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# DIAGNOSTIC FORUM

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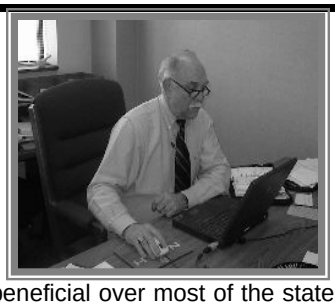
**A Quarterly Newsletter from the Animal Disease Diagnostic Laboratory**
**Purdue University, West Lafayette, Indiana 47907 (765-494-7440)**



## FROM THE DIRECTOR

H. Leon Thacker, DVM, PhD

Good day from Purdue ADDL. The arrival of summer is always a welcome time of the year in Indiana; the crops are in excellent condition for this time of year and, though we have had some dry weather,recent rains have been very welcome and beneficial over most of the state. At the time of the sending of the last Diagnostic Forum, we had encountered the toxic nephropathy problem in pet food but, at the time, the identity of the specific toxin was yet unknown. We now, however, know that the toxic principle is/was a nitrogen containing compound, melamine, which is a by-product of the manufacture of plastics. We have encountered only a few pets with pathology consistent with melamine toxicity but anecdotal and published reports of the pathology of the toxicity are quite specific for the diagnosis. Chemical analyses for the presence of the toxin are available, but the massive media coverage of the adulterated foods recalled as a result have been quite effective in stemming this unfortunate toxicity occurrence.

With the financial assistance of the Indiana Department of Homeland Security, an incinerator has been installed at the Heeke Southern Indiana Diagnostic Laboratory. Planning, engineering, funding and installation of the equipment has been delayed in this project, but the efforts have come to fruition, the equipment is in place and the initial firing is soon to come. The utilization of the tissue/carcass incinerator at the Heeke Laboratory will eliminate the necessity of finding alternative means of tissue and carcass disposal when the tissues and carcasses are unsuitable for sending to a rendering company. Additional biosecurity has also been effected in the West Lafayette laboratory with minor renovation of the mammalian and avian necropsy areas. Communicating door of the two areas has been installed, allowing discontinuance of direct traffic into the rooms from the common anteroom that formerly connected them.

The recent 2007 session of the Indiana General Assembly approved expenditure of $30 million to construct a biosafety level 3 (BSL-3) and biosafety level 3 Ag biocontainment building to be located near and attached to the southeast corner of the present ADDL-West Lafayette building. The use of this high security biocontainment facility will allow diagnostic procedures to identify highly infectious agents in the event of the introduction of a catastrophic disease agent to Indiana animal population(s) by accidental or intentional means. The ADDL presently has biosafety level 2 isolation capabilities which restricts working with such infectious agents as most of those included on the USDA foreign animal disease list. Occupancy of the new building is expected in approximately two years.

Evidence of the competency of our faculty, staff, and graduate students is frequently expressed. Our virology laboratory was recently identified as one of only three laboratories among 22 participants who correctly identified all proficiency tests taken to identify BVD virus by various means. Drs. Peg Miller, Pam Mouser, Dinesh Singh and Inge Langohr were all recently recognized for outstanding accomplishments as noted in the ADDL NEWS on page 3 following. Congratulations to each of you.

## Final Diagnosis

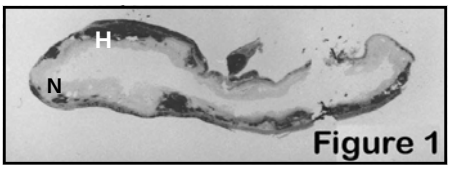
*Clostridium difficile* in a neonatal foal

In each issue, we feature a case submitted to ADDL

A 52 kg, reportedly 3-day-old brown Quarterhorse filly was presented with a history of hemorrhagic diarrhea. Other significant clinical findings included metabolic acidosis, gastric reflux, colic, and tachycardia. Abdominal ultrasound revealed dilated loops of intestine. Clinical pathology findings included hypoproteinemia, leucopenia, hypoglycemia, azotemia, hyperbilirubinemia and prolonged partial thromboplastin time. Due to poor response to fluid and antibiotic therapy, the filly was euthanized and presented for necropsy.

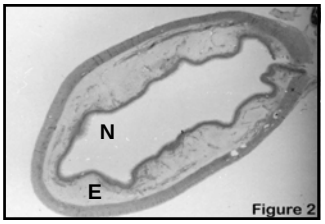
At necropsy, the filly was thin and moderately dehydrated. Skin of the lateral abdominal region was shaved bilaterally. The umbilicus was slightly swollen and congested. The abdominal cavity contained about two liters of straw-colored fluid that smelled like urine. Serosal surface of the small intestine (jejunum) along all its length, had multiple, variably sized (up to 5 cm in greatest dimension), oval to irregular depressed areas of necrosis, characterized as central pale tan to gray depressions surrounded by a dark pink to red hyperemic border that sharply demarcated these foci from the normal serosa. A few foci corresponded to mucosal ulcers that were characterized as pale tan to gray depressions on the mucosal surface. The ileum, cecum, and colon contained thick, pale brown to gray fluid. The cecum and large colon had moderate submucosal edema. Well-formed feces were not observed in the small colon and rectum.

In the left kidney, there were a few, up to 0.5 cm in greatest dimension, dark pink to red, wedge-shaped foci near the cranial pole that extended into the cortex on cut surface. Both lungs were non-collapsed, wet and heavy, and oozed a moderate amount of pink fluid from the cut surface. Cranioventral portions of the right lung were mottled dark red to pink. A gross diagnosis of mural enteritis with mucosal ulcers and serosal infarcts, abdominal effusions, and pulmonary edema was made. Microscopically, in the small intestine, there was circumferential coagulative necrosis of superficial mucosa (Figures 1,3) Bacterial rods, cocci and coccobacilli were occasionally noted within the necrotic mucosa. The lamina propria was infiltrated by neutrophils, karyorrhectic debris, and hemorrhage.

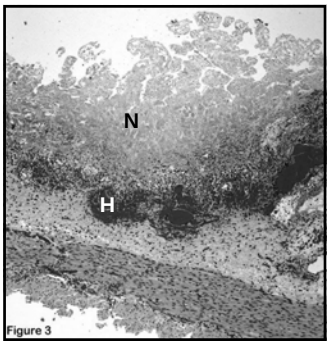



Small intestine; foal: Sub-gross view of a section showing diffuse superficial mucosal necrosis (N) and marked hemorrhage in the lamina propria and submucosa (H)

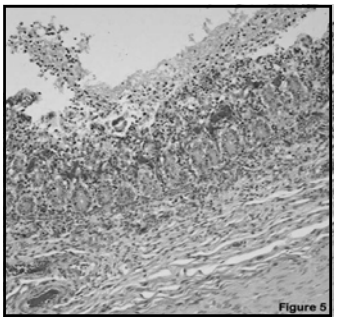
The submucosa was moderately expanded by edema (Figure 2) and congested blood vessels. A few blood vessels had fibrillar eosinophilic material in their walls and their lumina occluded by partially organized fibrin thrombi. In the colon, there was superficial mucosa necrosis (Figure 4)




Small intestine; foal: Subgross view of a section showing mucosal necrosis (N) and marked submucosal edema (E)




Small intestine; foal: Low power photomicrograph showing diffuse superficial coagulative necrosis (N) in the mucosa, congestion and perivascular hemorrhage (H)

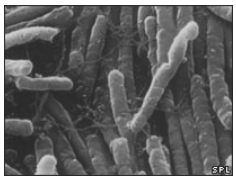



Colon; foal: Low-power photomicrograph showing fibrinonecrotic debris in the superficial mucosa and infiltration by neutrophils and lymphocytes in the lamina propria

The lamina propria and submucosa were expanded by edema, fibrin, congested capillaries, and increased numbers of lymphocytes and plasma cells. Subserosal connective tissue was expanded by edema, congested blood vessels and infiltration by neutrophils and macrophages, admixed with fibrin and karyorrhectic debris.

Other significant histologic lesions included pulmonary edema and adrenocortical congestion and hemorrhage. Histologic lesions in the small intestine and colon were consistent with a bacterial infection such as salmonellosis and/or clostridial infection. Bacteriological culture of pooled intestinal samples yielded *Clostridium difficile*, which tested positive for toxin A and B by polymerase chain reaction. Based on acute superficial mucosal necrosis and bacteriological culture, a final diagnosis of *C. difficile* infection was made. Bacteriological culture for *Salmonella* sp. was negative.

*Clostridium difficile* is recognized as an important human and veterinary pathogen, and is an important cause of antibiotic-associated diarrhea in adult humans and various age groups of a variety of animals. Syrian hamsters, rabbits, pigs, horses, and guinea pigs are most commonly affected. The organism is a gram-positive, anaerobic, spore-forming, rod-shaped bacterium, normally present in low numbers in the intestine of humans, many other mammals, birds and reptiles. Spores are also prevalent in the environment.



Pathogenic *C. difficile* elaborates a potent enterotoxin, toxin A, which causes widespread damage to the intestinal mucosa by acting synergistically with a cytotoxin, toxin B. Mucosal damage by toxin A paves the way for the toxin B. Receptors for toxin A are located on the brush border of the enteric epithelial cells. The combined action of these toxins results in necrosis of superficial epithelium and edema in affected areas of intestine. The toxin inactivates Rho and Rho-subtype GTPases in the cytoplasm leading to actin derangements and increased paracellular permeability, cell rounding, and cell death. Additional effects of these toxins include increased production of interleukin 8, which mobilizes and activates neutrophils.

Horses are susceptible to *C. difficile* infection as adults and foals. Affected adult horses develop necrohemorrhagic typhlocolitis, characterized by superficial mucosal necrosis, hemorrhage and submucosal edema in the cecum and ascending colon. The disease in foals is characterized by severe lesions in the small intestine which include necrohemorrhagic enteritis with severe necrosis of villi, mucosal ulceration and fibrin exudation. Gram positive rods may be observed adhered to the necrotic epithelium. Foals can also develop necrotizing colitis similar to adult horses. The disease in foals can develop immediately after birth, responds poorly to therapy, and is usually fatal. The most obvious clinical manifestation of the disease is diarrhea, which may be hemorrhagic. Foals are more susceptible to infection, most likely due to the lack of an established intestinal microflora. Diagnosis is made by bacterial culture, gross lesions and histopathology. Bacteriological culture of *C. difficile* is not highly sensitive and does not differentiate the pathogenic and non-pathogenic strains. Specific tests for *C. difficile* toxins used in the diagnostic laboratory include cell culture to detect the presence of biologically active toxin, and an ELISA assay to detect immunologically active toxin that may or may not be biologically active. PCR detection of *C. difficile* , as was done in this case, is highly sensitive and can discriminate between toxigenic and nontoxigenic strains of the organism by detecting its toxin producing genes.

-by Dr. Dinesh Singh, ADDL Graduate Student

**References**


1. Baverud V: 2002. *Clostridium difficile* infections in animals with special reference to the horse. A review. Vet Q 24(4): 203-219
2. Jones RL, Adney WS, Alexander AF, Shideler RK, Traub-Dargatz JL: 1988. Hemorrhagic necrotizing enterocolitis associated with *Clostridium difficile* infection in four foals. JAVMA. 193(1):76-9
3. Keel MK, Songer JG: 2006. Comparative pathology of *Clostridium difficile*-associated disease. Vet Pathol 43: 225-240.
4. Magdesian KG, Hirsch DC, Jang SS, Hansen LM, Madigan JE: 2002. Characterization of Clostridium difficile isolates from foals with diarrhea: 28 cases (1993-1997). JAVMA 220(1): 67-73.
5. Traub-Dargatz JL, Jones RL: 1993. Clostridia-associated enterocolitis in adult horses and foals. Vet Clin North Am Eq Prac 9:411-421.


# ADDL NEWS

**Dr. Peg Miller**, ADDL Pathologist, won the 2007 Samuel W. Thompson Lectureship Award as the Outstanding Lecturer for her four hour lecture on dog and cat diseases at the C.L. Davis Foundation's annual Symposium on Gross Morbid Pathology of Diseases of Animals.

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**Dr. Pam Mouser**, ADDL Pathology Graduate Student, was recognized as the Outstanding Graduate Student Teacher in the SVM Department of Comparative Pathobiolgy.

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At the 2007 School of Veterinary Medicine Honors and Awards banquet:

**Dr. Dinesh Singh,** ADDL Graduate Student, was recognized as a Nominee for the National Phi Zeta Research Award in Basic Science

**Dr. Ingeborg Langohr,** Graduate Student, was awarded the Dennis Sikes Scholarship in Veterinary Pathobiology.

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**Dr. Langohr** also won first place for her research presentation "Experimental reproduction of Porcine Circovirus Associated Disease by co-infection of Germ-Free Pigs with Ruminant pestiviruses and Porcine Circoviruses type 2", at the annual North Central Conference for Veterinary Laboratory Diagnosticians held in Ames, Iowa, June, 2007.

Purdue ADDL and Heeke ADDL will be closed on the following University holidays in 2007.

July 4……...Independence Day
September 3……..……...Labor Day
November 22-23……..……...Thanksgiving
December 24-25……..Christmas
December 31, January 1…….……..New Year

## Cocklebur Toxicosis

**Introduction**: Many large animal veterinarians play an integral role in establishing preventative herd health protocols for their clients. These protocols should include recommendations to perform routine examinations of pasture and crop fields for unwanted and potentially toxic plant species, as well as timely and appropriate control measures. Cocklebur toxicosis results from ingestion of the dicotyledonary stage or seedling stage of the common cockleburs. Cocklebur are ubiquitous throughout North America and can be fatal when consumed by livestock via pasture, feed contamination with bur seeds, in hay, or while grazing crop residues. Cases of cocklebur poisoning have been reported from cattle, sheep, swine and poultry.

**Plant characteristics:** The common cocklebur belongs to the genus *Xanthium*, with the most common species in the Midwest being *Xanthium strumarium*. Cocklebur is an annual plant that grows in many soil types including floodplains, moist rich soils such as shorelines of ponds and reservoirs, and disturbed soils of feedlots. Variation in morphology is common among cocklebur species, and they grow to an approximate mature size of 3-5 feet (1-1.5 cm) and usually have dark brown to purple spotted, stout stems. The plants have large, rough, glandular, triangular to shovel-shaped leaves with three main veins from the leaf base and measure 2-14" (5-35.5 cm) long and 1-8" (2.5-20 cm) wide. The female flowers are larger than the male flowers, and form a 3/4" (1.5 cm) egg-shaped bur near the base of the plant. Each bur contains two seeds which can stay dormant in the soil for many years. Intoxications with cocklebur are most common in the spring and summer following periods of rain coupled with warmer temperatures that encourage germination of the seeds. Pigs, ruminants, and horses are all susceptible to cocklebur toxicosis, especially in the early spring following germination of seeds and grazing in fields where the highly palatable young plants may be present.

**Toxic principle:** Carboxyactractyloside (CAT), a sulfated glycoside, is the principle toxin in cocklebur plants. CAT is structurally similar to adenosine diphosphate and, therefore, inhibits ADP translocase on the cytosolic side of the mitochondrial membrane. Inhibition of ADP translocase activity leads to diminished transport of ADP into the mitochondria and, hence, decreased oxidative phosphorylation and ATO formation. CAT is concentrated in embryonic and cotyledonary tissues of the cocklebur plant, resulting in the seed and two leaf cotyledonary stage of the plant being the most poisonous. While the toxin remains potent when these portions of the plant are preserved in hay, CAT is not present in the four-leaf stage and mature plant. Witte et al reported death in 6 of 70 yearling calves that had been fed round bale hay of foxtail and mature cocklebur plants. Cocklebur seeds can also contaminate grains used in concentrate rations fed to livestock.

**Clinical signs:** Consumption of as little as 0.75% of body weight of cotyledonary portions can cause death, with clinical signs occurring a few hours post cocklebur sprout or seed ingestion.The most commonly reported clinical signs of cocklebur intoxication in pigs and cattle include depression, weakness, anorexia, reluctance to move, opisthotonus, ataxia, hyperexcitability, spasmodic muscular activity, and an unusual gait with erect ears and head held high. Recumbency, paddling of limbs, convulsions followed by coma, and death can occur within hours to days of consumption. Onset of clinical signs is usually delayed in cattle with a functional rumen as compared to non-ruminants.

**Pathology:** Burrows and Tyrl report that distinct clinical pathology changes include a ten-fold or greater increase in the serum concentration of the liver enzymes AST and SDH accompanied by a marked hypoglycemia to as low as 10 mg/dl, especially in calves. Grossly,serosanguinous ascites, proteinaceous pericardial and pleural effusions, a swollen and congested liver, edema of the gall bladder wall, hepatic capsule, and subserosa of the small intestine along with fibrin tags on visceral surfaces are commonly present.

The most characteristic histologic lesion is severe, diffuse, centrilobular hepatocellular necrosis. Other lesions include para-central hepatic necrosis, renal tubular necrosis, and inconsistent neuronal degeneration and cerebral edema.

Prior to discovery of the CAT toxin and its related signs, reported lesions from cocklebur consumption included oral ulcers and other mechanical injury along with excessive salivation. As long haired dogs, cattle and sheep groomed burs out of their coats, spines or other parts of the bur would break off and traumatize oral mucosa resulting in ulcers similar to those caused by grass awns. Additionally, the bur remnants could become embedded in granulation tissue, making visualization of the foreign particles difficult and potentiating subsequent distant draining wounds or abscesses.

**Diagnosis:** Diagnosis of cocklebur intoxication is based on the combination of evidence of consumption of cocklebur cotyledonary tissues and/or burs in stomach contents, clinical signs, characteristic histopathologic lesions, and clinical pathology findings. Conklin and Hooser both report that diagnostic tests, including assays for CAT such as thin layer chromatography (TLC) reported in literature, have not produced reliable, repeatable results and, thus, no practical diagnostic test for cocklebur intoxication exists at this time.

**Treatment:** Carboxyatractyloside is a potent toxin that disrupts cellular function, and no treatment exists to antagonize the ADP/ATP mitochondrial imbalance caused by CAT. Supportive treatments reported in the

literature, while helpful, are not curative and include increasing GI motility and clearance of the toxin with mineral oil and neostigmine, offering fatty substances with milk and lard to prevent absorption, and giving activated charcoal as an absorbent (not in concert with fatty substances). IV glucose can be administered to counteract the severe hypoglycemia. Use of neostigmine or physostigmine IM has been reported to temporarily alleviate muscular problems and, as such, can be a diagnostic aid.

Populations of *Xanthium* spp. plants should be eliminated with mowing, cultivation, and/or herbicide use to most effectively prevent cocklebur intoxication.

**Case Report:** In July 2003, a case of cocklebur toxicosis was diagnosed at ADDL in a herd of cattle in Indiana where 29 of 98 yearling calves died. While some animals were found dead, others exhibited depression, anorexia, muscle tremors, ataxia, aggression, became recumbent and died within a few hours of showing clinical signs. Lesions reported included a swollen liver with acute diffuse centrilobular necrosis. Many intact burs, along with partially ruminated corn shucklage, were present in the rumen. Histologically, marked centrilobular necrosis, hemorrhage and congestion were evident in the liver. Diagnosis of cocklebur intoxication was made based on evidence of exposure found at necropsy along with characteristic clinical signs, clinical pathology, and histologic findings in affected cattle. Cocklebur plants with burs had contaminated the primarily corn shucklage ration fed to the animals. The producer reportedly continued to feed this ration to the remaining animals; however, prior to feeding, placed large tube socks over his boots and walked through the bur-contaminated shucklage. If burs were collected via attachment to the socks, that portion of the feed was discarded.

**Sample collection with a suspected toxicosis:** Recall that if a toxicosis of any kind is suspected and the toxin/toxicant is unknown, it is important to obtain a complete history and perform a thorough physical examination and/or necropsy. If the animal is alive freeze and save all available vomitus and urine, 5 ml of whole blood in both red top clot and purple top EDTA tubes. Serum should be collected and stored in a plastic tube. At necropsy, fix representative tissues in 10% neutral buffered formalin. Save samples for virology, bacteriology or other ancillary tests if appropriate, and freeze and save the following tissues: liver (100g), stomach/rumen contents (100g or all available), brain (1/2 if organophosphate toxicosis is suspected), kidney (1/2), urine (all available), and an eyeball or ocular fluid (if nitrate toxicosis is suspected). A minimum of one pound of feed and one quart of water should also be submitted for toxicologic examination.

-by Emily Blough, Class of 2007
-edited by Dr. Dinesh Singh, ADDL Graduate Student

**References:**


1. Burrows GE and RJ Tyrl: 2001. Toxic Plants of North America. Iowa State Press, Ames, Iowa
2. Conklin B: (undated): FSO Project: A Year in Review. Development of New Thin Layer Chromatography (TLC) Testing Methods for Diagnosing Cocklebur Poisoning in Livestock. Obtained from Dr. Steve Hooser, Purdue ADDL Toxicologist, June, 2006.
3. Hooser SB: Fall, 2005. Applied Food Animal and Equine Toxicology Class Notes. CPB 518
4. Hooser SB: 2006. personal communication
5. Knight AP and RG Walter: 2001. A Guide to Plant Poisoning of Animals in North America. Teton NewMedia, Jackson, Wyoming
6. McGavin MD et al: 2001. Thomson's Special Veterinary Pathology, 3rd ed., St. Louis: Mosby
7. Isweuker GD: 1996. The National Veterinary medical Series for Independent Study: Toxicology. Williams and Wilkins, Philadelphia, PA
8. Purdue Extension: 2004. Forage Field Guide, West Lafayette, IN
9. Radostits OM et al: 2000. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 9th ed., W.B. Saunders, New York.
10. Smith BO: 2002. Large Animal Internal Medicine, 3rd ed, St. Louis, Mosby.
11. Stiles PI: 2003. Investigation of Multiple Deaths in a Beef Cattle Herd. Purdue University School of Veterinary Medicine Research Seminar, November 6.
12. Witte ST et al: 1990. Cocklebur toxicosis in cattle associated with the consumption of mature *Xanthium strumarium*. J Vet Diag Invest 2(4): 263-7.

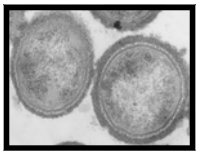

******PLEASE NOTE******

**Invoicing Changes**

In the first quarter of 2007, Purdue University launched the OnePurdue program. This business operations system was designed to unify the various legacy computer systems into a single entity better able to serve Purdue's employees, students, and customers. That's the upside.

The downside is that it also changed the look of ADDL invoices. We at ADDL do not have any control over this function and are working on a solution to give you the information you were accustomed to seeing on an invoice. In the near future, when a final report is issued, a "Billing Summary Report" will also be issued which will contain the line item details that were available to you in the previous invoice format. It will be sent in the same manner as the final report (fax, email, or US mail).

NOTE: What ADDL sends is not an invoice and is to be used for your information only. The actual invoice will still come from Purdue University.



## *Actinobacillus pleuropneumoniae in Swine*

*Actinobacillus pleuropneumoniae* (APP) causes respiratory disease in swine throughout the world. It has high morbidity and mortality rates and can cause severe economic losses to swine producers. APP severely damages the lungs of growing pigs and can linger on as a chronic infection, leading to poor weight gain and serving as a source of future outbreaks. Swine producers must rapidly control APP outbreaks and use preventative measures to control this disease.

*Actinobacillus pleuropneumoniae* is a non-motile, gram-negative coccobacillus respiratory pathogen. Two biovars of APP exist with biovar 1 containing 13 different serovars and biovar 2 containing 2 separate serovars. Serovars differ in the way they exert their pathogenic effects on the pig and the severity of infection that they induce. Although serovars 1,5 and 7 are reportedly the most common types of APP found in the United States, swine practitioners have noted all types of serovars infecting swine herds and some believe that there is not a single or predominant type of APP serovar seen most often in the field. Serovar 1 of biovar 1 is reported to be the most virulent type, and biovar 1 strains usually cause higher morbidity than biovar 2 strains, but any serovar of APP can cause significant damage to swine respiratory systems, especially if a secondary bacterial or viral disease exists.

APP outbreaks are generally seen from late fall to early spring when the environmental temperature greatly fluctuates or during harsh weather conditions. Growing pigs are most likely to be affected when they are 12-16 weeks old, but the disease can occur in all ages of swine. The stress of moving and mixing animals often causes the disease to break out among the subclinical pigs, and then the bacteria is passed directly from pig to pig or via aerosol over very short distances. Some vertical transmission from sow to piglet can occur, but it is not nearly as common as the direct contact from incoming infected animals. *Actinobacillus pleuropneumoniae* bacteria can survive in a mucus covered environment outside of the pig, but if it is exposed to the open, the bacteria are quickly killed by environmental conditions.

APP quickly colonizes the host animal, first by attaching to the epithelial cells of the tonsils and then moving to the lower respiratory tract where it creates the majority of damage. As the bacteria multiplies, it releases particles of an outer membrane that contain lipopolysaccharides (LPS) and cytotoxins. Neutrophils arriving as the beginning of an inflammatory response are attracted by the LPS and are then destroyed by the cytotoxins. As the neutrophils are destroyed, lysozymes are released, damaging nearby tissue. The tissue damage can progress rapidly, and some animals that are infected with APP will die within 4-12 hours of exposure. The LPS and cytotoxins not only help the bacteria damage the host cells, they also prevent the bacteria from being destroyed by impairing phagocytosis and complement activity.

The amount and severity of clinical signs shown by affected swine vary with dose of bacterial exposure during APP outbreaks, but all types of infection can be seen on one farm during any time of the outbreak. In general, lower doses of bacterial exposure lead to subclinical animals and higher doses of exposure cause clinical disease. Subclinical animals are often chronic carriers and most clinical cases are acutely affected, although some peracute infections occur. If an animal is peracutely affected, the most common sign is sudden death. However, some peracute infections manifest with septicemia-like signs, such as extreme hyperthermia, anorexia, mild diarrhea, cyanosis of extremities, and severe dyspnea. Acutely infected pigs also show hyperthermia, though not as extreme, congested extremities, depression, anorexia, dyspnea, coughing, open-mouth breathing, and condition loss. These pigs usually progress in disease severity and die a few days after the beginning of the clinical signs or resolve and become chronic carriers. The chronic carriers are the hardest to identify and are usually the cause of disease outbreaks. Some signs, such as decreased appetite, decreased weight gain, and exercise intolerance may be seen, but many chronic carriers show no obvious clinical signs and are only identified with testing during APP outbreaks.

Necropsies of acutely infected swine are the best way to diagnose an outbreak of *Actinobacillus pleuropneumoniae.* Pleuropneumonia that is fibrinohemorrhagic and necrotizing is usually seen bilaterally in the lungs. It is often focal and found in the caudal lung lobes, but it can be diffuse and may be seen in any or all parts of the lung lobes. The lungs may be darkened and consolidated, and pleural adhesions and abscesses are often found in chronic cases. Fibrin is commonly found throughout the surface of the lungs, and peracutely affected animals may have foamy, bloody exudate in the trachea and bronchi. When viewing the animal's clinical signs and gross lesions, additional differential diagnoses tend to include diseases that affect the respiratory system and show signs of septicemia such as *Actinobacillus suis*, *Haemophilus parasuis,* and *Salmonella cholerasuis.* Definitive diagnosis is usually confirmed with the aid of histopathology and bacterial cultures.

Microscopic examination of APP lesions usually shows severe fibrinohemorrhagic pneumonia that is often suppurative and necrotizing. Vasculitis, pleuritis, and bronchiolitis are often additional components of the disease. Neutrophils are seen more often in an early course of APP, while, later in the disease, macrophages are increased and fibrosis is more severe. APP is easily grown on blood agar with a *Staph epidermidis* nurse streak. NAD is needed for the growth of most of the serovars, but not for the biovar 2 types. The bacteria is most often cultured from affected lung samples, but it can be found in spleen

and liver samples as well.

Although a combination of clinical signs, gross observation of necropsy lesions, histopathology review, and bacterial culture is the most common way to diagnose *Actinobacillus pleuropneumoniae*, other tests can be performed, many of which are particularly useful at identifying subclinical carrier animals. ELISA, immuno-fluorescence, ring precipitation, coagglutination, latex agglutination, and counter immunoelectrophoresis are all tests that diagnostic labs perform to look for APP. PCR is frequently used to serotype the bacteria and is often required to determine which type or types of serovars are involved on each farm. The tests that are chosen for each farm are usually determined by the individual outbreak characteristics and the diagnostic lab's capabilities and resources.

Many options are available for treatment of APP, but best results are seen when the animals are treated immediately after the onset of clinical signs. If treatment is delayed, the number of chronic cases that develop is often increased and it becomes harder to eradicate the disease from the farm. Injectable antibiotics are the best way to treat *Actinobacillus pleuropneumoniae* because the infected swine are often anorectic and would not be able to consume adequate amounts of feed or water medications. An appropriate treatment regimen would be to treat all clinically ill pigs and all pigs that are in contact with the clinical pigs on the first day signs are observed, then treat only clinically ill pigs on subsequent days. Even with treatment, producers should except high morbidity and mortality.

Outbreaks of *Actinobacillus pleuropneumoniae* cause severe economic losses to swine operations. Not only do the producers have to deal with the mortality and cost due to increased growth rates, they also must calculate the cost of vaccines, medications, and losses at slaughter from infection site abscesses or pleural adhesions. Prevention is the best way to avoid the effects of APP infection and many of the methods used to prevent this disease are also effective at preventing other disease outbreaks. Strict biosecurity measures are the best way to avoid introduction of APP, including isolation of incoming animals and purchase of stock from APP-free herds. Management and environmental conditions should be optimized to avoid stressing the pigs whenever possible. Mild APP infections can be controlled with medicated feed, but these treatments usually only help eliminate residual infections and cannot be used to treat in the face of an outbreak. Vaccines are available for APP, but many cause side effects such as infection site swelling and abscesses, pyrexia and lethargy. The best method for prevention is to employ biosecurity and management practices to control the introduction of the bacteria.

*Actinobacillus pleuropneumoniae* can cause severe disease in a swine herd and lead to significant financial difficulty of a swine operation if an outbreak occurs. The disease is readily diagnosed by veterinarians and diagnostic labs and can be treated. However, preventative measures should be put in place before an outbreak occurs so that financial losses are minimized.

-by Betsy Brownfield, Class of 2007
-edited by Dr. Pam Mouser, ADDL Graduate Student

**References:**


1. Dr. Karen Lehe, Swine Veterinary Practitioner, personal communication
2. Farruggio et al. Diagnosis and Treatment of *Actinobacillus pleuropneumoniae* in Swine: An Update. Article from swine resource folder compiled by Dr. Sandy Amass and Dr. Darryl Ragland, Purdue University Production Medicine
3. Fedorka Cray P et al: 1993. *Actinobacillus pleuropneumoniae* Part 1—History, Epidemiology, Serotyping and Treatment. The Compendium 15 (10): 1447-1452.
4. Gottschalk M and Taylor D: 2006.*Actinobacillus pleuropneumoniae*. Diseases of Swine. Blackwell Publishing, Ames, IA 563-575.
5. Marstellar T and Fenwick B: 1999. *Actinobacillus pleuropneumoniae*. Disease and Serology. Swine Health Production 7(4): 161-165.
6. Veterinary Clinical Sciences 510 notes. Swine Production Medicine, Fall 2005.



**Mary Woodruff,** Virology Supervisor, attending the Association of Veterinary Microbiologists annual meeting in Madison, WI, April 2007.

**Dr. Roman Pogranichniy,** Head of Virology/Serology, traveled to the Czech Republic to establish a cooperative program and collaborations with the Veterinary School.

**Dr. Roman Pogranichniy,** was invited to participate in the National Pork Board PRRS genetic resistance/ susceptibility Task Force meeting in Des Moines, IA, May, 2007

**Drs. Roman Pogranichniy, Ingeborg Langohr,** and **Hueling Wei** attended the North Central Conference for Veterinary Laboratory Diagnosticians in Ames, Iowa, June, 2007

% of Micro-organisms Resistant to Selected Antibiotics from July-Dec. 2006 and Jan.-June 2007. Data provided by the Bacteriology lab, ADDL, Purdue University

| Antibiotic | Beef | | | | | | | | Dairy | | | | | | | | | | Swine | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | E. coli | | Man. haemolitica | | Past. multocida | | Salmonella sp. | | E. coli | | Man. haemolitica | | Past. multocida | | Staph. aureus | | Salmonella sp. | | Haemophilus sp. | | E. coli | | Salmonella sp. | | Strep. suis | |
| | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. |
| Ampicillin | 18 | 44 | 33 | 0 | 25 | 0 | nt | 100 | 60 | 59 | 33 | 50 | 0 | 20 | 66 | 66 | 64 | 29 | 16 | 2 | 63 | 56 | 40 | 60 | 4 | 6 |
| Ceftiofur | 0 | 15 | 0 | 0 | 25 | 17 | nt | 100 | 34 | 43 | 0 | 0 | 0 | 0 | 9 | 0 | 50 | 29 | 0 | 2 | 28 | 22 | 23 | 40 | 2 | 4 |
| Chlortetracycline | 27 | 65 | 0 | 0 | 0 | 17 | nt | 100 | 82 | 79 | 0 | 0 | 0 | 0 | nt | 0 | 64 | 29 | 2 | 5 | 93 | 76 | 80 | 73 | 76 | 86 |
| Clindamycin | 100 | 100 | 100 | 100 | 100 | 100 | nt | 100 | 100 | 100 | 100 | 0 | 100 | 100 | nt | 0 | 100 | 100 | 25 | 38 | 99 | 100 | 100 | 100 | 90 | 88 |
| Enrofloxacin | 0 | 8 | 0 | 0 | 0 | 17 | nt | 100 | 20 | 14 | 0 | 0 | 0 | 0 | nt | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 5 |
| Erythromycin | 100 | 100 | 33 | 0 | 100 | 50 | nt | 100 | 100 | 100 | 33 | 0 | 67 | 0 | 0 | 8 | 100 | 100 | 0 | 17 | 1 | 100 | 0 | 100 | 1 | 86 |
| Florphenicol | 27 | 47 | 0 | 50 | 50 | 17 | nt | 100 | 80 | 62 | 0 | 0 | 33 | 0 | nt | 0 | 80 | 29 | 20 | 4 | 98 | 47 | 97 | 40 | 85 | 2 |
| Gentamicin | 0 | 12 | 0 | 0 | 25 | 33 | nt | 100 | 54 | 36 | 0 | 75 | 0 | 0 | nt | 0 | 4 | 7 | 0 | 4 | 99 | 12 | 93 | 0 | 54 | 7 |
| Neomycin | 0 | 18 | 67 | 50 | 75 | 17 | nt | 100 | 73 | 61 | 33 | 75 | 100 | 60 | nt | 0 | 56 | 7 | 0 | 0 | 15 | 38 | 13 | 7 | 6 | 25 |
| Oxytetracycline | 45 | 71 | 67 | 50 | 75 | 33 | nt | 100 | 85 | 86 | 33 | 25 | 100 | 60 | nt | 0 | 68 | 29 | 14 | 21 | 53 | 77 | 17 | 73 | 27 | 95 |
| Penicillin | 100 | 100 | 33 | 0 | 100 | 100 | nt | 100 | 100 | 100 | 33 | 50 | 100 | 100 | 100 | 100 | 100 | 100 | 34 | 100 | 95 | 100 | 90 | 100 | 86 | 18 |
| Spectinomycin | 36 | 35 | 100 | 0 | 50 | 50 | nt | 100 | 82 | 69 | 100 | 100 | 100 | 40 | nt | nt | 100 | 64 | 50 | 23 | 99 | 61 | 100 | 100 | 9 | 24 |
| Sulphachloropyridazine | 9 | 45 | 0 | 0 | 0 | 0 | nt | 100 | 80 | 83 | 0 | 0 | 33 | nr | nt | 47 | 71 | 0 | 27 | 0 | 82 | 45 | 87 | 33 | 61 | 59 |
| Sulphadimethoxine | 9 | 60 | 33 | 50 | 50 | 50 | nt | 100 | 71 | 63 | 0 | 25 | 67 | 4 | 85 | 50 | 84 | 93 | 42 | 18 | 57 | 69 | 93 | 93 | 16 | 69 |
| Sulphathiazole | 9 | 50 | 0 | 0 | 50 | 50 | nt | 100 | 84 | 83 | 0 | 0 | 67 | nr | nt | 0 | 80 | 0 | 16 | 17 | 85 | 51 | 80 | 67 | 65 | 68 |
| Tiamulin | 63 | 90 | 33 | 0 | 50 | 0 | nt | 100 | 95 | 97 | 0 | 0 | 0 | 0 | nt | 0 | 100 | 100 | 59 | 0 | 79 | 99 | 77 | 100 | 63 | 14 |
| Tilmicosin | 100 | 100 | 33 | 50 | 100 | 50 | nt | 100 | 100 | 100 | 33 | 0 | 67 | 20 | nt | 0 | 100 | 100 | 33 | 7 | 99 | 100 | 100 | 100 | 25 | 89 |
| Triple Sulfa | 0 | 22 | 0 | 0 | 0 | 0 | nt | 100 | 67 | 51 | 0 | 0 | 0 | 0 | nt | 0 | 28 | 14 | 3 | 4 | 96 | 20 | 100 | 13 | 86 | 5 |
| Tylosin | 100 | 100 | 100 | 100 | 100 | 75 | nt | 100 | 100 | 100 | 100 | 100 | 100 | 100 | nt | 0 | 100 | 100 | 2 | 67 | 27 | 100 | 17 | 100 | 2 | 91 |
| number of isolates | 11 | 52 | 3 | 2 | 4 | 6 | 0 | 1 | 76 | 87 | 3 | 4 | 3 | 10 | 13 | 12 | 25 | 14 | 64 | 56 | 175 | 131 | 30 | 15 | 160 | 84 |

nt - not tested

% of Micro-organisms Resistant to Selected Antibiotics from July-Dec. 2006 and Jan.-June 2007. Data provided by the Bacteriology lab, ADDL, Purdue University

| Antibiotic | Canine | | | | | | | | | | Equine | | | | | | | | | | | | Feline | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | E. Coli | | Enterococcus sp. | | Psc. acruginosa | | Staph. aureus | | Staph. intermedius | | E. Coli | | Salmonella sp. | | Staph. aureus | | Staph. epidermidis | | Strep. equi | | Strep. zooepidemicus | | E. Coli | | Enterococcus sp. | | Psc. aeruginosa | | Staph. aureus | |
| | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. | Jan.-June | July-Dec. |
| Amikacin | 1 | 3 | 63 | 58 | 0 | 7 | 0 | 5 | nt | 0 | 0 | 5 | 0 | 0 | 11 | 50 | 0 | 3 | 0 | 50 | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 |
| Amoxycillin/Clauvulinic acid | 26 | 29 | 4 | 32 | 100 | 100 | 11 | 9 | nt | 0 | 10 | 11 | 0 | 0 | 22 | 0 | 37 | 24 | 50 | 0 | 0 | 0 | 6 | 14 | 16 | 0 | 100 | 100 | 0 | 0 |
| Ampicillin | 53 | 55 | 4 | 32 | 100 | 100 | 65 | 63 | nt | 0 | 35 | 25 | 28 | 0 | 57 | 100 | 62 | 35 | 50 | 0 | 0 | 0 | 39 | 29 | 16 | 100 | 100 | 100 | 50 | 100 |
| Cefazolin | 33 | 33 | 74 | 79 | 100 | 100 | 11 | 9 | nt | 0 | 15 | 14 | 14 | 0 | 22 | 0 | 37 | 24 | 0 | 0 | 0 | 0 | 8 | 7 | 50 | 100 | 100 | 100 | 0 | 0 |
| Cefotaxime | 29 | 32 | 82 | 84 | 100 | 100 | 14 | 9 | nt | 0 | 10 | 11 | 0 | 0 | 33 | 0 | 37 | 17 | 0 | 0 | 0 | 0 | 13 | 7 | 83 | 100 | 100 | 100 | 0 | 0 |
| Cefpodoxime | 29 | 27 | 89 | 90 | 100 | 100 | 12 | 11 | nt | 0 | 10 | 14 | 0 | 0 | 22 | 0 | 37 | 28 | 0 | 0 | 0 | 0 | 6 | 7 | 66 | 100 | 100 | 100 | 0 | 0 |
| Ceftiofur | 27 | 26 | 76 | 74 | 100 | 100 | 10 | 9 | nt | 0 | 5 | 9 | 0 | 0 | 22 | 0 | 28 | 28 | 0 | 0 | 0 | 0 | 8 | 7 | 0 | 100 | 100 | 100 | 0 | 0 |
| Cephalothin | 40 | 39 | 70 | 84 | 100 | 100 | 9 | 9 | nt | 0 | 15 | 16 | 14 | 0 | 22 | 0 | 25 | 17 | 0 | 0 | 0 | 0 | 13 | 7 | 60 | 100 | 100 | 100 | 0 | 0 |
| Chloramphenicol | 19 | 16 | 11 | 11 | 83 | 83 | 2 | 0 | nt | 0 | 15 | 11 | 28 | 0 | 0 | 0 | 12 | 7 | 0 | 0 | 0 | 0 | 6 | 14 | 50 | 0 | 100 | 50 | 0 | 0 |
| Clindamycin | 100 | 100 | 82 | 83 | 100 | 100 | 22 | 31 | nt | 0 | 100 | 100 | 100 | 100 | 11 | 0 | 62 | 48 | 50 | 0 | 0 | 0 | 100 | 100 | 0 | 100 | 100 | 100 | 0 | 0 |
| Enrofloxacin | 33 | 27 | 19 | 37 | 33 | 35 | 5 | 11 | nt | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 10 | 50 | 100 | 6 | 0 | 0 | 7 | 100 | 100 | 0 | 50 | 0 | 0 |
| Erythromycin | 92 | 100 | 56 | 37 | 100 | 100 | 20 | 30 | nt | 0 | 85 | 100 | 100 | 100 | 22 | 0 | 62 | 41 | 50 | 0 | 6 | 12 | 93 | 100 | 33 | 100 | 100 | 40 | 0 | 0 |
| Gentamicin | 24 | 23 | 26 | 37 | 8 | 7 | 6 | 7 | nt | 0 | 15 | 18 | 14 | 0 | 22 | 50 | 25 | 17 | 0 | 0 | 0 | 0 | 3 | 7 | 33 | 0 | 0 | 0 | 0 | 0 |
| Imipenem | 1 | 0 | 4 | 32 | 0 | 3 | 34 | 53 | nt | 0 | 0 | 0 | 0 | 0 | 22 | 50 | 37 | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 100 | 0 | 0 | 33 | 100 |
| Marbofloxacin | 32 | 27 | 8 | nr | 0 | nr | 5 | 11 | nt | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 7 | 17 | nr | 0 | nr | 0 | nr |
| Orbifloxacin | 33 | 27 | 7 | 37 | 33 | 41 | 5 | 9 | nt | 0 | 0 | nr | 0 | 0 | 0 | 0 | 0 | nr | 0 | nr | 0 | nr | 0 | 7 | 33 | 100 | 100 | 50 | 0 | 0 |
| Oxacillin + 2% NaCl | 99 | 99 | 93 | 90 | 100 | 100 | 10 | 9 | nt | 0 | 100 | 100 | 100 | 100 | 22 | 0 | 37 | 17 | 50 | 0 | 0 | 0 | 100 | 93 | 0 | 100 | 100 | 100 | 0 | 0 |
| Penicillin | 100 | 100 | 7 | 32 | 100 | 100 | 97 | 100 | nt | 0 | 100 | 100 | 100 | 100 | 11 | 100 | 71 | 35 | 50 | 0 | 0 | 0 | 100 | 100 | 83 | 100 | 100 | 100 | 100 | 100 |
| Rifampin | 94 | 96 | 4 | 26 | 100 | 100 | 5 | 2 | nt | 0 | 100 | 98 | 100 | 100 | 0 | 0 | 12 | 0 | 50 | 0 | 6 | 0 | 84 | 86 | 50 | 100 | 100 | 100 | 0 | 0 |
| Tetracycline | 39 | 38 | 44 | 42 | 92 | 100 | 29 | 28 | nt | 0 | 35 | 23 | 14 | 0 | 22 | 50 | 25 | 35 | 0 | 0 | 47 | 8 | 13 | 14 | 33 | 100 | 100 | 100 | 0 | 0 |
| Ticarcillin | 46 | 52 | 4 | 32 | 17 | 7 | 44 | 17 | nt | 0 | 25 | 23 | 28 | 0 | 57 | 0 | 37 | 17 | 0 | 0 | 0 | 0 | 39 | 29 | 33 | 100 | 100 | 0 | 50 | 0 |
| Ticarcillin/Clavulanic Acid | 20 | 23 | 4 | 32 | 8 | 7 | 9 | 9 | nt | 0 | 0 | 2 | 14 | 0 | 22 | 0 | 37 | 17 | 0 | 0 | 0 | 0 | 3 | 7 | 17 | 100 | 0 | 0 | 0 | 0 |
| Trimethoprim/Sulphamethoxazole | 30 | 36 | 11 | 16 | 92 | 93 | 17 | 5 | nt | 0 | 65 | 34 | 14 | 0 | 33 | 50 | 12 | 17 | 0 | 0 | 0 | 0 | 10 | 14 | 16 | 100 | 100 | 50 | 0 | 0 |
| number of isolates | 147 | 128 | 27 | 19 | 12 | 29 | 65 | 57 | 0 | 2 | 20 | 44 | 7 | 4 | 9 | 2 | 8 | 29 | 2 | 2 | 17 | 25 | 35 | 14 | 6 | 1 | 1 | 2 | 3 | 3 |

nt - not tested

## Diagnostic Forum

**DIAGNOSTIC FORUM is a quarterly newsletter of current services, regulations and research projects involving the ADDL which may be of interest to Indiana veterinarians and animal owners. It is our intention that the information provided will serve you. Please send your comments, suggestions, requests and questions to: Diagnostic Forum Editor, Animal Disease Diagnostic Laboratory, 406 S. University, Purdue University, West Lafayette, IN 47907.**

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Animal Disease Diagnostic Laboratory
406 S. University St.
Purdue University
West Lafayette, IN 47907

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