viruses isolated from four of twelve 5 fish kidney/spleen pools were identified as Infectious Pancreatic Necrosis Virus (IPNV) or an antigenically similar "aquatic birnavirus" which was cross-reactive with polyclonal IPNV anti-sera using fluorescent antibody (FA) techniques.

Subsequently, a sample of this lot of fish were euthanized, necropsied and examined histologically by pathologists at the ADDL. The results of these tests were equivocal for the diagnosis of Infectious Pancreatic Necrosis Virus infection. However, kidney samples collected at necropsy and submitted for VI and FA techniques were positive for IPNV or an antigenically similar "aquatic birnavirus" which was cross-reactive with polyclonal IPNV anti-sera utilizing the same previous mentioned techniques for these fish.

In early December, summer run skamania steelhead trout from this same facility also had six of twelve 5 fish kidney/spleen pools which tested positive for Infectious Pancreatic Necrosis virus or an antigenically similar "aquatic birnavirus" which was cross-reactive with polyclonal IPNV anti-sera utilizing the same previously mentioned techniques. These fish had previously tested negative during a routine annual fish health inspection (August, 1998) using the same methods.

Adult broodstock of "London strain" rainbow trout being kept at Curtis Creek Trout Rearing Station near Howe, Indiana for spawning during the winter months were sampled for their annual fish health inspection in December, 1998. Four of fourteen 5 fish ovarian fluid pools were positive for Infectious Pancreatic Necrosis Virus or an antigenically similar "aquatic birnavirus" which was cross-reactive with polyclonal IPNV anti-sera utilizing the same previously mentioned techniques.

Adult steelhead broodstock were sampled throughout the spawning season (January and February, 1999) at Bodine State fish Hatchery in Mishawaka, Indiana. A total of seven out of forty-two 5 fish kidney/spleen pools (representing 210 fish from a total of 338 females that spawned) were positive for Infectious Pancreatic Necrosis Virus or an antigenically similar "aquatic birnavirus" which is cross-reactive with polyclonal IPNV anti-sera utilizing the same previously mentioned techniques. To date, hatched offspring from both the steelhead from Bodine State Fish Hatchery and the rainbow trout from Curtis Creek Trout Rearing Station have not tested positive for the presence of this pathogen. Additionally, no increased morbidities or mortalities or lesions indicative of IPN disease have been observed in any of the hatcheries where "IPN positive" fish have been identified.

Based upon the importance of IPNV being listed as a restrictive disease agent, and the potential ramifications of large numbers of production fish which may need to be destroyed due to a lack of alternative stocking in non-Great Lakes watersheds and basins, coupled with the wording that "every effort should be made not to release these fish into waters of the Great Lakes basin" as stated in the model program, diagnostic assistance was requested from the Washington Animal Disease Diagnostic Laboratory in Pullman, Washington to confirm the identity of this viral isolate. This laboratory was able to confirm each of these three initial IPNV isolates using very

similar laboratory methods as those used in the virology laboratory of the ADDL. Subsequently, the electron microscopy laboratory of the ADDL has confirmed this isolate as a birnavirus based upon its characteristic size and ultrastructural morphology.

A management decision was made to destroy the entire year's stock of approximately 110,000 of the winter run steelhead trout in order to prevent violation of the control policy and model program of the Great Lakes Fish Health Committee. Factors involved in this decision-making process included the short time period prior to stocking of these fish and the potential jeopardizing of good culture and husbandry practices for arriving chinook and coho salmon production fish in order to minimize exposure of this viral agent to other salmon stocks.

These issues were discussed at great length by the Great Lakes Fish Health Committee at its annual meeting in March, 1999 in Winnipeg, Canada. Several key questions were posed to this committee as asked by mutual agreement of agency fishery chiefs in Illinois, Indiana, Wisconsin and Michigan. The outcome of the decision-making process at this meeting indicated that more research regarding the potential pathogenicity of this isolate needed to be done in order for proper risk assessment modeling procedures to be initiated to ensure the best possible outcome of these "IPN positive" fish. It was also noted that the control policy and model program of this committee should be revised as soon as possible. Much discussion centered around the issue of disease versus pathogen detection.

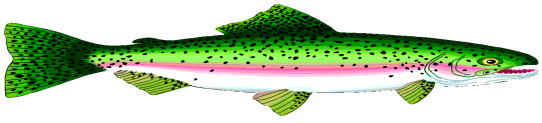
Beginning in late 1999 and early 2000, Drs. White, Kanitz and Sakamoto in the School of Veterinary Medicine of Purdue University and at the Animal Disease Diagnostic Laboratory of Purdue University, including support staff trained in aquaculture species will conduct research to try to focus on the following issues regarding this isolate:

- Serotyping and its significance.
- Pathogenicity as determined by experimental infection of health fish.
- Virulence factors associated with disease propagation such as age susceptibility, water temperatures, etc.
- Improvement of current diagnostic testing procedures for this agent.

Once these issues are addressed, models for risk assessment can be more completely developed to understand the significance of this isolate. This will allow fishery chiefs and hatchery managers within the Great Lakes basin to make educated, informed and science-based decisions regarding these current findings rather than having to destroy hundreds of thousands of fish in order to be in compliance with the current control policy and model program of the Great Lakes Fish Health Committee due to the presence of this isolate within seemingly otherwise health fish.

So, the answer to this question regarding whether or not Indiana's salmonids have IPNV is currently unresolved. However, one fact that is certain is that more research work needs to be done regarding this viral isolate to better answer this question.

- by M. Randy White, DVM, PhD



Pythiosis in Dogs

Pythiosis is an infectious disease caused by a fungus-like organism, *Pythium insidiosum*, that naturally inhabits wetlands, ponds, and swamps. In dogs, the disease usually is manifested by gastroenteritis or dermatitis. Presumably dogs become infected by ingesting, or swimming in contaminated water. Pythiosis is endemic in states that border the Gulf of Mexico, but has been diagnosed in dogs from southern Indiana (with no history of travel outside the state). Dogs with gastrointestinal pythiosis often have a history of retrieving objects, such as sticks, from water and then chewing on them; young male retriever-type dogs are particularly at risk. Dogs with open skin wounds are probably predisposed to acquiring cutaneous pythiosis.

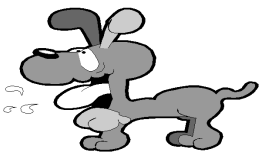
Gastrointestinal pythiosis is usually a fatal disease. Pathologically, the organism is highly invasive and angiotropic, so the infection can be complicated by disruption of vascular supply. The infection typically involves the full-thickness of the stomach or intestine and sometimes extends to adjacent organs. For example, pythiosis of the pylorus or duodenum can extend to the pancreas, omentum, lymph nodes, or contiguous viscera. The tissue response is typically a combination of macrophages, multinucleated giant cells, lymphocytes, plasma cells, and eosinophils. *Pythium* hyphae are usually found in necrotic foci, surrounded by inflammatory cells and sometimes coated by proteinaceous material that is probably derived from eosinophil granules. In H&E-stained sections, this coating (termed a Splendore-Hoeppli reaction) allows for microscopic visualization of the outline of the otherwise non-staining *Pythium* hyphae. Necrotic foci and granulomas associated with these organisms may be grossly visible as yellow granules. Extensive fibrosis often results in stenosis of the affected segment of gut producing clinical signs referable to the site of infection. For example, pythiosis of the gastric pylorus often results in projectile vomiting, and pythiosis of the duodenum in obstruction.

Lesions of cutaneous pythiosis usually develop on the limbs, tail, or face. They are typically firm or spongy (depending on the degree of fibrosis) and ulcerated, often with a draining fistulous tract. Clinically, these lesions may resemble acral lick granulomas.

Because both the organism morphology and the inflammatory reaction are so characteristic, the diagnosis of pythiosis is usually made by microscopic examination of affected tissue(s). A diagnostic biopsy sample of gastrointestinal tissue usually requires the submucosa (i.e. a biopsy of only the mucosa may not provide a diagnostic sample). Cytology and histopathology of the sample is recommended. The organism produces non-septate hyphae, 4 to 8 microns in diameter. They are best visualized in histologic sections stained with Gomori's methenamine silver (GMS). Culture and immunohistochemistry are ancillary techniques that are usually reserved for research.

Successful treatment of dogs with pythiosis is difficult. Although morphologically resembing fungi, the cell wall - plasma membrane of Pythium - differs biochemically from that of fungi. So it is essentially resistant to antifungal drugs. The only treatment option available for a potential cure is surgery and the goal is complete resection of the affected tissue. In the gastrointestinal tract, pythiosis clinically mimics an invasive carcinoma so aggressive surgical extirpation must be attempted. But the prognosis is poor. For cutaneous pythiosis involving extremity, amputation may be necessary.

- by Yumi Yuasa, ECFVG
- edited by Evan Janovitz, DVM, PhD



Histopathology Service

The diagnosis of many intestinal diseases requires histopathology. Since the mucosa is the most commonly affected layer of the gut in these diseases, the pathologists must critically evaluate it. Unfortunately, the mucosa is also the layer most easily disturbed by improper handling. In order to provide pathologists the best possible sample of intestine and, therefore, maximize chances of providing you an accurate diagnosis, a few guidelines are recommended when submitting intestine for histopathology:

DO:

1. Select at least three (3) segments of gut for submission.
2. Cut segments 1 to 2 cm (or 1/2 to 1 inch) in length.
3. Allow formalin to rinse ingesta out of lumen.
4. Be certain formalin can remain in contact with mucosa during shipping.

DO NOT:

1. Limit your samples to one (1) segment of gut.
2. Cut segments greater than 2 cm (1 inch).
3. Leave lumen filled with ingesta.
4. Rub mucosa with knife or fingers.
5. Crush specimen in shipping package.

- by Dr. Evan Janovitz, DVM, PhD

Helpful Hints for Submission of Tissue to the Histotechnique Laboratory

From time to time, the histotechnique lab receives tissues which are improperly packaged or fixed. This may result in problems in tissue processing which may delay or adversely affect the results of a particular case. In order to prevent such problems, I have listed some "dos and don'ts" which may be helpful.

DO fix tissues thoroughly in 10% neutral-buffered formalin solution for a minimum of 24 hours prior to shipping.

If tissues are large (greater than 1 cm in greatest dimension), please incise through the tissue at 1 cm intervals for more rapid fixation.

It is **NOT** necessary to place each separate tissue sample from a necropsy (i.e. liver, spleen, kidney, heart, brain) into individual plastic bags for tissue identification purposes.

It **IS** necessary to place tissue samples from a similar anatomic location (i.e. mammary gland, skin) into individual containers if you wish to identify each sample separately.

DON'T place tissue samples in glass bottles since many of these bottles will break prior to arrival to the laboratory.

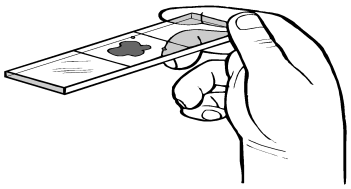
DON'T place large tissue samples into narrow-mouth bottles. Although these tissues will usually go into these type bottles, they are much more difficult to take out of these bottles after fixation.

DON'T allow drying out of tissue samples. Ship tissue samples with enough formalin solution to avoid drying out. Once samples dry out they are very difficult to process.

Please take the time to fill out each submission form completely. We do read this information provided on each form.

DO give us a call if you have any questions about tissue sample submission.

- by M. Randy White, DVM, PhD



Caprine Arthritis-Encephalitis Virus

Caprine arthritis-encephalitis virus (CAEV) is a lentivirus in the family *Retroviridae*. It is antigenically similar to ovine progressive pneumonia virus (OPPV). The seroprevalence of CAEV in United States dairy goat herds ranges from 38% to 81%. Both OPPV and CAEV cause life-long persistent infections in sheep and goats, respectively. With both viruses monocytes and macrophages are the primary target cells for infection. Generally, less than 35% of infected goats will show clinical signs, and the time from infection to clinical expression may be years.

Clinical Signs

CAEV infection can be manifested in a variety of clinical forms; arthritis is the clinical form most commonly seen. The most frequently affected joint is the carpal joint, but virtually all joints have microscopic evidence of infection. The arthritic form occurs in mature goats, usually over one year of age. The onset may be sudden or insidious. Signs may include: joint swelling, evidence of pain, stiffness, abnormal posture or weight loss. Dramatic synovial hyperplasia and distention of joints by yellow fluid may be observed grossly at necropsy. The neurologic form of CAEV is most often seen in kids 2 to 6 months of age. Clinical signs of this form may appear as a slowly developing paresis and paralysis of the limbs. Limb involvement may be asymmetrical. In over 50% of cases other neurologic signs may also be seen, such as blindness, abnormal pupillary light response, nystagmus, head tremors, head tilt, circling, or cranial nerve deficits. In the early form of the neurologic disease, kids remain bright and alert. CAEV may also present with mammary gland involvement. This is characterized by a firm udder and hypogalactia/agalactia in young does. CAEV can cause a chronic interstitial viral pneumonia, which results in progressive respiratory distress and weight loss.

Transmission

The major mode of transmission of CAEV is through ingestion of virus-infected colostrum or milk. Antibodies to CAEV are also ingested with the virus in the colostrum/milk; however, these antibodies do not appear to be protective. Antibodies from passive immunity are no longer detectable after 6 months of age. Horizontal transmission between mature goats is possible, as an increase in seroprevalence with age is observed among commingled infected and non-infected animals. Other potential routes of transmission include: in-utero, vaginal contact during birth, dam's saliva/respiratory secretions, cross milk contamination between does, blood contaminated veterinary equipment, or venereally. Infection of CAEV in sheep does not appear likely under natural conditions, although infection can be induced experimentally.

Serologic Diagnosis

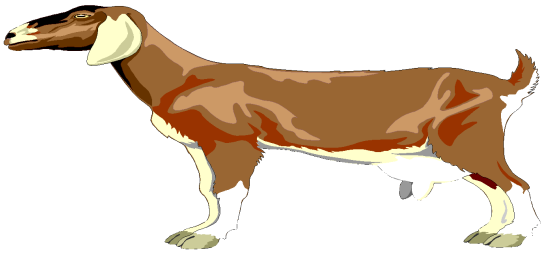
Serology is currently the most widely used means of detecting CAEV. The anti-CAEV antibody AGID test is commonly employed. Sensitivity of this test depends on the antigen used. The CAEV gp135 surface glycoprotein is more sensitive than the CAEV p28 core protein. The AGID test may give false-negative results, but it has a high specificity. The ELISA tests are generally considered more sensitive, but less specific than the AGID. Indirect ELISAs may give false-positive results, because there can be contamination of the antigen preparation with proteins that are cross-reactive. An indirect ELISA which detects CAEV antibodies in milk has recently been developed. However, knowing the CAEV status before lactation begins is most useful for planning any changes that might be made in kid rearing. PCR has also been used to detect viral DNA/RNA in infected animals; however, this test is not currently available for routine diagnostics.

Current Recommendations

The following recommendations have been made to help control CAEV in goat herds.

1. Kids should be removed from their dams immediately after birth to prevent contact with nasal secretions and nursing.
2. Feed kids only pasteurized milk and heat treated colostrum. CAEV can be inactivated by heating colostrum at 56 degrees C (133 degrees F) for 60 minutes. Temperatures lower than this failed to prevent disease transfer, and temperatures greater than 59 degrees C denature immunoglobulines. Using cow's milk and colostrum is acceptable if it is also heat treated and pasteurized to prevent other neonatal infections.
3. Establish a serologic surveillance program. Testing every 6 months appears sufficient in small herds with low seroprevalence.
4. Cull or separate seropositive animals. Separation consists of solid partitions or 2-3 m of space. Feed bunks and waterers should not be shared between groups.
5. Milk seropositive does after seronegative does.
6. When possible do not breed seropositive animals to seronegative animals.
7. Avoid iatrogenic transmission by disinfecting equipment.

- by Chris Cyr, Class of 1999
- edited by Marlon Rebellato, DVM



Disseminated Intravascular Coagulation

Disseminated Intravascular Coagulation (DIC) is a syndrome characterized by massive activation and consumption of coagulation proteins, fibrinolytic proteins and platelets. It is not a primary disease, but a disorder secondary to numerous triggering events such as bacterial, viral, rickettsial, protozoal, parasitic diseases, heat stroke, burns, neoplasia and severe trauma. In 41 cases of disseminated intravascular coagulation, 39% were secondary to malignancy, 30% from pancreatitis, 4% from sepsis, 14% from chronic active hepatitis and 12% secondary to heat stroke. Disseminated intravascular coagulation can be traced to platelet activation and/or the release of thromboplastins into the circulation from tissue damage from various conditions listed above. It can be acute, subacute or chronic and can be localized or generalized. Its severity is related to the rate of release of thromboplastin, the duration of exposure to the causative agent, the ability of the liver to replace consumed coagulation factors, and the ability of the bone marrow to restore platelets.

Tissue necrosis, inflammation, red cell or platelet damage, or endothelial damage induced by antigen-antibody or endotoxin initiates the coagulation process which ultimately forms a clot. Simultaneously, the fibrinolytic system is also activated. Plasmin, the active protease in fibrinolysis, degrades fibrinogen and fibrin, producing fibrin degradation products which in turn prevent fibrin polymerization. Plasmin can also degrade coagulation factors. Thus, the bleeding tendency in disseminated intravascular coagulation patients is a consequence of the depletion of coagulation factors and platelets and the anticoagulant properties of fibrin degradation products.

Coagulation disorders are characterized by spontaneous hemorrhage and/or excessive bleeding after surgery or trauma. Hemorrhage into the central nervous system may cause acute onset of neurological dysfunction or

sudden death. Bleeding in association with disseminated intravascular coagulation is usually severe and occurs from mucosal surfaces and into body cavities. In addition, signs of the underlying disease are usually present. Other signs include shock, petechiae and ecchymoses of the skin and mucous membranes. The organ or organs involved with their accompanying signs and clinical effects must also be taken into consideration.

Laboratory diagnosis is usually based on the triad of prolonged prothrombin time (PT), thrombocytopenia, and hypofibrinogenemia. The finding of fibrin-fibrinogenemia degradation products (FDP's), schistocytes (fragmented erythrocytes) in blood smears, and decreased concentrations of coagulation factors (factors V and VIII and antithrombin III) aid in the diagnosis. Also APTT and thrombin clotting time (TCT) are prolonged.

Therapy should be approached in a logical and sequential fashion. The most important measure involves removal of the inciting cause. When this is not possible, specific treatment may be indicated. Fluid therapy is used to correct hypovolemia; prevent or alleviate vascular stasis; and dilution of thrombin, FDP's and activators of fibrinolysis. Drugs for inhibiting coagulation are indicated if the patient manifests direct evidence of bleeding, thrombosis, or organ dysfunction. Heparin potentiates the action of plasma antithrombin III. When patients with DIC bleed, replacement of some or all blood constituents is indicated to replenish depleted coagulation factors and platelets. Plasma infusion is preferred, but whole blood may be given if red cell infusion is necessary. The infusion of red cells carries the risk of hemolysis and exacerbation of DIC.

Normalization of the screening coagulogram (PT, APTT, and FDP's) usually denotes successful therapy. The return of the normal fibrinogen concentration is a reliable long-term indicator of heparin therapy.

- by Arthur Newman, ECFVG
- edited by Victoria Owiredu-Laast, DVM