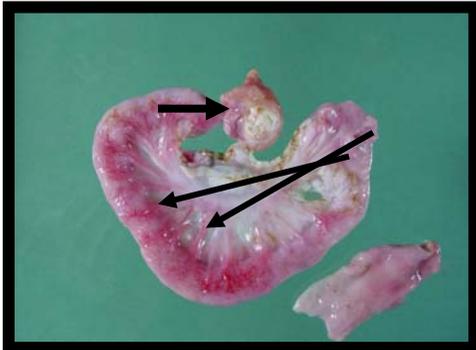

FINAL DIAGNOSIS: Johne's Disease in a Pygmy Goat

In each issue, we will feature a case submitted to ADDL that we hope will be of interest to you.

A 2-year-old pygmy goat with a clinical history of progressive weight loss was submitted for necropsy to the Animal Disease Diagnostic Laboratory. Two animals (including this one) out of 25 goats were presenting with

similar clinical history.

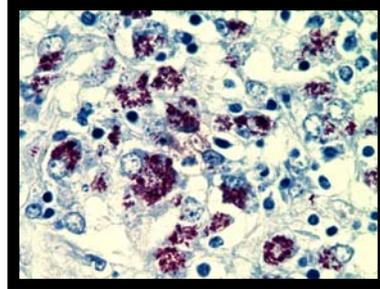
On necropsy examination, the animal had adequate muscle mass but decreased body fat. The remaining body fat was often gelatinous (serous atrophy of fat). The mesenteric lymph nodes were firm, nodular and enlarged (2-10 cm in diameter; normally they should be 1 cm in diameter) and, on cut surface, contained granular



Gross picture of ileum and mesenteric lymph node. The shorter arrow shows the mesenteric lymph node containing caseous exudate. The long arrows show ectatic lymphatics. Arrowhead demonstrates the roughened appearance of the ileal mucosa.

to caseous exudate. Mesenteric lymphatics were markedly distended. The serosa of the jejunum and ileum was hyperemic. The mucosa of the distal jejunum and ileum had a granular appearance and was moderately thickened.

On light microscopic examination, the normal architecture of the mesenteric lymph nodes was disrupted by multifocal to coalescing granulomas that contained necrotic mineralized centers. Lymphoid sinuses and paracortical areas were more severely affected. Macrophages and multinucleated giant cells often had intracytoplasmic acid fast positive bacilli (Ziehl-Neelsen staining). The remaining lymphoid tissue was depleted of lymphoid cells. The lamina propria of the distal jejunum and ileum was diffusely infiltrated by many macrophages and multinucleated giant cells containing acid fast bacilli, and fewer lymphocytes and eosinophils. The villi were often blunted. Intestinal lymphatics were ectatic and contained variable infiltration by macrophages. In the liver, there was granulomatous inflammation around blood vessels of the portal triads.



Myriads of intraplasmic bacilli within macrophages and multinucleated giant cells consistent with *Mycobacterium avium paratuberculosis* 100X

The clinical history of progressive weight loss observed in this goat, in conjunction with the gross and histologic findings of granulomatous lymphadenitis, enteritis and hepatitis, and bacterial culture results were consistent with Johne's disease. Although the epidemiology and pathogenesis of Johne's disease in large and small ruminants are similar, sheep and goats do not develop diarrhea due to the greater efficiency of electrolyte and fluid absorption by the colon; however, pygmy goats are an exception to the course of disease in other small ruminants in that some animals develop explosive diarrhea and die unexpectedly. This disease, considered to be one of the most serious diseases affecting dairy cattle is characterized by chronic body wasting and hypoproteinemia. Young animals (approximately six months of age) are most susceptible to *Mycobacterium avium paratuberculosis* (*M. paratuberculosis*) infection. Milk, semen, urine, intrauterine secretions, and contaminated feed can transmit this agent to susceptible animals. Within the enteric mucosa of infected animals, M cells transport this bacterium to Peyer's patches. *M. paratuberculosis* replicates within macrophages; it resides in phagosomes and phagolysosomes inhibiting production of superoxides and preventing fusion of lysosomes and phagosomes. Iron is also important for this organism to survive. *M. paratuberculosis* produces the lipid-soluble siderophore **mycobactin (iron transporter)** and the water soluble siderophore **exochelin (separates the iron from ferritin)** which help obtain iron from the infected cell. The bacterial cell wall protects the organisms within phagolysosomes. Infected macrophages travel through the lymphatic to the lymph nodes, spleen and liver, causing granulomatous inflammation. In the small intestine and colon there is granulomatous inflammation within the lamina propria causing crypt atrophy. Malabsorption of amino acids and plasma protein, resulting in negative nitrogen imbalance, protein-losing

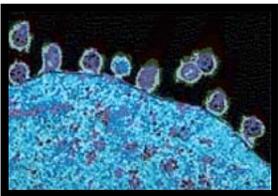
enteropathy and emaciation, are the result of crypt atrophy. PCR, serologic tests (antibodies against *M. paratuberculosis* including complement fixation, agar gel immunodiffusion test and ELISA) and fecal bacterial cultures are currently available for the diagnosis of this disease.

-by Dr. Ingrid Pardo, ADDL Graduate Student

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- 2) Manning EJ, Steinberg H, Krebs V, Collins MT: 2003. Diagnostic testing patterns of natural *Mycobacterium paratuberculosis* infection in pygmy goats. Can J Vet Res 67(3): 213-218.
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Equine Infectious Anemia



Equine infectious anemia (EIA) is a persistent infection of horses and other equids caused by a lentivirus (family Retroviridae). Common names for EIA include swamp fever, mountain fever, and malarial fever. EIA is clinically characterized by recurring episodes of fever, hemolytic anemia, icterus, depression, edema, and weight loss. Unlike other lentiviral infections, most horses with EIA eventually progress from a clinical stage of viremia and fever to an asymptomatic stage. Infected animals mount strong humoral and cell mediated immune responses but are unable to completely clear the virus, resulting in persistent infection. Currently, no effective treatments or vaccines for EIA are available.

EIA is a bloodborne disease. Transmission to horses most often occurs during interrupted feeding of large hematophagous insects of the family *Tabanidae* (deerflies and horseflies). Flies serve as mechanical vectors, and the likelihood of transmission is highest when flies feed on febrile, viremic horses. Iatrogenic transmission may result from use of contaminated blood transfusions, hypodermic needles, or surgical instruments. Transplacental transmission from dam to foal has also been described. EIAV actively replicates in many tissues with particular tropism for cells of the monocyte-macrophage system. Acute disease is associated with

extensive viral replication within tissue macrophages.

The three clinical stages of EIA are acute, chronic, and asymptomatic. The clinical stage may be influenced by virus strain, infective viral dose, and individual host factors. Thrombocytopenia is the earliest and most consistent hematologic abnormality associated with acute EIA. Other signs of acute infection include fever, lethargy, petechial hemorrhages, and anemia. Chronic EIA is characterized by recurring episodes of fever, depression, anemia, icterus, lymphadenopathy, petechial hemorrhages, dependent edema, and weight loss. In most horses, the severity and frequency of the clinical signs decrease over time, and surviving animals eventually enter the asymptomatic stage of infection. Asymptomatic animals remain infective for life. The asymptomatic stage is reversible. Recrudescence of clinical disease is associated with immunosuppression, including administration of exogenous corticosteroids.

Gross lesions of EIA infection vary depending on the clinical stage of the disease. Lesions associated with active infection include generalized lymphadenopathy, hepato-splenomegaly, anemia, widespread hemorrhages, and edema. Common microscopic lesions include hepatic lipidosis, hepatocellular necrosis, hemosiderosis, and perivascular lymphocytic infiltration of most tissues. Necropsy of horses during the asymptomatic stage of infection is often unremarkable.

Two serologic diagnostic tests, the Coggins agar gel immunodiffusion test (AGID) and a competitive enzyme-linked immunosorbent assay (cELISA) are recognized by the United States Department of Agriculture (USDA) as valid and reliable for the diagnosis of EIA. However, the Coggins AGID test is considered the international standard for diagnosis of EIA, with accuracy at least 95%. Both tests detect antibodies to the viral p26 core protein, which stimulates a strong humoral response in most infected horses. The cELISA test will detect antibodies somewhat earlier and at lower concentrations than the AGID test, but false positive tests have occurred, and positive cELISA test results are confirmed using the AGID test.

Control of EIA focuses on identification and elimination of carrier animals. Most US states require horses to have a negative EIAV test result within six months of entry, and all EIAV-positive horses must be reported to the Indiana Board of Animal Health. The options for EIAV-positive animals include euthanasia, permanent identification and lifelong quarantine, slaughter, or shipment to a research facility. Interstate travel of EIAV-positive animals is prohibited by federal law except for three conditions: 1) the return of the animal to its origin, 2) the transport of the animal to slaughter, and 3) movement of the animal to a research facility or diagnostic laboratory. Fly control and prevention of

iatrogenic infections are also essential for minimizing the spread of EIA.
 - by Casey Shake, Class of 2006
 -edited by Dr. Kim Maratea, ADDL Graduate Student

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On the Road

Dr. Ching Ching Wu attended a meeting on antimicrobial susceptibility testing in Miami, FL, January, 2006.

Dr. Roman Pogranichniy and **Steve Vollmer**, Computer Systems Manager, attended a National Animal Health Laboratory Network IT meeting in Sacramento, CA, January, 2006.

Dr. Leon Thacker attended the annual Indiana Veterinary Medical Association meeting in Indianapolis, IN, January, 2006.

Dr. Leon Thacker attended a meeting of the American Association of Veterinary Laboratory Diagnosticians Accreditation Committee in Las Vegas, NV, February, 2006.

Dr. Greg Stevenson attended the annual meeting of the American Association of Swine Veterinarians in Kansas City, MO, March 2006.

ADDL NEWS

Congratulations to **Alice Hardebeck, Cheryl Parker** and **Brenda Turner**, ADDL Serology technicians, who successfully completed Pseudorabies check tests administered by the National Veterinary Services Laboratory, Ames, Iowa.

Several ADDL employees recently celebrated anniversaries as Purdue employees; **Dr. Robert Everson**, Chemist (30 years), **Paula Brost**, Histopathology Laboratory Supervisor (30 years), **Barb Million**, Molecular Diagnostics technician (15 years), **Sharon Albregts**, Research technician (25 years) and **Christina Wilson**, Assistant Chemist (10 years). Congratulations and thanks for your service to ADDL.



Eosinophilic Myositis in Cattle

Eosinophilic myositis (EM) is a collective term used to describe an inflammatory condition grossly characterized by focal, green, muscular lesions in clinically healthy cattle. The most frequently affected tissues are striated skeletal muscle, esophagus and heart. Carcasses of animals exhibiting EM must undergo trimming of affected tissue prior to entering the food chain. Severely affected carcasses are condemned. It has been reported that 5% of carcasses may be condemned in severely affected areas; thus, a high prevalence of EM may account for severe economic losses to producers and processors.

Etiology: The exact cause of EM is unknown. *Sarcocystis cruzi* is the most commonly implicated pathogen, although its ability to consistently cause muscle lesions in affected cattle has not been proven.



Sarcocystis spp. are protozoa that have an obligatory two-host life cycle consisting of an intermediate host (prey) and a final host (predator). With regard to *S. cruzi*, cattle (the intermediate host) ingest the protozoal sporocysts from plant material contaminated with feces of dogs (the definitive host). Initially, two schizogonic generations of the multiplying parasite occur in the vascular endothelium of the infected intermediate host. The merozoites resulting from the second generation schizonts enter the skeletal and

cardiac muscle tissue forming sarcocysts over a period of several months. The dog becomes infected by consuming undercooked beef containing the encysted parasite.

Clinical findings: Subclinical *Sarcocystis spp.* infections are quite common in farm animals; however, clinical disease does not normally occur. When clinical signs are present they are usually non-specific and may include protracted fever, anorexia, decreased milk production, diarrhea, abortion and weakness. Severely affected cardiac muscle may lead to acute death. Acute clinical disease in cattle is referred to as Salmeny disease which is associated with ingestion of massive numbers of infective *Sarcocystis* sporocysts.

Clinical diagnosis: At present, there is no practical antemortem assay available for the diagnosis of EM. An immunofluorescent assay for *S. cruzi* antibodies detected significantly higher IgG fluorescence values in bovine carcasses condemned for EM when compared with those which passed inspection, but a distinct cause and effect relationship could not be determined between the parasite and the presence of EM.

Postmortem diagnosis: EM is most commonly diagnosed during postmortem examination of the affected animal. Gross lesions occur primarily in the heart and striated skeletal muscle and are characterized by focal, firm, greenish gray discoloration. Histologically, the lesions consist of extensive multifocal areas of muscle fiber degeneration and necrosis, with occasional mineralization, atrophy and fibrosis. There is, in addition, a marked inflammatory infiltrate composed predominantly of eosinophils, which accounts for the green appearance of the affected tissues on gross examination.

Pathogenesis: The inability of *S. cruzi* to consistently produce clinical disease or to cause EM in infected cattle has led many pathologists to doubt that this parasite is the actual cause of the inflammatory condition. The exact pathogenic mechanism of EM is not yet completely understood, but some authors have shown that there may be a relationship between EM and a type-1 hypersensitivity reaction to sarcocysts.

Treatment/Prevention: Since most cases of EM are diagnosed postmortem from subclinical infections, an effective treatment plan is not yet available. Prevention of the condition by preventing the occurrence of sarcocystosis in cattle may be attempted in areas of high incidence of EM; interrupting the life cycle of *S. cruzi* may, however, prove difficult to accomplish as feces from both domestic and wild canids can easily contaminate feed and water consumed by cattle. Further research is needed to completely establish the pathogenesis of EM, including the possible association with host-dependent, sarcocystic-specific, type 1 hypersensitivity reactions. This may allow for decreased incidence of EM and its adverse economic effects on cattle production.

-by Dr. Jason Hammelman, Class of 2004
-edited by Dr. Ingeborg Langohr, ADDL Graduate Student

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ADDL Schedule

Purdue ADDL and Heeke ADDL will be closed on the following University holidays in 2006.

May 29.....Memorial Day
July 4.....Independence Day
September 4.....Labor Day
November 23-24.....Thanksgiving
December 22-26..... Christmas

Purdue Animal Disease
Diagnostic Laboratory
West Lafayette, IN





Heeke Animal Disease
Diagnostic Laboratory
Dubois County, IN

Diagnosing *Blastomyces dermatitidis* In the Small Animal Patient

Systemic fungal infections are significant causes of disease because they can gain entry through a single portal and then disseminate to multiple organ systems. A common fungal agent found not only in Indiana, but throughout the Mississippi, Missouri, and Ohio River valleys is *Blastomyces dermatitidis*. Blastomycosis is most commonly acquired from spore inhalation and colonization of the respiratory tract. Following inhalation, fungal spores are transformed from the mycelial to the yeast phase at normal body temperatures. Dogs and cats can both be affected, but blastomycosis is much more common

in dogs. Dogs of any breed or age could become infected if they are in an area with the right environmental conditions for the fungal spores to develop (generally moist, acidic soil rich in decaying vegetation). However, most infected canines have a signalment described as being a young adult, large sporting breed, ranging from 2-5 years of age. There are many clinical signs associated with such a systemic fungal disease. Some of the more common signs that owners notice include anorexia, depression, weight loss, cough, dyspnea, ocular discharge, lameness, and draining cutaneous lesions. Physical exam findings may further include fever, increased lung sounds, lymphadenopathy, uveitis, glaucoma, bone involvement (commonly in the elbow and stifle regions) and cutaneous nodules, papules, or plaques of varying sizes and shapes which can be draining.

Blastomycosis affects a variety of organ systems; however, the clinical pathology data such as those on complete blood count (CBC) and serum chemistry panel can be very non-specific. One change that can be found, but does not necessarily have to be present, is evidence of chronic inflammation. Most dogs have leukocytosis with mild left shift, monocytosis and lymphopenia. Anemia of chronic inflammation is possible, but many other disease processes can cause this non-specific finding as well. The most common findings that can be found on serum chemistry in an animal with blastomycosis are hypoalbuminemia, hyperglobulinemia, and hypercalcemia. One of the most definitive ways to diagnose blastomycosis is by identifying the yeast organisms retrieved from affected sites by aspirates, impression smears, or biopsy. Cytologic evaluation, which can be performed on body cavity and transtracheal lavage fluid, skin, and organ aspirates, is one of the diagnostic tools that is beneficial when diagnosing blastomycosis. It is a quick and inexpensive way to arrive at a diagnosis. (Modified) Wright's Giemsa stain will adequately stain most fungal organisms in the cytologic preparation. Characteristic features of intralésional *B. dermatitidis* yeast forms include a thick basophilic cell wall, broad-based budding, are 5-20µm in size, and extracellular location. Cytologic analysis will also provide the type of inflammatory response present, which ranges from pyogranulomatous to granulomatous and can have multinucleated giant cells present. Aspirates of infected lymph nodes, or even exudates or aspirates from the dermal lesions, can contain the fungal organisms. Other common diagnostic procedures performed in dogs with severe respiratory signs include lung aspirate, transtracheal wash, or bronchoalveolar lavage to help find organisms. Blastomycosis can also be diagnosed through histopathology of tissue samples. There are special immunohistochemical staining techniques that can aid in the identification of blastomycosis in formalin-fixed tissue. Thoracic radiographs, which can display diffuse or nodular interstitial patterns in the lungs, can help determine how severe blastomycosis infection is, and what course of treatment should be pursued once the organism has been identified.

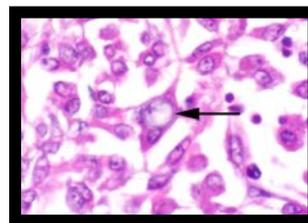
If you are highly suspicious of blastomycosis, but are unable to find the organism, it may be appropriate to conduct serologic or PCR testing. A variety of serologic tests are available, but agar-gel immunodiffusion (AGID) testing is the most commonly used for *B. dermatitidis*. This is an antibody test; thus, it can be negative either early in the course of the disease process or in immunosuppressed animals. AGID testing can become negative with treatment or remain positive even with clinical resolution depending on the antibody titer in a particular animal. PCR testing is also available for the diagnosis of blastomycosis, but this testing modality is less useful clinically for this particular fungal agent than for other fungi because of the good quality of the above mentioned diagnostic tests. Culture of *B. dermatitidis* is dangerous and should only be done by trained personnel.

Treatment of blastomycosis involves long term use of antifungal medications. Other supportive treatments and prognoses are highly variable depending on what organ systems are affected. The best way to maximize the success of treatment is to detect infections early in the disease process.

-by Stacy Amundson-Johnson, Class of 2006
-edited by Dr. Ingeborg Langohr, ADDL Graduate Student

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Histopathologic appearance of
Blastomyces dermatitidis in tissue.
H&E



5-year old female Rottweiler with disseminated blastomycosis of the eye (A), digit (B), skin (C), and muzzle (D,E). Photos provided by Dr. Kim Maratea, ADDL Graduate Student and Sam Royer, Purdue University School of Veterinary Medicine Medical Illustrations

News from the Virology and Serology Sections

Dr. Roman Pogranichniy and the Serology and Virology Sections of are pleased to announce the following new tests now available at ADDL.

- Hemagglutination and inhibition assay (HI) for the detection of antibodies to **H1N1 and H3N2 influenza virus** in swine serum. Tests will be performed at the beginning of each week
- ELISA for detection of antibodies for **IBR** in bovine serum samples. Tests run daily.
- **gB cELISA** for screening and detecting antibodies to PRV. This is a USDA-licensed test on serum and plasma
- Virus neutralization (VN) for detection of antibodies to **BRSV**.
- Virus neutralization (VN) for the detection of antibodies to Porcine enterovirus sergroups 1-7
- IFA for the detection of antibodies to **Porcine circovirus**
- Antigen capturing ELISA for the detection of **canine parvovirus** and **rotavirus** in feces

A list of all tests offered in the virology and serology section can be found on the updated sample submission form as well. These forms can be downloaded from our website at www.addl.purdue.edu (Click on Submission forms on left navigation bar).

If you have questions or comments, please contact Dr. Pogranichniy at 765-494-7440.

Muzzle

Muzzle

