Fall 1999

FROM THE ASSISTANT DIRECTOR

W. G. VanAlstine, DVM, PhD

The summer of 1999 has been a busy one for the ADDL faculty and staff. The serology laboratory was busy with testing involving the 4-H fairs, which typically triples their caseload for two months. In addition, they participated in a federal PRV serology check test and scored 100% on multiple samples using 4 different tests placing them as one of the most accurate labs in the country for PRV testing. In October, the bacteriology laboratory was acknowledged in a National Johnes Workshop held in San Diego as having scored the BEST in the United States in identifying Johne's Disease in samples sent for check testing and received kudos for preparing the best media for Johne's testing. The toxicology laboratory was busy with drug testing for 'performance enhancing drugs' in grand and reserve champion of each animal species at the 1999 State Fair. You'll be glad to know that Hoosier animals were drug free. Our computer section continues to battle the Y2K deadline. Our new computer system should be up and running by Dec 30 with continued development of the new system well into the year 2000. Streamlined reports and online results are some of the options we hope to offer you in the coming year. We will likely experience some changes in our email addresses over the next several months and new addresses can be found on our website which continues to be located at http://www.addl.purdue.edu/ The ADDL just completed a five-year external accreditation review site visit by the American Association of Veterinary Laboratory Diagnosticians. Initial reports from the visit team were very encouraging and some recommendations will be forthcoming. A final report of the accreditation process will not be completed until February 2000.

Inside this issue, please see the articles on new fees, practitioner sabbatics, and the new name of our SIPAC laboratory.

Practitioner Sabbatical

Veterinarians are encouraged to follow their own cases to the ADDL or arrange to spend time with us on necropsy or in one of our other laboratories. Several veterinarians have spent a few hours to a few days in the ADDL and have found their experience to be interesting and useful. The "practitioner's viewpoint" always enriches the learning environment of the veterinary students rotating through ADDL.

I will be happy to provide you a letter to document your time spent in the ADDL. Some practitioners are planning to use documented time spent in the ADDL as part of their Continuing Education requirements for licensure. Please contact the laboratory director if you want to arrange a sabbatical of any length at the ADDL.

W.G. VanAlstine
Assistant Director, ADDL

Front Office Changes

Linda Yankovich has accepted the position as Assistant to the ADDL Director. She continues to oversee the reception area and the front office of the ADDL. Her replacement at the front desk is Shellie Rodarmel who will work with Barb Ward, Mary Fran Nelson and Karen Vankley to answer the phone, accession cases, and direct email inquiries to the appropriate diagnosticians.

SIPAC Lab Changes Name

The Southern Indiana Purdue Agricultural Center (SIPAC) on East Purdue Farm Road in Dubois County was officially renamed the Purdue University Dennis H. Heeke Animal Disease Diagnostic Laboratory at a ceremony held in early September.

Mr. Heeke was a poultry farmer in Southern Indiana and was involved in southern Indiana politics as a state lawmaker for 34 years. A strong advocate of Purdue and Indiana agriculture, he was instrumental in recognizing the need for a facility in southern Indiana and helped secure funding to establish the lab which opened in 1969.

New Tests

ADDL continues to expand its service to Indiana veterinarians. Following is a list of new tests available.

E. coli K88 or K99 antigen…… 5.00ea

PCR tests……15.00ea
- *Clostridium perfringens* genotyping
- *Listeria monocytogenes*
- Avian *Mycoplasma*
- *Bartonelle henselae*
- *Lawsonia (non-porcine)*
- *Johne's* (PCR on culture)
- Turkey corona virus
- Multiple swine enterics panel…….30.00
  - Includes *Salmonella*
    Lawsonia intracellularis
    Brachyspira hyodysenteriae
    Brachyspira pilisicoli
- *E. coli* typing **…….**30.00

A complete fee schedule appears on pages 9 and 10 of this issue.

Dictyocaulosis in Dairy Cattle

With the advent of management changes in the dairy industry from confinement to intense grazing, previously discarded diseases might recur. One such disease to consider is dictyocaulosis, caused by the parasite *Dictyocaulus viviparous*. This disease is predominantly a problem in areas which have a mild climate, a high rainfall and abundant permanent grass, and has been reported in several states including Indiana. Dictyocaulosis is prevalent among calves and heifers during their first grazing season, while older animals in general are immune. To attain and maintain this immunity the incidence of infection with this parasite has to be high. Therefore, naïve cows or cows who did not maintain their immunity could become (re)infected with *D. viviparous* after being turned out on pasture.

D. viviparous has a direct life cycle. The adult worms live in the trachea and bronchi and produce eggs containing fully developed larvae (ovo-viviparous). These eggs are coughed up, swallowed, and hatch during their transport through their transport through the gastrointestinal tract. These first stage larvae develop to infective third-stage larvae on the pasture. Ingested L3 larvae penetrate the intestinal mucosa and mould to L4 larvae in the mesenteric lymph nodes. These larvae subsequently travel via the lymph and blood to the lungs where they end up in the alveoli by breaking the capillaries. The final molt occurs in the bronchioles and the emerging young adults move up the bronchi. The pre-patent period is 3-4 weeks.

Within an affected group of cattle, the clinical signs may vary. A few animals are affected mildly; an intermittent cough, especially when exercised. The majority of the animals are affected moderately; a frequent cough, tachypnea and increased lung sounds. A few animals are affected severely; a deep harsh cough, tachypnea, dyspnea, open-mouth breathing, increased lung sounds, salivation, anorexia and sometimes mild pyrexia.

Dictyocaulosis can be diagnosed by several techniques. The Baermann sedimentation technique is a sensitive method to detect first stage larvae of *D. viviparous* in the feces. These larvae are large, slow-moving and contain dark food granules. Unfortunately, this technique is of no diagnostic value during the pre-patent infection. A better diagnostic procedure during the pre-patent period is a transtracheal wash, in which eggs of *D. viviparous*, (immature) adults, eosinophils, neutrophils and macrophages may be observed.

During post-mortem examination various gross changes can be noticed, depending on the stage of infection. Atelectasis may be discerned due to the blockage of small bronchi and bronchioles, in response to the inflammatory response provoked by the presence of L4 larvae in the alveoli. The adult worms produce an inflammation in the larger airways, which may result in consolidation of the dorsocaudal aspects of lung lobes. In severe cases, an extensive pulmonary edema and interstitial emphysema may be noticed. The adult worms can be observed either directly in the trachea and bronchi or after flushing the respiratory tract with water. The adults are slender, thread-like worms up to 8 cm long.

In conclusion, with the advent of intense grazing management in the dairy industry, dictyocaulosis needs to be included in the differential diagnosis for pneumonia in dairy cattle. An accurate diagnosis of *D. viviparous* can be made based on the history of pasture grazing, clinical signs and the various diagnostic procedures.

-by Josef Steenbergen, Class of 1999
-edited by Victoria Owiredu-Laast, DVM

Canine Babesiosis

Background

Canine babesiosis is a disease caused by the intraerythrocytic protozoan parasites *Babesia canis* and *Babesia gibsoni*. Babesiosis is transmitted by ticks to susceptible canine hosts. *Rhipicephalus sanguineus* is the most common tick vector in the United States. Splenectomized dogs, immunocompromised dogs and young dogs between the ages of two and eight months are most susceptible to infection. Canine babesiosis occurs worldwide. Within the United States it is most common in the southeast. Although canine babesiosis is considered uncommon in the U.S., it is of clinical significance due to its morbidity and mortality. It is an important differential when history and clinical signs are consistent with infection and other more common diseases have been ruled out.

Pathogenesis and Clinical Signs

Animals are affected after an infected tick bites and feeds on a susceptible host for a minimum of three days. When the babesia organism is introduced into the host, it attaches to erythrocyte membranes and is endocytosed. Hemolytic anemia and hypotensive shock are typical clinical syndromes of infection. Hemolytic anemia results from direct erythrocyte damage by the parasite, and both intravascular and extravascular immune-mediated destruction of red blood cells. Infection can produce thrombocytopenia, the mechanism of which consists of immune-mediated destruction and sequestration in the spleen. Physical examination reveals splenomegaly, lymphadenomegaly, fever and less frequently, lethargy, vomiting, hematuria, and icterus. Hypotensive shock results from the release and production of vasoactive amines and cytokines which produce vasodilation. It most often occurs in puppies with the peracute form of the disease. Death may occur and is seen most often in *B. gibsoni* infections and in puppies affected with B. canis and B. gibsoni. Chronic infections, subclinical carrier states and atypical canine babesiosis may also occur.

Diagnosis

Infection with B. canis or B. gibsoni is definitively diagnosed by demonstration of the parasites on red cells. Blood smears may be stained with Diff-Quik or preferably Wright's or Giemsa stain. Stained smears demonstrate 2.4µm x 5.0µm, piriform-shaped, intraerythrocytic parasites which are usually paired (*B. canis*), or 1.0µm x 3.2µm pleomorphic, single to multiple, intraerythrocytic organisms (*B. gibsoni*). Blood samples collected from the peripheral capillary beds in the tip of the ear or the nail bed are most likely to reveal parasites. The feathered edge and monolayer of the smear should be closely examined for parasitized red blood cells. Practitioners may read their own smears and/or submit them to a nearby veterinary diagnostic laboratory. Babesia organisms may be a challenge to find in chronic infections or in asymptomatic carrier animals. Submission of a blood sample for serologic testing is an important adjunct diagnostic tool to help rule in the disease particularly when titers are significantly elevated. Serologic testing is available at Louisiana Veterinary Medicine Diagnostic Laboratory and at the University of Illinois Laboratories of Veterinary Diagnostic Medicine. Serologic tests which are currently available include the indirect fluorescent antibody test (IFA), which is used most frequently, and a more recently developed dot ELISA test.

Treatment

The most effective drugs used in the treatment of canine babesiosis include diminazene aceturate, phenamidine isethionate, and imidocarb dipropionate which

are not available or approved for use in the United States. Treatment of canine babesiosis in the U.S. is therefore mostly aimed at treating symptoms. The majority of babesia cases diagnosed in dogs in the U.S. are caused by the less virulent strains of *B. canis* and dogs frequently recover from these infections naturally with supportive therapy. Clindamycin has been successfully used to treat canine babesiosis and may be considered in refractory or more severe and virulent infections.

Prevention

Prevention of canine babesiosis is mostly aimed at controlling the vector. It is an important aspect since treatment is not always successful. The environment should be treated to decrease tick numbers, dogs should be treated to control tick infestations, and ticks should be removed from parasitized animals as quickly as detected.

Recently, a vaccine which minimizes the severity of infection was developed. The vaccine is reported to be 70 to 100% effective in diminishing the pathologic effects which typically ensue upon infection. The vaccine is currently available in Europe where canine babesiosis is more common life-threatening disease.

Blood transfusion poses a significant risk to recipient animals, therefore it is recommended that donor animals be tested for infection with babesia organisms. Splenectomy prior to testing significantly improves the likelihood of finding organisms in a blood sample from an infected donor.

Conclusion

It is important for practitioners to keep less common diseases such as Babesiosis in the list of differential diagnoses for acute hemolytic anemia, shock and icterus. It is equally important that practitioners know how to diagnose diseases which are less common and that they be diligent in their efforts to find a cause for disease when the more common possibilities have been ruled out. Animals are traveling more often today than ever before, making exotic diseases more common and more likely to spread to new areas. Canine babesiosis is an important diagnostic consideration which practitioners should not neglect to consider.

-by Kristen Ripberger, Class of 2000
-edited by Armando Irizzary, DVM

Encephalomyocarditis Virus in Pigs

Encephalomyocarditis virus (EMCV) has been recognized as a swine pathogen responsible for sporadic outbreaks for the last thirty years. It is classified in the genus *Cardiovirus* in the family Picornaviridae. The EMCV group is generally regarded as a rodent virus with the principle vectors being rats and mice. The group can naturally infect a wide range of vertebrate species, including swine, which is the most susceptible of the domesticated animals. To date there is no clear evidence to support the role of EMCV as a pathogen in livestock other than swine.

The traditional manifestation of the disease in swine is characterized by acute onset of disease with sudden deaths due to myocardial failure in pre-weaning pigs. Anorexia, listlessness, trembling, staggering, paralysis, or dyspnea may also be observed. Younger pigs are usually more susceptible with mortality reaching up to 100% in the unweaned animals. Post-weaning pigs generally develop subclinical infections. EMCV has been recovered from stillborn and mummified fetuses demonstrating that transplacental infection may occur. Infected pregnant sows may have near-term abortions and low farrowing rates. Infertility problems seen in these animals are attributed to fetal mummifications after intrauterine deaths.

Oral transmission through either rodent feces or carcasses appears to be the most likely route of transmission. * Virus

strain, viral dose, history and susceptibility of the individual animal all appear to affect the course of infection.

It is important when making a diagnosis to differentiate EMCV from the other reproductive diseases. History can be an important component to the diagnosis of EMCV especially when it is one of reproductive failure with high preweaning mortality. This can be seen in sows of any parity, which can be used to distinguish from porcine parvovirus, which is primarily manifested in gilt litters. Dyspnea, due to heart failure, seen grossly as white necrotic lesions in the heart, is characteristic of EMCV but should be differentiated from Selenium/Vit. E deficiency. * Virus isolation and identification is considered necessary for a definitive diagnosis. Since there is no transmission of maternal immunoglobulins across the placenta in pigs, detection of antibody specific to EMCV from stillborn or large mummified fetuses is significant for fetal infection.

EMCV has no treatment, however, there is an apparently effective inactivated vaccine available in the United States. Energy should, therefore, be utilized to control the rodent population on the farms as well as minimize stress among the affected pigs. Disinfection can be accomplished using mercuric chloride or iodine-based solutions when necessary.

-by Jason K. Huff, Class of 2000

The virus seems to replicate in the intestine and cause a viremia within 2 days of infection. The highest titers are obtained from the head, but spleen, mesenteric lymph nodes, liver, pancreas and kidney also contain virus.

*Other gross lesions may include hydropericardium, hydrothorax, pulmonary edema and ascitis. The most common microscopic lesions are necroses of myocardial fibers with mononuclear cell infiltration. Perivascular accumulation of mononuclear cells can also be observed in the meninges and brain.

-edited by Marlon Rebelatto, DVM, MS

Neospora Caninum

Neospora caninum is an organism that is capable of causing abortions in cattle, horses, goats, and possibly others. The organism was first reported in dogs in 1988 and has been since identified as a major cause of bovine abortion in California. The organism is very similar to *Toxoplasma gondi*. The definitive host was recently reported to be the dog. The intermediate hosts include cattle, goats, sheep, horses, and deer. The dog ingests *Neospora* infected tissues from an intermediate host and develops a subclinical to mild intestinal infection. The organism undergoes sexual replication and is shed as oocysts in the dog's feces. The intermediate host ingests these oocysts in contaminated feed. A systemic infection develops and the parasite localizes and becomes dormant in specific tissues, especially the brain. The infection is life long and latent unless the animal is pregnant. The fetus is nearly always infected when the mother is infected.

There are three consequences of fetal infection. The first is fetal death and mid-gestational abortions. The second is congenital CNS disease ranging from mild to severe. The third is a latent infection in the fetus (vertical transmission.)

Cow to cow transmission (horizontal transmission) does not appear to occur at this time. Cows throughout the U.S. and the world have antibodies to *Neospora caninum*. The biggest problem area in the U.S. is the California dairy industry, however, abortions do occur in beef cattle as well.

A highly sensitive and specific ELISA is available to identify those cows that have been exposed. However a positive result does not indicate the cow will abort. The majority of latently infected cows do not abort and pass the infection on to their fetuses. The risk of abortion is highest in first calf heifers and cows that are stressed or sick. Most of the abortion storms seem to occur in naïve cows

that are exposed to oocyst-contaminated feed during pregnancy.

The list of differential diagnoses for abortions and abortion storms in ruminants includes *Leptospirosis*, Bovine Viral Diarrhea, Infectious Bovine Rhinotracheitis, *Campylobacter*, and *Neospora*. To confirm a *Neospora*-associated abortion the organism must be demonstrated in the fetal tissues. This is often difficult because the fetal infection can occur up to one month prior to the abortion and the fetal tissues are often very autolyzed. Fetal serology and immunohistologic examination of fetal tissues (particularly brain and spinal cord) are the best method of diagnosis. In addition, other causes such as IBR, BVD and Leptospirosis need to be ruled out. Paired sera from the cows do not appear to be diagnostic because the cows do not have an increased antibody titer at the time of abortion.

At the present time there are no vaccines available. There are suggested control measures. The first is to protect the feed and water sources from canid fecal contamination, the second is to buy seronegative replacements. Finally, one should promptly remove aborted fetuses and placentas.

Neospora is estimated to cost the California dairy industry $35 million dollars per year. This can be a very costly disease and considered part of a differential list of abortions in ruminants in other states besides California.

-by Bryan Wolfe, Class of 1999
-edited by Brad Njaa, DVM, MVSc

Bordetella Bronchiseptica in Cats

Until recently, *Bordetella bronchiseptica* was considered an uncommon cause of respiratory disease in cats, and then only as a secondary agent. Today, it appears to be a primary respiratory pathogen in cats. Challenges to specific-pathogen-free kittens have produced clinical signs; however, prevalence of the disease and its pathogenesis in natural infections is not known.

Several studies have created data that suggest exposure to *B. bronchiseptica* is common. In selecting cats suitable for their study, Jacobs et al. (1993) had a difficult time finding cats that were seronegative for the organism. A second study showed the possibility of carrier cats with the initiation of shedding of the organism after a stressful event; shedding began after parturition. In this study, the kittens of the queens did not develop clinical disease and did not seroconvert. The possibility however exists that if the kittens become infected with another respiratory pathogen, such as feline calicivirus or feline herpesvirus, they could also be exposed to *Bordetella*, which could then opportunistically invade and possibly increase the severity of respiratory disease in the kitten.

The clinical signs seen with primary *Bordetella* infections include fever, listlessness, sneezing, ocular and nasal discharges, submandibular lymphadenopathy, increased lung sounds, and coughing. Unfortunately, these signs are seen with many respiratory pathogens. *Bordetella bronchiseptica* can also be associated with other respiratory agents, such as feline rhinotracheitis virus and feline calicivirus. To diagnose *B. bronchiseptica*, oropharyngeal and/or nasal swabs are needed for bacterial culture. Transtracheal washes have also been used. Isolation of the organism is commonly successful if the cat is showing clinical signs.

Carrier cats, on the other hand, are more difficult to diagnose, because the organism is shed only intermittently.

So where is Bordetellosis being seen? There have been reports of outbreaks in catteries, shelters and multi-cat households. Many other respiratory diseases thrive in these environments as well, so it is not surprising that this is where *B. bronchiseptica* is also being found. A focus has been made on the prevention of Bordetellosis. Recently an intranasal vaccine for cats has been developed and licensed. However, there is still much research to be done to better understand this disease, its etiology and prevalence.

-by Cathy Berquist, Class of 2000
-edited by Lavun Anothayanontha, DVM

Gastric Dilatation-Volvulus in Dogs

Acute gastric dilatation-volvulus (GDV) is a life-threatening condition, with fatality rates ranging from 10% to 60%.[1] The animals most commonly affected by GDV include older, large or giant breed, deep-chested dogs, including Great Danes, German Shepherds, Standard Poodles, and large mixed breed dogs. Early diagnosis, medical stabilization, surgical intervention and post-operative monitoring are important factors in reducing the mortality rate.

Gastric dilatation-volvulus is the result of accumulation of gas, fluid, or a combination of the two in the stomach. Factors responsible for causing dilatation include aerophagia, exercise after ingesting a meal, and overeating. The stomach distends with gas or fluid, and rotation along the axis of the esophagus and cardia follows. The rotation is generally in the clockwise direction (when viewed in dorsal recumbency), and can be up to a maximum
of 360°. A less common fate is a counter-clockwise rotation, to a maximum of 90°. In addition to the accumulation of gas and/or fluid, there is often an outflow obstruction due to a mechanical or functional abnormality.[2]

The clinical signs associated with GDV are restlessness, anxiousness, respiratory dysfunction, hypersalivation, retching, abdominal distension and frequent attempts to vomit. The animal may present weak, collapsed, or comatose, depending on the degree of shock. Signs related to hypovolemic shock are pale mucous membranes, prolonged capillary refill time, rapid, weak, thready pulses, and tachypnea. Diagnosis of GDV is based upon clinical signs, inability to pass a gastric or nasogastric tube effectively, and consistent radiographic findings.

The major life threatening abnormality associated with GDV is shock. Shock is due to compression of the caudal vena cava, from distension of the stomach, and the portal vein, from distension and rotation of the stomach. As a result, there is decreased tissue perfusion, which leads to hypoxia and ischemia of tissues. Ischemia of cardiac muscles can result in arrhythmias, and ischemia of abdominal organs can lead to necrosis/death of affected organs/tissues.

The first priority for treatment of GDV is cardiovascular stabilization. Dogs that have persistent circulatory collapse are thought to be at greater risk of dying than those dogs that are stabilized.[1] Hosgood, et al, suggests that intravenous therapy with 7% sodium chloride plus 6% dextran initially, followed by 9% sodium chloride, is superior to 9% sodium chloride alone. Once the animal is stabilized the stomach is decompressed using orogastric intubation or needle gastrocentesis. Radiographs are taken prior to surgery to determine if a volvulus is present. Broad spectrum antibiotics are administered prophylactically. The use of corticosteroids remains controversial, but have shown to be beneficial in instances of septic or endotoxic shock.

The timing of surgery also remains controversial, as there are advantages and disadvantages to early and late surgical

intervention. The surgical techniques used for repair of GDV include tube gastrostomy, circumcostal gastropexy, belt-loop gastropexy, and permanent incisional gastropexy. Complications associated with tube gastrostomy are peritonitis (due to premature removal or loosening of the tube), cellulitis (due to gastric content leakage), and alteration of gastric myoelectric activity. There is some evidence suggesting gastropexy procedures lead to chronic bloaters by altering gastric emptying.[3]

Perfusion of the tissues is maintained perioperatively and post-operatively, using an intravenously administered, balanced electrolyte solution. Perioperative and post-operative monitoring of the patient for perfusion, as well as abdominal distension, are important. The parameters used to assess tissue perfusion include capillary refill time, blood pressure, peripheral pulse pressures, arterial blood gas, urine output, PCV, and total protein. Abdominal distension is monitored due to the potential of re-bloating following surgery.

There are many complications that can occur postoperatively, most of which are secondary to the initial problems associated with GDV. Cardiac arrhythmias, usually of ventricular origin, tend to occur in the first 12-36 hours following surgery. A continuous ECG is recommended to monitor for arrhythmias, and anti-arrhythmic drugs (lidocaine, procainamide) are used when the arrhythmia is responsible for poor tissue perfusion. Additional complications include disseminated intravascular coagulation, sepsis caused by gastric leak or aspiration pneumonia, protein loss, gastric ischemia and esophagitis.

Additional medical options available for the treatment of GDV include: a lipid peroxidase inhibitor to prevent lipid peroxidation secondary to reperfusion injury; cisapride, metoclopramide, erythromycin, and ranitidine to facilitate gastric emptying; and vasoactive intestinal peptide to facilitate eructation and lower esophageal sphincter tone in animals which are chronic bloaters. The effect of metoclopramide on gastric emptying in dogs with GDV has been studied, and the results suggest there is no improvement of gastric emptying.[4]

Left untreated, GDV can lead to multiple organ failure, circulatory shock and death.[4] Factors which contribute to a higher mortality rate include gastric necrosis, gastric resection, splenectomy and pre-operative cardiac arrhythmias.[5] It is therefore important to be familiar with the clinical signs of GDV in order to arrive at an early diagnosis, stabilize the patient as soon as possible, surgically correct the volvulus, and medically manage any additional complications.

-by Elizabeth Natz, Class of 1999
-Edited by Brad L. Njaa, DVM, MVSc